



AALBORG UNIVERSITY
DENMARK

Aalborg Universitet

Investigations on tympanometric determination of middle ear pressure

Gaihede, Michael

Publication date:
2014

Document Version
Early version, also known as pre-print

[Link to publication from Aalborg University](#)

Citation for published version (APA):
Gaihede, M. (2014). Investigations on tympanometric determination of middle ear pressure: PhD thesis. Aalborg Universitetshospital.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- ? Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- ? You may not further distribute the material or use it for any profit-making activity or commercial gain
- ? You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

Investigations on tympanometric determination of middle ear pressure

PhD thesis

Michael Gaihede, MD

Department of Otolaryngology, Head & Neck Surgery,
Aalborg University Hospital and
Department of Clinical Medicine, Faculty of Health Sciences,
Aalborg University

Investigations on tympanometric determination of middle ear pressure
PhD thesis

Michael Gaihede, MD
Department of Otolaryngology, Head & Neck Surgery,
Aalborg University Hospital and
Department of Clinical Medicine, Faculty of Health Sciences,
Aalborg University

ISBN 978-87-90880-50-7

To Mathilde, Kathrine, and my parents

Denne afhandling er antaget til forsvar for Ph.D. graden ved Det Sundhedsvidenskabelige Fakultet, Aalborg Universitet, fredag den 6. juni 2014.

Supervisor

Prof. Asbjørn M Drewes, MD, DMSc, PhD,
Department of Medical Gastro-Enterology and Mech-Sense
Aalborg University Hospital
Aalborg, Denmark

Evaluation committee

Associate Prof. Jens B Frøkjær, MD, PhD (chairman)
Department of Radiology
Aalborg University Hospital
Aalborg, Denmark

Prof. Preben Homøe, MD, DMSc, PhD,
Department of Otolaryngology and Maxillofacial Surgery
Køge University Hospital
Køge, Denmark

Prof. Magnus von Unge, MD, PhD
Department of Otorhinolaryngology
Akershus University Hospital
Oslo, Norway

Copyrights

Illustrations and tables in this thesis originating from the original papers and other sources have been reused by permissions from the publishers: Wolters Kluwer Health – Lippincott, Williams & Wilkins; Elsevier; and Springer.

List of papers

This thesis was based on the following papers:

- I. Gaihede M, Kjær D. Positional changes and stabilization of middle ear pressure. *Auris Nasus Larynx* 1998;25:255-259.

Investigations on changes in *body position* and the resulting *time-dependent changes* in middle ear pressure including definition of point of repeatability or “*steady state*”.

- II. Gaihede M. Middle ear volume and pressure effects on tympanometric middle ear pressure determination: Model experiments with special reference to secretory otitis media. *Auris Nasus Larynx* 2000;27:231-239.

Investigations on the influence of *middle ear volume and pressure* on tympanometric determination of ME pressure.

- III. Therkildsen AG, Gaihede M. Accuracy of tympanometric middle ear pressure determination. The role of direction and rate of pressure change with a fast modern tympanometer. *Otology & Neurotology* 2005;26:252-6.

Investigations on the *direction and rate of pressure changes* on tympanometric determination of ME pressure.

- IV. Gaihede M, Bramstoft M, Thomsen LT, Fogh A. Accuracy of tympanometric middle ear pressure determination. Dose-dependent overestimation related to the viscosity and amount of middle ear effusion. *Otology & Neurotology* 2005;26:5-11.

Investigations on the influence of *middle ear effusion's viscosity and amount* on tympanometric determination of middle ear pressure.

Preface

The foundation of this thesis was formed by a series of biomechanical experiments of the middle ear system conducted predominantly during the 1990'ies. These biomechanical experiments investigated the viscoelastic properties of the middle ear system, and the interpretations of the results were highly dependent on the definition of the neutral position of the tympanic membrane. The neutral or resting position of the tympanic membrane is assumed to be at the position, where the ear canal pressure is identical to the middle ear pressure, and thus, the results were susceptible to the prevailing middle ear pressure. Hence, the biomechanical experiments required determination of the middle ear pressure which was determined by tympanometry. However, this also lead to a focus on more limitations in middle ear pressure determination which are inherited by tympanometry, and it turned out that the biomechanical experiments could be used to analyse these limitations. In diseased middle ears underpressures are often present, and these pressures are determined primarily by tympanometry in both clinical and basic research. Thus, tympanometric limitations have a broader interest and importance than only related to the biomechanical experiments.

During my later clinical work training for otosurgery, it became increasingly clear that middle ear underpressure is an immensely important factor affecting the majority of our otosurgical patients; this comprises both primary cases presenting with sequelae from underpressures, as well as the post-operative courses of patients after otosurgical reconstructions. The overall regulation of middle ear pressure is basically still unknown, but measurements of the exact pressures are prerequisite for an improved understanding of these mechanisms. This problem can be approached by the introduction of more reliable methods for accurate measurements as well as by the analysis of tympanometric limitations.

Thus, there has been a constant clinical inspiration to investigate methods for measurements of middle ear pressure as well as the basic and clinical aspects of middle ear pressure regulation. During the course of my work these aspects has become a continued focus including physiological, neurophysiological, epidemiological, radiological, and histological investigations as well as clinical research. The current thesis focuses on a series of problems related to tympanometric determination of middle ear pressure and its importance in basic research.

Michael Gaihede, January 2014

Acknowledgements

I am much indebted to Jens Ulrik Felding, MD, DMSc, for his introduction to middle ear biomechanics and otitis media research. The origin of the previous biomechanical experiments have been based on Jens's attempt to assess accurately the mechanical sequelae of secretory otitis media, i.e., the consequences atrophy and myringosclerosis on the tympanic membrane mechanics and hearing. This early introduction has subsequently been followed by Jens' continuous encouragement and inspiring discussions during the entire range of my research over the years whenever my studies have taken new directions.

From the early start encouragement and support was also given by late Professor Ole Elbrønd, MD, DMSc, previous Chairman of Otolaryngology – Head and Neck Surgery, Aarhus University Hospital, who himself had an interest in the mechanical properties of the middle ear system. During my later specialist training, Finn Rasmussen, MD, previous Chairman of Otolaryngology, Holstebro Hospital, also provided valuable support.

After completion of my specialist training continued encouragement and support has been given by Jørn Rosborg, MD, Kjell Tveterås, MD, and Karin Lambertsen, MD, previous Chairmen of Otolaryngology – Head and Neck Surgery, Aalborg University Hospital; latest Henrik Jacobsen, MD, Chairman of Otolaryngology – Head and Neck Surgery, Aalborg University Hospital, has also supported my work by providing the highly valuable and indispensable time for research along with the clinical work.

In particular I am much obliged to my fellow partner in otosurgery, Kjell Tveterås, MD, who has endured the most during my periods of absence from clinical work as well as he is acknowledged for sharing his profound clinical experience with me during many valuable discussions; especially whenever the latest ideas may have become too vivid, his sharp and sound judgements have often been appreciated by bringing back the focus to clinical realities.

Acknowledgements are also attributed especially to my previous colleagues, who participated directly in the works included in this thesis. Dorthe Kjær, MD, Anette G Therkildsen, MD, Lene T Thomsen, MD, Mads Bramstoft, MD, and Aksel Fogh, MD, provided valuable contribution to these papers. In particular, Aksel provided the essential access to the many patients with secretory otitis media in his ENT-practice in Hjørring.

In addition, my many other colleagues are acknowledged for their patience during the daily work and during my periods of absence. The benefits from both basic and clinical research conducted by clinicians relate to its inspiration from and proximity to problems relevant for our patients; however, it also demands many challenging and daily compromises between the clinical and the scientific work. The extent of the current studies and all the other studies forming both their backgrounds as well as our proceeding investigations would not have been possible without the understanding and acceptance from my colleagues.

For a continuous encouragement and substantial support during the later years while finalising this thesis, I must express my sincere gratitude to Professor Asbjørn Mohr Drewes, MD, DMSc, PhD, Department of Medical Gastro-Enterology and Mech-Sense, Aalborg University Hospital; this support has been crucial for the final compilation of the aspects of my research which constitute this thesis. During this period I am also grateful for the support and fellowships provided by Professor Lars Hvilsted Rasmussen, MD, PhD, Chairman of Clinical Institute, Aalborg University. My old friend, Jan Nørgaard, MD, DMSc, PhD, is acknowledged for his contribution of the final proof reading and valuable suggestions.

Finally, I must express my gratitude to all those patients and colleagues, who took part over the years by lending their ears to my research; these investigations have not been possible without their participation.

In many of the promising ongoing and new studies extending from the current papers, I am much indebted to my new group of younger researchers involved in both basic and clinical studies: Olivier Cros, MSc, Simona Padurariu, MSc, and Suzan Al Kole, MD. In addition, the ongoing works of Mikkel A Bruhn, MD, Janus BB Jespersen, MD, Chris L Jacobsen, MD, and Pernille F Jensen, MD, are appreciated.

Further, in the ongoing studies I am also much indebted to our external collaborations in the different fields of research:

- Professor Magnus Borga, tech.dr., Professor Hans Knutsson, tech.dr., and Research Engineer Mats Andersson, tech.dr., Department of Biomedical Engineering, Linköping University Hospital.

- Professor Dr. Joris JJ Dirckx, Laboratory of Biomedical Physics, Antwerp University.
- Dr.Ir. Manuel Dierick, assistant professor and Elin Pauwels, MD, Department of Physics and Astronomy, Ghent University.
- Professor Alexander Huber, dr. med. and Christof Rösli, dr.med., Department of Otorhinolaryngology, Head & Neck Surgery, Zurich University Hospital.
- Professor Asbjørn M Drewes, MD, DMSc, PhD, Department of Medical Gastro-Enterology and Mech-Sense, Aalborg University Hospital.
- Anja Brüggman, MD, PhD and Giedrius Lelkaitis, MD, Institute of Pathology, Aalborg University Hospital.
- Jens Brøndum Frøkjær, MD, PhD and Yousef Yavarian, MD, Department of Radiology, Aalborg University Hospital.
- Rikke B Nielsen, MSc, PhD and Mette Nørgaard, MD, PhD, Department of Clinical Epidemiology, Aalborg University Hospital and Århus University Hospital.
- Jens H Wanscher, MD and Christian Faber, MD, PhD, Department of Otolaryngology, Head & Neck Surgery, Odense University Hospital
- Jesper Carl, medical physicist, PhD, Department of Oncology, Aalborg University Hospital

I hope we can continue the projects together in the future in order to benefit from the advantages of a multidisciplinary approach to middle ear research which altogether aims at the improved understanding and treatments of our many patients with middle ear diseases and hearing impairments.

Michael Gaihede, January 2014

Funding sources

- Region Nordjyllands Sundhedsvidenskabelige Forskningsfond, 2010.
- Forskningsinitiativet, Region Nord, 2008.
- Forskningsadministrationen, Aalborg Universitetshospital, 2006.
- Oticon Fonden, 2000.
- Fonden for Lægevidenskabelig Forskning i Ringkøbing, Ribe og Sønderjyllands Amter, 1997.
- Statens Lægevidenskabelig Forskningsråd, 1996.

Abbreviations

- ET = Eustachian tube
- ME = middle ear
- OM = otitis media
- PPD = peak pressure difference
- PVR = pressure-volume-relationship
- SOM = secretory otitis media
- TM = tympanic membrane
- VT = ventilation tube

- P_{ec} = ear canal pressure
- P_m = middle ear pressure
- V_m = middle ear volume (tympanum + mastoid)
- ΔV_{muc} = change in volume of the middle ear mucosa
- ΔV_{tm} = change in volume by displacements of the TM

Pressure units

- $1000 \text{ Pa} = 10 \text{ daPa} = 1 \text{ kPa}$
- $1 \text{ mmH}_2\text{O} = 0.98 \text{ daPa}$; or $1 \text{ daPa} = 1.02 \text{ mmH}_2\text{O}$
- $1 \text{ mm Hg} = 13.6 \text{ mmH}_2\text{O} = 133 \text{ Pa}$

Contents

List of papers	iii
Preface	iv
Acknowledgements.....	v
Abbreviations	viii
Pressure units	viii
Contents.....	ix
1. Introduction	1
1.1 Physiology of pressure regulation	4
1.2 Clinical aspects of ME underpressures.....	6
1.3 Measurements of ME pressure.....	9
1.3.1 History of tympanometry	10
1.3.2 Basic principles of tympanometry	12
1.3.3 Tympanometric pitfalls	13
1.3.4 Monitoring ME pressure by tympanometry	17
1.4 Hypotheses.....	18
2. Aims.....	19
3. Methods and Materials.....	20
3.1 Monitoring positional changes in ME pressure (Paper I)	20
3.2 Middle ear modelling (Paper II).....	20
3.3 Peak pressure difference with a modern tympanometer (Paper III)	22
3.4 Peak pressure difference in patients with ME fluid (Paper IV)	22
3.5 Statistical notes.....	22
4. Results.....	24
4.1 Monitoring positional changes in ME pressure (Paper I)	24
4.2 Middle ear modelling (Paper II).....	25
4.3 Peak pressure difference with a modern tympanometer (Paper III)	26
4.4 Peak pressure difference in patients with ME fluid (Paper IV)	26
5. Discussion.....	29
5.1 Monitoring of middle ear pressures.....	29
5.2 Modelling experiments.....	34
5.3 Peak pressure difference.....	38
5.4 The "true" middle ear pressure and pathogenetic events	40
5.5 Middle ear pressure and otosurgery.....	44
6. Future studies	47
7. Conclusions.....	53
8. Summary in English	55
9. Summary in Danish / Resumé på dansk	57
10. Referencer	59
11. Appendices Papers I - IV.....	65

PROSPERO: - *Dost thou hear?*

MIRANDA: - *Your tale, Sir, would cure deafness!*

"The Tempest" by William Shakespeare, 1610-11.

1. Introduction

The hearing is one of the most important senses in humans, and diseases in the ear as well as their treatments represent an important field in the medical sciences in order to preserve good hearing in patients. The peripheral parts of the hearing system consist mainly of the ear canal, the tympanic membrane (TM), the ossicles, and the middle ear (ME) cavity (the tympanum and the mastoid); the basic function of these structures is sound transmission and impedance matching, so that the air borne sound pressures from the outside are effectively transferred to the fluid filled inner ear.

Diseases of the ear can result in a variety of structural and functional changes that may result in hearing impairment; however, one immensely important aspect of ME physiology is the overall regulation of the ME pressure. In a normal ear the pressure of the ME cavity is equal or close to ambient pressure, so that no pressure gradient exists across the TM, and the sound transmission is optimal (Shanks & Shelton, 1991; Sadé & Ar, 1997; Doyle, 2000). In diseased ears, however, underpressures are frequently encountered leading to a series of ME conditions resulting in decreased hearing and demand for otosurgical procedures. In order to understand the overall mechanisms of ME pressure regulation in both normal and diseased ME's measurements of these pressures are of evident importance. The vast majority of both clinical and basic studies on ME pressure have been based on measurements by tympanometry.

The origin of the research papers included in this thesis has been based on a previous series of biomechanical experiments of the ME system in a clinical setting, where the pressure-volume-relationship (PVR) has been recorded. This comprises recordings of the pressure changes in the ear canal (P_{ec}) as a function of a cyclic bidirectional volume displacement of the TM (ΔV_{tm}) (Felding et al., 1995; Gaihede et al., 1995a,b; Gaihede et al., 1997; Gaihede, 1999a,b), i.e.,

$$P_{ec} = f(\Delta V_{tm}).$$

A PVR recording from a normal ear is illustrated in Fig. 1, where its biomechanical variables also have been explained. Based on its relationship to a similar tradition of biomechanical studies from Lund in the 1970'ies (Ingelstedt et al., 1967; Elner et al., 1971a,b), where the volume-pressure-relationship of the ME system has been investigated (i.e., $V_{tm} = f(P_{ec})$), the

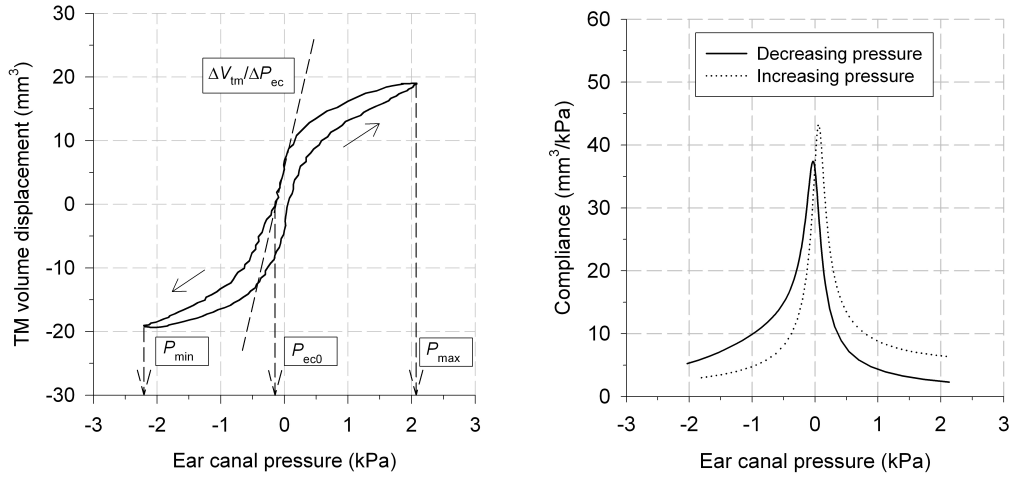


Fig. 1. Left panel: The PVR of a normal ME system, where the ear canal pressure changes is shown as a function of volume displacement of the TM; the arrows illustrate the direction of the cyclic pressure changes. The PVR shows a non-linear relationship with hysteresis. The variables determined for the recording are: 1) the hysteresis determined by the energy equivalent of the circumscribed area (μJ); 2) the compliance determined by the maximal slope of the tangential line at volume displacement = 0 mm^3 (mm^3/kPa); 3) the pressure range determined by the difference between the maximal and the minimal ear canal pressure ($P_{\max}-P_{\min}$)(kPa); and 4) the P_{ec0} determined by the ear canal pressure at the neutral position of the TM = ΔV_{tm} (kPa).
Right panel: The derived PVR functions: Compliance as a function of the ear canal pressure; the maximal compliance is found close to an ear canal pressure around 0 kPa.

PVR has been illustrated with the dependent variable (P_{ec}) on the x-axis, and the independent variable (ΔV_{tm}) on the y-axis (Fig. 1). These experiments also exhibit an important and close relationship to tympanometry which is illustrated by the derived functions of the volume-pressure-relationship (Fig. 1), i.e.,

$$dV_{tm}/dP_{ec} = f(P_{ec}).$$

These derived functions describe the compliance of the ME system as a function of the P_{ec} , and thus, they describe similar properties and appearances as tympanometric recordings. The close relationship between the PVR method and tympanometry is explained in more details below and later during the outlining of tympanometric principles and pitfalls. An overview of the anatomical structures and a definition of the ME system are depicted in Figure 2.

The biomechanical PVR experiments in human subjects were all preceded by tympanometry and this method became important for several reasons. *First*, it provided a pressure testing of

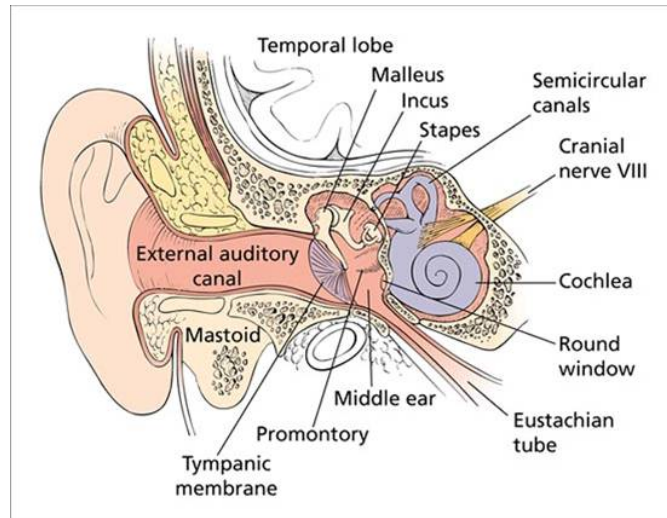


Fig. 2. Diagram of the normal ME with annotation of structures. The *ME system* is defined by the entity of the TM and the ME comprising the ossicles (malleus, incus stapes) including their ligaments and muscles, and the stapes footplate's connection to the oval window. The ME contains both the tympanum and the mastoid air cell system, and the ME volume as well as the prevailing ME pressure form part of the ME system.

the TM ensuring its normal strength for safety. *Second*, it provided an established method as a point of reference for comparison with the biomechanical experiments. *Third*, some questions raised during the biomechanical experiments were conveniently answered by tympanometric studies. *Fourth*, tympanometry contains more methodological limitations, which were drawn to attention during the course of the biomechanical experiments, and which could be analyzed based on these experiments.

Thus, the overall purpose of this thesis was to describe a series of clinical and methodological problems related to determination of ME pressures encountered during the previous series of biomechanical and tympanometric experiments. In the following sections, the background of these problems is explained preceded by an overview of the basic principles behind the physiology of pressure regulation and the clinical aspects related to ME underpressures. Finally, methods of ME pressure measurements are reviewed with an emphasis on tympanometry and its inherited problems related to both measurements and monitoring of ME pressure.

1.1 Physiology of pressure regulation

In ME physiology, the biomechanical properties of the ME system plays an important role for two major aspects. First, these properties determine the impedance of the ME system, and hence, the sound energy transfer to the inner ear. Second, these properties also determines the response of the ME system to static pressure loads of the TM and the ossicular chain; such static pressure loads may cause structural changes of the TM discussed below, and they may cause an increased impedance resulting in a decrement transfer of sound energy which is perceived as a hearing loss (Doyle, 2000). Thus, the maintenance of a ME pressure close to ambient pressure is a prerequisite for normal function of the ME.

A distinction is made between acoustic or “dynamic pressures” on one side and “static pressures” on the other side. The dynamic pressures relate to sound pressures with higher frequencies in the hearing range and smaller amplitudes in the range of μPa 's, whereas the static pressures relate to slower pressure changes with amplitudes in the range of kPa 's. The denominations “static” often refers to conditions which in a strict sense are not static, since the pressures may actually vary over time, but very slowly compared to dynamic or acoustic pressures. Hence, the denomination “quasi-static” is also used; in practice, quasi-static and static are often used interchangeably (Dirckx et al., 2013).

Static pressures are related to both 1) *environmental* changes in the ambient pressure as encountered in aviation, high buildings, diving, and others, as well as 2) *physiological* changes in ME pressure. These physiological changes include minor temporal pressure variations (Bylander et al., 1984; Grøntved et al., 1989; Knight, 1991; Tideholm et al., 1996) and pressure increase during sleep (Bylander et al., 1984; Hergils & Magnuson, 1985; Shinkawa et al., 1987; Tideholm et al., 1999), but also more sudden pressure changes in response to alterations in body position (Grøntved et al., 1989; Knight & Eccles, 1991), nose blowing including the Valsalva manoeuvre and sniffing (Magnuson, 1981; Sakikawa et al., 1995; Tideholm et al., 1996). However, in the clinical context, the formation of sustained negative ME pressures in diseased ears are most important.

Traditionally, the regulation of ME pressure is determined by two major factors: 1) an overall net gas absorption due to a continuous passive gradient driven gas exchange by diffusion

between the ME air and the ME mucosa blood compartments and 2) a gas supply provided by intermittent active muscle-assisted openings of the Eustachian tube (ET) (Ars & Ars-Piret, 1994; Sadé & Ar, 1997; Doyle, 2000). The continuous gas absorption results from differences in the partial gas pressures between the ME air and the ME mucosal blood compartments; under normal conditions, the partial pressures of CO₂, O₂, and H₂O are close to or in equilibrium with the partial pressures in mixed venous blood (Felding, 1998), but a gradient for N₂ around 6.4 kPa remains which is responsible for a continued slow gas absorption (Doyle, 2000). The details of gas exchange are beyond the scope of this thesis, but many factors are involved like the diffusion properties of the gases and the mucosal barrier; further, the vascular surface area and the blood perfusion of the mucosa play an important role. There is an ongoing discussion whether ME gas exchange is primarily determined by the diffusion properties or the perfusion properties of the mucosa (Dirckx et al., 2013). However, the overall effects are dominated by the N₂ gradient leading to a constant net gas absorption which is counter-balanced in normal ears by an intermittent air supply from ET openings.

In this context, it follows that underpressures have been interpreted as a depletion of the normal gas supply from the ET due to an impairment of its function, so that an abnormal ET function have been considered to play the major role in the development of ME underpressures. Thus, an impaired ET function plays a significant role in a series of clinical conditions related to underpressures, i.e., otitis media (OM) including its sequelae. Moreover, an impaired ET function is closely related to upper respiratory tract infections and hypertrophic adenoids, as well as it may result from other conditions (Ars & Ars-Piret, 1994; Sadé & Ar, 1997; Doyle, 2000).

Whereas the ET openings are considered an active regulatory process of the ME pressure changes, there are additional passive regulatory factors also. Hence, the mere volume of the ME cavity has been attributed the role of a pressure “buffer” or a “gas reserve”, where larger volumes will demand less frequent or effective ET openings to equilibrate any pressure changes (Doyle et al., 2000; Dirckx et al., 2013). Moreover, while the ME cavity is predominantly surrounded by rigid bony walls, the TM represents a flexible part of the cavity, and thus, it may also act as buffer of pressure changes (Dirckx et al., 2013). This also means that the TM is subject to a sustained pressure load in ears with underpressures; in such cases, the TM may become a “pressure victim” causing a series of clinical conditions discussed below.

From an overall perspective the regulation of ME pressure has been suggested to include a central neural feedback control based on peripheral mechano-receptors in the TM and the ME cavity with afferent connections (NIX) to brain stem regulatory centres in the nucleus of the solitary tract (Eden et al., 1990). This nucleus is connected to brain stem motor nuclei activating efferent motor connections (NV and NX) to the tensor veli palatini and the levator veli palatini muscles which are the primary muscles responsible for ET openings. The details of these mechanisms are beyond the scope of this thesis, but a review has been reported recently (Dirckx et al., 2013).

1.2 Clinical aspects of ME underpressures

From an otosurgical point of view, the development of ME underpressures is probably the single most important pathogenetic factor in ME diseases being involved in a larger range of ME conditions. These conditions can be described roughly by two major groups: 1) secretory OM (SOM), and 2) chronic OM or sequelae to OM with various stages of TM retractions including cholesteatoma. Selected conditions are illustrated in Figure 3.

In the first group, SOM has traditionally been explained by the *hydrops ex vacuo* theory formed by Politzer (1867). This classic theory also emphasizes on the ET dysfunction and consists of four steps 1) the constant net absorption of ME gas, 2) a resultant ME underpressure, 3) an increased permeability of the mucosal vasculature to fluids, and 4) the transudation of mucosal fluid into the ME space (Fig. 3B). This condition leads to hearing loss, and it is especially frequent in childhood, where 80 % of all children will experience at least one episode before the age of 4 years (Zielhuis et al., 1990). Similarly, treatments of this condition are very frequent and primarily include insertion of a ventilation tube (VT) into the TM. In Denmark the incidence of VT insertions amounts to 375 cases per 10,000 children (<16 years) with a peak incidence at 1.5 years of age (Jespersen et al., *in press*). Moreover, VT insertions amount to include 30 % of children with at least one episode before the age of 10 years, while 43 % of these children will also experience their second VT insertion (Bruhn et al., *in press*). While the VT's seem to have a rationale by equilibration of the ME pressure and relieve the ME fluid as well as the hearing loss, it is also commonly related to complications. Thus, permanent TM perforations are often encountered especially in cases with repeated insertions of VT's and/or the insertion of T-tubes used in recurrent cases, where TM perforations can be found in 24 % (Strachan et al., 1996).

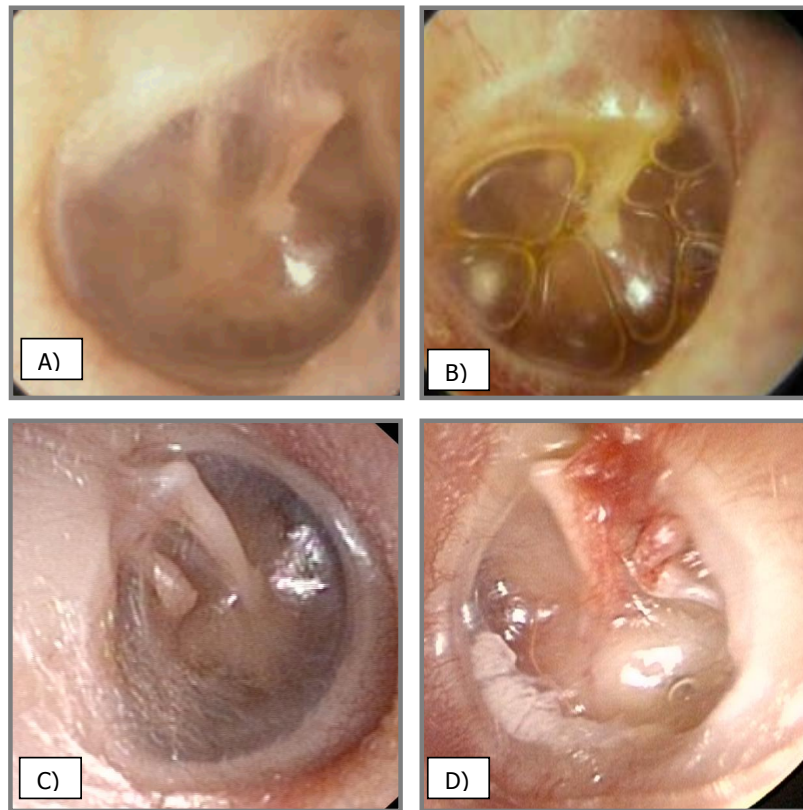


Fig. 3. Otomicro-photographs of the TM at selected ME conditions: A) normal TM; B) TM with fluid in the ME cavity and slight retraction; C) TM with atrophy and retraction posteriorly with adhesion to the incudo-stapedial joint; D) ME atelectasis, where the TM is retracted over large areas and adherent to the ME medial wall (promontorium) as well as the lower part of the crus longum of incus, the stapes head, and its tendon.

Otosurgery is needed in these cases in order to close the perforation by a reconstruction of the TM, because the perforation otherwise poses a significant long-term risk of recurrent infections as well as various degrees of decreased hearing loss. Thus, it is recommended that these children are referred for surgery later in childhood from around the age of 7 years.

In the second group, varying degrees of TM retractions are found explained by prolonged periods of ME underpressures together with the formation of weak spots in the TM lamina propria. These spots often present themselves by clinical otomicroscopy as thin atrophic parts of the TM, where its lamina propria is degenerated or absent, and thus, the mechanical strength of the TM is decreased or lost. The retractions may be restricted to small distinct areas (Fig. 3C) or present larger in the form of atelectasis, where wide parts of the inner surface of the TM adheres to the medial wall of the tympanum and the ossicles (Fig. 3D). The

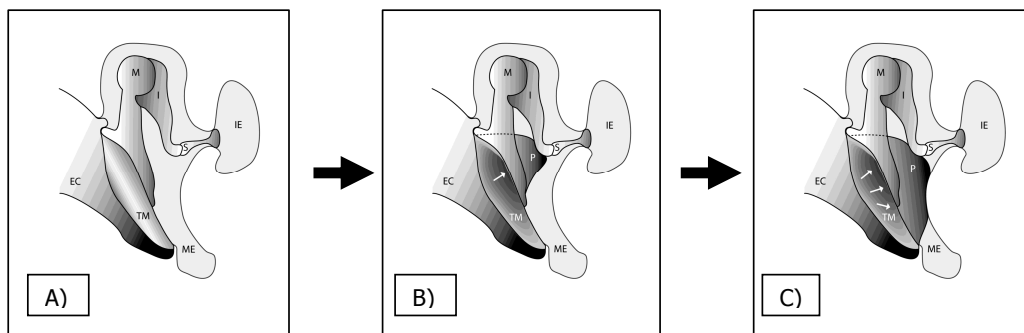


Fig. 4. Cross sectional diagrams of the tympanic membrane (TM) and the middle ear (ME) cavity depicting various stages of retraction. A) Normal TM position with normal aeration of the ME cavity; B) smaller distinct retraction pocket (P) of the posterior part of the TM with contact to the long process of the incus (I); C) a pronounced retraction or almost atelectasis of the TM with contact to the long process of the incus and the medial wall of the ME cavity. EC = ear canal; M = malleus; S = stapes; IE = inner ear. The diagrams B) and C) corresponds to the otomicro-photographs C) and D) in Figure 3, respectively. Reprinted by permission of Springer.

sequential steps of these pathogenetic events are schematically illustrated in Figure 4. Further progression and enlargement of such retractions into the ME results in formation of an acquired cholesteatoma with accumulation of epithelial debris and recurrent infections (Ars et al., 1989; Ars, 1995; Sadé & Ar, 1997; Tos et al., 1984; Sudhoff & Tos, 2000).

Cholesteatoma is a more serious condition reported with an incidence of primary acquired cases around 9 per 100,000 inhabitants per year in Denmark (Djurhuus et al., 2010; Kole et al., 2013). The cholesteatoma contains erosive properties causing a considerable risk for degeneration of ME structures and related complications. First of all, disruption of the ossicular chain is found in 50 % of the cases; such erosions result in a more serious and irreversible hearing loss (Kole et al., 2013). The erosion may also affect other structures (facial nerve, horizontal semicircular canal, cochlea, and meninges) which altogether amount to 11 % of the cases (Kole et al., 2013). These conditions may pose serious risks to patients such as facial palsy, vertigo, deafness, meningitis, and acute mastoiditis.

Otosurgery is also needed in these cases in order to remove the retraction pocket and/or the cholesteatoma, so that its further progression can be prevented; moreover, surgery aims at the reconstruction of the TM with a stable grafting material as well as in cases of disruption the reconstruction of the ossicular chain with reshaped autologous ossicles or artificial prostheses, so that hearing may be restored or improved.

Altogether, these two major otosurgical groups of ME conditions are characterized by being affected by the same significant pathogenetic factor at various points in the disease history, namely ME underpressure. In a 1-year cohort of 295 hospital-based consecutive otosurgeries, 79 % of the otosurgical procedures could be referred to either of these two groups; in other words, underpressures are a pathogenetic factor involved in the disease history in 79 % of otosurgical cases (Rasmussen et al., 2008). Further, for the primary cases at 1-year follow-up, it was found that 41 % still presented with problems related to underpressures (TM retraction = 34 %; cholesteatoma = 3 %; active VT = 4 %) (Rasmussen et al., 2008). Thus, the postoperative course is also susceptible to formation of underpressures. It should be noted that the series of otosurgical procedures in this context do not include ME implants as well as the insertion of VT's; treatments with VT's are minor procedures organized in private ENT practices in Denmark. Hence, the series comprised a hospital-based cohort of classical otosurgical procedures.

From a historical point of view, ME underpressure has been recognized early in otologic research, since Politzer formed the *hydrops ex vacuo* theory (Politzer, 1867). However, in the context of otosurgery, the significance of underpressures has not been fully recognized before the early 1950'ies related to the emerging principles of myringoplasty and tympanoplasty with functional reconstructions of the TM and the ossicles (Zöllner, 1955; Wullstein, 1956). Thus, it was encountered that the success of these new otosurgical principles, where the TM by its reconstruction became intact, depended strongly on the continued aeration of the ME, and thus, the function of the ET (Zöllner, 1955; Wullstein, 1956); in other words, the long-term stability of these reconstructions has been found susceptible to the postoperative development of underpressures.

1.3 Measurements of ME pressure

From a clinical perspective the evidence of ME underpressures seems obvious, when retractions of the TM are observed by otomicroscopy (Fig. 3). However, in order to understand the formation and the course of these underpressures, valid experimental and clinical methods are needed to measure the pressure difference between the ME cavity and the ambient pressure.

Various methods have been described, and in principle the ME pressure can be determined either directly or indirectly. Direct methods are relatively few and include puncture of the mastoid (Flisberg et al., 1963; Hergils et al., 1990), puncture or perforations of the TM (Buckingham & Ferrer, 1973; Sadé et al., 1976; Tideholm et al., 1996), or the insertion of a transducer into the ME from the ET (Takahashi et al., 1987). These methods are all obviously limited by various ethical, technical and/or methodological problems. Additional methods include pressure chamber experiments which are quite resourceful and complicated to operate, and their overall relevance is limited (Thomsen, 1960; Elnor et al., 1971b).

The indirect method based on tympanometry is by far the most commonly used in clinical otology as well as in both clinical and basic research. On account of its essential part of the current investigations as well as its immense and wide importance a historical review is given on tympanometry in the following section followed by descriptions on its basic principles, its sources of inaccuracies, and problems related to monitoring the ME pressure.

1.3.1 History of tympanometry

Acoustic impedance measurements of the ME system were introduced in clinical research by Metz in the 1940'ties primarily in search for a method to separate conductive and sensory-neural hearing disorders (Metz, 1946). While this purpose did not meet his expectations, Metz found that the impedance was influenced by pressure loads on the TM in accordance with van Dishoeck, who described optimal hearing at a normal tension of the TM by means of the pneumophone (van Dishoeck, 1941). Based on these observations, Thomsen demonstrated by experiments in a pressure chamber that the impedance was minimal, when the ear canal pressure was equal to the ME pressure, and hence, the ME pressure could be determined indirectly and objectively by the "pressure balance method" (Thomsen, 1960).

However, the mechano-acoustic impedance bridge developed for Metz' experiments was complicated to manoeuvre and could not be kept airtight. Hence, it was not until the more manageable electro-acoustic impedance bridge was developed that further research progressed (Therkildsen & Scott Nielsen, 1960). In this instrument the ear coupler could be held air tight, and further experiments were altogether made more feasible. Thus, the first tympanogram could be demonstrated, where the impedance was described as a function of continuous changes in the ear canal pressure (Therkildsen & Thomsen, 1959).

The studies by Metz reported also on the impedance in attempt to quantify the elastic properties of the ME system in different otological conditions; in otosclerotic ears they showed the impedance was higher than in normal ears, but a significant overlap was also found (Metz, 1946). These findings were confirmed by Therkildsen and Thomsen (1959), as well as other changes in impedance related to different middle ear conditions including flat curves in SOM and TM perforations (Therkildsen & Thomsen, 1959). In addition, the appearances of more flat curves with high impedance were reported in cases with thickening of the TM, while steep curves with low impedance were found in atrophic TM's; however, these TM changes were often found concurrently, and the outcome of the resulting impedance was reported unpredictable (Therkildsen & Thomsen, 1959).

Despite these limitations, the improvements made by the introduction of the electro-acoustic bridge and its subsequent commercial availability created the foundations for a larger range of experiments including its wider clinical application in the 1960'ties, but in particular the 1970'ties and 80'ties have provided a vast amount of literature with a great optimism for an objective differentiation between various middle ear disorders (Lidén et al., 1970; Jerger, 1970; Shanks & Shelton, 1991). However, in more of these studies it was also recognised that a number of procedural variables and measurements errors were inherited in tympanometry resulting in a poor accuracy and precision, some of which still remain unclarified (Lidén et al., 1970; Jerger, 1970). In the 1990'ties further attempts have been made to increase its diagnostic accuracy by the introduction of multiple frequency tympanometry, but this approach has not gained wider practice (Shanks & Shelton, 1991). Moreover, the impedance method was also found valuable for investigations of both the stapedial reflexes and the ET function, but these aspects are beyond the scope of this thesis.

In summary, tympanometry has been established as a valuable tool for evaluation of the ME function in conjunction with audiometry and otomicroscopy (Lidén et al., 1970; Jerger, 1970; Shanks & Shelton, 1991). Hence, tympanometry is still an indispensable tool in clinical otology which also has been implemented within recent years in general practices used for screenings of children with SOM (Felding, 2000; Johansen et al., 2000). The interest of tympanometry and the number of studies on its clinical applications as well as its accuracy has diminished within the last two decades, although more methodological questions and pitfalls still exist. Tympanometry is often applied in both clinical and basic research including

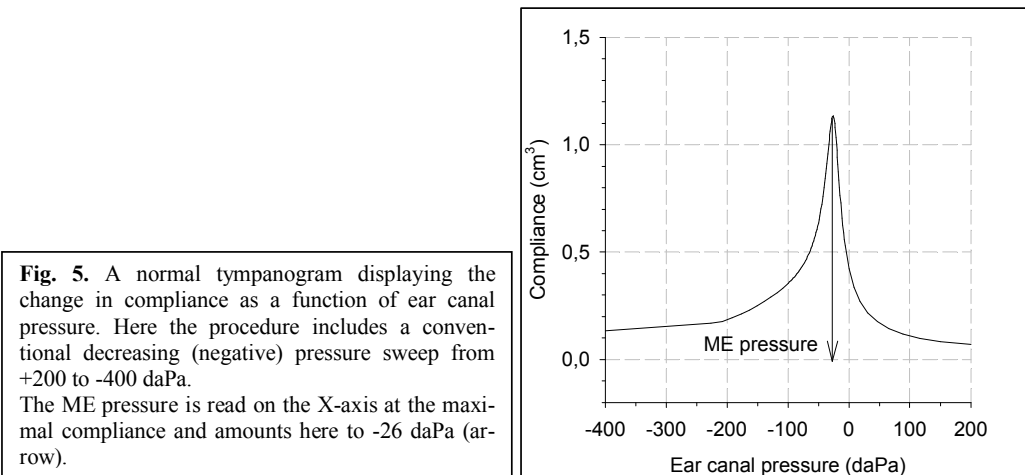
animal experiments (Alper et al., 2003; Yilmaz et al., 2008), and thus, due to its wide range of research applications, further studies on its methodological limitations including its accuracy and precision are still important.

1.3.2 Basic principles of tympanometry

The impedance of the ME system describes the opposition of the system towards the transfer of sound energy, and in accordance with electric impedance it is frequency dependent (Shanks & Shelton, 1991). The admittance is the reciprocal of impedance, and in most cases today the admittance rather than the impedance is used in practice; moreover, low frequency probe tones around 220 Hz are used, where the admittance is dominated by its compliance component (Shanks & Shelton, 1991). Thus, by definition tympanometry describes the relationship between the admittance or compliance of the ME system as a function of ear canal pressure changes (Fig. 5) (Shanks & Shelton, 1991).

In practice, an ear probe connects the instrument to the outer ear canal; it contains three channels: 1) one delivering the probe tone from a miniature loudspeaker, 2) another connecting to a pump delivering monitored pressure variations to the ear canal, and 3) another containing a microphone for measuring the sound intensity of the probe tone (Shanks & Shelton, 1991). Thus, changes in the sound intensity can be measured, while a continuous pressure sweep is introduced into the ear canal resulting in a cyclic pressure load of the TM. In our clinic, the conventional pressure sweep includes a pressure from +200 to -400 daPa (a decreasing or negative pressure sweep), but other pressure ranges as well as positive pressure sweeps have also been reported in the literature.

Tympanometry primarily determines the ME pressure and the static compliance of the ME system; this static compliance expresses an acoustic equivalent of compliance in comparison to the physical compliance determined by the PVR ($\Delta V_{tm}/\Delta P_{ec}$). Moreover, the gradient and/or the tympanometric width describe the steepness of the curve (Shanks & Shelton, 1991). Thus, these latter measures are only derived variables, and they play no role in our current studies. Determination of the ME pressure is based on the observation that the admittance or compliance reaches a maximum, when the ear canal pressure is equal to the ME pressure, i.e. when no pressure difference exists between the ear canal and the ME cavity. In this condition, the transfer of sound energy or the admittance is also maximal which is



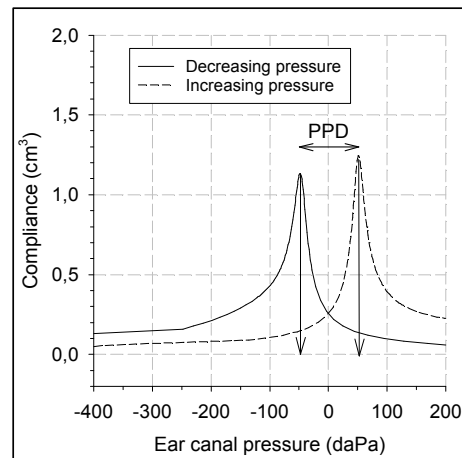
reflected by a minimal sound intensity recorded by the ear probe (Fig. 5) (Therkildsen & Thomsen, 1959; Thomsen, 1960; Shanks & Shelton, 1991). Traditionally, ME pressure has been measured in mmH₂O, while modern instruments use the equivalent daPa (1 mmH₂O = 0.98 daPa ≈ 1 daPa = 10 Pa).

1.3.3 Tympanometric pitfalls

Whereas the basic principles of tympanometric determination of ME pressure seem sound and well documented, there are various factors that can introduce sources of inaccuracies. These are related to both the physical parameters of the ME system as well as the specific tympanometer.

ME volume and TM elasticity. The procedural pressure load in the ear canal during a tympanometric recording causes a volume displacement of the TM (ΔV_{tm}) which inevitably results in a small change of the ME volume itself (V_m). This volume change ultimately also results in a change in the actual ME pressure according to Boyle's Law; in other words, the procedure of measuring alters the pressure we aim to measure. The error is small in ME's with a normal V_m and a normal TM compliance or elasticity, since the ΔV_{tm} will be relatively small in comparison with the V_m . However, in ears with a small ME volume and/or a flaccid TM, the ΔV_{tm} will be relatively larger and the resulting pressure changes will be accordingly larger (Flisberg et al., 1963; Ingelstedt et al., 1967; Elnor et al., 1971a,b). Unfortunately, studies reporting a valid agreement between direct and tympanometric measurements of ME

Fig. 6. A bidirectional tympanogram, where a decreasing (negative) pressure sweep results in a peak at -50 daPa, and a increasing (positive) pressure sweep at +50 daPa. Thus, the PPD is 100 daPa, and the true ME pressure represents the mean pressure = 0 daPa.



pressure have generally only included few and/or normal test subjects (Thomsen, 1960; Flisberg et al., 1963; Takahashi et al., 1987; Hergils et al., 1990); thus, these sources of inaccuracies in determination of the ME pressure have not been fully addressed.

Peak pressure difference. Tympanometric determination of the ME pressure exhibits a directional sensitivity, so that a negative pressure sweep (from positive to negative pressure) leads to more negative estimates of ME pressure, whereas a positive sweep (from negative to positive pressure) leads to more positive estimates. This can be expressed by the peak pressure difference (PPD), where the PPD is defined by the difference between the ME pressure determined by the positive sweep minus the negative sweep (Fig. 6). The phenomenon has been attributed to an intrinsic hysteresis explained by the viscoelastic properties of the ME system (Ivarsson et al., 1983; Decraemer et al., 1984; Shanks & Wilson, 1986; Kobayashi et al., 1987; Hergils et al., 1990).

Moreover, the PPD may also be affected by the rate of the ear canal pressure change during the recording; thus, in some studies faster rates of pressure change have been reported to result in numerically larger estimates of ME pressure (Ivarsson et al., 1983; Feldman et al., 1984; Shanks & Wilson, 1986; Kobayashi et al., 1987; Hergils et al., 1990). This has been illustrated in Figure 13 for two studies with available data (Shanks & Wilson, 1986; Kobayashi et al., 1987). This phenomenon is explained by a phase delay, i.e. a delay between the actual pressure change and its subsequent registration by the pressure transducer of the instrument. This factor is influenced by the resistance of the tube, i.e. it correlates to its

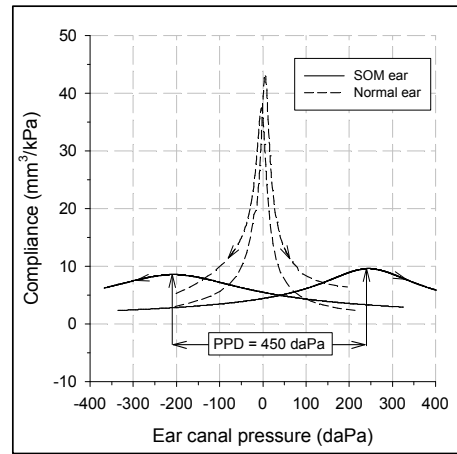
diameter and the length between the transducer and the ear canal. Hence, it can be diminished by constructing the tympanometer with a transducer close to the ear canal, for instance in the headset of the instrument (Decraemer et al., 1984). It follows that since this factor is related to the instrument itself, it should also be specified for each instrument especially in scientific investigations using tympanometry.

Thus, the PPD represents a source of inaccuracy, since separate results are measured depending on the direction of the pressure change as well as possibly the rate of pressure change depending on the instrument. It has been shown that this inaccuracy can be accounted for, because it corresponds to $0.5 \times \text{PPD}$, so that the exact ME pressure is equal to the mean of the pressures determined in the two directions (Fig. 6) (Kobayashi et al., 1987; Decraemer et al., 1984; Hergils et al., 1990). Since the PPD in normal ears has been found in the order of 10 to 30 daPa in some studies, this difference may not seem significant (Decraemer et al., 1984; Hergils et al., 1990), but in other studies it has been reported increasing to 50-70 daPa depending on the rate of pressure change (Shanks & Wilson, 1986; Kobayashi et al., 1987).

Moreover, a few casuistic cases have demonstrated that the PPD may be increased in diseased ears to more 100's daPa; in one case with otalgia after swimming and a normal TM, the PPD was 400 daPa, but the phenomenon was not explained (Kobayashi et al., 1987). Accordingly, the error determining the ME pressure in such cases may be as large as 200 daPa, which causes seriously misleading results unless a bidirectional recording is performed, and the mean ME pressure is calculated from the PPD (Kobayashi et al., 1987; Decraemer et al., 1984; Hergils et al., 1990).

Peak pressure difference in SOM ears. In the previous biomechanical PVR experiments in the human ME system, its viscoelastic properties have been measured. This included the hysteresis which has been quantified in normal ears as well as in selected diseased ears and expressed in units of μJ 's (Gaihede, 1999a, b). Thus, it has been demonstrated in a case of SOM with a large amount of mucoïd ME fluid that the hysteresis can be increased by a factor of four (from 20 to 80 μJ) (Gaihede, 1999a). Considering the viscous properties of such fluid in contact with the TM this finding may not be surprising, though it has not been demonstrated earlier.

Fig. 7. The derived compliance functions of the PVR from a normal ear and a case with mucoid ME fluid, where compliance is depicted as function of ear canal pressure. In the normal ear the PPD is 8 daPa, whereas in the ear with fluid the PPD is markedly increased to 450 daPa (peaks occurring at -210 daPa and +240 daPa). Reprinted by permission of Wolters Kluwer Health – Lippincott, Williams & Wilkins.



Since the PPD has been related to hysteresis as outlined above, it follows that the PPD should also increase markedly. This is illustrated in Fig. 7, where the derived compliance function, i.e. the $dV_{tm}/dP_{ec} = f(P_{ec})$, has been depicted for a normal and a SOM ear (Gaihede & Kabel, 2000)(IV). As explained earlier (Fig. 1) this derivative represents the compliance of the ME system as a function of the ear canal pressure, and thus, it is equivalent to tympanometric recordings as well as the curves are similar (Fig. 7). Thus, based on the derivative of the PVR recordings, the PPD can be determined similar to tympanometry; in the normal ear it amounts to 8 daPa, whereas in the SOM ear it amounts to 450 daPa. In terms of the previous tympanometric evidence discussed above, these findings suggest that the error of a routine one-way tympanometry in this case will amount to 225 daPa (0.5×450) (Kobayashi et al., 1987; Decraemer et al., 1984; Hergils et al., 1990).

Thus, from these basic biomechanical PVR experiments in normal and SOM ears, it can be suggested that similar bidirectional tympanometric recordings of ME pressure in SOM ears will result in a markedly increased PPD, and thus, a markedly increased inaccuracy for ME pressure determination. Further, since the viscosity of the ME fluid seem directly related to the increased hysteresis and the PPD, it can also be suggested that the PPD should be related to both the degree of its viscosity and its amount, so that a dose-response relationship would be expected.

Altogether, these sources of inaccuracies are important, since they may substantially affect our perception of the range of true pressures especially in diseased ME's, and hence, influence our ideas of pathogenetic events related to ME diseases (Sadé & Ar, 1997).

1.3.4 Monitoring ME pressure by tympanometry

Monitoring of ME pressure over time is important in order to understand its overall variation and long-term regulation; thus, repeated measurement of pressure has been performed in a number of studies. Most often tympanometry has been employed in these studies, but for practical reasons the intervals have been from 3 to 15 min (Bylander et al., 1984; Grøntved et al., 1989; Knight, 1991). Further, most of these studies are limited by only monitoring the pressure over 7-hours periods (Grøntved et al., 1989; Knight et al., 1991), whereas only one study included monitoring a full 24-hours period (Bylander et al., 1984). However, these studies all confirm that ME pressure is subject to minor temporal fluctuations without any overall specific patterns, except that pressure increases during sleep (Bylander et al., 1984). In accordance, the ME pressure has been shown to be positive in the morning in normal ears (Hergils & Magnuson, 1985; Shinkawa et al., 1987).

Monitoring of the ME pressure has also described various changes on a smaller time scale. For instance, in more of these studies a pressure increase has been observed on a scale of few min's related to changes in body position from erect or sitting to supine position (Grøntved et al., 1989; Knight & Eccles, 1991). This has been explained by an increment in the volume of the ME mucosa (ΔV_{muc}) based on experiments, where its volume has increased in response to similar change in body position, and these volume changes have been attributed to a congestion of the mucosa (Ingelstedt et al., 1967; Andreasson et al., 1976). Such congestion is directly related to an increased pressure in the jugular veins, when the body position is changed to supine (Ingelstedt et al., 1967; Rundcrantz, 1969; Jonson & Rundcrantz, 1969).

In previous mechanical experiments measuring the PVR of the ME system, a similar fast and instant increase in ME pressure has been reported amounting to around 14 daPa after reaching the supine position followed by an impression of an additional slower and smaller pressure increase (Gaihede et al., 1995b). Since these PVR experiments inherently have been performed in the supine position, and since the ME pressure affected the recordings, these

aspects should be accounted for in order to interpret the results (Gaihede et al., 1995a; Gaihede, 1999a,b). This problem may apply to any ME experiments in the supine position, where the ME pressure and/or mucosa volume may influence the results. Thus, it has been necessary to determine both the magnitude of the total ME pressure increase as well as its time course in order to identify a potential stable phase, where conditions with a stable and repeatable ME pressure can be identified.

Summary of tympanometric pitfalls and monitoring. It has been pointed out that the tympanometric measurements of ME pressure are susceptible to the volume of the ME cavity and the compliance of the TM, and that these problems have not been quantitatively addressed earlier. Moreover, the tympanometric rate and direction of pressure change may also introduce inaccuracies due to phase delay and hysteresis; these problems are reflected by variations in the PPD. Whereas the phase delay relates to the actual instrument, the hysteresis is related to the viscoelastic properties of the ME system. Further, experimental evidence has suggested that the hysteresis properties may be significantly increased in SOM ears, which may also be reflected by an increased PPD. Finally, experiments involving the supine position may be susceptible to temporal pressure changes; thus, supine test conditions seem unstable, and measurements of the ME pressure are not repeatable. These problems have been summarised in the hypotheses below.

1.4 Hypotheses

- ME pressure in normal ears is susceptible to changes in body position from sitting to supine position and a steady state with repeatable conditions can be obtained within few minutes.
- Tympanometric ME pressure determination is affected by physical parameters of the ME such as the ME volume due to the inherited procedural volume displacement of the TM (ΔV_{tm}) by tympanometry.
- Tympanometric ME pressure determination is affected by instrument variables such as the direction and the rate of pressure change; this is reflected by increasing PPD for higher rates of pressure change.
- Tympanometric ME pressure determination is affected by the viscosity and the amount of ME fluid in cases with SOM; this is reflected by an increased PPD in SOM ears.

2. Aims

1. To determine the short-term changes in ME pressure in response to changes in body position from sitting to supine position in a group normal ears in order to
 - 1.1. quantify the total positional changes in ME pressure by tympanometry, and to
 - 1.2. define the time period needed to obtain stable and repeatable conditions

2. To develop a ME mechanical model, where ME parameters of pressure and volume can be controlled, and where realistic tympanometric recordings can be obtained, in order to investigate
 - 2.1. the accuracy of tympanometric ME pressure measurements at various defined exact pressures, and
 - 2.2. the effect of changes in the ME air volume on the accuracy of tympanometric ME pressure measurements

3. To determine the effects of instrument parameters on tympanometric ME pressure measurements; this include the effects of both
 - 3.1. the tympanometric pressure change rate, and
 - 3.2. the tympanometric pressure change direction

4. To determine the effects of ME fluid properties on tympanometric ME pressure measurements. This include the effects of both
 - 4.1. the viscosity of ME fluid, and
 - 4.2. the amount of ME fluid

3. Methods and Materials

3.1 Monitoring positional changes in ME pressure (Paper I)

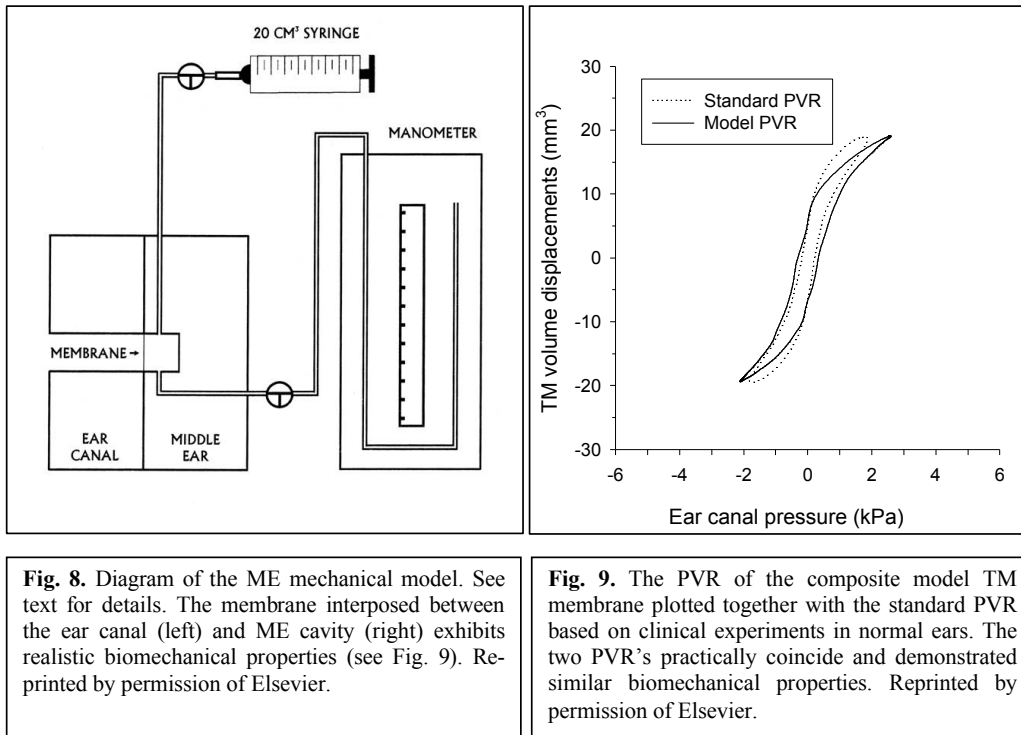
In order to investigate ME pressure changes in relation to positional changes attaining the dorsal supine position, serial tympanometry was performed at 15 s intervals over a period of 120 s in a group of 20 healthy adult volunteers. All subjects were investigated on both right and left ears; a period of at least 10 min separated these experiments. A set of ten trials was obtained, where the first trial was obtained in the sitting position, the next was obtained immediately after assuming the supine position, and the following eight trials at intervals of 15 s until 120 s, where the experiment was aborted (I).

The tympanometer was an automatic Madsen Electronics Zodiac Middle Ear Analyzer 901 with a 226 Hz probe tone. Range and direction of measurements was +200 to -400 daPa with a rate of pressure change set at 400 daPa/s (I). This tympanometer was used during all the proceeding experiments; in some of these experiments the rate of pressure was altered, which has been specified accordingly.

3.2 Middle ear modelling (Paper II)

The ME model was constructed by two pieces of Plexiglas, where one had a cylindrical hole in its middle representing the ear canal, and the other a smaller cylindrical cavity of 0.83 cm³ representing the ME cavity (Fig. 8). The latter also had two connections: 1) to a reservoir of 20 cm³ air and 2) to a manometer. Thus, both the model ME air volume and ME air pressure could be controlled and altered. Between the two cylinders a membrane could be mounted in order to represent the TM.

Whereas these parts of the model were rather simple, the clinical background and the construction of the model membrane were more complex. The normal PVR of the ME system exhibits both non-linearity and hysteresis (Fig.1); based on these earlier clinical experiments in 39 normal volunteers (Gaihede, 1999b), substantial amounts of high-resolution pressure-volume data have been provided for the basis of a set of mathematical computations presenting an averaged expression of the PVR (Gaihede et Kabel, 2000). This averaged or “standard PVR” has been used to define the biomechanical properties of the normal ME



system, and thus, this standard can constitute a template for a realistic model of the TM membrane (Fig. 9) (II).

Basically, a composite membrane was needed in order to create non-linearity, as well as some kind of viscous layer in order to create hysteresis similar to a normal PVR of the ME system (II). By examining more possibilities we found that it could be achieved by combining two elastic materials with different elastic properties (latex and polyethylene) into a double-layered membrane with a thin smear of Vaseline between these layers. By repeated trials of firmer and looser suspensions of either layer of this model membrane and various amounts of Vaseline smear, the characteristics illustrated in Fig. 9 could be obtained; the model PVR coincided well with the standard PVR as well as its mechanical properties were similar to the standard PVR (II).

Once constructed this model membrane was very stable lasting for many hours of experiments without changing its mechanical properties. Moreover, when the ear probe of the tympanometer was inserted into the ear canal part, it produced realistic tympanometric recordings with a compliance of 0.33 cm^3 (II).

Thus, tympanometric experiments could be performed, where variations in ME volume between 1 and 21 cm³ were introduced, as well as variations in ME pressure between -170 and +85 daPa. The tympanometer and the procedure were identical to the previous study (I), and triplicate measurements were obtained at each set of experimental conditions (II).

3.3 Peak pressure difference with a modern tympanometer (Paper III)

In order to characterize the tympanometer used in these studies, a separate series of experiments were made to determine the PPD and any correlation with the rate of pressure change.

Thus, bidirectional tympanometries were performed at four rates of pressure changes: 50, 100, 200, and 400 daPa/s in a group of 38 healthy adult volunteers. In all subjects recordings were obtained from both right and left ears. The PPD was calculated by the difference between the positive and the negative pressure sweep (III).

3.4 Peak pressure difference in patients with ME fluid (Paper IV)

In order to determine the PPD in ears with ME fluid, both a group of children with SOM were investigated, and a group of normal children; thus, the material consisted of a test group as well as an age-matched control group.

The test-group comprised 56 children with SOM and ME fluid admitted for insertion of VT's in an ENT private practice. Bidirectional tympanometries at a rate of 100 daPa/s were performed immediately before the surgery, and only cases with well-defined pressure peaks were included, so that cases with type-B tympanograms were excluded (IV). After incision of the TM, the ME fluid was evacuated and semi-quantified according to its viscosity (serous, seromucoid, or mucoid) and its amount (small, medium, or large) (IV). The control group comprised 28 age-matching normal children with no upper air way infection or OM recruited from a local kindergarten (IV).

3.5 Statistical notes

The precision of measurements describes the variation between repeated measures, while accuracy describes their distance from the true value. Both precision and accuracy of

measurements are important aspects describing the reliability of methods. In clinical research the precision or repeatability is often determined by test-retest measurements for practical and ethical reasons. Thus, we have adopted the approach described by Bland & Altman (1986), where test-retest results are analysed by calculating the distribution of their differences and testing this difference against 0. The variation of the differences around 0 can be used as a measure of repeatability; thus, the $\pm 2SD$ range of the differences or their 95 % confidence range defines the coefficient of repeatability (Bland & Altman, 1986).

In clinical research paired organs are sometimes investigated like for instance right and left ears. In this case data cannot be pooled from either side for statistical analysis, because either side is a dependent variables related to the contralateral side; this violates the assumption of independency of data sampling used in many statistical tests. Moreover, pooling of data leads to a false inflation of sample size which may lead to false statistical significance (Altman & Bland, 1997). One solution is to determine the mean value of right and left ear, before statistical tests are performed, or to perform the tests separately for right and for left ears.

In more of the experiments included in this thesis, the right and left ears were investigated at the same time. The data have been described and tested separately for the right and left sides to avoid violation of the presumption of independent data sampling and false inflation of sample size. However, if the tests showed similar results for the right and left side data, the data may have been pooled and statistics of the pooled data have been reported for simplicity.

4. Results

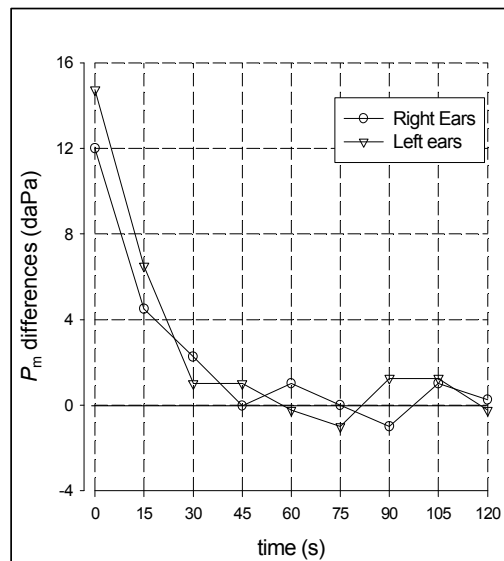
4.1 Monitoring positional changes in ME pressure (Paper I)

These experiments revealed a biphasic increase in ME pressure in response to a change from sitting into dorsal supine position, where an initial larger increase was followed by a slower and minor increase. The total increase amounted to 22 daPa (mean; SD = 12.1) over a period of 120 s (I).

The minor increase reached a stable phase over time, which indicated that a *steady state* was achieved. This is illustrated in Fig. 10, where the means of the differences between one and the preceding trial have been plotted for both right and left ears (Bland & Altman, 1986). The two curves coincide and the differences approach to 0 daPa for increasing time. For an overall analysis, the differences between trials at 30 and 15 s were not significantly different from 0 ($2p > 0.05$), while for trials at 45 and 30 s the insignificance was considerably larger ($2p > 0.6$). Thus, in practice the ME pressure could safely be considered stable or repeatable after 30 s, i.e. a *steady state* was achieved at this point and it remained stable for at least another 90 s.

The initial as well as the total increase in ME pressure was not correlated to the magnitude of the prevailing ME pressure or to the height of the subject (all p -values > 0.9).

Fig. 10. Stabilisation of ME pressure after assuming the supine position. The mean of differences between one and the preceding trial is plotted against time. *Steady state* is reached after 30 s. The SD's vary between 9.8 (at 0 s) and 3.4 (at 75 s) daPa.



4.2 Middle ear modelling (Paper II)

Various experimental pressures of the ME model are plotted against the tympanometric ME pressure recordings for the various ME volumes in Figure 11. All plots show a significant linear correlation with a systematic overestimation of the tympanometric ME recordings. This was reflected by the slopes of the regression lines; the mean slope amounted to 2.3 (II).

Moreover, in the ME model the tympanometric measurements of ME pressure were analysed for various ME volumes between 1 and 21 cm³. An example is illustrated below in Figure 12. In this experiment the actual model ME pressure was 0 kPa, where the tympanometric estimates for larger volumes were slightly negative around -10 daPa, while for smaller volumes less than 5 cm³ the estimates decreased exponentially, so that for infinitely small volumes, an infinitely large negative ME pressure could be predicted by the regression line curve fit (II). Similar exponential decrements in the tympanometric estimates of ME pressure at smaller ME volumes < 5 cm³ were found for all model ME pressures; altogether five sets of experiments were analysed at different ME model pressures (range -85 to +85 daPa) (II).

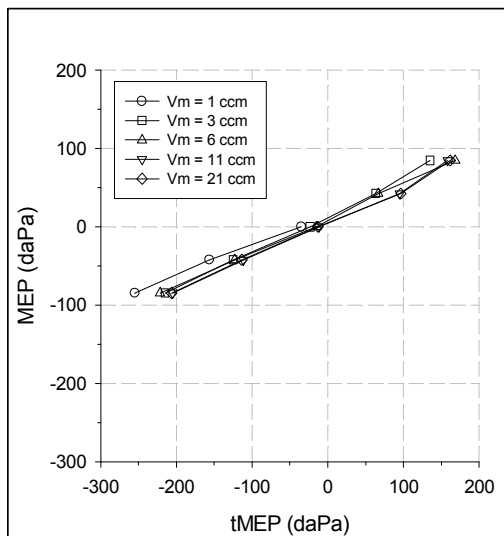


Fig. 11. The model ME pressure (MEP) plotted vs. the tympanometric ME pressure (tMEP) for the various ME volumes (V_m).

For all V_m 's there is a systematic overestimation of the actual ME pressure. This corresponded to the slope of the regression lines; the overall slope of the five correlations was 2.3 (range 2.1 to 2.6). Reprinted by permission from Elsevier.

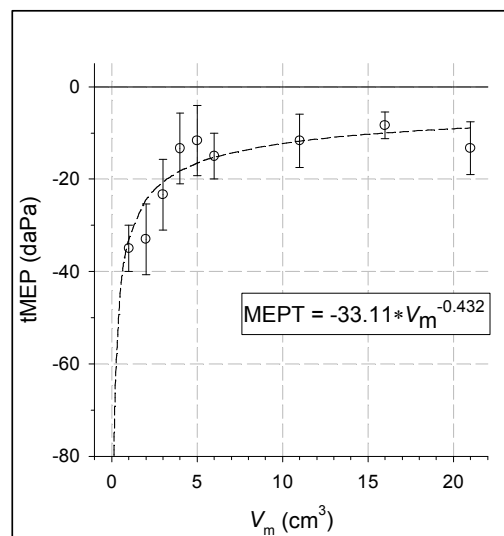


Fig. 12. Tympanometric ME pressure (tMEP) plotted as function of ME volume (V_m) with normal ME model pressure = 0 daPa.

An exponential function is indicated, and the regression line curve fit suggests that tMEP is approaching to $-\infty$ daPa, when V_m is approaching to 0 cm³. Reprinted by permission from Elsevier.

4.3 Peak pressure difference with a modern tympanometer (Paper III)

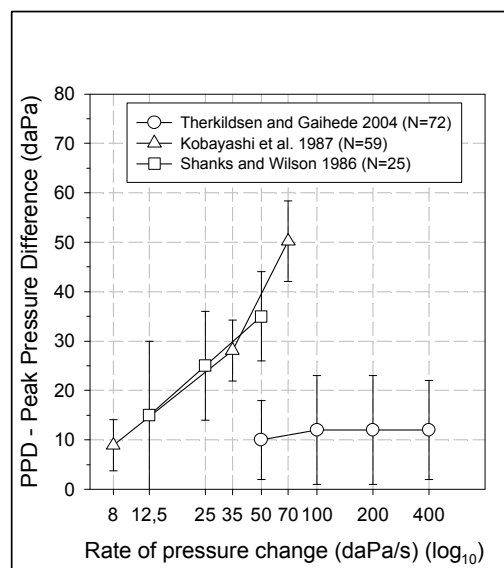
The tympanometric recordings for ME pressure determined for the four rates of pressure changes in both negative and positive directions showed that negative pressure sweeps consistently resulted in more negative pressures (-16 to -20 daPa) than positive pressure sweeps (-6 to -8 daPa) (III). However, the ME pressures were similar for all four rates of pressure change, and there were no significant differences between them. In accordance, the PPD also did not show any variation related to the rate of pressure change and the mean PPD remained between 10 and 12 daPa; the PPD's of the experiments are depicted in Fig. 13 together with two previous studies using older instruments (Shanks & Shelton, 1986; Kobayashi et al., 1987) (III).

Thus, whereas the tympanometers of the older studies demonstrated rate dependent changes in PPD, i.e. phase delay, the present tympanometer showed constant values, and the PPD only varied between 10 to 12 daPa (III). The results from right and left ears have been pooled in this analysis for simplicity, since the results did not differ between right and left ears.

4.4 Peak pressure difference in patients with ME fluid (Paper IV)

The overall PPD's of the control and the test groups are displayed in Table 1, and the PPD is

Fig. 13. The PPD as a function of rate of pressure change. Results from previous studies using older tympanometers show increasing PPD for increasing rates (mean and SD's), whereas our study using a modern tympanometer showed no influence; the mean PPD varied between 10 and 12 daPa. Reprinted by permission of Wolters Kluwer Health – Lippincott, Williams & Wilkins.



found significantly increased in the test group (mean = 69 daPa) compared with the control group (mean = 10 daPa). Further, the PPD range is found much larger in the SOM group amounting to 205 daPa (IV).

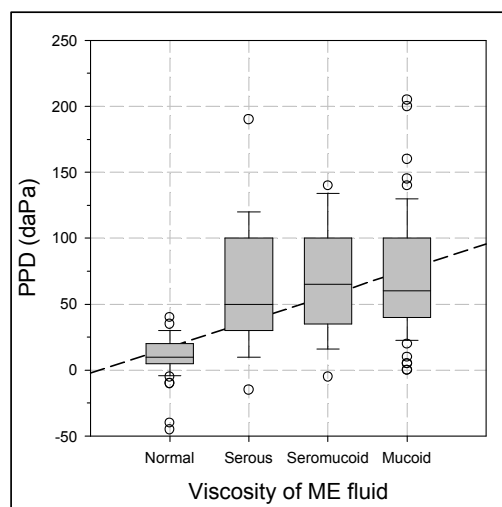
Table 1. PPD distributions in normal and SOM ears.

	Control / Normal (daPa)	Test group / SOM (daPa)
Mean	10	69
SD	15	45
Minimum	-45	-15
Maximum	40	205
N	51	98
Rank Sum Test*	$P < 0.001$	

* Mann-Whitney Rank Sum Test was used to compare the two groups.

Additional analysis included stratification of the PPD according to the properties of the ME fluid. For both the viscosity and the amount of fluid significantly positive correlations were found, so that the PPD increased for both increasing viscosity as well as increasing amount of fluid ($p < 0.0001$ for both correlations) (IV). The correlation between the PPD and the viscosity of ME fluid is depicted in Fig. 14; the correlation between PPD and the amount of ME fluid displayed a similar pattern (IV). Combined analysis with multiple linear regression analysis of the PPD against both viscosity and amount of ME fluid resulted in a regression coefficient for viscosity = 8.3 ($p = 0.1$) and for amount = 13.1 ($p = 0.02$).

Fig. 14. The relationship between PPD and viscosity of the ME fluid. The dashed line indicates the linear regression analysis ($r = 0.629$; $p < 0.001$; $N = 149$). Boxes illustrate medians, 25 and 75 percentiles, whereas whiskers 10th and 90th percentiles ($N = 51$, 19, 15, and 64, respectively). Reprinted by permission of Wolters Kluwer Health – Lippincott, Williams & Wilkins.



It should be noted that the data from right and left ears have been pooled in the current analyses, but the same results have been obtained for the separate analyses of right and left ears. Thus, our approach can be justified for reasons of simplicity (IV).

5. Discussion

5.1 Monitoring of middle ear pressures

The tympanometric short-term monitoring of changes in ME pressure related to a change in body position from the sitting to the supine position revealed a smaller pressure increase over the time course, and stable conditions in terms of repeatability between trials were obtained (I). The pressure response showed a biphasic response with an initial faster and larger pressure increase followed by a slower and smaller increase; this response corresponded to a similar biphasic increase in ΔV_{muc} within few s reported by Andréasson et al. (1976). Thus, these coinciding biphasic responses corroborated the hypothesis that the pressure increase was resulting from an increased congestion of the mucosa in response to changing the body from the sitting to the supine position. The two phases have been attributed to an immediate congestion of the venules, followed by slower congestion of the capillaries and eventually redistribution of intercellular fluids (Ingelstedt et al., 1967; Andréasson et al., 1976). Further, these changes in congestion have been directly related to an increased pressure in the jugular veins, when the body position is changed to supine (Rundcrantz, 1965; Jonson & Rundcrantz, 1969). Finally, the later phase may also be explained by a creep due to the viscoelastic properties of the mucosa including the venules (I). Altogether, repeatable conditions with a stable ME pressure has been demonstrated from 30 to 120 s, and thus, this time interval should be considered for ME experiments in the supine position which may be susceptible to changes in ME pressure and/or mucosa volume (Gaihede, 1999a,b).

The current tympanometer did not exhibit any phase delay, and the error of the intrinsic hysteresis was very small (III); thus, this source of measurements error can be disregarded. Additional factors that may affect the recordings during the time course are 1) gas exchange and 2) openings of the ET. However, the SD's of the distributions of the later differences were smaller (I) and found in range with the precision of the tympanometer (Gaihede & Marker, 1998). Thus, this variation can be explained merely by measurement variations, and it can be concluded that gas exchange as well as ET openings were not likely to have any effects during this short time scale. In general, the ET can be considered closed in the supine position (Rundcrantz, 1965; Ingelstedt et al., 1967). Moreover, whereas the compliance of the TM

increases in response to serial tympanometries, it has been demonstrated that the measurements ME pressures are not susceptible to repetitive measurements (Gaihede, 1996).

The total mean increase in ME pressure found in our study corresponded well to the results of Grøntved et al. (1989), who reported a similar increase over 3 min's between sitting and supine position. Contrary, Knight & Eccles (1991) has found a very small increase in normal subjects, but comparison was made between averaged values sampled over 20 to 30 min at intervals of 10 min; this would tend to blur any details of the course of pressure changes and due to a longer time course, ME gas exchange as well as ET openings may also affect the results. Most recently, Cinamon et al. (2009) corroborated our results by reporting a similar pressure increase. Further, this study also confirms the hypothesis of a venous filling can explain the pressure increase, since retesting some of the subjects after assuming the erect position immediately again results in pressures identical to the initial pressure; i.e. the venous congestion is instantly reversed upon changing back to erect position (Cinamon et al., 2009).

Diseased ears. Whereas the results discussed above relate to normal ears, Grøntved et al. has found larger increments in response to changing body position in subgroups of more negative ME pressures (1989). This indicate that the increments may be correlated to the magnitude of the ME pressure itself, and since it can be assumed that subjects with larger negative ME pressures also may represent inflammatory conditions, the pressure response in such ME's may differ from normal ME's. In accordance, Knight and Eccles also found larger increments in subjects with signs of upper respiratory tract infection (1991). Thus, a correlation between the pressure increments and the initial pressure would seem likely. We attempted to demonstrate such a correlation, but it was not be supported in our normal study group which is limited by including very few subjects with abnormal pressures (4 ears with ME pressure < -95 daPa) (I). Altogether, positional pressure changes in inflamed ears with abnormal ME pressures should be further investigated in order to define both the total pressure increase as well as any short term stable and repeatable conditions.

Body height. Since the increase in venous pressure has been suggested to play a major role for the volumetric increase in the ME mucosa, it may seem similarly related to the body height of the subjects. However, we have not been able to confirm this idea, since the correlation between the pressure increase and the body height was not significant ($p \geq 0.9$) (I). These

results may be limited by only including adult subjects; if children had been included the range of heights would have been larger, and a correlation may have been evident. However, the total increment of ΔV_{muc} , and thus, the increment of ME pressure, is probably a complex result of changes in height as well as changes in the vascular tone (I).

Sampling density. The sampling density of the pressure data is important in order to obtain a detailed analysis of the pressure changes. The current sampling density of four recordings per minute ($4/60 \text{ s} = 0.07 \text{ Hz}$) was not possible to increase further due to the manual operation of the tympanometer and registration of pressure data. Most tympanometric studies have reported longer durations between trials of 3 to 15 min's (Bylander et al., 1984; Grøntved et al., 1989; Knight & Eccles, 1991).

Long-term monitoring of ME pressure. The overall variation and regulation of ME pressure should be investigated by methods with an accurate long-term monitoring of pressure fluctuations at high sampling frequencies, and preferably with a direct approach to the ME cavity in order to avoid the inaccuracies related to tympanometry.

Tideholm et al. have reported a direct method, where a small transducer is mounted into an ear mould which is fitted air tight into the ear canal in subjects with either a TM perforation or a VT inserted (1996). This method allowed dense automatic sampling of pressure data at 1.25 Hz and monitoring up to 27-hours periods. In principle, these direct recordings should have the advantage of more detailed recordings compared with manual tympanometric trials (I). However, the vast amount of data collected by Tideholm et al. also constituted a limitation by displaying numerous pressure fluctuations due to a dense sampling, so that they introduced a sliding mean over 5 min's periods; this approach obviously will tend to cancel out details of short-term pressure changes (1996, 1999). Thus, in one of their studies the aspects of both positional changes and sleep have been investigated, where they have been unable to demonstrate any significant increase in ME pressure in response to changes into the supine position (Tideholm et al., 1999). However, they reported that positive ME pressures are developed during sleep and maintained until the morning (Tideholm et al., 1999); this confirmed previous reports on an apparently bidirectional gas exchange which tend to revert from gas absorption during awake conditions to gas excretion during sleep (Bylander et al., 1984; Hergils & Magnuson, 1985; Shinkawa et al., 1987).

However, in the context of an overall regulation of ME pressure, where a neural feedback mechanism plays a role, the physiological afferent input to the brain stem may depend on mechano-receptors in the ME cavity and the TM (Eden et al., 1990; Dirckx et al., 2013). Thus, the methodological demand of either a TM perforation or a VT insertion may hamper the afferent input needed for such a feedback mechanism, because the pressure load of the TM has been removed, and so, the regulatory mechanism cannot function on physiological terms.

More recently, another method with a trans-mastoid approach has been reported. This method comprises a catheter inserted into the mastoid in patients operated for parotid tumours, where the antero-lateral aspect of the mastoid is exposed in the surgical field; thus, drilling a small hole for the catheter is a simple acceptable procedure allowing access to the mastoid cavity while leaving the TM and the ME unaffected (Jacobsen et al., 2007). The catheter is connected to a transducer and a sampling unit which allows a highly accurate and dense registration of pressure measurements (10 Hz) for up to 48-hours periods (Jacobsen et al., 2007). However, similar to the experience by Tideholm et al. (1996), the amounts of data collected by such experiments are very large, and despite log-notes with registrations of the subject's activities, the vast occurrences of pressure variations are very difficult to interpret related to these activities. Figure 15 displays an example of a full recording over 22 hours from after surgery until the next morning; a dense and large variation in pressures is found during the entire period. Thus, while the details of the numerous pressure variations may contain valuable information, they may still confuse the overall interpretations of the pressure changes.

Consequently, another approach has been adapted from Jacobsen et al. (2007), where specific experiments with limited time frames have been focused on; thus, the counter-regulation of experimental pressure changes in smaller 10 min time frames has been reported (Gaihede et al., 2010a), as well as detailed analysis of the pressure and time parameters characterising ET openings and pressure equilibrations (Gaihede et al., 2013a). Such limited and well-defined experiments can be valuable in order to proceed with full 24- or 48-hours monitoring, since they may form a basis for pattern recognition and automatic analysis of the full recordings. However, the trans-mastoid approach also has its limitations, since diseased ears with possible sclerotic changes of the mastoid cannot be included due an increased risk of injury to the fa-

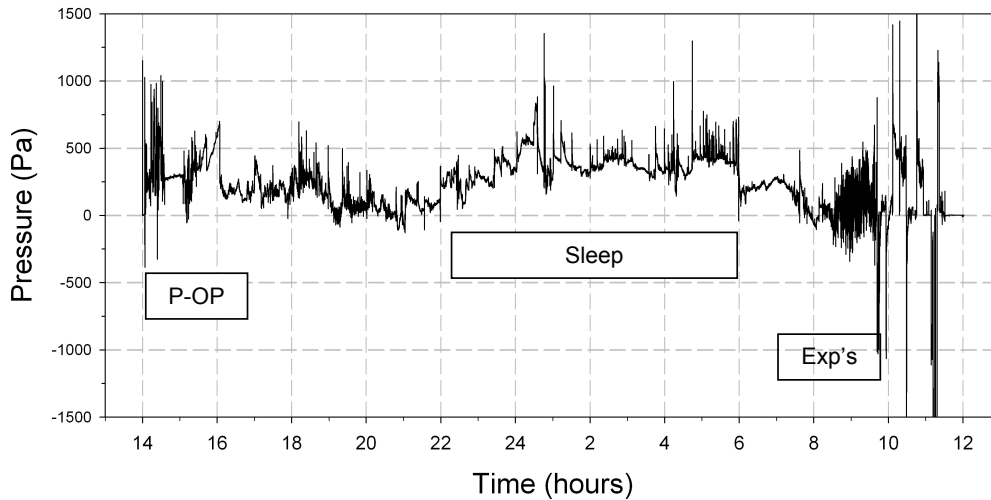


Fig. 15. Long-term monitoring of ME pressure via trans-mastoid access to the ME cavity; the full recording here amounts to approximately 22 hours. The recording starts at 14.00 hours after parotid surgery and continues through all night until the next morning at around 12.00 hours. The first two hours are in the post-operative care unit (P-OP), and the afternoon is dominated by rest and sleep after surgery. During the night period (sleep) the pressure shows an increase to higher positive pressures around 400-500 Pa. In the morning the pressure approaches to 0 Pa, and later multiple larger pressure deviations are seen due to the pressure experiments conducted in the subjects (Exp's) (Gaihede et al., 2010; 2013a). Note that no particular periods are found with a negative ME pressure

cial nerve, and hence, only subjects with normal ears have been included (Jacobsen et al., 2007; Gaihede et al., 2010a; Gaihede et al., 2013a).

Summary. Short-term monitoring of ME pressure have been demonstrated in response to a change in body position from sitting to supine in normal subjects, and a total pressure increase has been determined together with the time of stable pressure conditions. These observations should be taken into account in ME experiments which may be susceptible to positional changes in the ME pressure and/or the ME mucosal volume.

Long-term pressure monitoring should be applied in order to understand the overall pressure variation and regulation. For obvious reasons, tympanometry is not very practical, and 24-hour monitoring using this approach has only been reported in one study; further, the sampling density is not very high. Thus, current methods with accurate and direct monitoring including dense sampling of the pressure are more reliable for this purpose; however, the

methods should also pursue physiological conditions with an intact TM, as well as methods should allow subjects with various ME disorders to be included.

Short-term experiments are still valuable in order to interpret the full long-term pressure variations, and such experiments can include both direct methods and tympanometry (I). It should be noted that serial tympanometric trials can be justified, when the purpose is to monitor changes in ME pressure, where the differences between trials are analysed, because any errors due to the tympanometric procedure or the ME parameters can be considered constant between each trial (I).

5.2 Modelling experiments

The ME model. The application of models may have the advantages of controlling more physical parameters, but it also demand accurate and realistic model properties. Thus, modelling of the ME system should include its non-linear and viscoelastic properties (Gaihede, 1999b); this has been achieved by the double-layered membrane with Vaseline smear interposed (II). These properties have not been documented in similar models reported, where only mono-layered membranes have been used (Okitsu et al., 1985; Kobayashi et al., 1987; Cinamon & Sadé, 2003). Such membranes inevitably exhibit only linear deformation characteristics without hysteresis, and so their properties do not coincide with the characteristics of the normal PVR; this means that realistic volume displacements may not be achieved, and hence, the effects on the prevailing ME pressure are not realistic. The previous clinical studies on the PVR in normal ears provided an exact template for such a non-linear model with hysteresis expressing the averaged normal human PVR (the standard PVR) (Gaihede, 1999b; Gaihede & Kabel, 2000), and the model membrane exhibited realistic static pressure properties, since it coincided graphically with the standard PVR in the entire range of pressure variations as well as it had the same mechanical variables (II).

The area of the model membrane was constructed in accordance with the normal TM (Dirckx & Decraemer, 1992). However, in contrast to the normal TM, the model membrane was not cone shaped but flat, as well as it was not attached to any ossicles. However, the most important part is that the membrane tracked the volume-displacement relative to the ear canal

pressures, and hence, it resulted in realistic volume and pressure changes within the model ME cavity (II).

ME pressure. The accuracy of ME pressure determination by tympanometry was investigated by comparing the exact controlled ME model pressure with the tympanometric estimate. The ME model demonstrated a marked linear overestimation of the exact ME pressure, when measured by tympanometry (Fig. 10) (II). Similar overestimation has been found in other studies, as well as it has been found for both negative and positive pressure sweeps including temporal bone models (Peterson & Lidén, 1970; Renvall et al., 1975) and in normal subjects (Hergils et al., 1990). The study from Renvall et al. allows for determination of the slope of the regression line amounting to 1.4 (1975); thus, it is in the same order of magnitude of our results (II).

Since the current tympanometer did not exhibit any phase delay this factor can be ruled out (III), and the overestimation has been attributed to hysteresis (Decraemer et al., 1984; Kobayashi et al., 1987; Hergils et al., 1990). However, the inaccuracy reflected by the slope of the regression line has not been accounted for in any of these studies. The viscoelastic properties of the ME system is reflected also by the horizontal distance between the PVR curves in the negative and the positive direction, and this distance is often found quite narrow around its origin, whereas it increases towards its pressure extremes. This observation is present in individual PVR recordings (Gaihede, 1999a,b), but it can also be seen in the standard PVR (Figs. 1 and 9) (Gaihede & Kabel, 2000); thus, similar to the hysteresis, this overestimation is subject to individual variation which can explain the different slopes of the regression lines in different studies (II, Renvall et al., 1975).

ME volume. In the model the air volume behind the model membrane was altered between 1 and 21 cm³. Hence, tympanometric measurements of ME pressure could be obtained for various ME volumes which have been illustrated in Fig. 11. In this experiment the “true” ME pressure was 0 kPa, but for larger volumes the estimate was slightly negative, which corresponds to findings in normal ears, and which can readily be explained by the negative direction of pressure change as well as the minor inherited hysteresis of the MES (III; Decraemer et al., 1984). However, for smaller volumes less than 5 cm³, the estimates seemed to decrease exponentially, so that for infinitely small ME volumes, an infinitely large negative

ME pressure could be predicted from the regression line curve fit (II). This suggested that despite a normal ME pressure around 0 daPa, the tympanometric ME pressure estimate would be a very large negative pressure merely due to the decrease in ME volume, when it becomes less than 5 cm³. Moreover, the additional experiments with various deviations of model pressures showed the same response, so that especially negative model pressures were further overestimated in the negative direction by tympanometry (II).

Similarly, Elnor et al. (1971b) reported that the accuracy of indirect measurements of ME pressure decreased below a critical value of 6 cm³. Since this volume relates to the expandable air volume of the ME cavity, a similar decrease in the tympanometric ME pressure estimates would be expected, if this air volume has been replaced by an inexpandable ME fluid. Alternatively, increased thickness of the mucosa or mucus in the antrum may plug the opening between the tympanum and the mastoid which also will decrease the air volume behind the TM (Suetake et al., 1990). It follows, that high negative ME pressures may be measured by tympanometry due to inherited methodological errors, when patients display SOM and/or ME fluid. Moreover, similar errors may apply in patients with sclerotic mastoids as well as in children, who have smaller ME volumes due to an immature size of the mastoid (Rubensohn, 1965). The range of ME volumes applied in the model were in range with previous findings for normal ears (Ingelstedt et al., 1967; Elnor et al., 1971b; Park et al., 2000).

The ME model may have been improved by including additional model membranes with different elastic properties in order to investigate the effects of the TM mobility on the ΔV_{tm} , and hence, on the ME pressure itself. The model membrane employed in the current model exhibited tympanometric compliance of 0.33 cm³, which represents a rather low compliance, but within range of normal values (Shanks & Shelton, 1991). This means that the errors encountered by this study are likely to be larger, if more flaccid membranes have been constructed and investigated (Flisberg et al., 1963; Ingelstedt et al., 1967; Elnor et al., 1971a,b). The construction of the current model membrane was a quite time consuming iterative process with multiple attempts to loosen or tighten the two elastic layers as well as to apply different amounts of Vaseline (II). Thus, we did not pursue the construction of additional model membranes; there are no reports available in the literature with quantitative analysis of the TM compliance and its effects on tympanometric accuracy. Further

improvements of the model also include a smaller ME cavity, so that the apparent exponential decrease of pressure estimates at even smaller volumes may be further explored (II).

Subsequent studies. In response to the inaccuracies reported in this model, two proceeding papers have been investigating the same aspects in both a mechanical and an animal model, where the exact ME pressures have been compared with their tympanometric estimates (Cinamon & Sadé, 2003; Alper et al., 2003). The model employed by Cinamon & Sadé (2003) only included a mono-layered elastic membrane and they did not account for its mechanical properties. Thus, non-linear behaviour and hysteresis is unlikely to have been incorporated into this model; nevertheless, they confirmed more of our findings. A similar linear overestimation of the tympanometric estimate of ME pressure was found, and further, they reported that smaller ME model volumes resulted in very high erroneous negative tympanometric estimates. They concluded that tympanometry cannot determine the ME pressure accurately in diseased ears, where the mastoid is small and/or the pressures slightly negative. Moreover, they underlined the controversy of the important interests of attaining accurate pressures in such diseased ear, and the tympanometric inaccuracy specifically of under these conditions (Cinamon & Sadé, 2003).

In contrast, the model presented by Alper et al. (2003) concluded that tympanometry accurately estimated their experimental underpressures; moreover, they reported a similar linear overestimation of the tympanometric estimates of ME pressure. However, despite various theoretical considerations on the effects of the ME volume, their experimental setup disregarded investigations of this factor (Alper et al., 2003). Thus, their results do not justify a rejection of the hypothesis that smaller ME air volumes will seriously affect the accuracy of tympanometric estimates of ME pressures (II). Altogether, investigations of the ME volume including experimental volume alterations is complicated in both a monkey model as well as in human subjects, because the range of the ME volume is unlikely to be small enough to result in the exponential decrease of the tympanometric estimates (Alper et al., 2003; Hergils et al., 1990). It may have been explored by replacing the ME air by filling isotonic NaCl into the ME, but obviously this may cause risk of infection and possible other complications. Thus, modelling is still useful for certain aspects and tympanometric B-curves have demonstrated in both temporal bone and plastic models merely by filling the model ME with

water (Peterson & Lidén, 1970; Renvall et al., 1975; Okitsu et al., 1985). Most recently, Yilmaz et al. reported similar results in a rodent model (2008).

Summary. In conclusion, the results from the current model have suggested both a linear overestimation of tympanometric ME pressures and an exponential overestimation for small ME volumes. Thus, it has been hypothesized that normal or negative ME pressures are likely to be highly overestimated in the negative direction in diseased ears with a small ME volume and/or presence of ME fluid. These findings have been corroborated directly in one study, whereas another study made the attempt, but did not address the volume problem directly; no other studies have been reported on this problem. Further validation in human subjects is difficult due to current methodological limitations and for ethical reasons.

5.3 Peak pressure difference

The PPD found in the current studies for normal ears showed a rather constant smaller value independent of the rate of pressure change (III), and thus, the PPD represented the intrinsic hysteresis of the MES (Decraemer et al., 1984). The hysteresis is a general feature found in biomechanical testing, and it relates to the viscoelastic properties of biological tissues; thus, it also represents an inevitable factor of the mechanical properties of the MES (Decraemer et al., 1984). This hysteresis is also reflected by the circumscribed area of the PVR measurements, where the hysteresis can be measured directly and expressed in μJ for individual ears (Fig. 1); hysteresis shows biological variation as well as it may be altered in diseased ears (Gaihede et al., 1997; Gaihede, 1999a,b). The derived compliance functions of the PVR illustrated in Figure 7 from a normal ear also displays this hysteresis reflected by a smaller intrinsic PPD (Gaihede & Kabel, 2000).

Thus, the inaccuracy of determining the ME pressure with our current tympanometer was accordingly very small (III). Compared to the previous studies with rate dependent PPD's (Shanks & Wilson, 1986; Kobayashi et al., 1987), this improvement is explained by the technological advances introduced at that time by the MEA 901 tympanometer from Madsen Electronics, where the pressure transducer has been incorporated into the headset; thus, the distance between the actual pressure changes and its recordings has been brought to a minimum (Decraemer et al., 1984). It can be concluded that our instrument represented a

minimal PPD which only reflected the intrinsic hysteresis of the MES, and which was not related to the rate of pressure change (III). These observations were valuable for the interpretations of the other parts of this thesis.

The PPD was shown significantly increased in cases with ME fluid compared to normal age-matched ears (Table 1) (IV). Since our tympanometer did not show any phase delay, this increased PPD could only be explained by the increased viscoelastic properties of the MES represented by the ME fluid. Further, this hypothesis has been corroborated by the significant dose-response correlations found for both the amount of the fluid as well as its viscosity (Fig. 14) (IV). Based on these observations, the inaccuracy of determining the ME pressure in SOM ears may be moderate on average, but since the range included a maximum of more 100's daPa, larger errors around 100 daPa have also been suggested. However, the estimate was only based on the cases with less severely affected ears, because all cases with B-type tympanograms at the time of surgery were excluded (IV); B-type tympanograms have flat curves, so that no peaks are present, and thus the ME pressure cannot be identified (Shanks & Shelton, 1991). It was estimated that B-type recordings was found in around 50 % the patients at the VT insertion (IV); this is in accordance with Moody et al. (1998). Thus, the current results are a low estimate representing only the 50 % less severely affected ears. In essence, though only based on a casuistic report, the results based on the PVR measurements and its derivatives may seem a more accurate estimate of the PPD in ears with SOM, where the inaccuracy amounts to 225 daPa (Fig. 7) (Gaihede, 1999a)(IV). It should be noted that an age-matched control groups was included in this study (IV), because the elastic as well as the viscoelastic properties of the ME system may be subject to age-related changes (Gaihede & Koefoed-Nielsen, 2000).

It has not been possible to identify additional studies, where the influence of the properties of ME fluid on tympanometric ME pressure measurements has been investigated. In stead the influence of these properties on the hearing impairment has been reported. Thus, both the viscosity and the amount of ME fluid plays an important role for the resulting hearing loss in SOM ear, however, the latter plays the most prominent role (Majima et al., 1988; Hartley et al., 2003). We attempted a multiple linear regression analysis of the PPD against both viscosity and amount of MEE which indicated that the influence of the amount of effusion is more important than the viscosity (IV). This is in agreement with the findings of the amount

also being more important for the hearing loss than the viscosity (Majima et al., 1988; Hartley et al., 2003), but the conclusion should be taken with caution due to statistical limitations, since our independent variables were categorical (IV). The analysis may be improved by a higher number of observations increasing the numbers in subgroups. In addition, objective determination of the viscosity by a viscometer could improve the analysis of data, but this is hampered by generally very small amounts of ME fluid. This obstacle can be overcome by a dilution technique, or by quantifying the contents of glycoprotein, since it correlates to the viscosity (Carrie et al., 1992; Sichel et al., 2003). However, the amount of fluid will still remain difficult to quantify accurately, since it can be concealed in the adjacent spaces of the ME, and thus, remain undetected (IV).

Summary. The inherent PPD represented by the tympanometer in these investigations has been found insignificant, whereas the PPD in ears with ME effusion has been found significant larger, and hence, it constitutes a considerable directional sensitivity, which overestimates the true ME pressure. This error correlates to both the viscosity and the amount of the effusion. These findings have not been described earlier, though the rheological properties of ME fluid have been discussed in attempt to explain the disagreement between the negative tympanometric values of ME pressures and the directly measured pressures in a group of SOM ears (Sadé et al.; 1976). The accuracy may be improved by determining the mean ME pressure from bidirectional recordings in such ears, but in general this attempt has not gained much focus in the literature. Finally, it should further be noted that the group investigated here reflected only the milder cases of the SOM children, and thus, the error is likely to amount to more 100's daPa.

5.4 The "true" middle ear pressure and pathogenetic events

From the current studies on the accuracy of tympanometric ME pressure a series of errors have been investigated and quantified which altogether suggest that erroneous high negative ME pressures can result simply from the combined effects of 1) the directional sensitivity, 2) a small ME air volume, and 3) the increased hysteresis due to the ME fluid. It is important to note that these errors do not challenge the existence of ME underpressures, since these seem evident by observations of the TM in diseased ears (Fig. 4C,D). Unfortunately, these errors apply to the conditions in diseased ears, where the exact pressures are important for our

understanding of the pathogenetic events, for instance the *hydrops ex vacuo theory*. However, while demonstrating these errors, our results do not provide information about the exact pressures in these ears.

In a series of patients with SOM and high negative tympanometric ME pressures, direct measurements of pressures have demonstrated only normal or slightly negative ME (Buckingham & Ferrer, 1973; Sadé et al., 1976; Takahashi et al., 1991). Further, Bratmo et al. has reported relatively low underpressures in ears with a chronic TM perforation measured by an ear canal transducer (2003); their results have been outlined in Table 2. Various methodological limitations may hamper the results (Alper et al., 2003; Gaihede et al., 2010a), but basically they support the interpretations of the current studies (II, III, IV), since higher underpressure of more 100's daPa are almost absent. Hence, the significance of high ME underpressures may further be questioned.

Table 2. Direct measurements of ME pressure in selected studies.

Study	Patient group	ME pressure (daPa)*
Buckingham & Ferrer, 1973 (N=108)	Serous OM	-7 (-1.6 to -45.5)
Sadé et al., 1976 (N=36)	SOM	-1.7 (5 to -12.1)
Takahashi et al., 1991 (N=25)	SOM	-53.2 (SD=57.9)
Brattmo et al., 2003 (N=23)	Chronic TM perforation	-64 (SD=68)

*) The range of data is reported by the interval in parentheses; or the standard deviation is reported similarly depending on availability.

Exudate or transudate? Traditionally, the ME fluid found in SOM ears has been interpreted as a transudate in accordance with Politzer's *hydrops ex vacuo* hypothesis (1867). However, a transudate will only form at underpressures around -50 to -90 daPa or more negative, whereas an exudate is merely based on an inflammatory reaction and do not depend on any hydrostatic pressure gradient (Sadé & Ars, 1997). It follows from the preceding discussion that the actual underpressures may not be high enough to cause such a transudation. Thus, the current results on tympanometric accuracy may in stead point to an exudate being more likely a part of the pathogenesis in SOM which also has been supported by the biochemical composition of the fluid found in SOM (Sadé & Ars, 1997).

Experimental underpressures in humans have reported by a trans-mastoid approach, where transudation appeared between -2.7 and -4.1 kPa (Flisberg et al., 1963); however, while these pressures have been measured directly, they may still not be physiological pressures. Similar underpressures have been induced by inflation of the ME in monkeys with O₂ and CO₂ resulting in pressures around -6 kPa; these pressures resulted in subsequent fluid formation in the ME recorded by MRI studies (Swarts et al., 1995). In a similar study, the ET in monkeys has been functionally obstructed by muscular injection of botulinum toxin, and ME underpressures as well as ME fluid determined by MRI studies developed over a time course of weeks; a critical value of -2.7 kPa was reported in order to provoke the formation of fluid (Alper et al., 1997). These observations have been proposed as evidence for the *hydrops ex vacuo* theory; however, in the first study, the gas compositions were non-physiological, and in both studies the ME pressure was determined by tympanometry, and thus, the magnitude of the ME pressure may be erroneous or not physiological (II, IV). True transudates are indeed well known in certain cases with barotraumas to the ME ear, where larger underpressures have been shown to affect the ME for instance in relation to aviation; however, this aetiology is unlike the situation in SOM (Sadé & Ars, 1997).

In terms of the efficacy of VT insertions to relieve the ME fluid in patients with SOM, the mechanism has been attributed to the equilibration of the ME pressure. However, an alternative explanation has been proposed, where the propulsion of ME fluid by the epithelial cilia towards the ET orifice creates an underpressure in the ME cavity which at a certain point inhibits its further propulsion and clearance; in this context, the insertion of a VT relieves the underpressure and allow for the continued propulsion of the fluid (Sadé & Ars, 1997). Other factors may be related to the mucosa of the ME, since it has been demonstrated that the absorption of water and Na⁺ is enhanced by a relative hyperoxic atmosphere of the ME cavity; this corresponds to the situation, where a VT allows the access of atmospheric air to the ME (Portier et al., 1999).

Morphological aspects. It follows, that if high underpressures are questioned, then other factors may be involved in the development of TM degeneration and atrophy. Traditionally, Tos et al. (1984) has documented a correlation between TM atrophy and the frequency of SOM, and thus, explained TM degeneration by the pressure load onto the TM during periods of disease. Based on the discussion above, an alternate explanation of degeneration and

atrophy may be intrinsic factors within the TM. Various degrees of submucosal oedema have been reported in the TM in ears with SOM (Møller, 1981; Sano et al., 1994; Berger et al., 1996). These observations are likely to be associated also with an increased tension within the TM, i.e. an increased intrinsic shear stress; such changes may similarly cause degeneration of the lamina propria of the TM (II). Further, the microscopical appearance of TM atrophy is characterized by areas of fragmentation as well as absence of the lamina propria connective fibres, and inflammatory changes are found with oedema, lymphocytes, and plasmocytes (Sadé, 1993). It was concluded that inflammation plays a role in the loss of the lamina propria which corresponded to a loss of the mechanical skeleton of the TM; this turns the ME into a collapsible gas-pocket, where retraction and atelectasis can prevail (Sadé, 1993). Finally, Magnuson et al. have shown that repeated static pressures administered to the TM result in structural changes of the rat lamina propria (1995). However, the pressure loads used in these experiments were around 3 kPa, and this may not be considered physiological. Altogether, there is more evidence of intrinsic factors that may contribute to the development of TM atrophy in diseased ears; these factors may constitute the primary problem which ultimately results in the situation, where only smaller negative ME pressures can cause retraction of the TM and atelectasis of the ME.

Summary and the role of tympanometry. The tympanometric sources of inaccuracies in ME pressure determination in diseased ears question the existence of higher underpressures. This has been corroborated by few studies reporting on direct measurements, where only smaller underpressures are found; these are in general less negative than -100 daPa. This may point to the ME fluid is formed by an exudate and not a pressure dependent transudate. Further, the structural degeneration of the TM leading to atrophy may primarily be explained by intrinsic inflammatory factors and not a high pressure load.

While tympanometry may not be accurate in measuring the exact ME pressure in diseased ears, the method is still immensely valuable in the overall evaluation of otological patients; for instance, a normal tympanometric ME pressure around -50 to 50 daPa is still likely to indicate an exact pressure and normal aeration of the ME. It should also be noted that flat B-curves found in tympanometry have a high accuracy in the detection of ME fluid and diagnosis of SOM (Ovesen et al., 1993; Watters et al., 1997). So tympanometry is a valuable tool for screening patients for SOM, as well as it has other valuable applications such as

determining the TM compliance, the stapedia reflexes, and the ET function (Shanks & Shelton, 1991).

5.5 Middle ear pressure and otosurgery

SOM. Despite the previous discussion on the mechanisms of VT insertions in SOM, the surgical treatment by VT's in patients with sustained disease is still the only established method; medical treatment with for instance oral or intranasal steroids with or without the combination of antibiotics may lead to a quicker relief of SOM on a short term scale, but the long term effects lack (Thomas et al., 2010).

Pressure protection and otosurgery. As previously outlined the ME system is adapted to a remarkably wide-ranging series of pressures from low acoustic pressures (μPa 's) to high static pressures (kPa's). This adaptation is reflected by the notable transition of the ossicular chain vibration pattern, where: 1) acoustic pressures result in *piston like displacements* of the stapes foot plate transmitting the sound pressures to the inner ear, and where 2) static pressures result in *tilting displacements* of the stapes foot plate with decoupling the inner ear. Thus, the complexity of the ossicular system ensures an optimal transmission of acoustic pressures, while still protecting the inner ear from the static pressures with high amplitudes (Hüttenbrink, 1988). This protection also applies to the situation of otosurgery with an intact ossicular chain, where the subtle manipulation by the surgeon may otherwise harm the inner ear. Further, the annular ligament of the stapes footplate may comprise an additional protective factor, because the biomechanical properties of this ligament display a rather steep sigmoid pressure-volume characteristic (Direkx et al., 2006). This means that the ligament exhibits a very high compliance at smaller acoustic pressures, while it gradually exhibits a very high stiffness at higher static pressures, and thus, it also contributes to decoupling the inner ear at static pressures; this protective factor remains in cases of disruption of the ossicular chain. In addition, the hysteresis of the ME system also contributes to a protection of the ME from changes in ambient pressures, because the hysteresis corresponds to an energy loss, where the energy is dissipated as heat into the tissues (Gaihede, 1999a,b).

Techniques of otosurgical reconstructions. It follows that the acoustic as well as the static properties of the ME system should be considered in the otosurgical reconstruction of ME

structures (Hüttenbrink, 1988). As stated earlier, the classical works on otosurgical functional reconstructions also recognised that the results depended highly on the post-operative course demanding a continued aeration of the ME, i.e., the absence of sustained ME underpressures (Zöllner, 1955; Wullstein, 1956). Consequently, more techniques have been reported, where various attempts have been made in order to avoid the formation of new retraction pockets, atelectasis or cholesteatomas, and thus, the need for revision surgeries.

Grafting materials with a higher stiffness than conventional fascia or perichondrium have been searched for in order to resist the prolonged pressure loads and ensure favourable long-term results, while still complying with reasonable acoustic properties for an optimal hearing. During the recent decades cartilage grafts have become increasingly popular. The optimal compromise between its static and acoustic properties can be obtained by slicing the full thickness cartilage into thinner layers (Zahnert et al., 2000), and techniques of various forms have been reported (Beutner et al., 2009).

Moreover, obliteration of the mastoid with bone or soft tissue materials has been increasingly applied; this surgical technique is used in relation to cholesteatoma surgery, and it is related to lower recurrence rates (Tahahashi et al., 2007; Vercruyse et al., 2009; Edfeldt et al., 2013). It has been proposed that the favourable outcome of obliteration is based on a substantial reduction of the size of the ME surface area, and thus, its capacity for gas absorption and/or inflammation (Vercruyse et al., 2009). Recent clinical experiments have suggested that the normal mastoid has its own separate role in pressure regulation (Gaihede et al., 2010a); thus, it follows from a basic point of view that the impaired pressure regulation found in for instance cholesteatoma ears may be explained by a diseased mastoid, and thus, the mastoid obliteration will improve the outcome for the patient by removing the major source of the underpressures (Tauris et al., *in press*).

Summary. Insertion of VT's is still the primary treatment of SOM. The outcome of otosurgical reconstructions in cases with chronic OM and sequelae related to underpressures are susceptible to the post-operative formation of underpressures. Grafting the TM with stiffer materials like cartilage as well as obliteration of the mastoid seem to target specific problems related to these underpressures.

However, further improvements of our knowledge of the multiple factors involved in the overall pressure regulation are mandatory in order to optimize the long-term results of otosurgery in patients suffering from the various clinical conditions of ME underpressures. This also includes the aspects of the TM elasticity which determines the outcome of specific ME pressures; thus the precipitation of retractions and atelectasis is a balance between ME pressure and TM compliance. An important aim would be to identify patients at risks of these conditions, before the structural degeneration of the ME structures are taking place, because this may enable to maintain an intact ossicular chain and normal hearing.

6. Future studies

The mechanisms of the overall regulation of ME pressure are still unclarified and many questions remain to be answered. Improved direct measurements monitoring the pressures over long-term time scales should be pursued in order to understand the daily pressure fluctuations in normal ears as well as the formation and course of the underpressures in diseased ears. The current methods should still focus on limited well-defined experiments with smaller time-frames in order to provide data for future pattern recognition and automated analysis of larger long-term data sets. In particular, such studies should maintain physiological conditions with an intact TM. The future advent of smaller pressure transducers may pose advantages by being able to be positioned into the ME cavity itself.

Direct measurements of ME pressure with high resolution in both time and pressure have revealed small constant pressure oscillations synchronous to the heart rate, and hence, they reflect the vascular pulsation of the ME mucosa. The amplitude of these pressure variations is subject to variation, so that smaller amplitudes around 6 Pa can be found during sleep, while around 20 Pa after a Valsalva manoeuvre (Jacobsen et al., 2007). In the light of the ongoing studies reported below, where changes in the mucosal blood flow and congestion may be considered a new important factor in pressure regulation, changes in these pressure oscillations may reflect changes in the blood flow; thus, these high frequency oscillations may be interesting to correlate to the lower frequency changes in ME pressure.

Neurophysiological studies are also relevant in order to improve our understanding of the pressure regulation, since it seems related to a neural feedback control as mentioned earlier. The literature contains relatively few studies corroborating this idea (Dirckx et al., 2013), but direct connections between the ME and the ET muscles have been reported in an important paper by Eden et al. (1990); they demonstrated that electric stimulation of the tympanic nerve resulted in EMG activity of the ET muscles in primates. Recently, clinical experiments have measured the evoked brain potentials in response to static pressure stimulation of the TM and ME in normal subjects in attempt to establish related neural activities. In one study distinct early brain stem and cerebellar neural activities have been demonstrated which are different from acoustic pressure stimulation (Sami et al., 2009) (Fig. 16). Another study applying connectivity analysis of these neural activities has demonstrated distinct links between γ -activity

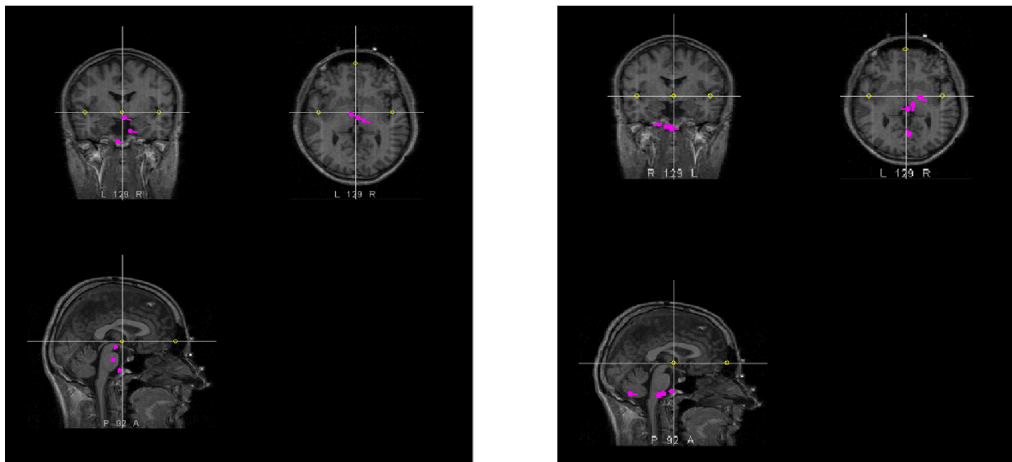


Fig. 16. The early pressure evoked brain potentials from a 64-channel EEG has been analyzed by source localisation and superimposed onto a standard MRI brain map model.
Left panel: the brainstem neural activities in response to acoustic pressures (white noise); the activity pattern extend from the lower part to the higher part of the brainstem.
Right panel: the neural activities in response to static pressures (2 kPa); the activity pattern extend from the lower part of the brainstem into the cerebellar hemisphere. Thus, acoustic and static pressures result in distinctively different neural activity patterns.

of cerebellar locations and β -activity of motor cortices in response to static pressures; these findings are suggestive of a sensory-motor feedback (Gaihede et al., 2010b). Thus, specific afferent neural pathways as well as central neural activity patterns exist related to stimulation by static pressures. Future studies should also include diseased ears, and further attempts to establish any related efferent motor activities are highly relevant; this may be accomplished by simultaneous EMG of the ET muscles in human experiments (Alper et al., 2012).

In previous clinical investigations with direct measurements of the ME pressure smaller experimental pressure deviations have been introduced and the resulting counter-regulations have been studied (Gaihede et al., 2010a). In these experiments the counter-regulation shows two patterns: 1) step-wise fast pressure changes towards ambient pressure and 2) gradual slower pressure changes in both negative and positive directions; while the step-wise responses can readily be attributed to ET openings, the gradual responses must be explained by another mechanism. Since the experiments have been performed in awoken subjects, where only gas absorption with a negative pressure change should prevail (Doyle, 2000), the pressure changes in the positive direction cannot be explained by gas exchange. Thus, an additional mechanism must also play a role. The mastoid has not gained much attention in physio-

logical research, but it comprises by far the largest part of the entire ME, and it seems an obvious aim for further studies aiming at the explanation of the gradual pressure changes. Thus, the mastoid has become a focus of ongoing research.

The tympanum of the ME is a relatively small ($<1 \text{ cm}^3$) and smooth walled cavity, whereas the mastoid contains an irregular branching structural arrangement with multiple air cells, so that its surface area-to-volume-ratio is higher (Fig. 17). Based on a normal mastoid volume and surface area (Park et al., 2000; Swarts et al., 2010), it can be calculated that an increase in mucosal thickness of only $0.6 \text{ }\mu\text{m}$ can effectively change the ME pressure by 1 kPa (Magnuson, 2003). Thus, it has been suggested that volumetric changes of the mastoid mucosa based on changes in its congestion may be part of the ME pressure regulation in humans (Magnuson, 2003), and it follows that such volumetric changes as well as resulting pressure changes will be gradual and that they can function in both positive and negative directions. In fact, a series of older biomechanical studies investigating the volume-pressure-relationship of the ME system have reported smaller changes in ΔV_{muc} which originally have been perceived

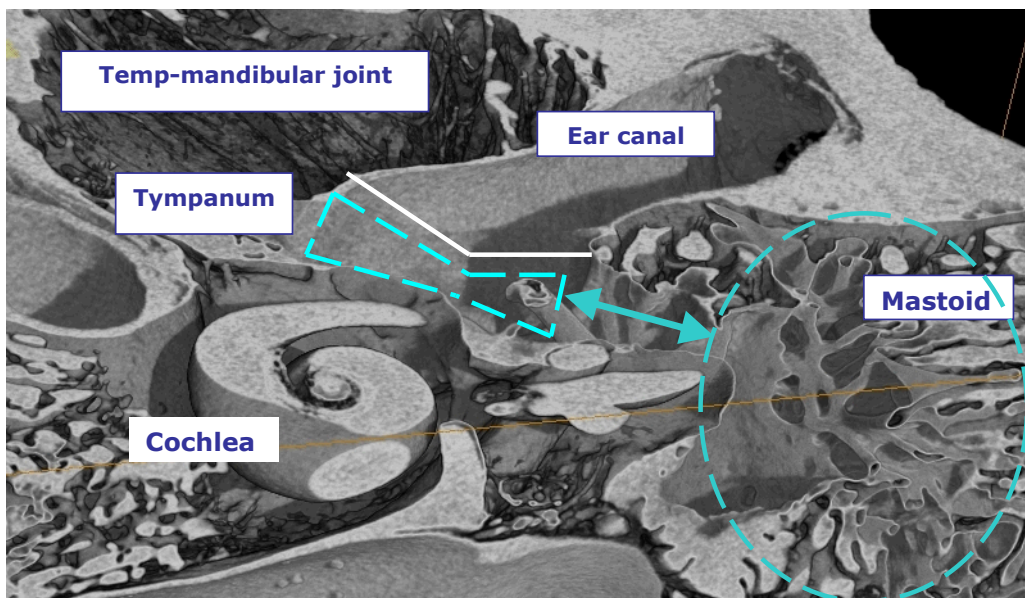


Fig. 17. Micro-CT scanning of the temporal bone with the temporo-mandibular joint, ear canal, cochlea, tympanum, and mastoid outlined. The TM has been illustrated by the white line. In contrast to the more regular smooth walls of the tympanum (squared blue area), the mastoid consists of numerous ramifications and air filled cellular compartments (oval blue area). This increases the surface area of the mastoid significantly including its surface area-to-volume-ratio. The mastoid is openly connected to the tympanum by the “antrum” (blue double arrow) and these compartments share same pressure (*Courtesy by Olivier Cros, engineer*).

as smaller sources of measurement errors (Ingelstedt et al., 1967; Elnor et al., 1971a). Moreover, it has also been demonstrated that changes in the actual ME pressure can induce similar changes in ΔV_{muc} (Andreasson et al., 1976), as well as it has also been suggested to explain the positional changes in ME pressure discussed previously in this thesis (I).

In diving mammals a similar mechanism has been suggested, where the ME mucosa contains sinusoid venules assembled in cavernous structures; these structures may expand or shrink, while adapting the ME pressure to high ambient pressures in diving, and thus, protect the ME and the inner ear from these high pressures (Sassu & Cozzi, 2007). While the pressure regulation in these animals represents extreme conditions, the same mechanism may still contribute to pressure regulation also in land-living animals including humans.

Similar mucosal structures has not been described for the human ME, but most recently we have pursued further ultra-structural studies of the mastoid based on micro-CT-scanning and histological immunostaining of its mucosa. These studies have shown multiple micro-channels between the surface of the mastoid bone and the air cells which constitute a vast and additional vascular supply to the mastoid mucosa (Cros et al., 2013). Further, immunostaining of the mucosa has shown a dense vascular presence with numerous venules, where some appear to represent sinusoids (Gaihede et al., 2013b) (Fig. 18). Thus, the mastoid seems to have a high vascular supply which may both alter the gas exchange by changes in its perfusion, and may enable volumetric changes in the congestion of mucosal capacitance vessels. Hitherto, this last mechanism has not been considered a part of the traditional factors in ME physiology, but it may still play an important additional role in pressure regulation. This needs to be further corroborated in more detailed functional and ultra-structural studies.

The lining of the ME consists of respiratory epithelium similar to other areas of the airways. Thus, the tympanum itself contains pseudo-stratified columnar epithelium with cilia as well as goblet cells and smaller glands, while the mastoid epithelium is lower with a flat or cuboidal mono-cellular surface with a loose connective tissue with frequent blood vessels (Ars et al., 1997). Histological investigations on the mastoid are few, but based on our preliminary demonstration of sinusoidal venules (Gaihede et al., 2013b), it may also be proposed that the mastoid share more structural similarities with the nose, where sinusoids are common (Widdicombe, 1997). In the nose, the inflammatory responses to various stimuli have been investi-

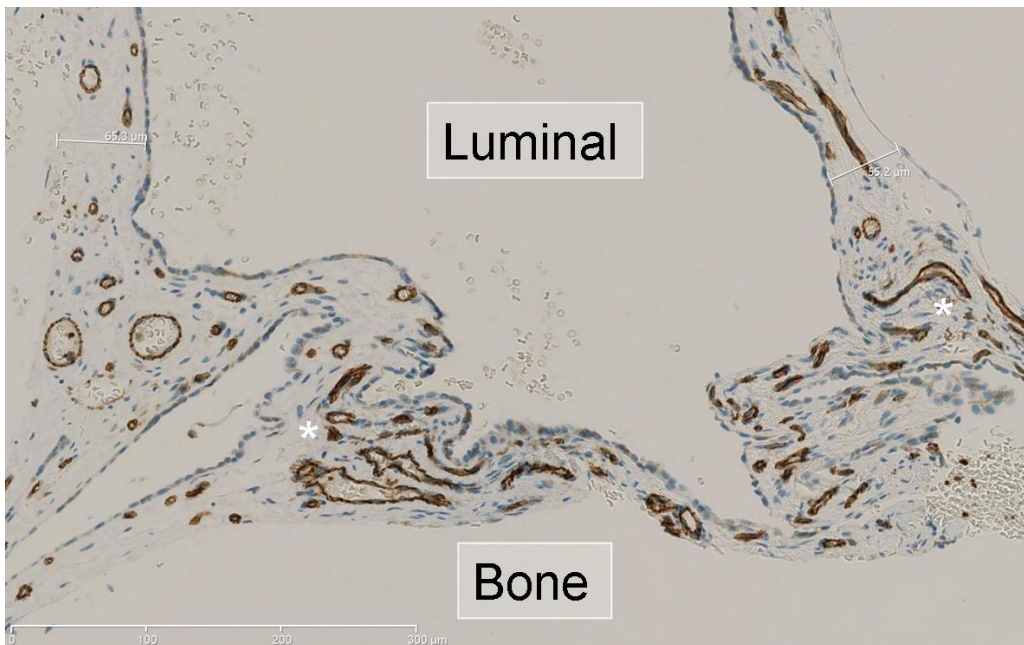


Fig. 18. Histological section of a normal mastoid mucosa with immunostaining CD31 marking the endothelial tissue with brown (20 \times). The vascular elements are numerous appearing mostly as thinned walled venoles more of which are dilated (marked with asterisk). The thickness of the mucosa varies between 55 to 65 μ m.

gated for many years, where fenestrations in both capillaries and post-capillary venoles play an important role: they can act by a fast and vast extravasation of fluid and inflammatory mediators into the tissue and the lumen of the nose (Widdicombe, 1997). It follows, the fluid of the ME cavity may indeed turn out to consist of an inflammatory exudate rather than a transudate, and more similarities between the inflammatory responses of the mastoid and the nose may exist.

Altogether, the mastoid represents the largest part of the ME with a very large mucosa surface; however, the mastoid has traditionally been considered a passive volume of air which may be explained in part by its relative inaccessibility, and thus, evading from experimental approaches. The current aspects of emerging new knowledge seem promising for future research in order to obtain an improved understanding the basic physiology of the larger part of the ME cavity. Such attempts include further structural studies with micro CT scanings and functional studies by improved resolutions of MRI as well as PET-CT scanings that may

elucidate for instance changes in blood flow and congestion of the mucosa (Swarts et al., 2013).

Summary. Future studies on ME physiology and pressure regulation still include the traditional factors of ME gas exchange and the ET function. Methods for direct monitoring of ME pressures have been reported, and improvements are warranted in order to extend on these aspects avoiding the limitations of tympanometry. Moreover, recent neurophysiological reports of an overall neural feedback control system also point to a distinct role in pressure regulation, which seem an important focus for more intensive studies. Finally, the mastoid seems to play an immensely significant role in pressure regulation. While more of these new emerging areas up to now have been conducted only in normal cases, the investigations in diseased ears still await for a better understanding of the series of ME disorders resulting from underpressures. Overall these scopes of future research should ultimately improve our treatment strategies of patients with dysregulation of ME pressure, so that good and stable hearing can be maintained.

7. Conclusions

Pressure changes in the ME in response to body change into supine position have been characterized in terms of its increment and time course including its stabilisation in normal ears. The time course coincides with previous reports of ΔV_{muc} in response to similar position change, and thus, the pressure increment can be attributed to a volumetric increase of the ME mucosa due to congestion. These observations are relevant in ME experiments, where the ME pressure and/or the mucosa may affect the results, as well as the characteristics of the pressure changes may be used to identify similar changes by pattern recognition in automated analysis of full 24-hour recordings of ME pressure. Serial tympanometry, where differences between trials are analysed to study changes in ME pressure, can be justified, since errors of measurements are likely to be constant. Monitoring pressure changes by tympanometry is however limited by its sampling density and impractical for long-term experiments.

Studies in the mechanical model showed that actual deviating ME pressures are overestimated by tympanometry; the difference is defined by a linear overestimation of tympanometric pressures compared to actual pressures. Thus, actual underpressures are measured more negative by tympanometry. Moreover, actual smaller underpressures seem to be exponentially overestimated in the negative direction, when ME air volume decreases below its normal values. Small ME air volumes can be found per se in smaller children, but this may also refer to the conditions, where the air volume of the ME is reduced by the presence of ME fluid or thickened mucosa due to inflammatory conditions. Thus, it has been hypothesized that very high underpressures may be obtained by tympanometry in cases with a normal or only slightly negative ME pressure, when the ME air volume is reduced due to replacements by fluid or mucosa.

The contribution to the directional ambiguity of tympanometric measurements of ME pressure represented by the PPD in normal ears has been diminished to a minimum by the construction of the modern instrument. Thus, the PPD determined here corresponded only to the inherited hysteresis of the ME system due to its viscoelastic properties, and the error related to this phenomenon is negligible in normal ears. It followed that the ME pressure determination was not susceptible to the rate of pressure change chosen on the instrument. However, similar investigations of the PPD in cases with SOM and ME fluid showed significantly higher PPD's

which suggested significantly higher errors in pressure determination. This is readably explained by the properties of the ME fluid, because the PPD correlated to both the viscosity and the amount of the fluid in a positive dose-response relationship.

Altogether, the combined effects of ME fluid contribute to an increased PPD and replacement of the ME air which both will result in higher underpressures as estimated by tympanometry in comparison with the actual pressures. It follows that tympanometric measurements of ME pressure could be suggested to be highly inaccurate in diseased ears, where the exact pressures are important to study in order to analyse the pathogenetic events. Further, it could be hypothesized that the mechanism of ME fluid in SOM is more likely an inflammatory exudate rather than a transudate. Finally, it has also been hypothesized that intrinsic inflammatory changes in the TM may be responsible for its degeneration and development of atrophy, rather than high negative pressures causes degenerative changes.

The overall pressure regulation is an important aspect of the ME physiology, and the occurrence of underpressures found in more ME conditions must be perceived as an impaired regulatory mechanism. Measurements of ME pressure is still a very important method in order to investigate and understand these pressure variations in both normal and diseased ears. Tympanometry has here been presented with a series of limitations, and direct physiological methods with long-term detailed sampling should be pursued. The basic pressure regulatory mechanism explaining the pressure variation involves a series of factors, where the ME gas exchange and ET function has traditionally been investigated. However, recent studies have also corroborated the existence of a neural brainstem centre involved in feedback control to ME pressure, as well as the mastoid has been suggested to play an important role by changes in blood perfusion and/or congestion. The completion of these factors still awaits and an approach of multidisciplinary research is mandatory in order to achieve an improved understanding of the complexity of ME pressure regulation. Such scientific advances should enable an adaptation of treatment strategies, so that stable hearing and improved quality of life can be obtained in our patients.

8. Summary in English

Middle ear (ME) pressure is a significant factor in the physiology of the ME and in clinical otology, because the development of underpressures are very frequent and related to the pathogenesis of both secretory otitis media and a series of otitis media sequelae which causes decreased hearing. In order to understand the pathogenesis of these conditions, accurate measurements and monitoring of ME pressure are imperative, and tympanometry is by far the most commonly used method in both clinical and basic research. However, based on biomechanical studies of the ME system, tympanometry has more inherent methodological errors, which affects its accuracy as well as monitoring pressures in relation to positional changes have demonstrated unstable pressures. On this background, a series of studies have investigated factors which affect tympanometric measurements of ME pressure.

ME pressure displays daily fluctuations in normal ears; on a short term scale positional changes has been demonstrated, so that the pressure increases in the supine position. This has been explained by congestion of the ME mucosa due to an increased hydrostatic pressure. These pressure increments have been monitored in normal ears by tympanometry, and the total increment and its time course including a stable phase have been described. These aspects should be taken into account, when ME experiments are performed in the supine position, as well as the results may contribute to analyse long-term daily pressure fluctuations. Serial tympanometry analysing the differences between trials can be considered reliable, since methodological errors are most likely constant.

Tympanometric estimates of ME pressure may be sensitive to the pressure change direction during recordings due to instrumental phase delay and intrinsic hysteresis of the ME system. In modern tympanometers the instrumental delay is practically zero and the intrinsic hysteresis in normal ears is insignificant. However, hysteresis has been shown significantly increased in diseased ears with ME fluid leading to a significant overestimation of the negative pressures. Moreover, a linear overestimation of tympanometric pressure estimates has been demonstrated in a ME model, so that smaller negative pressures are overestimated. Finally, in the same model, decrements of the ME air volume contributes further to an exponential negative overestimation of the tympanometric pressures. Altogether these findings suggest that measurements of ME pressure by tympanometry are highly inaccurate in diseased ears.

The true ME pressure cannot be obtained by the current investigations, and so methods of direct measurements must be applied. A few studies with direct measurements have reported only smaller underpressures which corroborate the current interpretations of the methodological errors and limitations of tympanometry. The determination of the exact ME pressures in diseased ears is important to understand the pathogenetic events, specifically if the ME fluid in secretory otitis media is an exudate or a transudate. Based on our findings, we propose that an inflammatory exudate is most likely.

Tympanometry is still a valuable tool in clinical otology, and the method is very accurate in detecting middle ear fluid and diagnosis of secretory otitis media, but the determination of middle ear pressure must be regarded highly inaccurate in diseased ears.

9. Summary in Danish / Resumé på dansk

Mellemøretryk spiller en stor rolle i mellemørets fysiologi og klinisk otologi, fordi forekomsten af undertryk er hyppig og relateret til udviklingen af sekretorisk mellemørebetændelse og en række følgetilstande til mellemørebetændelse generelt, som medfører nedsættelse af hørelsen. En øget forståelse af sygdomsmekanismerne bag undertryk forudsætter nøjagtige målemetoder med mulighed for monitorering af trykændringer over tid, og tympanometri har hyppigt været anvendt her ifm. både klinisk og basal forskning. Imidlertid har en række biomekaniske undersøgelser af mellemøret vist, at tympanometri har en række indbyggede målefejl, som medfører unøjagtigheder, ligesom monitorering af tryk ved stillingsændringer har vist ustabile tryk. Der er på den baggrund foretaget en række undersøgelser af faktorer, som påvirker tympanometriske målinger af mellemøretryk.

Mellemøretryk udviser daglige variationer i normale ører; et eksempel er positions-betingede ændringer, hvor trykket stiger i liggende stilling. Det er forklaret ved kongestion af mellemørets mucosa pga. et øget statisk tryk. Disse tryk-stigninger er blevet monitoreret i normale ører vha. tympanometri, og den samlede stigning såvel som en tidsafhængig stabil fase er bestemt. Disse forhold er af betydning, når mellemøre eksperimenter foretages i liggende stilling, og resultaterne kan anvendes i undersøgelser af normale daglige trykvariationer. Seriel tympanometri med analyse af forskelle mellem målingerne kan anses for pålidelig, fordi metode fejl er konstante.

Tympanometriske bestemmelser af mellemøretryk påvirkes af trykændringens retning ifm. måleproceduren pga. fase forskydning og hysteres. I moderne tympanometre er måleusikkerheden ved fase forskydning fundet neglignel, og hysteres hos normale har vist et sporadisk bidrag. Imidlertid er hysteresen markant forøget hos patienter med væske i mellemøret; det medfører en markant overestimering af mellemøretrykket i negativ retning. Dertil kommer, at model forsøg har vist en lineær overestimering af mellemøretrykket. Slutteligt er i samme model vist, at formindskelse af mellemørets luft rumfang medfører eksponentielt faldende negative mellemøretryk ved tympanometri. Samlet set peger disse resultater på, at tympanometri er meget unøjagtig ved trykmålinger i syge mellemører.

Det sande mellemøretryk kan ikke bestemmes ved disse undersøgelser, og der må henvises til metoder med direkte målinger af trykket. Et mindre antal studier har anvendt sådanne direkte målinger og har kun fundet mindre undertryk; det underbygger herværende fund, hvor høje negative mellemøretryk må fortolkes som metode fejl og begrænsninger i tympanometri. Målinger af de nøjagtige tryk i syge mellemører er vigtige for at forstå sygdomsmekanismerne, særligt om mellemøre sekret ifm. sekretorisk mellemørebetændelse er et exudat eller et transudat. På baggrund af herværende fund må et exudat anses for mest sandsynligt.

Tympanometri er fortsat et værdifuldt redskab i udredning af patienter med sygdomme i mellemøret, og metoden har stor nøjagtighed ved påvisning af væske i mellemøret og diagnose af sekretorisk mellemørebetændelse, men bestemmelse af mellemøretrykket må anses for unøjagtige og upålidelige hos patienter med sygdom i mellemøret.

10. Referencer

1. Alper CM, Seroky JT, Tabari R, Doyle WJ. Magnetic resonance imaging of the development of otitis media with effusion caused by functional obstruction of the Eustachian tube. *Ann Otol Laryngol* 1997; 106: 422-431.
2. Alper CM, Banks JM, Philp KD, Doyle WJ. Tympanometry accurately measures middle ear underpressures in monkeys. *Ann Otol Rhinol Laryngol* 2003; 112: 877-884.
3. Alper CM, Swarts JD, Singla A, Banks J, Doyle WJ. Relationship between the electromyographic activity of the paratubal muscles and Eustachian tube opening assessed by sonotubometry and videoendoscopy. *Arch Otolaryngol Head Neck Surg* 2012; 138: 741-746.
4. Andréasson L, Ingelstedt S, Ivarsson A, Jonson B, Tjernström Ö. Pressure dependent variation in volume of mucosal lining of the middle ear. *Acta Otolaryngol (Stockh)* 1976; 81: 442-449.
5. Ars B, Decraemer W, Ars-Piret N. The lamina propria and cholesteatoma. *Clin Otolaryngol* 1989; 14: 471-475.
6. Ars B, Ars-Piret N. Middle ear pressure balance under normal conditions. Specific role of the middle ear structure. *Acta Otorhinolaryngol Belg* 1994; 48: 339-342.
7. Ars, B. Tympanic membrane retraction pocket. *Acta Otorhinolaryngol Belg* 1995; 49: 163-171.
8. Ars B, Wuyts F, van de Heyning P, Miled I, Bogers J, Van Marck E. Histomorphometric study of the normal middle ear mucosa. *Acta Otolaryngol (Stockh)* 1997; 117: 704-707.
9. Altman DG, Bland JM. Units of analysis. *BMJ* 1997; 314: 1874.
10. Berger G, Sachs Z, Sadé J. Histopathologic changes of the tympanic membrane in acute and secretory otitis media. *Ann Otol Rhinol Laryngol* 1996; 105: 458-462.
11. Beutner D, Hüttenbrink K-B, Stumpf R, Beleites T, Zahnert T, Luers J-C, Helmstaedter V. Cartilage tympanoplasty. *Otol Neurotol* 2009; 31: 105-110.
12. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurements. *Lancet* 1986; 1: 307-310.
13. Bratmo M, Tideholm B, Carlborg B. Chronic tympanic membrane perforation: middle ear pressure and tubal function. *Acta Otolaryngol (Stockh)* 2003; 123: 569-574.
14. Bruhn MA, Jespersen JBB, Tveterås K, Nielsen RB, Nørgaard M, Gaihede M. Cumulated incidence of ventilation tube insertion and its correlation to subsequent ear surgery. In "Proceedings of the 10th International Symposium on Recent Advances in Otitis Media", New Orleans, USA, June 6-9th, 2011. *In Press*.
15. Buckingham RA, Ferrer JL. Middle ear pressures in Eustachian tube malfunction: Manometric studies. *The Laryngoscope* 1973; 83: 1585-1593.
16. Bylander AK, Ivarsson A, Tjernstrom O, Andreasson L. Middle ear pressure variations during 24 hours in children. *Ann Otol Rhinol Laryngol* 1984; Suppl 120: 33-35.
17. Carrie S, Hutton DA, Birchall JP, Green GGR, Pearson JP. Otitis media with effusion: Components which contribute to the viscous properties. *Acta Otolaryngol (Stockh)* 1992; 112: 504-511.
18. Cayé-Thomasen P, Hermansson A, Bakaletz L, Hellström S, Kanzaki S, Kerschner J, Lim D, Lin J, Mason K, Spratley J. Panel 3: Recent advances in anatomy, pathology, and cell biology in relation to otitis media pathogenesis. *Otolaryngol Head Neck Surg* 2013; 148: E37-51.
19. Cinamon U, Sadé J. Tympanometry versus direct middle ear pressure measurement in an artificial model: is tympanometry an accurate method to measure middle ear pressure? *Otol Neurotol* 2003; 24: 850-853.
20. Cinamon U, Russo E, Levy D. Middle ear pressure change as a function of body position. *The Laryngoscope* 2009; 119: 347-350.
21. Cros O, Borga M, Pauwels E, Dirckx JJ, Gaihede M. Micro-channels in the mastoid anatomy. Indications of a separate blood supply of the air cell system mucosa by micro-CT scanning. *Hear Res* 2013; 301: 60-65.
22. Decraemer WF, Creten WL, Van Camp KJ. Tympanometric middle ear pressure determination with two-component admittance meters. *Scand Audiol* 1984; 13: 165-172.
23. Dirckx JJJ, Decraemer WFS. Area change and volume displacement of the human tympanic

- membrane under static pressure. *Hear Res* 1992; 62: 99-104.
24. Dirckx JJJ, Buytaert JAN, Decraemer WF. Quasi-static transfer function of the rabbit middle ear measured with heterodyne interferometer with high-resolution position decoder. *JARO* 2006; 7: 339-351.
 25. Dirckx JJJ, Marcusohn Y, Gaihede M. Quasi-static pressures in the middle ear cleft. In: Puria S, Fay RR, Popper AN, Eds. *The Middle Ear: Science, Otosurgery, and Technology*. Springer Handbook of Auditory Research, Vol. 46, Springer, New York 2013; pp 93-133.
 26. Doyle WJ. Middle ear pressure regulation. In: Rosowski JJ, Merchant SN, Eds. *The function and mechanics of normal, diseased and reconstructed middle ears*. The Hague, the Netherlands: Kugler, 2000: p3-21.
 27. Djurhuus BD, Faber CE, Skytthe A. Decreasing incidence rate for surgically treated middle ear cholesteatoma in Denmark 1977-2007. *Dan Med Bull* 2010; 57: A4186 (1-5).
 28. Eden AR, Laitman JT, Gannon PJ. Mechanisms of middle ear aeration: Anatomic and physiologic evidence in primates. *The Laryngoscope* 1990; 100: 67-75.
 29. Edfeldt L, Strömbäck K, Kinnefors A, Rask-Andersen H. Surgical treatment of adult cholesteatoma: long-term follow-up using total reconstruction procedure without staging. *Acta Otolaryngol* 2013; 133: 28-34.
 30. Elnér Å, Ingelstedt S, Ivarsson A. method for studies of the middle ear mechanics. *Acta Otolaryngol (Stockh)* 1971a; 72: 191-200.
 31. Elnér Å, Ingelstedt S, Ivarsson A. Indirect determination of middle ear pressure. *Acta Otolaryngol (Stockh)* 1971b; 72 :255-261.
 32. Felding JU, Gaihede M, Elbrønd O. The biomechanical characteristics of the middle ear system measured by a new method I: Instrumentation. *Acta Otolaryngol (Stockh)* 1995; 115: 408-413.
 33. Felding JU. Middle ear gas – its composition in the normal and in the tubulated ear. A methodological and clinical study. *Acta Otolaryngol (Stockh) Suppl* 1998;536:1-57.
 34. Felding JU. Sekretorisk otitis media i almen- og otologpraksis. *Månedsskr Prakt Lægegerm* 2000; 10: 1317-1322.
 35. Feldman RM, Fria, TJ, Palfrey CC, Dellecker CM. Effects of rate of air pressure change on tympanometry. *Ear Hear* 1984; 5: 91-95.
 36. Flisberg K, Ingelstedt S, Örtengren U. On middle ear pressure. *Acta Otolaryngol (Stockh)* 1963; Suppl. 182: 43-56.
 37. Gaihede M, Felding JU, Elbrønd O. The biomechanical characteristics of the middle ear system measured by a new method II. Clinical application and normal material. *Acta Otolaryngol (Stockh)* 1995a; 115: 414-421.
 38. Gaihede M, Felding JU, Elbrønd O. Biomechanical characteristics of the middle ear system measured by a new method III: Comparisons to tympanometric measurements. *Acta Otolaryngol (Stockh)* 1995b; 115: 522-527.
 39. Gaihede M. Tympanometric preconditioning of the tympanic membrane. *Hear Res* 1996; 102: 28-34.
 40. Gaihede M, Lildholdt T, Lunding J. Sequelae of secretory otitis media: Changes in middle ear mechanics. *Acta Otolaryngol (Stockh)* 1997; 117: 382-389.
 41. Gaihede M, Marker F. Agreement between two tympanometers. A methodological study of instrument comparison. *Scand Audiol* 1998; 27: 113-119.
 42. Gaihede M. Mechanical properties of the middle ear system investigated by its pressure-volume relationship. Introduction to methods and selected preliminary clinical cases. *Audiol Neurootol* 1999a; 4: 137-41.
 43. Gaihede M. Mechanics of the middle ear system: Computerized measurements of its pressure-volume relationship. *ANL* 1999b; 26: 383-99.
 44. Gaihede M, Kabel J. The normal pressure-volume relationship of the middle ear system and its biological variation. In: Rosowski JJ, Merchant SN, Eds. *The Function and Mechanics of Normal, Diseased and Reconstructed Middle Ears*. The Hague: Kugler Publications, 2000; 59-70.
 45. Gaihede M, Koefoed-Nielsen B. Mechanics of the middle ear system: Age-related changes in viscoelastic properties. *Audiol Neurootol* 2000; 5: 53-58.

46. Gaihede M, Dirckx JJJ, Jacobsen H, Aernouts JEF, Søvsø M, Tveterås K. Middle ear pressure regulation – Complementary active action of the mastoid and the Eustacian tube. *Otol Neurotol* 2010a; 31: 603-11.
47. Gaihede M, Sami SAK, Drewes AM. Connectivity analysis of suggestive brain areas involved in middle ear pressure regulation in humans. *Hear Res* 2010b; 263: 245.
48. Gaihede M, Padurariu S, Jacobsen H, De Greef D, Dirckx JJJ. Eustachian tube pressure equilibration. Temporal analysis of pressure changes based on direct physiological recordings with an intact tympanic membrane. *Hear Res* 2013a; 301: 53-59.
49. Gaihede M, Cros O, Padurariu S. The role of the mastoid in middle ear pressure regulation. In: Takahashi H, Ed. “*Cholesteatoma and Ear Surgery – An Update*”. Proceedings of the 9th International Conference on Cholesteatoma and Ear Surgery, Nagasaki, Japan, June 3-7th 2012. Kugler Publications, Amsterdam, The Netherlands, 2013b: pp 17-20.
50. Grøntved A, Krogh HJ, Christensen PH, Jensen PO, Schousboe HH, Hentzer E. Monitoring middle ear pressure by tympanometry. A study of middle ear pressure variation through seven hours. *Acta Otolaryngol (Stockh)* 1989; 108: 101-106.
51. Hartley DEH, Moore DR. Effects of conductive hearing loss on temporal aspects of sound transmission through the ear. *Hear Res* 2003; 177: 53-60.
52. Hergils L, Magnuson B. Morning pressure in the middle ear. *Arch Otolaryngol* 1985; 111: 86-89.
53. Hergils LG, Magnuson B, Falk B. Different tympanometric procedures compared with direct pressure measurements in healthy ears. *Scand Audiol* 1990; 19: 183-186.
54. Hüttenbrink KB. The mechanics of the middle-ear at static pressures. The role of the ossicular joints, the function of the middle-ear muscles and the behaviour of stapedial prostheses. *Acta Otolaryngol (Stockh)* 1988; Suppl. 451:35.
55. Ingelstedt S, Ivarsson A, Jonson B. Mechanics of the human middle ear. *Acta Otolaryngol (Stockh)* 1967; Suppl. 228: 1-58.
56. Ivarsson A, Tjernström Ö, Bylander A, Bennrup S. High speed tympanometry and ipsilateral middle ear reflex measurements using a computerized impedance meter. *Scand Audiol* 1983; 12: 157-163.
57. Jacobsen H, Dirckx JJ, Gaihede M, Tveterås K. Direct measurements and monitoring of middle ear pressure. In: Huber A, Eiber A, Eds. “*Middle Ear Mechanics in Research and Otolology – Proceedings of the 4th International Symposium*”, Zurich, Switzerland, June 2006. World Scientific Publishing Co. Pte. Ltd., Singapore, 2007, pp 26-35.
58. Jerger J. Clinical experiments with impedance audiometry. *Arch Otolaryngol* 1970; 92: 311-324.
59. Jespersen JBB, Bruhn MA, Gaihede M, Tveterås K, Nørgaard M, Nielsen RB. Incidence of ventilation tube treatments. The largerst numbers of VT insertions in the world? In “*Proceedings of the 10th International Symposium on Recent Advances in Otitis Media*”, New Orleans, USA, June 6-9th, 2011. *In Press*.
60. Johansen ECJ, Lildholdt T, Damsbo N, Eriksen EW. Tympanometry for diagnosis and treatment of otitis media in general practice. *Family Practice* 2000; 17: 317-322.
61. Jonson B, Rundcrantz H. Posture and pressure within the internal jugular vein. *Acta Otolaryngol (Stockh)* 1969; 68: 271-275.
62. Knight LC. Temporal changes in unilateral middle ear pressure under basal conditions. *Clin Otolaryngol* 1991; 16: 543-546.
63. Knight LC, Eccles R. The effect of postural change and upper respiratory tract infection on middle ear pressure. *Acta Otolaryngol (Stockh)* 1991; 111: 1075-1082.
64. Kobayashi T, Okitsu T, Takasaka T. Forward-backward tracing tympanometry. *Acta Otolaryngol Stockh Suppl* 1987; 435: 100-106.
65. Kole SA, Nieland PF, Søvsø M, Tveterås K, Gaihede M. Incidence of middle ear cholesteatoma with analysis of its locations, extensions, and complications during 1993 to 2009. In: Takahashi H, Ed. “*Cholesteatoma and Ear Surgery – An Update*”. Proceedings of the 9th International Conference on Cholesteatoma and Ear Surgery, Nagasaki, Japan, June 3-7th 2012. Kugler Publications, Amsterdam, The Netherlands, pp. 297-301.
66. Lidén G, Peterson JL, Björkman G. Tympanometry. *Arch Otolaryngol* 1970; 92: 248-257.
67. Magnuson B. Tubal opening and closing ability in unilateral middle ear disease. *Am J*

-
- Otolaryngol* 1981; 2: 199-209.
68. Magnuson B. Functions of the mastoid cell system: auto-regulation of temperature and gas pressure. *J Laryngol Otol* 2003; 117: 99-103.
 69. Magnuson K, Hellström S, Magnuson B. Structural changes in the rat tympanic membrane following repeated pressure loads. *Eur Arch Otorhinolaryngol* 1995; 252: 76-82.
 70. Majima Y, Hamaguchi Y, Hirata K, Takeuchi K, Morishita A, Sakakura Y. Hearing impairment in relation to viscoelasticity of middle ear effusions in children. *Ann Otol Rhinol Laryngol* 1988; 97: 272-274.
 71. Metz O. The acoustic impedance measured on normal and pathological ears. Einar Munksgaard, Copenhagen 1946:1-254.
 72. Moody SA, Alper CM, Doyle WJ. Daily tympanometry in children during the cold season: association of otitis media with upper respiratory tract infections. *Int J Pediatr Otorhinolaryngol* 1998; 45: 143-150.
 73. Møller P. Tympanosclerosis of the ear drum. *Acta Otolaryngol (Stockh)* 1981; 91: 215-221.
 74. Okitsu T, Kobayashi T, Endo S, Shibahara Y, Sakuma M, Takasaka T, Kawamoto K, Kaneko Y. Tympanograms of otitis media with effusion: an experimental study. *ANL* 1985; 12 Suppl.: 222-224.
 75. Ovesen T, Paaske PB, Elbrond O. Accuracy of an automatic impedance apparatus in a population with secretory otitis media: principles in the evaluation of tympanometrical findings. *Am J Otolaryngol* 1993; 14:100-104.
 76. Park MS, Yoo SH, Hoon DH. Measurement of surface area in the human mastoid air cell system. *J Laryngol Otol* 2000;114:93-96.
 77. Peterson JL, Lidén G. Tympanometry in human temporal bones. *Arch Otolaryngol* 1970; 92: 258-266.
 78. Politzer A. Diagnose und Therapie der Ansammlung seröser Flüssigkeit in der Trommelhöhle. *Wien Med Wochenschr* 1867; 17: 244-247.
 79. Portier F, van den Abbeele T, Lecain E, Sauvaget E, Escoubet B, Huy PTB, Herman P. Oxygen modulates Na⁺ absorption in middle ear epithelium. *Am J Physiol* 1999; 276: 312-317.
 80. Rasmussen LS, Søvsø M, Söderberg O, Tveterås K, Gaihede M. Frequency of otosurgical procedures and its results related to middle ear pressure dysregulation. Poster presentation at "Kongres for Medicinsk Studenterforskning", Sandbjerg Gods, Danmark, 12-14. sept. 2008.
 81. Renvall U, Lidén G, Björkman G. Experimental tympanometry in human temporal bones. *Scand Audiol* 1975; 4: 135-144.
 82. Rubensohn G. Mastoid pneumatization in children at various ages. *Acta Otolaryngol (Stockh)* 1965; 60: 11-14.
 83. Rundcrantz H. Posture and Eustachian tube function. *Acta Otolaryngol (Stockh)* 1969;68:279-92.
 84. Sadé J, Halevy A, Hadas E. Clearance of middle ear effusions and middle ear pressures. *Ann Otol Rhinol Laryngol* 1976; Suppl 25: 58-62.
 85. Sadé J. Atelectatic tympanic membrane: Histologic study. *Ann Otol Rhinol Laryngol* 1993; 102: 712-716.
 86. Sadé J, Ar A. Middle ear and auditory tube: Middle ear clearance, gas exchange, and pressure regulation. *Otolaryngol Head Neck Surg* 1997; 116: 499-524.
 87. Sakikawa Y, Kobayashi H, Nomura Y. Changes in middle ear pressure in daily life. *The Laryngoscope* 1995; 105: 1353-1357.
 88. Sami SAK, Gaihede M, Nielsen LG, Drewes AM. Early static pressure related evoked potentials. Indications of central middle ear pressure control in humans. *Otol Neurotol* 2009; 30: 649-656.
 89. Sano S, Kamide Y, Schachern PA, Paparella MM. Micropathologic changes of pars tensa in children with otitis media with effusion. *Arch Otolaryngol Head Neck Surg* 1994; 120: 815-819.
 90. Sassu R, Cozzi B. The external and middle ear of the striped dolphin *Stenella coeruleoalba* (Meyen 1833). *Anat Histol Embryol* 2007; 36: 197-201.
 91. R Sederberg-Olsen JF, Sederberg-Olsen AE, Jensen AM. The prognostic significance of the air volume in the middle ear for the tendency to recurrence of secretory middle ear condition. *Int J Pediatr Otorhinolaryngol* 1983; 5: 179-187.
-

92. Shanks JE, Wilson RH. Effects of direction and rate of ear canal pressure changes on tympanometric measures. *J Speech Hear Res* 1986; 29: 11-19.
93. Shanks J, Shelton C. Basic principles and clinical applications of tympanometry. *Otolaryngol Clin North Am* 1991; 24: 299-328.
94. Shinkawa H, Okitsu T, Yusa T, Yamamuro M, Kaneko Y. Positive intratympanic pressure in the morning and its etiology. *Acta Otolaryngol (Stockh)* 1987 Suppl. 435: 107-111.
95. Sichel J-Y, Priner Y, Weiss S, Levi H, Barshtein G, Eliashar R, Elidan J. Characteristics of the type B tympanogram can predict the magnitude of the air-bone gap in otitis media with effusion. *Ann Otol Rhinol Laryngol* 2003; 112: 450-454.
96. Strachan D, Hope G, Hussain M. Long-term follow-up of children inserted with T-tubes as a primary procedure for otitis media with effusion. *Clin Otolaryngol* 1996; 21: 537-541.
97. Sudhoff H, Tos M. Pathogenesis of attic cholesteatoma: Clinical and immuno-histochemical support for the combination of retraction theory and proliferation theory. *Am J Otol* 2000; 21: 786-792.
98. Suetake M, Kobayashi T, Takasaka T, Shinkawa H. Is change in middle ear volume following ventilation tube insertion a reliable prognostic factor? *Acta Otolaryngol (Stockh)* 1990 Suppl. 471: 73-80.
99. Swarts JD, Alper CM, Seroky JT, Chan KH, Doyle WJ. In vivo observation with magnetic resonance imaging of middle ear effusion in response to experimental underpressures. *Ann Otol Rhinol Laryngol* 1995; 104: 522-528.
100. Swarts JD, Doyle BMD, Alper CM, Doyle WJ. Surface area-volume relationships for the mastoid air cell system and tympanum in adult humans: Implications for mastoid function. *Acta Otolaryngol* 2010; 130: 1230-1236.
101. Swarts JD, Alper CM, Luntz M, Bluestone CD, Doyle WJ, Ghadiali SN, Poe DS, Takahashi H, Tideholm B. Panel 2: Eustachian tube, middle ear, and mastoid--anatomy, physiology, pathophysiology, and pathogenesis. *Otolaryngol Head Neck Surg* 2013; 148 (4 Suppl.): E26-36.
102. Takahashi H, Hayashi M, Honjo I. Direct measurement of the middle ear pressure through the eustachian tube. *Arch Otolaryngol* 1987; 243: 378-381.
103. Takahashi H, Honjo I, Hayashi M, Fujita A, Kurata K. Middle ear pressures of children with otitis media with effusion. *Ann Otol Rhinol Laryngol* 1991; 100: 469-471.
104. Takahashi H, Iwanaka T, Kaieda S, Fukuda T, Kumagami H, Takasaki K, Hasebe S, Funabiki K. Mastoid obliteration combined with soft-wall reconstruction of posterior canal. *Eur Arch Otorhinolaryngol* 2007; 264: 867-871.
105. Tauris J, Al Kole S, Tveterås K, Gaihede M. Causative treatment of retraction cholesteatomas by obliteration of the mastoid. In "Proceedings of the 10th International Symposium on Recent Advances in Otitis Media", New Orleans, USA, 6-9th June 2011. *In Press*.
106. Therkildsen K, Thomsen KA. The influence of pressure variations on the impedance of the human ear drum. *J Laryngol Otol* 1959; 73: 409-418.
107. Therkildsen K, Scott-Nielsen S. An electroacoustic impedance measuring bridge for clinical use. *Arch Otolaryngol* 1960; 72: 339-346.
108. Thomas CL, Simpson SA, Butler CC, van der Voort J, Lewis R. Oral or topical steroids for hearing loss associated with otitis media with effusion in children (Review). *The Cochrane Library* 2010; 4: 1-25.
109. Thomsen KA. Investigations on the tubal function and measurements of the middle ear pressure in pressure chamber. *Acta Otolaryngol (Stockh) Suppl.* 1960; 140: 269-278.
110. Tideholm B, Jönsson S, Carlborg B, Welinder R, Grenner J. Continuous 24-hour measurement of middle ear pressure. *Acta Otolaryngol (Stockh)* 1996; 116: 581-588.
111. Tideholm B, Brattmo M, Carlborg B. Middle ear pressure: Effect of body position and sleep. *Acta Otolaryngol (Stockh)* 1999; 119: 880-885.
112. Tos M, Stangerup SE, Holm Jensen S, Sørensen CH. Spontaneous course of secretory otitis and changes of the eardrum. *Arch Otolaryngol* 1984; 110: 281-289.
113. van Dishoeck HAE. Measurement of the tension of the tympanic membrane and of the resistance of the Eustachian tube. *Arch Otolaryngol* 1941; 34: 596-602.
114. Vercruyse J-P, De Foer B, Somers T, Casselman J, Officiers E. Long-term follow-up after bony mastoid and epitympanic obliteration: radiological findings. *J Laryngol Otol* 2009; 124: 37-43.

-
115. Watters GWR, Jones JE, Freeland AP. The predictive value of tympanometry in the diagnosis of middle ear effusion. *Clin Otolaryngol* 1997; 22: 343-345.
 116. Widdicombe J. Microvascular anatomy of the nose. *Allergy* 1997; 52 (suppl. 40): 7-11.
 117. Wullstein H. Theory and practice of tympanoplasty. *The Laryngoscope* 1956; 66: 1076-1093.
 118. Yilmaz I, Cagici CA, Ozluoglu LN, Akkuzu B, Ozgirgin N, Sener M, Atas A. Effects of various densities of middle ear fluids on acoustic immittance: experimental study. *J Otolaryngol Head Neck Surg* 2008; 37: 130-136.
 119. Zahnert T, Hüttenbrink KB, Mürbe D, et al. Experimental investigations of the use of cartilage in tympanic membrane reconstruction. *Am J Otol* 2000; 21: 322-328.
 120. Zielhuis GA, Rach GH, van-den-Broeck P. The occurrence of otitis media with effusion in Dutch pre-school children. *Clin Otolaryngol* 1990; 15: 147-153.
 121. Zöllner F. The principles of plastic surgery of the sound-conducting apparatus. *J Laryngol Otol* 1955; 69: 637-652.

11. Appendices Papers I - IV



Positional changes and stabilization of middle ear pressure

Michael Gaihede ^{a,*}, Dorthe Kjær ^b

^a Department of Otolaryngology, Holstebro Central Hospital, 7500 Holstebro, Denmark

^b Department of Otolaryngology, Aarhus University Hospital, 8000 Aarhus C, Denmark

Received 7 July 1997; received in revised form 29 September 1997; accepted 14 November 1997

Abstract

Middle ear pressure has been shown to increase when body position is shifted from the erect to the supine position, which is explained by an increased volume of the middle ear mucosa due to an increased hydrostatic pressure. The increase in the volume of middle ear mucosa consists of a fast major response followed by slow minor increase, which is reflected by a similar pattern in the increase of middle ear pressure. Since many otological experiments may be performed with subjects in the supine position, it is of interest to analyse these changes in middle ear pressure, as results may be affected by changes in middle ear pressure. The present study investigated the middle ear pressure changes due to a shift in body position from sitting to supine at time intervals of 15 s over a period of 120 s in a group of 20 normal adults. The middle ear pressure was found to increase 22 daPa (mean; S.D. = 12.1), whereas a stable middle ear pressure was reached after 30 s, indicating a steady state concerning the increase in volume of the middle ear mucosa. Thus, it is recommended that experiments with subjects in the supine position should be carried out only after assuming the position for 30 s. The increase in pressure did not correlate to the prevailing middle ear pressure or to the body height. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Middle ear pressure; Supine position; Middle ear mucosa

1. Introduction

When body position is changed from the erect to the supine position, the middle ear pressure (MEP) determined by tympanometry increases

[1–3]. This can be explained by an increased pressure in the jugular veins in the supine compared to the erect position, which results in an increase of the volume of the middle ear mucosa (V_{muc}) due to congestion ultimately affecting the MEP [4,5]. Such pressure dependent changes in the volume of the middle ear mucosa (ΔV_{muc}) have previously been measured directly and amount to 5–25 μl [5]. In this experimental study

* Corresponding author. Department of Otolaryngology, Aalborg Hospital, P.O. Box 365, 9100 Aalborg. Fax: +45 99322938.

it was also revealed that the increase in V_{muc} consists of two phases: an initial fast and larger increment within few seconds followed by a slower and minor increment, which continues for 10 s or more [5]. These two phases have similarly been found in changes in MEP, when body position is changed (unpublished observation).

Experiments in middle ear research may often be performed with patients or subjects in the supine position for practical reasons. In this situation it becomes of interest to obtain a stable phase in MEP, since changes in MEP may affect the results of experiments. The questions, which have not previously been answered, are: (1) does MEP actually reach a stable phase, when body position is altered? (2) if a stable phase is found, when is it reached? Thus, the purpose of the present study was to investigate temporal changes in MEP after changing body position from sitting to supine position. This was accomplished by serial tympanometry in a group of normal adults.

2. Materials and methods

A total of 20 healthy adults with no previous history of otological disorders and normal otomicroscopy entered the study. The mean age was 41 years (S.D. = 12). All subjects were investigated bilaterally, and right and left ears were measured randomly as first or second ears. Between measuring each ear in a subject a resting period of 10 min was introduced, where the subjects were standing or sitting. The study was approved by the Regional Scientific Ethical Committee (1996/3616) and informed consent was obtained from each subject prior to the experiment.

The tympanometer applied was an automatic Madsen Electronics Zodiac Middle Ear Analyzer 901 with a 226 Hz probe tone. Range and direction of pressure change was +200–400 daPa, and rate of pressure change was 400 daPa/s. In all 40 ears, 10 tympanometries were recorded without removing the probe. The first tympanometry was recorded in the sitting position. Following this, nine recordings were performed at intervals of 15 s, starting immediately after assuming the dorsal supine position ($t = 0$ s).

3. Results

Fig. 1 shows the mean MEP for both right and left ears in the sitting and the supine position against time. Pressures for right ears are seen to be consequently lower than left ears. This is explained by three cases with larger negative MEPs (–165, –145, and –95 daPa, respectively) among the right ears, while only 1 left ear had a large negative MEP (–175 daPa). Otherwise all MEPs were higher than –65 daPa. Table 1 depicts the mean and the standard deviation (S.D.) of the MEP in the sitting position and for selected time intervals in the supine position. The S.D.s are large and no significant difference between the two sides was found (Table 1). Both sides display the same instantaneous larger increase followed by a minor increase and a subsequent stable phase. The total increase from the sitting position to the last trial in the supine position amounted to 19.7 and 24.3 daPa (S.D. = 10.6 and 13.4) for right and left ears, respectively, and there was no significant difference between the total increase of the two sides (paired t -test: $2P = 0.163$).

The stabilization of the MEP after assuming the supine position is more clearly analyzed, when

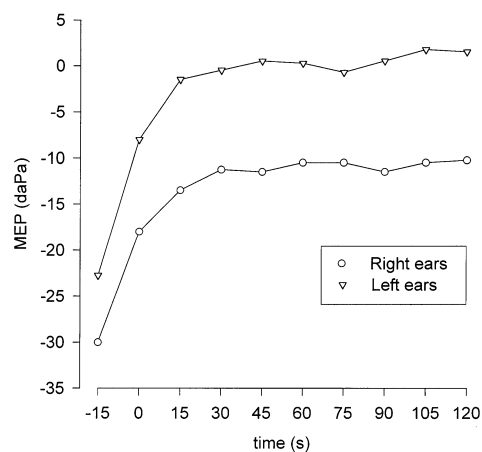


Fig. 1. MEP for right and left ears in the sitting position ($t = -15$ s) and the supine position at time intervals from 0 to 120 s ($t = 0$ s corresponds to MEP measured immediately after assuming the supine position).

Table 1
Middle ear pressure (daPa) for right/left ears in the sitting and the supine position at selected time intervals (s)

		Sitting position	Supine position			
			0	15	30	120
Right	Mean	−30.0	−18.0	−13.5	−11.3	−10.3
	S.D.	50.4	49.2	50.1	49.2	49.2
Left	Mean	−22.8	−8.0	−1.5	−0.5	1.5
	S.D.	39.9	43.8	44.0	43.2	44.5
2P		>0.6	>0.5	>0.4	>0.4	>0.4

$n = 20/20$; Right ears were tested against left ears (paired t -test).

the differences between one and the preceding trial are plotted against time. This is shown in Fig. 2 for right and left ears, and the curves are completely overlapping. For stabilization of the MEP, the difference between one and the preceding trial ideally should be 0, or for practical purposes the distribution of the differences should not deviate significantly from 0. This was tested by a one-sample t -test against 0, and $2P$ values are depicted in Table 2 together with the mean and S.D. of the differences for the whole material. For differences at 30 s $2P > 0.05$, i.e. the MEPs at 30 and 15 s are not significantly different from

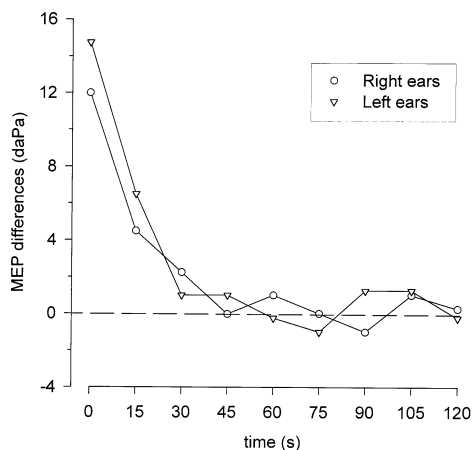


Fig. 2. Differences in MEP between one and the preceding trial against time for right and left ears. Complete overlap is seen for right and left ears.

each other. The $2P$ values are consequently larger than 0.05 for later trials (Table 2).

Fig. 3 shows the increase in MEP between the supine position at $t = 0$ s and the sitting position against the initial MEP in the sitting position for all cases. There was no correlation between the increase and the magnitude of the prevailing MEP ($P = 0.912$). Neither was the increase in MEP after stabilization ($t = 120$ s) found dependent of the prevailing MEP ($P = 0.905$). Similarly, we tested for any correlation between increase in MEP and body height. Neither the instantaneous ($t = 0$ s) nor the stable phase ($t = 120$ s) MEP increments were correlated to the height of the subjects ($P = 0.887$ and $P = 0.945$, respectively). These correlations were also tested for subgroups of right and left ears as well as the first and second ears separately, but with the same results.

Finally, we analyzed the subgroups of ears measured as first and second ears to see if the order of investigating the ears had any effects. Neither the absolute MEPs at various time intervals nor the differences showed any significant differences between first and second ears.

4. Discussion

The immediate and larger increase in MEP due to shift in body position followed by a slower and minor increase as seen in Fig. 1 corresponded well to the previous observations on increases in the V_{muc} consisting of two similar phases [5]. This can be explained by an immediate filling of the

Table 2
Distribution of MEP differences between one and the preceding trial (daPa)

<i>t</i> (s)	0	15	30	45	60	75	90	115	120
Mean	13.4	5.5	1.6	0.4	0.4	-0.5	0.1	1.1	0.0
S.D.	9.8	5.6	5.1	5.0	4.4	3.4	5.2	3.8	4.1
2 <i>P</i>	<0.001	<0.001	>0.05	>0.6	>0.5	>0.3	>0.8	>0.05	1

n = 40; Mean of differences are tested by a one-sample *t*-test against 0

venoles of the mucosa followed by slower capillary filling and possibly a redistribution of intercellular fluids [5]. The slow increase may also be reflecting the viscoelastic properties of the mucosa exhibiting a creep before steady state in the V_{muc} is achieved.

The point at which a stable MEP was reached, indicating a steady state in the increase of V_{muc} , is illustrated in Fig. 2, where differences between adjacent trials are approaching 0 gradually. The mean of the differences between trials at 30 and 15 s (1.6 daPa) was not significantly different from 0 (Table 2: $2P > 0.05$). However, the difference between MEPs at 45 and 30 s was found to be closer to 0 (0.4 daPa), and the insignificance was quite larger (Table 2: $2P > 0.6$). Hence, in order to achieve a stabilized MEP, experiments

with subjects in the supine position should not be performed before at least 15 or preferably 30 s. Further the present data showed that MEP was stable for a period of 90 s.

Changes in MEP in the present study is suggested to be due to changes in V_{muc} in response to changes in body position, but generally both gas exchange and pressure equilibration by the eustachian tube have to be considered [6]. However, when the pressure in the middle ear gas pocket was changed due to changes in V_{muc} , the relative partial pressures of dry middle ear gas were only slightly changed, which hardly resulted in any significant gas exchange over the middle ear mucosa. We have not instructed our subjects to avoid swallowing during the study, since this may cause an attention towards this usually involuntarily act, which may results in difficulties trying to avoid it over a period of 2 min. However, deglutitions were not very likely, since pressure equilibration by the eustachian tube is significantly impaired, when subjects are in the supine position [7]. Hence gas exchange and pressure equilibration by the tube could be considered insignificant in the present study.

Further evidence for this point of view was the magnitude of the S.D. of the distribution of the differences between trials (Table 2). The precision of measurements of the MEP can be determined by the S.D. of the distribution of the differences between two repeated measurements, which reflects the variation between measurements due to measurements errors [8]. For the tympanometer applied in the present study, the S.D. of the distribution of differences between two repeated measurements was 3.9 daPa [9]. The S.D.s of the distribution of differences between measurements in Table 2 at 30 s or later trials are ranging from

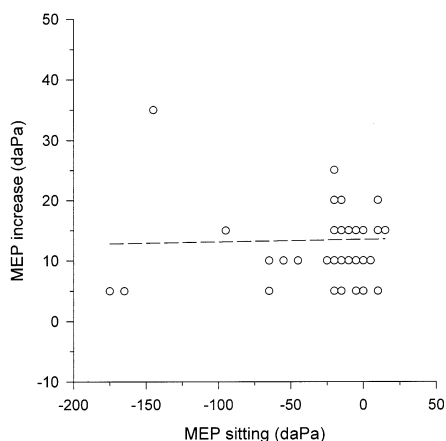


Fig. 3. Differences in middle ear pressure between the sitting position and the supine position ($t = 0$) against the prevailing middle ear pressure in the sitting position ($r(P) = 0.018$; $P = 0.912$; $n = 40$). Nine points are coinciding.

3.4 to 5.2 daPa. Thus, they are within range of purely errors of measurements.

The overall increase from the sitting position to the last trial in supine position amounted to 22.0 daPa (S.D. = 12.1). This corresponds to the findings of Grøntved et al. [2], where the majority of the subjects showed a mean increase of 23–25 daPa for a period of 3 min. Knight and Eccles [1] found a mean increase of only 2.1 daPa in a normal group, while in a group of subjects with upper respiratory tract infections, the mean increase was 22.6 daPa. This study, however, measured MEP at 10 min intervals, which increases the possibility of a significant influence by gas exchange and pressure equilibration through the Eustachian tube. Gaihede et al. [3] found a mean increase of 14 daPa (S.D. = 10) in a normal group, but stable values were not achieved.

Grøntved et al. [2] found a larger mean increase in MEP (58 daPa) in a subgroup with pronounced negative MEPs (< -250 daPa) compared to groups with less negative and normal MEPs. Hence the increase in MEP due to change in position may be correlated to the magnitude of the MEP. Contrary to this, we found no correlation between the increase in MEP and the actual MEP (Fig. 3). This may be explained by few subjects with high negative MEPs, since our group consisted of normal subjects only. The test group enrolled by Grøntved et al. [2] presented with complaints of hearing loss and all had initial MEPs < -150 daPa. Inflammatory changes in the middle ear mucosa may be present in ears with large negative MEPs, in which case their response may differ from normal subjects.

Jonson and Rundcrantz [4] found that the pressure increase in the bulb of the internal jugular vein due to changes in body position correlated to the distance from the right atrium to the bulb. Hence, the ΔV_{muc} and any resulting increase in MEP would be expected to correlate to the height of the subjects. Contrary to this, the increase in MEP was found independent of the body height. However, ΔV_{muc} are not determined by changes in venous pressure only, but also affected by changes in arterial pressure and the vascular tone [5].

These factors will complicate any relationship between the increase in MEP and height.

No differences in absolute MEP or pressure increase were found between ears measured as first ears versus second ears, which suggested that the period of 10 min between the experiments in each ear were sufficient to ensure that the V_{muc} had reverted to its initial state after being subject to increased pressure in the supine position.

Acknowledgements

This study was supported by Danish Medical Research Council. GN Danavox, Denmark, kindly supplied the Madsen Electronics Zodiac Middle Ear Analyzer 901 used in the experiments. J.U. Felding provided valuable discussions.

References

- [1] Knight LC, Eccles R. The effect of postural change and upper respiratory tract infection on middle ear pressure. *Acta Otolaryngol Stockh* 1991;111:1075–82.
- [2] Grøntved A, Krogh HJ, Christensen PH, Jensen PO, Schousboe HH, Hentzer E. Monitoring middle ear pressure by tympanometry. A study of middle ear pressure variation through seven hours. *Acta Otolaryngol Stockh* 1989;108:101–6.
- [3] Gaihede M, Felding JU, Elbrønd O. The biomechanical characteristics of the middle ear system measured by a new method III: comparisons to tympanometric measurements. *Acta Otolaryngol Stockh* 1995;115:522–7.
- [4] Jonson B, Rundcrantz H. Posture and pressure within the internal jugular vein. *Acta Otolaryngol Stockh* 1969;68:271–5.
- [5] Andréasson L, Ingelstedt S, Ivarsson A, Jonson B, Tjernström Ö. Pressure-dependent variation in volume of mucosal lining of the middle ear. *Acta Otolaryngol Stockh* 1976;81:442–9.
- [6] Ars B, Ars-Piret N. Middle ear pressure balance under normal conditions. Specific role of the middle ear structure. *Acta oto-rhino-laryngol belg* 1994;48:339–42.
- [7] Rundcrantz H. Posture and eustachian tube function. *Acta Otolaryngol Stockh* 1969;68:279–92.
- [8] Gaihede M, Ovesen T. Precision of tympanometric measurements. *J Speech Lang Hear Res* 1997;40:215–22.
- [9] Gaihede M, Marker F. Agreement between two tympanometers. A methodological study of instrument comparison. Accepted for publication in *Scandinavian Audiology*.



Middle ear volume and pressure effects on tympanometric middle ear pressure determination: model experiments with special reference to secretory otitis media

Michael Gaihede *

Department of Otolaryngology, Holstebro Central Hospital, DK-7500 Holstebro, Denmark

Received 19 April 1999; received in revised form 10 September 1999; accepted 17 September 1999

Abstract

Objective: Middle ear pressure (P_m) measured by tympanometry has revealed high negative values in patients with secretory otitis media (SOM) in contrast to direct measurement. This may be explained by errors in tympanometry caused by volume displacement of the tympanic membrane (TM) affecting the volume of the middle ear (V_m) and the P_m according to Boyle's Law. Such errors are susceptible to the size of V_m . **Methods:** A realistic middle ear model based on previous clinical studies of normal pressure-volume relations of the middle ear system (MES) was constructed. In this model non-linear behaviour and hysteresis of the MES was imitated and P_m as well as V_m could be controlled. **Results:** Tympanometrically estimated P_m decreased on average 38 daPa, when V_m was changed from 21 to 1 cm³. The decrease was most pronounced, when V_m became smaller than 5 cm³. Moreover, tympanometry showed a linear numerical overestimation of P_m by a factor 2.31 compared with model P_m . **Conclusion:** A curve fit was derived describing the tympanometric P_m as a function of V_m . This demonstrated that tympanometric P_m approached $-\infty$ daPa, when middle ear volume approached 0 cm³, which indicates that negative tympanometric recordings and B curves can be found in ears with normal P_m entirely due to very small V_m 's. This explains the discrepancy between direct and tympanometric measurements of P_m in SOM, since the effusion replaces the air filled expandable volume resulting in a very small 'functional' V_m . Numerical overestimation of P_m by tympanometry was explained by hysteresis, which reflected the viscoelastic properties of the MES. These results question the significance of negative P_m 's as a pathogenetic factor in SOM. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Tympanometry; Middle ear pressure; Middle ear volume; Secretory otitis media; Pathogenesis

1. Introduction

The existence of negative middle ear pressure (P_m) plays a central role as a pathogenetic factor in the ex vacuo theory explaining the development of secretory otitis media (SOM). This is partly based on numerous studies, where indirect tympanometric measurements have revealed high negative P_m 's in these patients [1]. P_m 's in the range of -100 to -400 daPa are common tympanometric findings and often alternate with type B tympanograms indicating P_m 's below -400 daPa and the presence of middle ear effusion.

However, the magnitude of these large negative P_m 's are not in accordance with studies of direct measurements. Buckingham et al. [2] found a mean P_m of only -6.5 mmH₂O in 136 ears with SOM. Measurements were made by a manometer and puncture of the tympanic membrane (TM), and the largest negative pressure amounted to -46 mmH₂O. These experiments were repeated by Sadé et al. [1], who found a mean pressure of -1.7 mmH₂O in 36 ears with SOM (range $+5$ to -12.06 mmH₂O). Later, Takahashi et al. [3] measured the P_m directly by a pressure transducer technique through the eustachian tube and found a mean pressure of -54.33 mmH₂O in 30 ears with SOM (range $+40$ to -185 mmH₂O). These studies not only question the magnitude of negative P_m 's as measured by tympanometry, but also indicates that positive pressures exist in SOM.

* Corresponding author. Present address: Department of Otolaryngology, Aalborg Hospital DK-9000 Aalborg, Denmark. Tel.: +45-99322911; fax: +45-99322938.

E-mail address: mgaihede@dadlnet.dk (M. Gaihede)

Human in vivo experiments have shown good agreement between direct and tympanometric measurements of P_m [4,5]. However, it has been pointed out that indirect measurements of P_m are influenced by the procedural pressure load, which results in a volume displacement of the TM (ΔV_{tm}) [6,7]. This ΔV_{tm} affects the actual P_m according to Boyle's Law. Hence, the measuring procedure itself creates pressure changes in the middle ear. The error introduced in this way is susceptible to both the compliance (mobility) of the TM and the size of the middle ear and the mastoid (V_m), but the error is small in normal ears [6,7]. When previous studies found good agreement between direct and indirect measurements of P_m , they were limited by comparing results only in few normal adult subjects [4,5], where normal V_m and compliance can be presumed. Since SOM predominantly affects children, and since the size of the mastoid only matures at the age of 15 years [8], cases with a small V_m may constitute a frequent problem in tympanometric determination of P_m . This could explain the discrepancy between direct and indirect measurements of P_m .

The purpose of the present study was to investigate the significance of the V_m in tympanometric measurements of P_m , which was accomplished by experiments in a middle ear model. Based on previous mechanical studies in 39 normal subjects [9], general expressions have been established describing the standard (= averaged) pressure–volume relationship (PVR) of the middle ear system (MES) [10]. This standard PVR describes the normal non-linear relationship between pressure changes in the ear canal and volume displacements of the TM, and it served as a templet for the construction of a realistic model membrane. Such a membrane was incorporated into a middle ear model, where tympanometric measurements could be performed under realistic conditions, and where both V_m and P_m could be controlled. The experiments included variations in P_m as well to investigate the accuracy of tympanometric measurements.

2. Materials and methods

The model was constructed by two pieces of plexiglass (Fig. 1). One piece had a hollow cylinder with a diameter of 9 mm and a length of 30 mm, which simulated the ear canal part of the model. The other piece had a small cavity of the same diameter and a depth of 13 mm (corresponding to 0.83 cm³) simulating the middle ear cavity. When the two pieces were held together by four screws like a cylinder head, a membrane could be interposed simulating the TM. The area of the membrane was 64 mm². From the cavity two narrow tubes (diameter 0.8 mm) were connecting to a manometer and a 20 cm³ syringe to control variation in P_m and V_m .

The mechanical behaviour of the model membrane and the standard PVR are illustrated in Fig. 2. The non-linear sigmoid shape and hysteresis of the standard PVR is well imitated by the model membrane. Non-linearity is a general biomechanical phenomenon, which is due to the anisotropy of biological tissues [11]. Anisotropic properties are explained by the complex of different tissue fibers and ground substance, which have different mechanical properties [11]. Hysteresis is another a general biomechanical phenomenon, which reflects the viscoelastic properties of biological tissues and results in different deformation characteristics depending on the direction of change [11]. This is seen in Fig. 2 by the two different curves describing the increasing and decreasing parts of both the standard and model PVR. Due to viscoelasticity the tissue deformation is accompanied by friction, and hysteresis expresses the equivalent energy, which is dissipated as heat [11].

Non-linear anisotropic properties were achieved by constructing a double layered membrane with two types of materials. One layer was made from a thin polyethylen film (0.04 mm), and the other from a piece of latex (ordinary examination glove). In this way two materials with different properties were connected: polyethylen being more stiff than latex. By attaching the polyethylen film loosely and the latex firmly to the model, a non-linear relationship was achieved, where small initial deformations reflected mainly the more elastic properties of the firmly attached latex, while increasing deformations resulted in recruiting the more stiff properties of the polyethylen. Hysteresis was imitated by imposing a thin viscous smear of vaseline between the two layers of the membrane.

The mechanical variables usually determined for the PVR [9] were also determined for the two PVR's in Fig. 2. Hysteresis was expressed by the energy equivalent of the area circumscribed by the curve (model = 23.69 μ J; standard = 20.78 μ J). Compliance was determined by the slope of the tangential line to the decreasing part of the PVR at $\Delta V_{tm} = 0$ kPa (model = 20.17 mm³/kPa; standard = 25.27 mm³/kPa). P_{range} described the difference between maximal and minimal P_{ec} (model = 4.69 kPa; standard = 3.70 kPa). Finally, P_{ec0} was determined by the P_{ec} at $\Delta V_{tm} = 0$ kPa for the decreasing part of the PVR (model = -0.35 kPa; standard = -0.19 kPa).

By adjusting the volume of the 20 cm³ syringe additional volume was included in the middle ear part of the model corresponding to various V_m 's. The volume of the syringe was changed between 0 and 20 cm³, corresponding to a V_m approximately between 1 and 21 cm³ due to the space of 0.83 cm³ in the model middle ear and dead space in tubes. The manometer was filled with 70% alcohol to decrease the viscosity, and pressures of the model middle ear were changed in

steps of 50 mm alcohol between +100 and –200 mm corresponding to a range of +85 to –170 daPa. The manometer was disconnected during tympanometric recordings to decrease dead space.

The tympanometer was connected to the model ear canal by a usual ear probe, and at each condition of varying V_m and P_m triplicate tympanometries were recorded. Tympanometry was performed by a Zodiac MEA 901 from Madsen Electronics with a probe tone frequency of 226 Hz. The routine clinical setup of the instrument was used, which included a pressure range and direction from +200 to –400 daPa, and rate of pressure change of 400 daPa/s. P_m was registered as the ear canal pressure at the peak of the tympanogram (daPa).

3. Results

Fig. 3 depicts the tympanometric estimates of P_m as a function of V_m for various preset P_m 's in the model. A general finding is that for all preset P_m -values a decrease in tympanometric P_m is found for V_m 's smaller than 5 cm³. For a normal model P_m (= 0 daPa) the tympanometric P_m is –13 daPa for a large V_m (21 cm³), whereas it decreases to –35 daPa at V_m = 1 cm³. The maximum decrease is found for model P_m = –85, where the tympanometric P_m changes from –207

(V_m = 21 cm³) to –255 daPa (V_m = 1 cm³). The corresponding mean change for all experiments was –38 daPa (SD = 12 daPa).

Another general finding is that model P_m is numerically overestimated by tympanometry; for instance, tympanometric P_m is found around –200 daPa for a model P_m of –85 daPa. More negative model P_m 's of –127 and –170 daPa resulted in tympanometric P_m 's below –300 daPa, which could not be quantitated on the tympanometer, or in type B tympanograms with no admittance maximum. Thus, these data have not been shown. Moreover, V_m 's of 1 and 2 cm³ at a model P_m = 85 daPa and 1 cm³ at 42 daPa resulted in tympanometric artefacts, and these measurements have also been omitted. Otherwise all recordings showed realistic tympanograms with well defined pressure peaks and a static compliance around 0.33 cm³.

In Fig. 4 the overestimation of tympanometric P_m 's is more clearly illustrated for selected V_m 's. In all cases with abnormal P_m 's ($P_m \neq 0$ daPa) the tympanometric P_m 's were numerically larger than the preset model values. For each V_m a linear correlation was found between tympanometric P_m and model P_m , where the slope of the regression lines varied between 2.60 and 2.10 daPa/daPa (mean = 2.31; SD = 0.21 daPa/daPa). This means that the tympanometric P_m is on average a factor 2.31 larger than the model P_m .

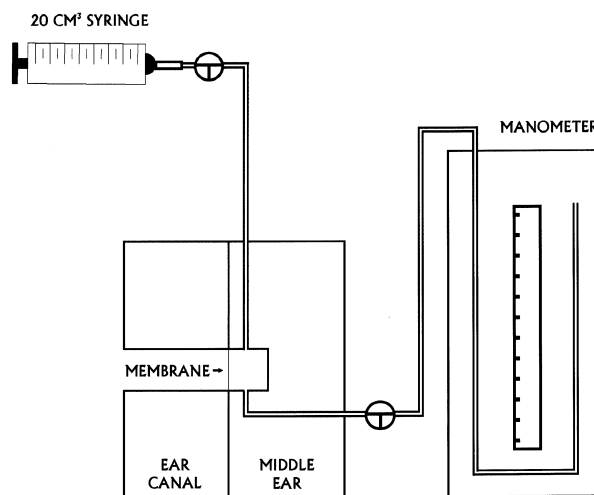


Fig. 1. Diagram of the middle ear model. The model membrane is interposed between the ear canal and the middle ear parts of the model. The middle ear is connected to a manometer, which is used to measure the P_m , and a 20 cm³ syringe used to regulate the V_m . Two three way stopcocks allow resetting of the P_m to ambient pressure and for an additional syringe to be connected for creating changes in model P_m .

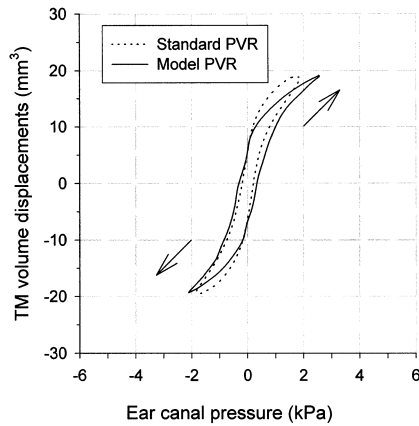


Fig. 2. The PVR of the model membrane is illustrated together with the standard PVR constructed from 76 ears. Arrows indicate direction of pressure and volume changes. The mechanical variables for each relationship are nearly identical—see text.

4. Discussion

4.1. General comments

The non-linear characteristics of the model PVR including the hysteresis behaviour coincided with the characteristics of the standard PVR (Fig. 2), which was also reflected by the similar mechanical variables for the two PVR curves. Minor discrepancies between the PVR's (Fig. 2) and the variables are insignificant, since they are included by the normal range [9,10]. Hence, a realistic anisotropic model with hysteresis had been constructed in agreement with normal pressure and volume relations of clinical experiments. This agreement was further corroborated by the subsequent realistic tympanometric recordings. The task of achieving a model membrane with these realistic characteristics was tedious and included many experiments, where the PVR was measured on different combinations of materials as well as different degrees of firm or loose attachments. However, once the model coincided with the standard PVR, it remained stable during the period of experiments.

The PVR recordings shown in Fig. 2 included an amplitude of $\pm 20 \text{ mm}^3$ resulting in a P_{range} of 3.7–4.7 kPa, which corresponded to approx. ± 1.8 to ± 2.4 kPa. The range of the tympanometer was 2 to -4 kPa exceeding that of the PVR's (Fig. 2). The present measurements of the PVR are only possible in the range of $\pm 20 \text{ mm}^3$ [9], and the standard PVR has only been defined in this range [10]. However, in Fig. 5 the PVR of the model membrane has been illustrated for abnormal P_m 's (± 85 daPa). These two asymmetric PVR's exhibit the same behaviour as in clinical measurements,

where the TM deviates from its neutral position [9]. Further, their mechanical characteristics corresponded to such clinical measurements (see legend) [9]. Hence, the model was reliable corresponding to a range similar to that found in Fig. 5 (± 6 kPa).

It is interesting that the agreement between the model and the standard PVR could be obtained despite the fact that the model membrane was plane compared with the cone shape of a normal TM. Further, the model membrane was fixed only at its periphery, whereas a normal TM is fixed also to the manubrium. The area of the model membrane was 64 mm^2 , which is in accordance with Dirckx et al. [12], who found an area of the human TM of 66.1 mm^2 .

Previous *in vivo* studies investigating the accuracy of indirect tympanometric measurements of P_m included measurements of the true direct P_m by means of introducing a pressure transducer through the eustachian tube [4] or puncturing the mastoid [5]. These studies only included few adult subjects for obvious ethical reasons. An alternatively realistic way to investigate the accuracy of tympanometry is human temporal bone experiments, where the P_m may be controlled [13,14]. However, the present approach has the advantages that it includes no ethical concerns, and it allows variation in V_m as well as P_m .

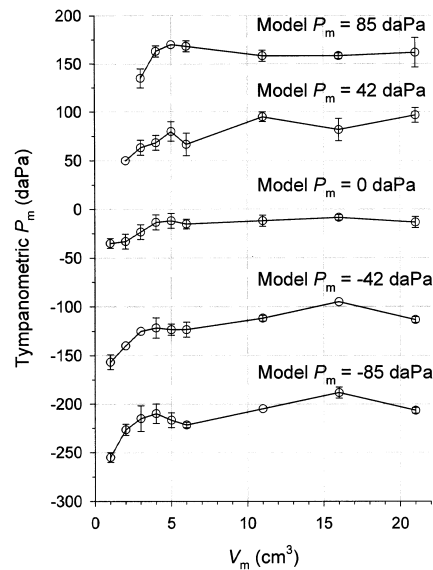


Fig. 3. Tympanometric P_m 's as functions of V_m for various preset P_m 's on the model. In general, a steeper decrease is found for V_m 's smaller than 5 cm^3 . Also tympanometric P_m 's are overestimated compared with model P_m 's. Points and error bars represent mean and S.D. of triplicate tympanometries.

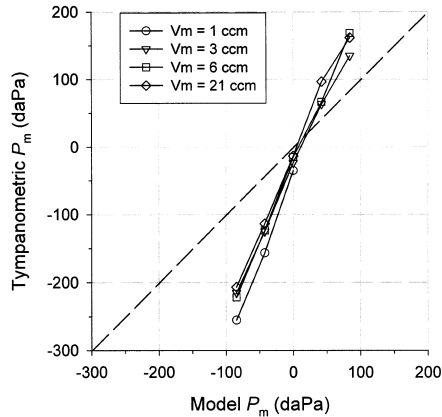


Fig. 4. Model P_m as a function of tympanometric P_m for selected V_m 's. The mean slope of the functions (2.31) reflects a linear numerical overestimation of tympanometric P_m compared with the actual model P_m . Dashed line represents line of identity, i.e. the ideal correlation. Points represent mean of triplicate tympanometries.

Since tympanometric pressure variations in the ear canal result in changes in P_m due to ΔV_{tm} , the effects on P_m are larger for TM's with a high compliance. This has experimentally been confirmed in tympanometric studies by Renvall et al. [14]. This factor was not investigated in our study, since only one model membrane was used. In accordance with a static compliance in the lower end of normal range (0.33 cm^3) the model membrane showed a lower compliance ($20.17 \text{ mm}^3/\text{kPa}$) measured by the PVR compared with the standard membrane ($25.27 \text{ mm}^3/\text{kPa}$). If our model membrane had a higher compliance, larger influence on P_m would be expected [6,7,14]. Future studies will elucidate the significance of compliance by incorporating model membranes with different mechanical properties.

4.2. Middle ear volume effects

The general result of a decrease in V_m from 21 to 1 cm^3 was that the tympanometric P_m 's showed a decrease ranging from -22 to -48 daPa , which was most pronounced below 5 cm^3 (Fig. 3). This can be explained by the increasing effects of ΔV_{tm} relative to the V_m resulting in a procedural decrease in the model P_m in accordance with previous studies [6,7,14].

The scaling is rather large in Fig. 3 in order to include all experiments. This tends to hide the nature of the functions, which seem exponential on smaller scales including only one experiment. In Fig. 6 this has been illustrated for the experiment, where the model $P_m = 0 \text{ daPa}$. In accordance with an exponential function a significant linear correlation was found between the

logarithmic values of the data samples ($P < 0.001$). By regression analysis a curve fit could be calculated and the equation has been inserted together with a graphical illustration of the function (Fig. 6). It is seen that the tympanometric P_m approaches asymptotically $-\infty \text{ daPa}$, when V_m approaches 0 cm^3 .

By extrapolation it can be calculated from the equation (Fig. 6) that a tympanometric P_m of -400 daPa corresponds to a V_m of 0.0031 cm^3 . If V_m becomes smaller, the tympanometric P_m will become more negative than -400 daPa , in which case the tympanogram will not be able to obtain an admittance maximum, when the lower pressure range of the instrument is -400 daPa . In other words a type B tympanogram will be recorded. Thus, in cases of a normal $P_m (= 0 \text{ daPa})$ either high negative P_m 's or type B tympanograms can in theory result entirely from a pronounced decrease in V_m .

Experiments of other model P_m 's than 0 daPa showed a similar exponential decrease in tympanometric P_m (Fig. 3), where a significant correlation was found between the logarithmic data sets (in all cases $P < 0.001$). In cases, where model P_m was lower than 0 daPa , the exponential effect of a decreasing V_m on tympanometric P_m was an even more negative result, since the measurements of P_m were generally more negative (Fig. 3). Contrary, when model P_m was larger than 0 daPa , this effect was counteracted by the tympanometric overestimation of P_m .

The V_m of normal ears has been reported to range from 4.6 to 9.5 cm^3 [15], but may vary up to more than 20 cm^3 [16]. Hence, the range of V_m 's applied in the present model are in accordance with previous studies. Elner et al. [7] reported that the accuracy of indirect determination of P_m decreases, when V_m becomes less than 6 cm^3 . This agrees with the general finding in Fig. 3 that the tympanometric P_m shows a steeper decrease, when V_m becomes smaller than 5 cm^3 . Hence, a V_m of $5\text{--}6 \text{ cm}^3$ seems to be a critical size with respect to indirect tympanometric measurements of P_m .

The model did not allow for smaller values of V_m than approx. 1 cm^3 due to the fixed size of the cavity in the model itself and dead space in tubes connecting to the 20 cm^3 syringe and the manometer. It would be interesting to achieve even smaller V_m 's, since the effect on tympanometric measurements become increasingly significant for the smallest volumes. Such experiments would justify the extrapolation of the curve fit (Fig. 6). Hence, a new model would benefit from a smaller middle ear cavity, but also from incorporating a pressure transducer to decrease the dead space.

4.3. Middle ear pressure effects

A significant linear relationship was found between tympanometric recordings of P_m and model P_m reflect-

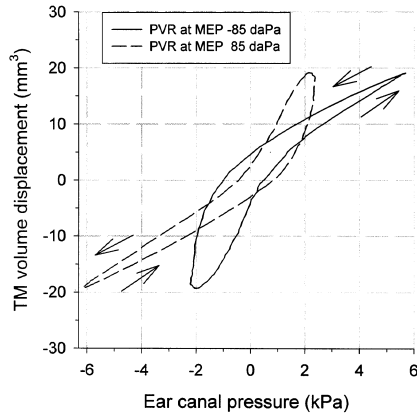


Fig. 5. The PVR of the middle ear model for two different model P_m 's. The mechanical variables for $P_m = -85$ daPa (full line) are: hysteresis = 42.31 μ J, compliance = 5.91 mm^3/kPa , $P_{\text{range}} = 7.79$ kPa, and $P_{\text{eco}} = -1.07$ kPa. For $P_m = +85$ daPa (dashed line): hysteresis = 37.51 μ J, compliance = 4.54 mm^3/kPa , $P_{\text{range}} = 8.54$ kPa, and $P_{\text{eco}} = -0.63$ kPa. The scaling of the figure is approximately the same as in Fig. 2. Arrows indicate direction of change.

ing a numerical overestimation of the former (Fig. 4). This means that negative values of model P_m gave more negative results by tympanometry and vice versa, and the error is reflected by the slope of the regression lines (mean slope = 2.31 daPa/daPa). Since a model P_m of -42 daPa gave tympanometric P_m 's around -100 daPa or more negative, this overestimation had a larger impact on tympanometric P_m 's for the abnormal model pressures ($P_m \neq 0$ daPa) than the isolated effect of a decreasing V_m . However, the effect of a decreasing V_m would be expected to be larger, if volumes smaller than 1 cm^3 were possible in the model.

Peterson and Lidén [13] and Renvall et al. [14] using a positive pressure sweep from -200 to $+200$ daPa found similar numerical overestimation of tympanometric P_m . Hergils et al. [5] generally found similar overestimation for both positive and negative sweeps, while the present study found it using a negative pressure sweep ($+200$ to -400 daPa). Hence, numerical overestimation of tympanometric P_m is a general finding, which is not influenced by the direction of pressure change. The data from Renvall et al. [14] allow for similar regression analysis, which shows a significant linear correlation with a slope of the regression line of 1.39, i.e. tympanometric P_m is on average a factor 1.39 larger than actual P_m .

This numerical overestimation of tympanometric P_m compared with the exact P_m is probably reflecting the tympanometric phenomenon that different pressure peaks are found in bidirectional tympanometry [5,17,

18]. When negative and positive pressure sweeps are recorded in the same ear, the negative sweep results in a more negative pressure peak, while the positive sweep results in a relative positive pressure peak. This phenomenon has been attributed to hysteresis reflecting the viscoelastic properties of the MES [5,17,18]. In tympanometry hysteresis results in a time dependent behaviour, where changes in TM position and P_m lag behind the changes in ear canal pressure [17].

Hysteresis itself relating to the viscoelastic properties of tissues is not susceptible to changes in strain rate over large ranges of variation [11]. In accordance with this Decraemer et al. [17] found no significant differences in tympanometric determination of P_m for different sweep rates. However, other authors find that determination of P_m is affected by the sweep rate, so that larger rates of ear canal pressure changes result in more extreme estimates of P_m [5,18,19]. This means that for a negative pressure change, the peak pressure will be found increasingly negative, when the sweep rate is increased, and vice versa. Hence, hysteresis in tympanometric recordings can be rate dependent [5,18,19].

However, in tympanometry two other factors influence the time dependent behaviour of hysteresis: compressibility of the media (air) and change of sweep direction. Since the tympanometer measures air pressure changes, a time delay may exist between actual pressure changes and its registration due to compressibility of the air. This problem can partly be overcome by placing the pressure transducer close to the ear probe [17,18]. The other problem is that changing the sweep direction on the tympanometer results in a time delay. Hence, hysteresis found in tympanometry reflects not only 'pure' hysteresis, but also instrument factors of time delay may contribute depending on the instrument and procedure. This can explain why some authors report that measurements are susceptible to sweep rates.

In the study by Renvall et al. [14] a pressure sweep of only 10 $\text{mmH}_2\text{O}/\text{s}$ was used, and the factor of overestimation is 1.39. When the present study found a factor of 2.31 using a faster rate of pressure change (400 daPa/s), it seems that the overestimation is rate dependent in accordance with the previous discussion.

Due to the hysteresis determined difference in peak pressures found in bidirectional tympanometry, it has been suggested that the average of the peak pressures should be taken for the exact P_m [5,17,18], but for normal ears the peak difference is only up to 50 daPa depending on the sweep rate [18]. This results in a small error of 25 daPa without any clinical implications. However, Kobayashi et al. [18] reported that the peak difference increased significantly in one case with abnormal P_m . In accordance with this finding, the hysteresis of the MES as measured by the PVR

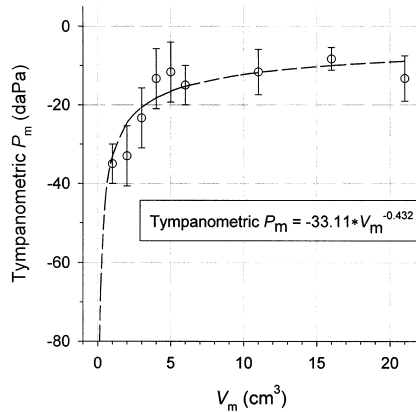


Fig. 6. Exponential decrease of tympanometric P_m as a function of V_m at model $P_m = 0$ daPa. The curve fit of the function is illustrated by the dashed line, and shows that tympanometric P_m approaches asymptotically $-\infty$ daPa, when the V_m approaches 0 cm³.

increases for deviating positions of the TM [9], and the model membrane used in the present study showed similar behaviour.

This has been illustrated in Fig. 5, where PVR recordings are shown for the model at P_m 's of 85 and -85 daPa. The hysteresis is approx. 2-fold increased for the two PVR's (see legend) compared with the PVR in Fig. 2, where $P_m = 0$ daPa. This is reflected by the increased distance between the increasing and decreasing curves in the major parts of the PVR's. Hence, increased hysteresis is found in cases of abnormal P_m both in tympanometry and PVR recordings, and this likely to explain the larger discrepancy between tympanometric and model P_m in these cases.

In summary, a linear numerical overestimation of tympanometric P_m was found compared with model P_m , which is in agreement with previous studies, where exact P_m is controlled and altered [5,13,14]. This overestimation is suggested to be explained by hysteresis of the MES, which is supported by the presence of similar time dependent behaviour in purely tympanometric studies, where P_m is affected by sweep direction [5,17,18] and rate [5,18,19]. The demonstration that hysteresis is increased in cases with abnormal P_m (Fig. 5) explains why tympanometric determination of such deviating pressures are accompanied by larger errors.

4.4. Results in relation to secretory otitis media

Tympanometric findings of high negative P_m 's in the range of -100 to -400 daPa in patients with SOM has supported the ex vacuo theory, where the negative pressure is held responsible for the middle ear effusion

[1]. These findings are not in accordance with direct measurements [1–3], and based on the present findings it is suggested that such high negative P_m 's are exaggerated or erroneous. In cases with smaller V_m 's tympanometry results in more negative recordings than the actual P_m , which may be a problem especially in children, since the mastoid is not fully developed until the age of 15 years [8]. More important, in cases of SOM the effusion of the middle ear cavity will replace the air filled expandable volume, i.e. the 'functional' V_m acting as pressure buffer becomes very small [20]. An additional factor may be blockage of the antrum by a mucoid plug, which also will decrease the functional V_m [21]. As a consequence, for very small V_m 's large negative P_m 's or type B curves can in theory be obtained by tympanometry, though the P_m itself is normal (Fig. 6). Any smaller preexisting negative P_m 's tend to enhance this effect due to hysteresis determined numerical overestimation (Fig. 4).

These suggestions are in agreement with previous experimental studies demonstrating that type B tympanograms can be obtained solely due to the presence of fluid in the middle ear cavity [13,14,22]. Hence, if the P_m in cases of SOM is normal or only slightly deviating from 0 daPa, then the tympanometric estimate of P_m only reflects the functional V_m . This means in other words that P_m measured by tympanometry in ears with SOM is likely to reflect only the combined effect of the amount of fluid in the middle ear cavity and the V_m itself. Thus, experiments monitoring changes in the P_m by tympanometry may simply reflect changes in the amount of fluid. Further, since a large V_m has been shown to be a positive prognostic factor in SOM [21], it can be suggested that ears with less negative tympanometric P_m 's may similarly indicate a better prognosis. However, this suggestion is complicated by the combined effect of the amount of the effusion and the V_m itself, and it will have to be confirmed in clinical studies.

Tympanometric recordings of type B curves have documented a high sensitivity and specificity regarding the detection of middle ear effusion, and hence, it is a valuable tool in SOM screening investigations [23,24]. It follows from the previous discussion that despite a normal P_m different ears with varying V_m 's and amounts of effusion are likely to result in similarly varying tympanometric P_m 's, which also means that the presence of an effusion may be of no significance in a large V_m . In accordance with this, tympanometric type B curves includes both false negative and false positive test results in detecting middle ear effusion [23,24].

Additional attention should be paid to the rheological properties of the middle ear effusion. Sadé et al. [1] suggested that the rheological properties of the effusion may be responsible for the high negative P_m 's and type B tympanograms found in SOM ears, when direct measurements in contrast showed a mean P_m of only

– 1.7 mmH₂O. In fact, preliminary experiments measuring the PVR in patients with SOM have shown that hysteresis can increase by a factor 4 compared with normal ears (80 vs. 20 μ J) [25]. This increased hysteresis of the MES is obviously a result of the effusion behind the TM and related to its viscoelasticity. In accordance with the previous discussion such increased hysteresis will also result in an increased numerical overestimation of tympanometric P_m . These findings, however, should be supported by investigating the PVR in more patients with SOM.

TM atrophy is a frequent sequelae of SOM, which has been attributed to long periods pressure load (due to high negative P_m) resulting in a depletion of fibers in the lamina propria [26]. Also related sequelae like TM retraction and atelectasis have been attributed to similar negative P_m 's. However, direct measurements of P_m in cases of chronic adhesive otitis media with atelectasis have revealed no pressure difference in 28 out of 101 ears, while the remaining 73 ears had a mean P_m of only – 7 mmH₂O [2]. Hence, the magnitude of the negative P_m is very small. The significance of such small pressures responsible for tissue remodelling and sequelae could be questioned. When further high negative P_m 's explaining TM atrophy can be questioned due to errors in tympanometry, it may lead one to look at intrinsic TM factors for an explanation.

There is general agreement that the mechanical properties of the TM is determined predominantly by the properties of the lamina propria with its elaborate interlacing fibers. It is also evident that shear stresses are present in the TM, since the membrane opens in response to myringotomy. According to Berger et al. [27] inflammation of the TM in SOM ears results in an equally increased thickness of the subepithelial and submucosal layers. Contrary to this finding, Møller [28] and Sano et al. [29] reported that the increased thickness of the TM predominantly is a result of an increased thickness of the submucosal layer.

Such increased thickness is likely to increase the tension in the tissue, i.e. the shear stresses may increase. Hence, it can be hypothesized that atrophy is a result of inflammatory induced increase of shear stresses within the TM, which result in a degenerative depletion of the fibrous elements of the lamina propria. Further, it may be of importance that if a unilateral inflammatory response in relation to the fibers of the lamina propria is predominant [28,29], the intrinsic stress vectors of the medial and lateral sides of the lamina propria are out of balance. Once atrophy is present, the TM has lost its mechanical skeleton and the middle ear cavity is turned into a collapsible gas pocket, which will respond to only small negative P_m 's by remodeling of the tissue resulting in retraction of the TM and atelectasis [30].

Considering the effective treatment of SOM with ventilation tubes, one has to look for an alternative

explanation than pressure equilibration between ear canal and middle ear, if the significance of negative P_m 's is questioned. This has recently been provided by Portier et al. [31], who found that middle ear epithelium enhances its absorption of Na⁺ and water in response to relative hyperoxia, i.e. clearance of middle ear fluid is likely to increase, when atmospheric air is present in the middle ear due to a ventilation tube.

The question remains whether small negative P_m 's as measured directly [1–3] are responsible for the effusion in SOM and its sequelae or inflammatory changes are responsible. Negative P_m 's have experimentally been shown to result in transudation, but the pressures applied were more pronounced than – 270 daPa [5] or – 600 daPa [32], which seem unphysiological. Since also positive P_m 's have been found in SOM, the significance of any negative P_m seems questionable, and it would be of interest to investigate directly monitored fluctuations of P_m .

5. Conclusions

The middle ear model was fitted with a realistic double layered membrane, which exhibited non-linear behaviour and hysteresis in agreement with normal clinical PVR recordings. This enabled tympanometric recordings under realistic and controlled circumstances. Changes in V_m resulted in exponentially decreasing tympanometric estimates of P_m for vol. < 5 cm³ due to the increased ΔV_{tm} in relation to V_m . Further, tympanometric estimates of P_m showed a linear numerical overestimation compared with exact P_m 's, which was explained by hysteresis.

Based on these findings high negative P_m 's measured by tympanometry in patients with SOM could be explained as methodological errors. This is in accordance with direct measurements of P_m in such patients, which have not been able to reproduce similar large negative pressures. The present study gives no answer to the exact P_m , which is of interest among others due to its significance as a pathogenetic factor in SOM, but in this context only directly measured pressures should be taken in account. Since direct studies have revealed very small negative and even positive P_m 's, one leads to question its significance in SOM.

Acknowledgements

The study has been supported by The Foundation for Medical Scientific Research at the Hospitals in Ringkøbing, Ribe, and Sønderjylland Counties. I am grateful to Torben Brask, M.D., for the loan of the middle ear model and valuable discussions. GN Danavox, Denmark, kindly supplied the Madsen Elec-

tronics Zodiac Middle Ear Analyser 901 used in this study.

References

- [1] Sadé J, Halevy A, Hadas E. Clearance of middle ear effusions and middle ear pressures. *Ann Otol Rhinol Laryngol* 1976;25 Suppl. 85:58–62.
- [2] Buckingham RA, Ferrer JL. Middle ear pressures in eustachian tube malfunction: manometric studies. *Laryngoscope* 1973;83:1585–93.
- [3] Takahashi H, Honjo I, Hayashi M, Fujita A, Kurata K. Middle ear pressures of children with otitis media with effusion. *Ann Otol Rhinol Laryngol* 1991;100:469–71.
- [4] Takahashi H, Hayashi M, Honjo I. Direct measurements of middle ear pressure through the eustachian tube. *Arch Otorhinolaryngol* 1987;243:378–81.
- [5] Hergils LG, Magnuson B, Falk B. Different tympanometric procedures compared with direct pressure measurements in healthy ears. *Scand Audiol* 1990;19:183–6.
- [6] Flisberg K, Ingelstedt S, Örtengren U. On middle ear pressure. *Acta Otolaryngol Stockh* 1963;182 Suppl.43–56.
- [7] Elnér Å, Ingelstedt S, Ivarsson A. Indirect determination of the middle ear pressure. *Acta Otolaryngol (Stockh)* 1971;72:255–61.
- [8] Rubensohn G. Mastoid pneumatization in children at various ages. *Acta Otolaryngol Stockh* 1965;60:11–4.
- [9] Gaihede M. Mechanics of the middle ear system: computerised measurements of its pressure–volume relationship. *Auris Nasus Larynx* 1999;26:383–99.
- [10] Gaihede M., Kabel J. The normal pressure-volume relationship of the middle ear system and its biological variation. (in press).
- [11] Fung YC. *Biomechanics. Mechanical Properties of Living Tissues*. New York: Springer-Verlag, 1993:242–320.
- [12] Dirckx JJJ, Decraemer WFS. Area change and volume displacement of the human tympanic membrane under static pressure. *Hear Res* 1992;62:99–104.
- [13] Peterson JL, Lidén G. Tympanometry in human temporal bones. *Arch Otolaryngol* 1970;92:258–66.
- [14] Renvall U, Lidén G, Björkman G. Experimental tympanometry in human temporal bones. *Scand Audiol* 1975;4:135–44.
- [15] Elnér Å. Indirect determination of gas absorption from the middle ear. *Acta Otolaryngol (Stockh)* 1972;74:191–6.
- [16] Andréasson L, Ingelstedt S, Ivarsson A, Jonson B, Tjernström Ö. Pressure dependent variation in volume of mucosal lining of the middle ear. *Acta Otolaryngol (Stockh)* 1976;81:442–9.
- [17] Decraemer WF, Creten WL, Van Camp KJ. Tympanometric middle ear pressure determination with two-component admittance meters. *Scand Audiol* 1984;13:165–72.
- [18] Kobayashi T, Okitsu T, Takasaka T. Forward-backward tracing tympanometry. *Acta Otolaryngol (Stockh)* 1987;435 Suppl.:100–6.
- [19] Feldman RM, Fria TJ, Palfrey CC, Dellecker CM. Effects of rate of air pressure change on tympanometry. *Ear Hear* 1984;5:91–5.
- [20] Ars B, Ars-Piret N. Middle ear pressure balance under normal conditions. Specific role of the middle ear structure. *Acta Oto Rhinol Laryngol (Belg)* 1994;48:339–42.
- [21] Suetake M, Kobayashi T, Takasaka T, Shinkawa H. Is change in middle ear air volume following ventilation tube insertion a reliable prognostic indicator? *Acta Otolaryngol (Stockh)* 1990;471 Suppl.:73–80.
- [22] Okitsu T, Kobayashi T, Endo S, Shibahara Y, Sakuma M, Takasaka T, Kawamoto K, Kaneko Y. Tympanograms of otitis media with effusion: an experimental study. *Auris Nasus Larynx* 1985;12(Suppl 1):222–4.
- [23] Ovesen T, Paaske PB, Elbrond O. Accuracy of an automatic impedance apparatus in a population with secretory otitis media: principles in the evaluation of tympanometrical findings. *Am J Otolaryngol* 1993;14:100–4.
- [24] Watters GWR, Jones JE, Freeland AP. The predictive value of tympanometry in the diagnosis of middle ear effusion. *Clin Otolaryngol* 1997;22:343–5.
- [25] Gaihede M. Mechanical properties of the middle ear system investigated by its pressure volume relationship. Introduction to methods and selected preliminary clinical cases. *Audiol Neurootol* 1999;4:137–41.
- [26] Tos M, Stangerup SE, Holm-Jensen S, Sørensen CH. Spontaneous course of secretory otitis media and changes of the eardrum. *Arch Otolaryngol* 1984;110:281–9.
- [27] Berger G, Sachs Z, Sadé J. Histopathologic changes of the tympanic membrane in acute and secretory otitis media. *Ann Otol Rhinol Laryngol* 1996;105:458–62.
- [28] Møller P. Tympanosclerosis of the ear drum. *Acta Otolaryngol (Stockh)* 1981;91:215–21.
- [29] Sano S, Kamide Y, Schachern PA, Paparella MM. Micropathologic changes of pars tensa in children with otitis media with effusion. *Arch Otolaryngol Head Neck Surg* 1994;120:815–9.
- [30] Sadé J. Atelectatic tympanic membrane: histologic study. *Ann Otol Rhinol Laryngol* 1993;102:712–6.
- [31] Portier F, van den Abbeele T, Lecaïn E, Sauvaget E, Escoubert B, Huy PTB, Herman P. Oxygen modulates Na⁺ absorption in middle ear epithelium. *Am J Physiol* 1999;276:312–7.
- [32] Schwartz JD, Alper CM, Chan KH, Seroky JT, Doyle WJ. In vivo observation with magnetic resonance imaging of middle ear effusion in response to experimental underpressures. *Ann Otol Rhinol Laryngol* 1995;104:522–8.

Accuracy of Tympanometric Middle Ear Pressure Determination: The Role of Direction and Rate of Pressure Change with a Fast, Modern Tympanometer

Anette G. Therkildsen and Michael Gaihede

Department of Otolaryngology, Head and Neck Surgery, Aalborg Hospital,
Aarhus University Hospital, Aalborg, Denmark

Hypothesis: Modern tympanometers run at higher rates of pressure change than older tympanometers, which increases the inaccuracy of determining the middle ear pressure.

Background: Tympanometric middle ear pressure may be susceptible to both the direction as well as the rate of pressure change, which is reflected by two different pressure peaks in a bidirectional recording. The resulting peak pressure difference results in an inaccuracy, which can amount to 25 daPa in older instruments with slower rates of pressure change. However, modern instruments often apply much faster rates, which may increase the peak pressure difference and thus the inaccuracy of middle ear pressure.

Methods: Middle ear pressure was measured for a negative and positive direction of pressure change at four different rates (50, 100, 200, and 400 daPa/s) in 38 normal adults. The peak

pressure difference was calculated by the middle ear pressure determined in positive minus negative direction.

Results: The mean peak pressure differences ranged between 10 and 12 daPa (standard deviation = 8–11) in the four groups and were independent of the rate of pressure change ($p = 0.321$).

Conclusion: The peak pressure differences found by the current tympanometer were consistently small for all rates of pressure change and were thus independent of the rate. This means that high rates can be used without decreasing accuracy, and the mean error is only 5 to 6 daPa, corresponding to the intrinsic hysteresis of the middle ear system. **Key Words:** Accuracy—Hysteresis—Middle ear pressure—Phase delay—Rate of pressure change—Tympanometry
Otol Neurotol 26:252–256, 2005.

Tympanometry is well established and widely used to evaluate middle ear status in clinical and scientific studies. Primarily, the elastic properties of the tympanic membrane and the middle ear pressure (MEP) are determined (1). From early reports on procedural variables, it is well known that estimates of MEP are susceptible to the direction of pressure change, so that negative pressure sweeps (positive to negative) result in more negative values, whereas positive pressure sweeps (negative to positive) result in more positive values (2–5). In addition, the rate of pressure change may also lead to differences, so that faster rates result in numerically larger estimates than slower rates (3–6).

These phenomena are illustrated in principle in Figure 1. In bidirectional tympanometry, two different peak pressures are found corresponding to the negative and positive directions of pressure change. For the slower

recording, the peak pressure difference (PPD) is only 20 daPa (Fig. 1A), whereas the PPD is 100 daPa for the faster recording (Fig. 1B). These features are explained by hysteresis and phase delay, and they raise the question of how to determine MEP most accurately. Hysteresis is related primarily to the viscoelastic properties of the middle ear system, whereas phase delay is related to the actual instrument and its rate of pressure change (discussed in detail below).

The PPD has mostly been reported in normal ears and found to be approximately 10 to 50 daPa (2–5). Because the actual MEP corresponds to the mean of the negative and positive direction of pressure change (MEP_{mean}), the error corresponds to $0.5 \times PPD$ (2,4,5). Thus, errors of 5 to 25 daPa can be anticipated, which may not be considered important, depending on the purpose. However, other complications can occur, which may increase the inaccuracy. First, previous reports on these aspects were based on older instruments, where only lower rates of pressure change were possible; the fastest instrument applied a rate of 180 daPa/s (5). In contrast, newer instruments may apply pressure rates up to 400 daPa/s, which in case of susceptibility to the pressure rate is

Address correspondence and reprints requests to Dr. Michael Gaihede, Department of Otolaryngology, Head and Neck Surgery, Aalborg Hospital, Aarhus University Hospital, DK-9000 Aalborg, Denmark; Email: mgaihede@aes.nja.dk

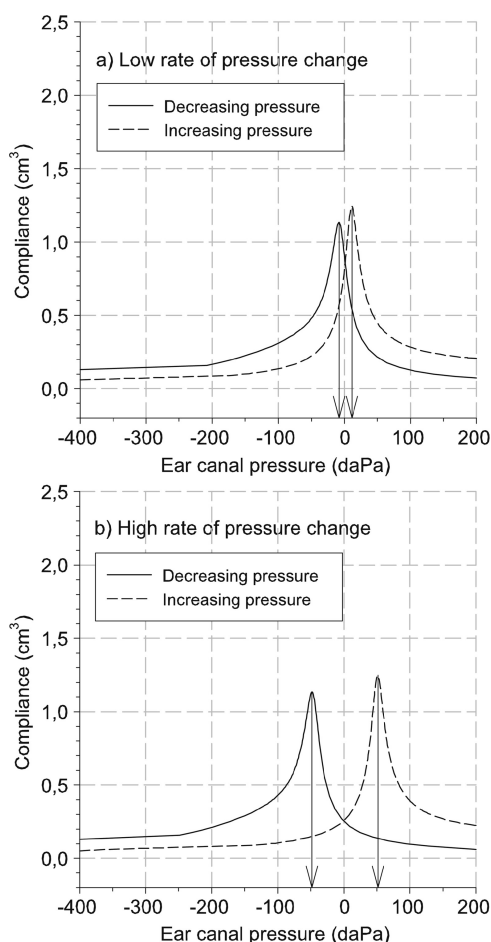


FIG. 1. Principles of bidirectional tympanometry. (A) At a low rate of pressure change, the peak pressures are found at -8 and $+12$ daPa (decreasing and increasing rates, respectively) (i.e., PPD = 20 daPa). (B) At high rates, the peak pressures are found at -48 and $+52$ daPa (i.e., PPD = 100 daPa). Arrows indicate the peak pressures on the abscissa.

likely to increase the PPD further and, consequently, increase the inaccuracy. It should also be noted that pressure rates are not always valid; discrepancies between the instrument specifications and the actual rates are not uncommon and thus should be checked when the PPD is investigated (5–7).

Second, recent preliminary tympanometric studies using a modern tympanometer at 100 daPa/s in patients with otitis media with effusion (OME) have found PPD up to 205 daPa explained by additional hysteresis or damping of the middle ear (ME) system because of the

effusion (8). Thus, more significant errors up to 100 daPa can be expected in some ears. However, both hysteresis and phase delay may contribute. Thus, it is important to determine any component of phase delay and susceptibility to pressure rate for the actual instrument to analyze the effects of the additional damping or hysteresis caused by the effusion, which can affect accuracy seriously (8).

Finally, previous experiments investigating the relationship between the tympanometric MEP (tMEP) and the actual MEP in a middle ear model with the same modern tympanometer at 100 daPa/s have suggested large errors in cases where the ME air volume is small (9). This situation applies to OME, where the effusion replaces the air volume, but also other middle ear conditions with a small air volume. The errors induced by this situation have been suggested to explain discrepancies between high negative tMEP and direct measurements of MEP being less negative in OME ears (9). Because the MEP is considered an important pathogenetic factor in OME (10) and because tympanometry is the most often used method to evaluate the MEP, these problems have gained recent attention from other authors (11,12). In such studies, the accuracy of the tympanometer itself is of obvious importance, where phase delay may play a role. This is especially of significance if studies are concerned with smaller negative MEPs greater than -100 daPa (12), because the PPD may then be relatively larger; the PPD can amount to 50 daPa, according to previous results (4).

In summary, many factors affect the accuracy of tympanometric estimates of MEP, and recent reports have brought new attention to these problems. Thus, we found it valuable to determine the PPD and any relationship to the rate of pressure change to evaluate the accuracy of tMEP in a modern instrument with higher pressure rates. This was accomplished by bidirectional tympanometries at different pressure rates in a group of normal ears. We are not aware of any more recent studies reporting on this problem.

PATIENTS AND METHODS

Thirty-eight normal adults with normal otomicroscopic findings and a negative history of previous ME disorders were included, and subjects were investigated in both ears (76 ears). All experiments were consequently started with a negative pressure sweep followed by a positive sweep at each rate of pressure change, and with an increasing sequence of rates (50, 100, 200, and 400 daPa/s) (i.e., in each ear, eight recordings were obtained). The mean age was 38 years (standard deviation = 11), and informed consent was obtained in each case.

An MEA 901 tympanometer (Madsen Electronics, Copenhagen, Denmark) with a 226-Hz probe tone was used for our experiments. This instrument has four rates of pressure change: 50, 100, 200, and 400 daPa/s, and it allows for recordings in both negative and positive directions. The pressure range was $+200$ to -400 daPa, and the MEP was determined as the ear canal pressure at the admittance peak of the tympanogram. In each case, the PPD was calculated by MEP measured by a positive pressure sweep minus MEP measured by a negative

sweep. Distributions of both the absolute MEP values and PPDs were determined. A significance level of 0.05 was chosen for the statistical analysis.

RESULTS

Table 1 displays the distributions of the absolute values of MEP for each direction and rate of pressure change. In all four groups of rate of pressure change, the distributions for the negative directions were consistently more negative than for the positive directions; this difference was in all cases significant (Table 1) ($p < 0.001$ in all four groups). The distributions of the MEP recorded in the same direction of pressure change were almost identical, and there was no significant difference in the groups of different pressure rates in the negative or the positive directions (Table 1) ($p = 0.268$ and $p = 0.989$, respectively).

In Table 2, the distributions of the PPD are depicted for each group of rate of pressure change; the distributions were almost identical and there were no significant differences between the groups (Table 2) ($p = 0.321$). The overall mean PPD was 12 daPa. Figure 2 displays the distributions of the PPD plotted against pressure rate, which include results from two previous studies (3,4).

DISCUSSION

The phenomenon of the PPD has been explained by two factors: phase delay and hysteresis (2–6). Although phase delay is related to the instrument, hysteresis is predominantly related to the viscoelastic properties of the ME system. In general, biomaterials display hysteresis characterized by different deformation curves in loading-unloading experiments, and to a large extent hysteresis is not susceptible to changes in strain or deformation rate (13). This means that the hysteresis component of the PPD is unlikely to change in response to increasing pressure rates. Phase delay is an instrumental and rate-dependent component, where a delay is found between, for instance, a pressure change and its subsequent registration (14). This is determined by the resistance of the tubes of the tympanometer, which can be high because of small diameter and long distance

between the pressure transducer and the site of pressure change, that is, the ear canal (the transducer inside the instrument of some older models versus in the headset of newer models). Because phase delay depends on the pressure rate, this explains why increasing PPD is found for increasing rates in some tympanometers (3–6). In accordance, this factor can be minimized or avoided by modifying the instrument, placing the transducer closer to the ear canal (2).

The current results of the actual MEP depicted in Table 1 are in accordance with a normal group of subjects, where a few subjects showed larger negative values up to -135 daPa. The difference between positive and negative pressure sweep was highly significant in all four groups of rate of pressure change (all $p < 0.001$), that is, the differences between the direction were systematic and could not be attributed to coincidental errors of measurements in accordance with previous reports (2–5). Furthermore, the results obtained in the four different groups of rate of pressure change in negative and positive directions were practically identical, and they did not differ significantly from each other (Table 1) ($p = 0.268$ and $p = 0.989$).

Correspondingly, the distributions of the PPD in the four groups of rate of pressure change were similarly very close to each other, and they did not show any significant difference (i.e., the PPD was independent of the rate in the range of 50 to 400 daPa/s) (Table 2) ($p = 0.321$). Consequently, the tympanometer showed no sign of contribution of phase delay. The mean PPDs were small, between 10 and 12 daPa, which indicate an inaccuracy of only 5 to 6 daPa on average. These results are compared with previous studies in Figure 2 (3,4).

The previous studies included in Figure 2 are the only results where the distributions of the PPDs were directly available for various pressure rates; both studies display increasing PPDs for increasing pressure rates (3,5). It is interesting to note that by extrapolation of their almost coinciding results to a pressure rate of 400 daPa/s, a PPD of 70 to 80 daPa can be suggested (Fig. 2); consequently, an error of 35 to 40 daPa can be anticipated. At the lowest rate, Shanks and Wilson (3) found a mean PPD of 15 daPa (at 12.5 daPa/s), whereas Kobayashi et al. (4) found a mean of 9 daPa (8 daPa/s). Both these results are

TABLE 1. Distribution of MEP (daPa) for each direction and rate of pressure change ($n = 72$)

	50 daPa/s		100 daPa/s		200 daPa/s		400 daPa/s	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Mean	-16	-6	-18	-6	-19	-7	-20	-8
SD	26	26	27	26	26	27	26	26
Minimum	-135	-120	-135	-115	-130	-115	-130	-115
Maximum	15	20	25	35	10	40	15	25
Wilcoxon ^a	$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$	
ANOVA ^b			$p = 0.268$ and $p = 0.989$					

^aWilcoxon signed-rank test.

^bKruskal-Wallis one-way analysis of variance on ranks: p values for testing results of groups of negative pressure change and positive pressure change, respectively.

SD, standard deviation; ANOVA, analysis of variance.

TABLE 2. Distribution of PPD (daPa) for each rate of pressure change (n = 76)

	50 daPa/s	100 daPa/s	200 daPa/s	400 daPa/s
Mean ^a	10	12	12	12
SD	8	11	11	10
Minimum	-10	-20	-10	-10
Maximum	30	60	60	50
ANOVA ^b	p = 0.321			

^aThe overall mean PPD = 12 daPa.

^bKruskal-Wallis one-way analysis of variance on ranks. SD, standard deviation; ANOVA, analysis of variance.

close to the present findings of 12 daPa and suggest that the instruments at low rates show no contribution of phase delay. In accordance, Decraemer et al. (2) found a mean PPD of 15 daPa using a modified instrument with no phase delay. Finally, mean PPDs of 22 and 28 daPa can be estimated from Hergils et al. (5) (at 31 and 180 daPa/s, respectively); however, their instrument showed phase delay, which accounted for the higher values.

Accuracy of tMEP can be improved by calculating the MEP_{mean} from a bidirectional recording, which has been experimentally verified in temporal bone (2), in a middle ear model (4), and in live human subjects (5), where the exact MEP was known. This approach was carried out for our group at 400 daPa/s, where the average MEP_{mean} was -14 daPa (standard deviation = 26; range, -123-18). This distribution likely to express a more accurate

description of the MEP suggests a slightly negative pressure in normal ears. The other groups of rate of pressure change showed similar distributions of the MEP_{mean}.

As pointed out by Decraemer et al., the contribution of the intrinsic hysteresis of the ME system cannot be avoided (2). According to the previous discussion, it amounts to 9 to 15 daPa, and the related inaccuracy corresponds to 4.5 to 7.5 daPa in normal ears. For most purposes, this can be considered negligible, but the hysteresis of the ME system is subject to individual variation (15), which is also reflected by the range of the PPD in Table 2, where it amounted to 60 daPa in some ears. This meant that the error in these cases amounted to 30 daPa.

Whereas the previous discussion has been concerned with normal ears only, the PPD may be significantly increased in diseased ears. Kobayashi et al. presented two pathologic cases, where a PPD over 400 daPa was demonstrated in one ear with otalgia and a normal-appearing tympanic membrane; in another ear with OME, an increased PPD of 45 daPa was found compared with normal ears recorded at the same rate (4). Recently, similarly increased PPD has been reported in a preliminary study of OME ears, where it amounted to approximately 200 daPa in some ears and, thus, significantly affected the accuracy of tMEP (8).

This study has now been extended, demonstrating significant positive correlations between the PPD and effusion properties (viscosity and amount) (16). In both studies, the same MEA 901 tympanometer as used currently was used (8,16), and based on our current results, it can be concluded that the instrument did not contribute to the PPD by any phase delay. Thus, the increased PPD must be attributed to an increased hysteresis or damping of the ME system caused by the properties of the effusion (8,16). In summary, the hysteresis may be insignificant in normal ears, although it is subject to individual variation, and it can be significantly increased in some abnormal ears, thus affecting accuracy seriously.

Rates of pressure change may not be valid (5-7). Thus, our rates were checked and found to be 60, 114, 200, and 391 daPa/s, respectively. These actual rates were very close to the specifications of our instrument, and the problem of disagreement may be of less importance in newer than in older instruments. If the older instruments were used at low rates to avoid phase delay and increase accuracy, the time needed to complete a recording from +200 to -400 daPa was 48 to 75 seconds (600 daPa/12.5 daPa/s and 600 daPa/8 daPa/s) (3,4). This is unpractical in a clinical setting and in children, whereas our tympanometer only took 1.5 seconds to complete a recording without phase delay to interfere with accuracy (600 daPa/400 daPa/s).

Finally, our tests were all made in the same sequence of direction and rate, and it may be argued that this could affect the results. However, whereas compliance measured by tympanometry is subject to increments over repeated trials (17), there is no evidence that tMEP is affected by the previous history of trials (10,17). Thus, there was no reason for randomizing the test sequence.

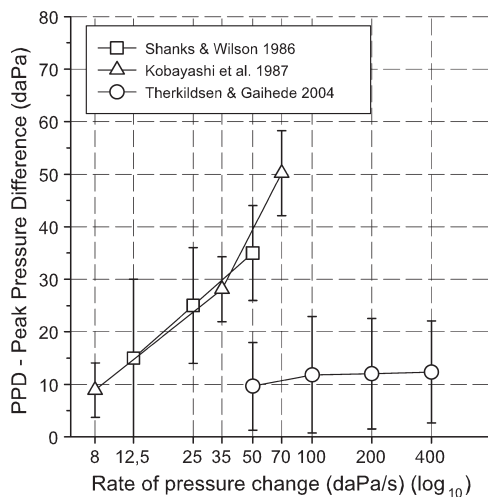


FIG. 2. The PPD as a function of rate of pressure change; current results show no variation in response to increasing rate varying between 10 and 12 daPa on average. Results from previous studies show increasing PPD for increasing rates. Points and error bars illustrate the mean and SD in all studies. The instrument used by Shanks and Wilson was a Grason-Stadler, Model 1723 (n = 25); that used by Kobayashi et al. was Teledyne TA-2C Impedance Meter (n = 59).

CONCLUSION

The currently investigated tympanometer showed consistently low PPDs of approximately 12 daPa for all groups of rate of pressure change ranging from 50 to 400 daPa/s. This meant that there was no sign of phase delay affecting the results. Moreover, the accuracy of measurements was not compromised by using fast rates, and the instrument only reflected the hysteresis component of the ME system. Moreover, the current tympanometer was suitable for further investigations of the PPD in diseased ears. If the PPD for a specific tympanometer is on the order of 10 to 15 daPa in normal ears, it corresponds to the intrinsic hysteresis of the ME system, whereas if the PPD is larger, phase delay is exhibited, and this instrumental factor should be accounted for, especially if the purpose is more basic experiments, where high accuracy is demanded (8–12,16).

REFERENCES

1. Shanks J, Shelton C. Basic principles and clinical applications of tympanometry. *Otolaryngol Clin North Am* 1991;24:299–328.
2. Decraemer WF, Creten WL, Van Camp KJ. Tympanometric middle ear pressure determination with two-component admittance meters. *Scand Audiol* 1984;13:165–72.
3. Shanks JE, Wilson RH. Effects of direction and rate of ear canal pressure changes on tympanometric measures. *J Speech Hear Res* 1986;29:11–9.
4. Kobayashi T, Okitsu T, Takasaka T. Forward-backward tracing tympanometry. *Acta Otolaryngol Suppl* 1987;435:100–6.
5. Hergils LG, Magnuson B, Falk B. Different tympanometric procedures compared with direct pressure measurements in healthy ears. *Scand Audiol* 1990;19:183–6.
6. Feldman RM, Fria TJ, Palfrey CC, Dellecker CM. Effects of rate of air pressure change on tympanometry. *Ear Hear* 1984;5:91–5.
7. Gaihede M, Felding JU, Elbrond O. Biomechanical characteristics of the middle ear system measured by a new method: III—comparison with tympanometric measurements. *Acta Otolaryngol (Stockh)* 1995;115:522–7.
8. Gaihede M, Lambertsen K, Bramstoft M, Kamarasukas A, Fogh A. Tympanometric hysteresis effect and errors in middle ear pressure determination: a preliminary study in children with secretory otitis media. *Acta Otolaryngol Suppl* 2000;543:58–60.
9. Gaihede M. Middle ear volume and pressure effects on tympanometric middle ear pressure determination: model experiments with special reference to secretory otitis media. *Auris Nasus Larynx* 2000;27:231–9.
10. Doyle WJ. Middle ear pressure regulation. In Rosowski JJ, Merchant SN, eds. *The Function of the Normal, Diseased and Reconstructed Middle Ears*. The Hague, The Netherlands: Kugler, 2000:3–21.
11. Alper CM, Banks JM, Philp KD, Doyle WJ. Tympanometry accurately measures middle ear underpressures in monkeys. *Ann Otol Rhinol Laryngol* 2003;112:877–84.
12. Cinamon U, Sade J. Tympanometry vs direct middle ear pressure measurement in an artificial model: is tympanometry an accurate method to measure middle ear pressure? *Otol Neurotol* 2003;24:850–3.
13. Fung YC. Bioviscoelastic solids. In Fung YC, ed. *Biomechanics: Mechanical Properties of Living Tissues*. 2nd ed. New York: Springer Verlag, 1993:242–73.
14. Olson WH. Basic concepts of instrumentation. In Webster JG, ed. *Medical Instrumentation*. Boston: Houghton Mifflin, 1978:1–47.
15. Gaihede M. Mechanics of the middle ear system: computerized measurements of its pressure-volume relationship. *Auris Nasus Larynx* 1999;26:383–99.
16. Gaihede M, Bramstoft M, Thomsen LT, Fogh A. Accuracy of tympanometric middle ear pressure determination in otitis media with effusion: dose dependent overestimation related to the viscosity and amount of effusion. *Otol Neurotol* 2005;26:5–12.
17. Gaihede M. Tympanometric preconditioning of the tympanic membrane. *Hear Res* 1996;102:28–34.

Accuracy of Tympanometric Middle Ear Pressure Determination in Secretory Otitis Media: Dose-Dependent Overestimation Related to the Viscosity and Amount of Middle Ear Fluid

*Michael Gaihede, *Mads Bramstof, *Lene T. Thomsen, and †Aksel Fogh

*Department of Otolaryngology, Head and Neck Surgery, Aalborg Hospital, Aarhus University Hospital, Aalborg, and †ENT Clinic, Hjørring, Denmark

Hypothesis: Tympanometric measurements of middle ear pressure in children with secretory otitis media are overestimated in a dose-response manner because of increased hysteresis explained by the viscosity and amount of middle ear fluid.

Background: Tympanometric middle ear pressure is important in evaluating children with secretory otitis media. These measurements are influenced by hysteresis appearing as a peak pressure difference in bidirectional tympanometry. This represents an inaccuracy of $0.5 \times$ peak pressure difference, which is only 5 to 25 daPa in normal ears. However, previous experiments found increased hysteresis, suggesting an inaccuracy of 225 daPa in secretory otitis media ears.

Materials and Methods: In 56 patients with secretory otitis media, bidirectional tympanometry was performed; Type B curves were excluded. The middle ear fluid was semiquantified subsequently at surgery according to viscosity (serous, seromucoid, or mucoid) and amount (small, medium, or large). A control group included 28 normal children. Peak pressure difference was calculated by the difference between middle

ear pressure determined by a positive and negative pressure sweep.

Results: Mean peak pressure difference was 10 and 69 daPa in the normal and secretory otitis media groups, respectively ($p < 0.001$). However, peak pressure difference ranged to 205 daPa in the secretory otitis media group and showed a significant positive correlation to viscosity and amount of the fluid (both $p < 0.0001$).

Conclusion: Peak pressure difference is significantly increased in secretory otitis media because of additional damping explained by the viscosity and amount of the fluid. The mean error was 5 daPa in normal ears and 35 daPa in secretory otitis media ears, but ranged to greater than 100 daPa. These results were only a low estimate of the inaccuracy, because patients with Type B tympanograms could not be included, and errors of more than 100 daPa can be anticipated. **Key Words:** Accuracy—Hysteresis—Middle ear pressure—Secretory otitis media—Tympanometry—Viscosity.

Otol Neurotol 26:5–11, 2005.

Indirect tympanometric determination of middle ear pressure (MEP) is a frequent procedure in both clinical and scientific contexts. In particular, tympanometry is a valuable diagnostic tool in cases with secretory otitis media (SOM), where it often indicates negative MEP in the range of -100 to -400 daPa or lower (Type B tympanograms). Such negative MEPs are considered a component of the pathogenetic events responsible for middle ear (ME) fluid (1). However, some controversies exist concerning the magnitude of these pressures, because the few studies recording the MEP directly in SOM ears were unable to reproduce such high negative MEPs (2–4). Methodological limitations of tympanometry in such

ears have been suggested to explain this disagreement by overestimation of the extant MEP (5). These problems have resulted in recent attention to investigating the accuracy of tympanometric MEP (tMEP) (6,7).

A series of factors affects the accuracy of tympanometry. Hysteresis is one of these, which is more or less negligible in normal ears, but it can increase inaccuracy in ears with disease. Hysteresis results in two different pressure peaks for negative (+ to $-$) and positive ($-$ to +) directions of pressure change, respectively (8–10). The resulting peak pressure difference (PPD) is a well-known feature, which consequently leads to a methodological ambiguity, raising the question of which procedural direction most accurately reflects the MEP. Previous studies have shown that the exact MEP corresponds to the mean MEP of the two directions (MEP_{mean}), and it follows that the inaccuracy corresponds to $0.5 \times$ PPD (8–10). In normal ears, the PPD ranges from 10 to 50 daPa,

Address correspondence and reprints requests to Michael Gaihede, M.D., Department of Otolaryngology, Head and Neck Surgery, Aalborg Hospital, Aarhus University Hospital, DK-9000 Aalborg, Denmark; Email: mgaihede@aaas.nja.dk

resulting in an inaccuracy of 5 to 25 daPa, which in most cases can be considered insignificant (8–10).

However, previous mechanical experiments investigating the pressure-volume relationship of the ME system (tympanic membrane [TM], ossicles, volume and mucosa of the ME, and MEP) have demonstrated that hysteresis can increase by a factor of 4 in SOM compared with normal ears (11). From these results, experimental tympanograms can be derived (12), which is illustrated in Figure 1. The recordings from the normal ear show almost coinciding pressure peaks for negative and positive direction (PPD = 8 daPa) (i.e., the hysteresis is very small). In contrast, in the SOM ear with a high hysteresis, the pressure peaks are wide apart from each other. Peaks appear at -210 and $+240$ daPa (i.e., PPD = 450 daPa). Thus, the PPD is significantly increased compared with the normal ear. Consequently, it has been suggested that tympanometric recordings can be similarly affected in SOM ears by increasing the PPD and consequently increasing inaccuracy to 225 daPa (12).

This hypothesis was tested by a preliminary tympanometric study reporting an increased mean PPD of 75 daPa in SOM ears, but ranging up to 205 daPa (12). The increased PPD was explained by an increased hysteresis due to an additional damping of the ME system by the ME fluid (i.e., the viscosity of the fluid increased the overall viscous properties of the system). Consequently, it was also likely to propose a dose-dependent relationship between PPD and the properties of the fluid (i.e., its

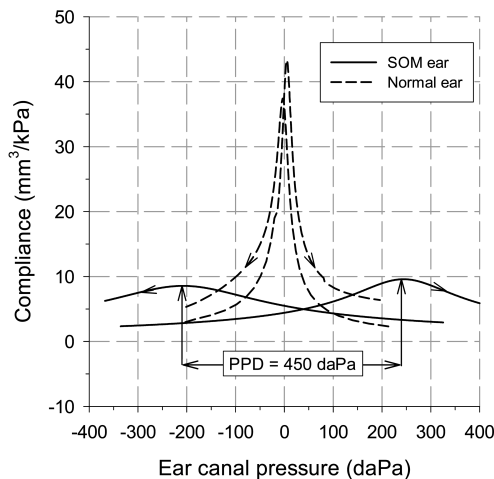


FIG. 1. Two cases of experimentally derived functions describing compliance as a function of ear canal pressure changes ($dV_m/dP_{ec} = f(P_{ec})$); these are analogous to bidirectional tympanometries. (*Small arrows*) Direction of pressure change. The normal ear (*dashed lines*) shows pressure peaks almost coinciding, that is, PPD is small (8 daPa). The SOM ear (*solid lines*) shows a wide distance between pressure peaks appearing at -210 and $+240$ daPa, that is, the PPD is 450 daPa (illustrated on the figure).

viscosity and amount). Thus, the purpose of the current study was to substantiate previous results and investigate the additional damping reflected by any dose-dependent relationship between PPD and the properties of the fluid. This was achieved by bidirectional tympanometry in a larger group of children with SOM and relating these findings to the fluid properties. Such results are relevant, because they will enhance our knowledge of factors affecting the accuracy of tMEP.

PATIENTS AND METHODS

The test group was recruited from a group of children submitted to the ENT clinic for insertion of ventilation tubes after 3 months of persistent SOM. Bidirectional tympanometry was performed before surgery, and if pressure peaks were identified, the subjects were included (i.e., cases with Type B tympanograms were excluded). At operation, the ME fluid was evacuated and semiquantified by the same surgeon according to its viscosity (serous, seromucoid, or mucoid) and amount (small, medium, or large). A total of 56 children were included in the test group; mean age was 4.2 years (standard deviation [SD] = 1.4 yr). Although most of the children had bilateral SOM, three were only affected unilaterally. Furthermore, a TM perforation was encountered in two ears and a cholesteatoma was found in one ear. In four ears, the tympanogram was technically impaired, and in four ears no ME fluid was found at myringotomy. This left a total test group of 98 ears.

The control group was recruited from a kindergarten and consisted of 28 children with no previous history of ME disorders and a normal otomicroscopy test and tympanogram. As in the test group, bidirectional tympanometry was performed; in 5 ears, sealing of the ear probe was incomplete, resulting in a total of 51 ears. Mean age was 4.4 years (SD = 1.8 yr). Informed consent was obtained from all children and parents, and the study was approved by the Scientific Ethical Committee of our county (VN 99/147).

An MEA 901 tympanometer (Madsen Electronics, Taastrup, Denmark) equipped with a 226-Hz probe tone was used at a rate of 100 daPa/s from $+200$ to -400 daPa and vice versa. The MEP was determined as the ear canal pressure at the peak of the tympanogram in both directions, and the PPD was calculated by the difference between MEP determined in the positive minus the negative direction. In some cases with low compliance (<0.1 cm³), the parameters including the MEP were not determined by the instrument, but if well-defined peaks could be presented with a proper scaling of the y axis, the MEP was determined manually from the recording.

The two groups were compared using a Mann-Whitney rank sum test. For correlation analysis, each ear in the SOM group was allocated a value of 1 to 3 for increasing viscosity and amount of fluid according to the surgeon's semiquantification, whereas all ears in the control group were allocated a value of 0. Correlation was analyzed by a Spearman rank order correlation test. Significance levels were 5%.

RESULTS

The distributions of the PPDs appear in Table 1 for an overall comparison. The mean PPD in the SOM group is significantly higher than in the normal group ($p < 0.001$);

TABLE 1. Distribution of peak pressure difference in normal and secretory otitis media ears

	Normal ears (daPa)	SOM ears (daPa)
Mean	10	69
SD	15	45
Minimum	-45	-15
Maximum	40	205
No.	51	98
Rank sum test	$p < 0.001$	

SOM, secretory otitis media; SD, standard deviation.

also, its variation and range are larger than in the normal group.

In Figure 2, the relationship between the PPD and viscosity of the fluid is depicted. A significant positive correlation was found between the variables ($r = 0.629$; $p < 0.0001$; $n = 149$). The relationship between the PPD and the amount of the fluid is shown in Figure 3, where a similarly significant positive correlation was found ($r = 0.627$; $p < 0.0001$; $n = 149$).

DISCUSSION

Hysteresis and tympanometry

Hysteresis is a general biomechanical feature of the viscoelastic properties of soft tissues (13), which is also found in the ME system (11). The tympanometric version of this property expressed by the PPD is similarly well known (8–10). However, the tympanometric PPD

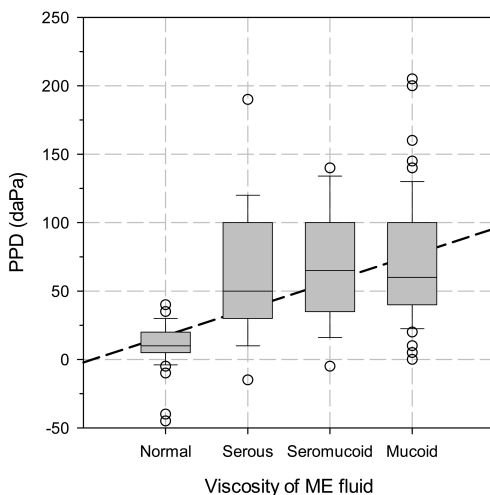


FIG. 2. The relationship between the PPD and the viscosity of the fluid; boxes indicate 25th and 75th percentiles and medians, and whiskers indicate 10th and 90th percentiles ($r = 0.629$; $p < 0.0001$; $n = 149$). The results of a linear regression analysis have been illustrated (dashed line). The number of observations in the groups are 51, 19, 15, and 64, respectively.

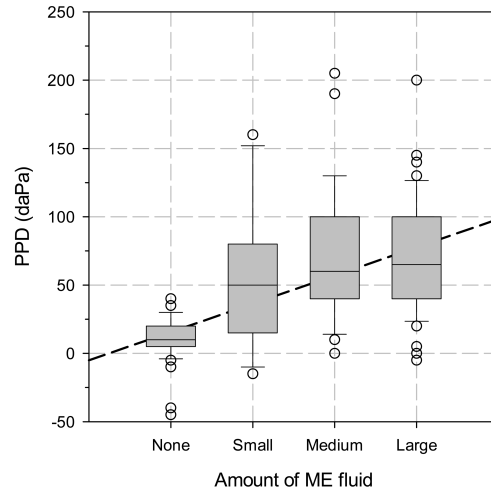


FIG. 3. The relationship between the PPD and the amount of the fluid; boxes indicate 25th and 75th percentiles and medians, and whiskers indicate 10th and 90th percentiles ($r = 0.627$; $p < 0.0001$; $n = 149$). The results of a linear regression analysis have been illustrated (dashed line). The number of observations in the groups are 51, 11, 31, and 56, respectively.

may consist of two components: 1) hysteresis itself due to the intrinsic viscoelasticity of the MES, and 2) phase delay related to the actual instrument. Although PPDs up to 30 to 50 daPa have been reported using instruments with phase delay (9,10), Decraemer et al. (8) found a PPD of 15 daPa using a modified instrument with no phase delay. This complies with a recent study using our current tympanometer, where the mean PPD was 12 daPa (SD = 10) in a normal adult group; in addition, the PPD was found constant in a range from 50 to 400 daPa/s (i.e., this instrument showed no phase delay) (14). Hence, a small PPD in the order of 12 to 15 daPa will always exist, representing the intrinsic hysteresis of the ME system (8,14).

Current results

The PPDs from normal ears discussed above are in range with our present control group with a mean of 10 daPa (SD = 15) (Table 1) (14). This represented a pediatric normal group with an age distribution similar to the SOM group. Because viscoelastic properties of the MES may change in relation to age, it was important that the control group matched the test group (15).

By an overall comparison, the mean PPD of 69 daPa in the SOM group was significantly higher than the mean of 10 daPa in the normal group (Table 1). As a result, a mean error of 35 daPa was indicated in the SOM group, whereas 5 daPa was indicated in the normal group. However, the SOM group was subject to much larger variation, with PPDs up to approximately 200 daPa (Table 1 and Figs. 2 and 3), and consequently, errors amounted to

approximately 100 daPa in these ears. Because the instrument did not contribute to any phase delay, the increased PPD could not be attributed to instrumental factors (14).

Therefore, the increased PPD is explained by increased hysteresis which must be explained by the additional viscosity or damping caused by the fluid behind the TM (11,12). In fact, Sadé et al. (2) have suggested that the rheologic properties of the fluid may play a role, when they tried to explain the discrepancy between tMEP in SOM ears and their direct recordings being less negative. Dose-response behavior between PPD and the fluid properties is indicated by the correlations in Figures 2 and 3 (both $p < 0.0001$). The normal group represented the small intrinsic damping of the ME system itself and the variation was small, although two outliers were found with larger negative PPDs. These are likely to represent cases of errors of measurement or they are explained by pressure equilibration between the two trials. Otherwise, the PPDs in both correlations show a wide range and overlap between the SOM subgroups.

This could be explained by the combined effects of the fluid properties. Our analysis was based on a rough stratification, because in any SOM subgroup of viscosity, all three subgroups of amount of fluid were included and vice versa. Therefore, it seemed obvious that the analysis would improve from a more strict stratification of data. In Table 2, the numbers of ears in each of these subgroups are depicted. The subgroups of mucoid and large amount of fluid represented the majority of the cases. By including ears with a large amount of fluid only, the correlation between viscosity and the PPD (Fig. 2) was not influenced by subgroups of small and medium amounts, but the overlap between subgroups of viscosity was unchanged. Similarly, including ears with mucoid fluid only, the correlation between amount of fluid and the PPD (Fig. 3) was not influenced by subgroups of serous and seromucoid fluid, but similar overlap was found. Further analysis will need more ears in the remaining subgroups than provided by the current material.

Another problem is the semiquantification of the fluid properties. All scores were made by the same surgeon, and a visual distinction between serous and mucoid fluid has been found valid (16). However, the scores still had a subjective element. An objective quantification of viscosity would ideally be to measure it using a viscometer, but the amounts of fluid were too small for our capabil-

ity. Alternately, a dilution technique might have been applied, where the amount can be increased sufficiently (16,17), or the glycoprotein content might have been determined, because it correlates to viscosity, providing an indirect measure (16). However, estimations of the amount of fluid may constitute a larger problem, because we do not know how much is left after evacuating the ME cavity; remnants may still be present in the attic and antrum. In accordance, studies describing increased air-bone gaps in response to the damping by ME fluid conclude that the amount is more important than viscosity itself in terms of the hearing loss produced by the fluid (18,19).

In some cases, the SOM subgroups showed PPDs close to the normal ears. These cases can be explained by a condition where the fluid in the ME cavity was categorized as a large amount, but where adjacent areas (attic and antrum) remained air-filled and thus did not contribute to the damping effects. In contrast, cases with high PPD because of a large amount of fluid may obtain lower scores because of displacement of the fluid from the ME cavity into these adjacent areas. This situation may occur in some ears, because surgery was performed under general anesthesia using a mask for ventilation. Therefore, a positive airway pressure existed, which could be transmitted to the ME cavity; by displacement of the fluid, the subsequent score at myringotomy will be too small. This may explain why in four cases no fluid was found at myringotomy, and also explains the high PPDs found in the subgroups of small and medium amounts of fluid (Fig. 3). The subgroups of viscosity will similarly be affected because of the rough stratification, where each group contained different amounts of fluid (Fig. 2). Additional volume factors are discussed in the section on the exact MEP.

In summary, the mean error in the SOM group may be considered moderately increased, though it ranged to 100 daPa in some ears. However, it is important to consider that our study group represented only less severely affected SOM patients, because cases with Type B tympanograms were excluded. We have no account of the number of children excluded during the study period, but our impression was less than half of the children could be included. This agrees with Moody et al. (20), who found identifiable peaks (Types A or C) in 47% of the ears in a similar group of children with SOM. Consequently, the actual extent of inaccuracy is difficult to determine by tympanometry, because it can only be determined in a smaller part of less severely affected cases. Thus, our results are only a low estimate of the inaccuracy. It should be noted that the experimental tympanogram suggested errors up to 225 daPa (Fig. 1) (12).

Additional statistical comments

In an attempt to analyze the combined effects of the fluid properties in more detail, we used a multiple linear regression analysis, where the PPD constituted the dependent variable and viscosity and amount of fluid constituted two independent variables. This analysis found

TABLE 2. Distribution of number of observations in the secretory otitis media subgroups

Amount of ME fluid	Viscosity of ME fluid			Total
	Serous	Seromucoid	Mucoid	
Small	8	0	3	11
Medium	8	8	15	31
Large	3	7	46	56
Total	19	15	64	98

ME, middle ear.

the regression coefficient to be 8.3 for viscosity ($p = 0.125$) and 13.1 for amount of fluid ($p = 0.019$). This is in agreement with the studies discussed above, where the amount of fluid rather than its viscosity was found important in damping of the ME system (18,19). However, this analysis should be taken with caution, because the independent variables were categorical and because the correlations may not be linear; in fact, the linear regression lines illustrated in Figures 2 and 3 were only meant to indicate the increasing relationships between PPD and fluid properties, whereas the exact nature of this relationship cannot be determined. More detailed studies are needed with an exact numerical quantification and more detailed stratification of the properties.

In statistical analysis, pooling of right and left ears is strictly not correct, because they are not independent variables; pooling of such data leads to a false inflation of sample size and possibly false significance in testing (21). In accordance, testing scores of viscosity and the amount of fluid in right versus left ears, they were not found to be significantly different (Wilcoxon signed rank sum test, $p = 0.098$ and 0.305 , respectively). However, for reasons of simplicity, our data were pooled, but analysis of right and left ears separately gave the same highly significant p values as the combined analysis (Table 1 and Figs. 2 and 3). Thus, our simplified approach did not conceal any insignificant analyses.

The exact MEP and additional factors of inaccuracy

The accuracy of tMEP can be increased by calculating MEP_{mean} from a bidirectional recording by which the effects of hysteresis and phase delay are avoided (8–10,14). We attempted this improvement, and results are shown in Table 3. The conventional MEP measured in a negative direction showed a mean of -89 daPa for normal ears and -159 daPa for SOM ears, whereas the MEP_{mean} showed a mean of -84 daPa for normal ears and -125 daPa for SOM ears. For both methods, the normal group would have been suspected to be less negative, but still the results were significantly less negative than in the SOM group (Table 3) ($p < 0.001$ and $p = 0.002$, respectively).

TABLE 3. Distributions of middle ear pressure measured in the negative direction and mean middle ear pressure calculated from a bidirectional recording

	Normal ears (daPa)		SOM ears (daPa)	
	MEP_{neg}	MEP_{mean}	MEP_{neg}	MEP_{mean}
Mean	-89^a	-84^b	-159^a	-125^b
SD	93	93	61	60
Minimum	-275	-270	-350	-15
Maximum	80	75	-20	-300
No.	51	51	98	98

^aRank sum test, $p < 0.001$.

^bRank sum test, $p = 0.002$.

MEP_{neg} , middle ear pressure measured in a negative direction; MEP_{mean} , mean middle ear pressure; SOM, secretory otitis media; SD, standard deviation.

Besides the increased hysteresis in SOM ears, there are additional factors that increase the inaccuracy of tMEP. The expandable free air volume behind the TM is of great importance, because in cases of a small volume (including the mastoid), the procedural volume displacement of the TM induced by tympanometry is relatively larger; this results in a change of the ME air volume, which ultimately affects the actual MEP (22). This problem has been investigated in a physical ME model, where a negative exponential relationship was suggested between the tMEP and ME air volume (5). Results indicated that tMEP approaches $-\infty$ daPa for an ME air volume approaching 0 cm^3 despite a normal MEP of 0 daPa in the model. Thus, it is suggested that a high negative tMEP can be obtained purely as a result from depletion of the ME air volume. This depletion can result from fluid in the ME cavity, edema of its mucosa, blockage of the antrum, or any combination; in SOM ears, all these factors may apply. Cinamon and Sadé (7) recently confirmed these results, reporting increasing inaccuracy for smaller air volumes in an ME model; although the specific relationship between tympanometric MEP and air volume was not addressed, their results indicate a similar exponential relationship at least for pressures less than -20 daPa (data from Fig. 2 of their article).

Furthermore, the extant MEP is numerically overestimated by tympanometry, reflected by the slope of a linear regression line describing the correlation between the actual and tMEP. This phenomenon has been demonstrated in mechanical (5,7) and animal models (6), where the slope varies between 1.1 and 2.6. One mechanical model found slopes larger and subject to variation between 2.1 and 2.6 but independent of different ME air volumes (5), whereas another model demonstrated slopes between 1.2 and 1.4, increasing for smaller ME volumes (7). Their range of investigation was limited to -50 to -100 daPa, but with a detailed sampling (7). A similar slope of 1.1 was found in a normal monkey model for a larger range of -400 to $+200$ daPa; this study did not account for the ME volume (6). Therefore, the majority of experiments found slopes on the order of 1.1 to 1.4. Whether numerical overestimation per se is related to the ME volume also remains uncertain. Finally, the compliance of the TM plays a role, because in ears with a flaccid TM, the tympanometric volume displacement is relatively larger, thus also affecting the actual MEP to a larger extent (22). We are not aware of any studies addressing this problem.

In summary, the interaction of these additional factors and our current results is complicated, but it is suggested that indirect tMEP cannot be considered accurate in SOM ears. Therefore, direct measurements are the ideal, which has been attempted in only a few studies. Sadé et al. (2) and Buckingham and Ferrer (3) punctured the TM and found a mean MEP of -1.7 and -6.3 daPa, respectively (2,3), whereas Takahashi et al. (4) used a transducer inserted into the ME via the eustachian tube, reporting a mean MEP of -53.2 daPa. These studies, however, may represent methodological problems result-

ing in less negative results than the extant MEP (6). Most recently, Brattmo et al. (23) found a mean MEP of -60 daPa by direct measurements in ears with chronic perforations mainly related to previous otitis media. These patients do not compare directly with SOM patients, but the magnitude of MEP compares well to Takahashi et al. (4). Concluding on these aspects, controversies exist concerning direct measurements, but none of these studies has demonstrated high negative MEP on the order of greater than 100 daPa as suggested by tympanometric results. On the basis of our current results and ME modeling (5,7), this discrepancy can be explained by inaccuracies of tMEP in SOM ears.

Clinical and scientific implications

Tympanometry has demonstrated a high accuracy in determining the MEP in normal ears based on comparison with direct recordings (6,10), and sensitivity and specificity detecting fluid in the ME is high (24). The inaccuracy described by this study refers mainly to ears with SOM but extends to cases of depletion of air volume (chronic otitis with small mastoids, atelectasis, blockage of the antrum). In a clinical context, this problem may not be significant, because decisions for interventions are based on a broader clinical assessment.

However, in scientific research, where results are involved in studies of gas exchange, exact results are demanded, and the tympanometer is not likely to be sufficiently accurate in ears with SOM. This constitutes a significant problem because of the role of negative MEP in the pathogenesis of SOM, where the fluid has been explained by a transudate driven by a pressure gradient of approximately 250 daPa between the mucosa and the ME cavity (1). Alternately, inflammatory changes responsible for impaired eustachian tube function may extend also onto the ME mucosa and result in an increased susceptibility to transudation. Therefore, the transudate could be driven by a less negative MEP. The high incidence of SOM and related sequelae obviously justify a search for the exact circumstances to offer a rational treatment strategy. The effect of ventilation tubes is well documented, but recurrences and sequelae are common problems.

Accuracy may be improved by calculating the MEP_{mean} from a bidirectional recording (8–10), although this procedure does not account for the depletion of ME air volume (5,7,18). Furthermore, it should be noted that classification of tympanograms used in some studies depend on the direction of recording: cases with increased PPD classification can be significantly affected. This can be accounted for by determining the MEP_{mean} , which is independent of direction of pressure change.

On the basis of the previous discussion, large PPDs may represent cases with coherent amounts of fluid extending to the attic and antrum; consequently, the ME air volume is small. Because small air volumes correlate to less favorable prognosis with higher recurrence rates of SOM (25,26), the PPD may also be a reliable prognostic factor, which is simple to determine.

CONCLUSION

We have demonstrated a significantly increased PPD in SOM ears resulting in a moderate average error of 35 daPa estimating the MEP by tympanometry, but amounting to more than 100 daPa in some SOM ears. The PPD showed a significant positive correlation to the viscosity and amount of fluid, where the latter probably played the major role. It should be noted that these errors constitute a small estimate of the errors, because our material represented a less severely affected group of patients. Therefore, combined with other sources of inaccuracies, the errors are suggested to be even larger, so that tympanometry seems inaccurate for determining the MEP in SOM and in other diseased ears. Our results partly explain the discrepancy between tMEP and direct measurements, where high negative pressures could not be demonstrated. The exact MEP cannot be estimated from our results, but further model experiments and direct measurements are needed. Such studies are highly relevant considering the central role of MEP in the pathogenesis of SOM and the high incidence of this condition and its sequelae.

Acknowledgments: The authors thank Michael Bjerregaard, M.A., audiologist, for providing the tympanograms for all SOM patients; and the children of the Fusingø Kindergarten, their parents, and staff for help in obtaining the tympanograms for the control material.

REFERENCES

1. Doyle WJ. Middle ear pressure regulation. In Rosowski JJ, Merchant SN, eds. *The Function of the Normal, Diseased and Reconstructed Middle Ears*. The Hague, The Netherlands: Kugler, 2000:3–21.
2. Sadé J, Halevy A, Hadas E. Clearance of middle ear effusions and middle ear pressures. *Ann Otol Rhinol Laryngol* 1976;85(Suppl 25):58–62.
3. Buckingham RA, Ferrer JL. Middle ear pressures in eustachian tube malfunction: manometric studies. *Laryngoscope* 1973;83:1585–93.
4. Takahashi H, Honjo I, Hayashi M, Fujita A, Kurata K. Middle ear pressures of children with otitis media with effusion. *Ann Otol Rhinol Laryngol* 1991;100:469–71.
5. Gaihede M. Middle ear volume and pressure effects on tympanometric middle ear pressure determination: model experiments with special reference to secretory otitis media. *Auris Nasus Larynx* 2000;27:231–39.
6. Alper CM, Banks JM, Philp KD, Doyle WJ. Tympanometry accurately measures middle ear underpressures in monkeys. *Ann Otol Rhinol Laryngol* 2003;112:877–84.
7. Cinamon U, Sadé J. Tympanometry versus direct middle ear pressure measurement in an artificial model: is tympanometry an accurate method to measure middle ear pressure? *Otol Neurotol* 2003;24:850–53.
8. Decraemer WF, Creten WL, Van Camp KJ. Tympanometric middle ear pressure determination with two-component admittance meters. *Scand Audiol* 1984;13:165–72.
9. Kobayashi T, Okitsu T, Takasaka T. Forward-backward tracing tympanometry. *Acta Otolaryngol Suppl* 1987;435:100–6.
10. Hergils LG, Magnuson B, Falk B. Different tympanometric procedures compared with direct pressure measurements in healthy ears. *Scand Audiol* 1990;19:183–6.
11. Gaihede M. Mechanical properties of the middle ear system investigated by its pressure-volume relationship: introduction to

- methods and selected preliminary clinical cases. *Audiol Neurootol* 1999;4:137–41.
12. Gaihede M, Lambertsen K, Bramstoft M, Kamarauskas A, Fogh A. Tympanometric hysteresis effect and errors in middle ear pressure determination: a preliminary study in children with secretory otitis media. *Acta Otolaryngol Suppl* 2000;543:58–60.
 13. Fung YC. Bioviscoelastic solids. In Fung YC *Biomechanics: Mechanical Properties of Living Tissues*. 2nd ed. New York: Springer Verlag, 1993:242–73.
 14. Therkildsen AG, Gaihede M. Accuracy of tympanometric middle ear pressure determination: the role of direction and rate of pressure change with a fast modern tympanometer. *Otol Neurotol* (in press).
 15. Gaihede M, Koefoed-Nielsen B. Mechanics of the middle ear system: age-related changes in viscoelastic properties. *Audiol Neurootol* 2000;5:53–8.
 16. Carrie S, Hutton DA, Birchall JP, Green GGR, Pearson JP. Otitis media with effusion: components which contribute to the viscous properties. *Acta Otolaryngol* 1992;112:504–11.
 17. Sichel J-Y, Priner Y, Weiss S, et al. Characteristics of the type B tympanogram can predict the magnitude of the air-bone gap in otitis media with effusion. *Ann Otol Rhinol Laryngol* 2003;112:450–4.
 18. Majima Y, Hamaguchi Y, Hirata K, Takeuchi K, Morishita A, Sakakura Y. Hearing impairment in relation to viscoelasticity of middle ear effusions in children. *Ann Otol Rhinol Laryngol* 1988;97:272–4.
 19. Hartley DEH, Moore DR. Effects of conductive hearing loss on temporal aspects of sound transmission through the ear. *Hear Res* 2003;177:53–60.
 20. Moody SA, Alper CM, Doyle WJ. Daily tympanometry in children during the cold season: association of otitis media with upper respiratory tract infections. *Int J Pediatr Otorhinolaryngol* 1998;45:143–50.
 21. Altman DG, Bland JM. Units of analysis. *BMJ* 1997;314:1874.
 22. Elnér Å, Ingelstedt S, Ivarsson A. Indirect determination of the middle ear pressure. *Acta Otolaryngol* 1971;72:255–61.
 23. Brattmo M, Tideholm B, Carlborg B. Chronic tympanic membrane perforation: middle ear pressure and tubal function. *Acta Otolaryngol* 2003;123:569–74.
 24. Ovesen T, Paaske PB, Elbrond O. Accuracy of an automatic impedance apparatus in a population with secretory otitis media: principles in the evaluation of tympanometrical findings. *Am J Otolaryngol* 1993;14:100–4.
 25. Sederberg-Olsen JF, Sederberg-Olsen AE, Jensen AM. The prognostic significance of the air volume in the middle ear for the tendency to recurrence of secretory middle ear condition. *Int J Pediatr Otorhinolaryngol* 1983;5:179–87.
 26. Suetake M, Kobayashi T, Takasaka T, Shinkawa H. Is change in middle ear volume after ventilation tube insertion a reliable prognostic factor. *Acta Otolaryngol Suppl* 1990;471:73–80.

