MIDDLE EAR STRUCTURE IN RELATION TO FUNCTION

The Rat in Middle Ear Research

Akademisk avhandling

Umeå 1985

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ABSTRACT

The present study was undertaken to evaluate the rat as a model for middle ear re-
search. The rat was chosen primarily because the gross structure of its middle ear
shows several similarities to that of man. It was considered of great importance to
make a thorough structural study of the rat middle ear and to compare the results
with those reported for the human middle ear. The thesis therefore includes indepen-
dent studies on various aspects of rat middle ear structure and function as well as a
review of the literature. The most pertinent findings in the experimental part of
this study were the following.

The rat Eustachian tube consists of a nasopharyngeal, and a cartilaginous and bony
portion. The orifice of the nasopharyngeal portion is composed of two soft tissue
lips, which appear to be opened mainly by the action of the salpingopharyngeal mus-
cle, but also by the levator and tensor veli palatini muscles. The cartilaginous por-
tion appears to be opened solely by the tensor veli palatini muscle. The tensor
tympani muscle seems to have no effect on the tube.

A ciliated and secretory epithelium lines the inferomedial walls of the tube
throughout its length. In the tympanic cavity these epithelial cell types extend as
two tracts – one anterior and the other inferoposterior to the promontory – which
communicate with the epitympanic/attic compartments. The remaining parts of the tube
and the tympanic cavity are covered by a squamous/cuboidal, non-ciliated epithelium.
The subepithelial loose connective tissue contains vessels, nerves, and connective
tissue cells, among these mast cells. The mast cells are confined to areas covered by
the ciliated epithelium, and in the floor of the bulla, in the pars flaccida, and
along the manubrial vessels. Glands are restricted to the Eustachian tube.

In the clearance/transport of serum-like material, from the epitympanum towards the
tube, hydrostatic forces appear to be important.

The tympanic membrane is vascularized from meatal and tympanal vessels. Meatal ves-
sels branch in the pars flaccida and along the handle of the malleus, where they are
localized directly beneath the outer, keratinizing, stratified, squamous epithelium.
Furthermore, meatal vessels form a vascular network at the junction between the fi-
brocartilaginous annulus and the tympanic sulcus. Tympanal vessels send branches to
the periphery of the pars tensa, where they run immediately beneath the tympanal,
simple, squamous epithelium. In the major portion of the pars tensa, no blood vessels
were found.

The rat stapedial artery is a thin-walled vessel with a wide lumen. Without branch-
ing, it runs through the tympanic cavity to the extratympanal regions it supplies. In
contrast to the corresponding artery in man, the rat stapedial artery persists
throughout life. The artery does not seem to be affected by the fluid produced during
experimentally induced otitis media with effusion.

The middle ear structure in the rat and in man show both similarities and differ-
ences. If the differences are kept in mind and considered, it would seem that the rat
is indeed a suitable model for experimental middle ear research.

Key words: rats; Eustachian tube; ear, middle; tympanic membrane; palatal muscles;
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by

NILS ALBIIN

To Carina

Cover: Drawing by

Karin Nordin.

All animals are equal
but some animals are more equal than others

George Orwell
1903-1950

ORIGINAL PAPERS

This thesis is based upon the following papers, which will be referred to by their Roman numerals.

I.   Albiin N, Hellström S, Salén B, Stenfors L-E and Söderberg O. The anatomy of the Eustachian tube in the rat. A macro- and microscopical study. Anat Rec 207(1983)513-521.

II. Albiin N, Hellström S, Stenfors L-E and Cerne A. The mucosa of the middle ear in man and rat. (Submitted to Ann Otol Rhinol Laryngol).

III. Albiin N, Hellström S and Stenfors L-E. Clearance of effusion material from the attic space. An experimental study in the rat. Int J Pediatr Otorhinolaryngol 5(1983)1-10.

IV. Albiin N, Hellström S, Salén B, Stenfors L-E and Wirell S. The vascular supply of the tympanic membrane. Anat Rec. In press.

V.   Albiin N, Hellström S, Salén B and Stenfors L-E. The stapedial artery in the rat - a microscopical study under normal conditions and in otitis media with effusion (OME). Acta Anat 115(1983)134-140.

CONTENTS

INTRODUCTION

As early as 1832, Shrapnell stated that "A correct knowledge of the
anatomy of the ear, is, however, not only of the utmost importance to
the surgeon from a pathological point of view, but it forms a most in-
teresting field of research in its connexion with the science of acous-
tics". Thus, in order to study the normal physiology and
pathophysiology of the middle ear, a thorough knowledge of its struc-
ture is required.

Studying a textbook in anatomy or otology may give the impression that
the structure and function of the middle ear are known in detail. This
is definitely not the case, however. Many fundamental questions remain
to be answered, e.g. what functions can be attributed to the tensor
tympani muscle and the pars flaccida of the tympanic membrane in middle
ear mechanics, and how does the Eustachian tube work? Studies that can
bring answers to these questions are difficult to carry out in man, for
several reasons, e.g. ethical, but also because the complex middle ear
is embedded in the temporal bone, and there is no easy access to this
portion of the ear. Therefore, animal models may provide an opportunity
to answer some of these questions.

This thesis will present some aspects of the basic structure of the rat
middle ear, and hopefully it may serve as a normal reference material
for those who are engaged in experimental studies in this species.
However, the ultimate goal is to obtain results that may prove applica-
ble to the human middle ear. It follows that findings made in an animal
model must be carefully analyzed against the background of what is
known of human middle ear structure and function. The present thesis
will thus start with a brief review of what is hitherto reported in the
literature concerning anatomy, embryology, and physiology of the human
and the rat middle ear.

AIMS OF THE STUDY

The rat as a model for middle ear research was introduced quite recently (Maeda et al., 1976; Kuijpers et al., 1979; Stenfors et al., 1979; Hellström et al., 1984). The rat was chosen as a model for middle ear research for several reasons, e.g. the anatomy of its middle ear appears to resemble that of man, it is easily accessible for surgical and experimental procedures, and it can be obtained at relatively low cost. However, our knowledge of the structure in relation to the function of the rat middle ear still has large gaps which need to be filled in.

Against this background the principal aims of the present thesis were:

1. To further characterize the structure of the rat middle ear in relation to function.

2. To review the literature on the anatomy, embryology, and physiology of the human and the rat middle ear.

3. To compare the findings in the rat middle ear with those described in man, in an attempt to elucidate whether the rat middle ear is an appropriate model for experimental middle ear research.

ANATOMY, EMBRYOLOGY, AND PHYSIOLOGY
OF THE HUMAN AND THE RAT MIDDLE EAR - A REVIEW

According to the 1983 edition of Nomina Anatomica the tympanic cavity is synonymous with the middle ear, and thus includes the Eustachian tube and the mastoid cells. However, in otological textbooks (Anson & Donaldson, 1973) the tympanic cavity is defined as the air-filled chamber of the middle ear - medial to the tympanic membrane - which communicates anteriorly with the nasopharynx (via the Eustachian tube) and posteriorly with the mastoid antrum and the mastoid cells (via aditus ad antrum). In this thesis the latter definition of the tympanic cavity will be used, as it is more practical and also more widely used among otologists.

ANATOMY OF THE HUMAN MIDDLE EAR

Historical Notes

According to Singer (1922) the auditory tube in animals was discovered by Alcmaeon about 500 B.C., and Hippocrates described the tympanic membrane about 100 years later (Saunders, Paparella & Miglets, 1980). Bartholomeo Eustachio (1520-1574) is accredited the first description of the membranocartilaginous tube connecting the nasopharynx with the tympanic cavity (Graves & Edwards, 1944). Falloppio described the shape of the tympanic cavity in 1562.

Eustachian Tube

The auditory, or Eustachian, tube extends from the nasopharynx, in a posterior, lateral, and superior direction, to the tympanic cavity. The tubal length is 31-38 mm and its angle with the horizontal plane is about 30-40° (Graves & Edwards, 1944).

Nasopharyngoscopy reveals that the nasopharyngeal opening is normally closed (Bryant, 1907; Rich, 1920; Simkins, 1943; Graves & Edwards, 1944; Honjo et al., 1984). It is characterized by two lips of soft tissue which together form a vertical slit (Bryant, 1907; Rich, 1920; Simkins, 1943; Graves & Edwards, 1944; Rood & Doyle, 1982). In material

fixed post mortem, on the other hand, the orifice is normally open and described as being funnel shaped and either oval, or triangular (Simkins, 1943; Graves & Edwards, 1944). The posterosuperior prominent boundary of the orifice, torus tubarius, is caused by the protrusion of the tubal cartilage. The salpingopharyngeal fold, which covers the salpingopharyngeal muscle, descends from the torus tubarius.

The nasopharyngeal two-thirds of the tube wall is cartilaginous and the tympanal one-third is osseous (Valsalva, 1717; Graves & Edwards, 1944; Proctor, 1967; Lim, 1974).

Recently the tubal cartilage has been described to be non-segmented, and to be firmly attached to the sulcus of the skull base in which it lies (Rood & Doyle, 1982). Furthermore, the cartilage is fixed to the osseous tube (Bryant, 1907; Graves & Edwards, 1944) and has been described to extend several millimetres into the bony canal (Lim, 1974; Rood & Doyle, 1982), or all the way into the tympanal orifice (Graves & Edwards, 1944; Sadé, 1985). The cartilage surrounds the tube medially and speriorly, and in cross section it has the appearance of a shepherd's crook (Graves & Edwards, 1944; Proctor, 1973).

The osseous portion of the tube is completely surrounded by bone. Its pharyngeal end is the narrowest part of the tube, the isthmus (Graves & Edwards, 1944). However, the lumen of the osseous portion widens only slightly towards the tympanic cavity (Graves & Edwards, 1944; Proctor, 1973).

The tympanal orifice of the tube opens about 4 mm above the lowest part of the floor of the tympanic cavity, a site which corresponds to the anterosuperior quadrant of the tympanic membrane (Graves & Edwards, 1944).

There are three muscles intimately associated with the Eustachian tube. These paratubal muscles are the tensor veli palatini, the levator veli palatini and the salpingopharyngeal muscle. The tensor tympani muscle should also be included in the group of paratubal muscles. However, this muscle will be discussed in the chapter on the tympanic cavity.

The tensor veli palatini muscle originates from the skull base, the tubal cartilage and the posterior portion of the membranous wall of the tube (the medio-inferior wall not supported by the cartilage). From its origin the muscle runs downwards and forward, rounds the hamulus of the medial pterygoid plate, and inserts into the aponeurosis of the soft palate (Bryant, 1907; McMyn, 1940; Graves & Edwards, 1944; Proctor, 1973; Rood & Doyle, 1978). The tensor veli palatini muscle is innervated by the mandibular branch of the fifth cranial nerve (Graves & Edwards, 1944; Holborow, 1975; Cantekin et al, 1979; Keller et al, 1984).

The levator veli palatini muscle arises from the base of the skull and from the medial lamina of the tubal cartilage (Bryant, 1907; Graves & Edwards, 1944; Proctor, 1973). Some fibers may originate from the tubal membranous floor (Proctor, 1973; Rood & Doyle, 1982). However, some authors (McMyn, 1940; Doyle & Rood, 1980) claim that this muscle normally has no tubal origin. The muscle passes inferiorly and medially, parallel to the tube, and inserts into the dorsal part of the soft palate. The source of innervation of the levator veli palatini muscle has yet to be established, although several nerves have been suggested - the accessory nerve (Williams & Warwick, 1980), the facial nerve (Ibuku et al., 1978), the hypoglossal or the vagus nerve (Keller et al., 1984).

The salpingopharyngeal muscle originates from the apex of the medial lamina of the tubal cartilage, and extends downwards and slightly backwards, and finally intermingles with the fibers of the palatopharyngeal muscle, in the lateral and posterior walls of the pharynx (McMyn, 1940; Simkins, 1943). Moreover, Proctor (1973) and Doyle & Rood (1980) add that it also inserts directly into the superior horn of the thyroid cartilage. This muscle is innervated by the pharyngeal branch of the vagus nerve (Graves & Edwards, 1944).

Tympanic Cavity

The tympanic cavity is very narrow in the lateromedial direction. Opposite the umbo of the tympanic membrane the transverse diameter measures only 2 mm. The vertical and anteroposterior diameters are about 15 mm each (Anson & Donaldson, 1973; Williams & Warwick, 1980).

The tympanic cavity can be divided into the mesotympanum, or the tympanic cavity proper (opposite the tympanic membrane), the epitympanum or the epitympanic recess, or the attic (above the membrane), and the hypotympanum, the hypotympanic recess (below the membrane).

At the upper part of the anterior wall of the tympanic cavity the Eustachian tube opens. Above the tympanal opening of the tube the tensor tympani muscle runs in its bony canal. A thin bony septum separates the canal from the Eustachian tube.

The tensor tympani muscle originates from the walls of its canal, the tympanal end of the tubal cartilage, and the adjacent part of the greater wing of the sphenoid. Moreover, the muscle has been described as being connected with the tensor veli palatini muscle (Proctor, 1973; Holborow, 1975; Rood & Doyle, 1978). The slender tensor tympani muscle ends posterolaterally in a rounded tendon, which turns laterally around the processus cochleariformis, and inserts into the root of the handle of the malleus. The tensor tympani muscle is innervated by the mandibular branch of the trigeminal nerve, serving both motor and proprioceptive functions (Candiollo, 1965).

The medial wall of the tympanic cavity forms the lateral wall of the inner ear. Characteristic features of the medial wall are the promontory (the lateral projection of the basal turn of the cochlea), the fenestra vestibuli or the oval window (occupied by the foot-plate of the stapes), the fenestra cochleae or the round window (closed by the secondary tympanic membrane), and the prominence of the facial nerve canal.

The lateral wall is formed mainly by the tympanic membrane, but also partly by the ring of bone in which the membrane is inserted.

The major taut portion of the tympanic membrane, the pars tensa, is thin and semi-transparent, and forms an angle of about 55° with the floor of the external auditory canal. The tympanic membrane is funnel-shaped. Thus, its centre, umbo, projects medially because of traction from the handle or the malleus which is firmly attached to the inner surface. The greater part of the circumference of the tympanic membrane

is thickened, forming the fibrocartilaginous ring, and is attached to the tympanic sulcus. This sulcus is deficient superiorly where it forms a notch, incisura Rivini. From the sides of the notch the anterior and posterior malleolar folds pass to the lateral process of the malleus. The small triangular portion of the membrane situated above these folds is lax, and is named the pars flaccida.

In the posterior wall of the tympanic cavity lies the entrance to the mastoid antrum as well as the pyramidal eminence housing the stapedial muscle. The roof of the tympanic cavity, the tegmen tympani, separates the cavity from the middle cranial fossa. The floor separates the cavity from the superior bulb of the internal jugular vein.

The ossicular chain is composed of the malleus, the incus and the stapes. As mentioned earlier the handle and the lateral process of the malleus are attached to the inner surface of the tympanic membrane. The malleolar head articulates posteriorly with the body of the incus. The long process of the incus articulates with the head of the stapes, the crura of which lead to the footplate, which in turn is attached to the oval window.

The epitympanum is the prism-shaped space in which the main portions of the malleus and incus are located. The epitympanum is about 6-8 mm in wide, and 5-6 mm in height (Kobrak, 1959). The epitympanum communicates with the mesotympanum through two small openings, the isthmus tympani anticus et posticus (see Embryology chapter). The small and specific mucosal cul-de-sac, situated medial to the pars flaccida and lateral to the neck of the malleus, is called Prussak's space (Anson & Donaldson, 1973). Posteriorly the epitympanum merges into the tympanic antrum through aditus ad antrum.

Tympanic Antrum and Mastoid Cells

The tympanic antrum, or antrum mastoideum, is located mainly posterior to the tympanic cavity, and communicates with the mastoid cells (cellulae mastoideae) within the mastoid process of the temporal bone.

Middle Ear Mucosa

In the second half of the 19th century the first reports on the nature
of the lining of the middle ear appeared. For almost a century there
was great controversy concerning the structure and origin of the middle
ear mucosa. The new and better histological techniques that have become
available during recent decades have contributed answers to some of the
questions raised. It is now generally agreed upon that the mucosal lin-
ing of the middle ear is continuous with the respiratory mucosa of the
nasopharynx. Thus, the respiratory mucosa extends through the Eusta-
chian tube into the tympanic cavity, which it lines completely. Poste-
riorly the mucous membrane lines the tympanic antrum and mastoid cells.
However, the epithelial layer varies throughout the middle ear cavity.
Thus, ciliated and secretory cells are confined to certain tracts - in
the walls of the Eustachian tube (Buch & Jorgensen, 1964; Sadé, 1966;
Lim, 1974; Tos & Bak-Pedersen, 1976), and anterior and inferior to the
promontory, extending into the epitympanum and hypotympanum, respec-
tively (Sadé, 1966; Lim & Shimada, 1971; Shimada & Lim, 1972; Lim et
al., 1973; Tos & Bak-Pedersen, 1976; Akaan-Penttilää, 1980). The re-
maining parts of the middle ear are lined by a squamous/cuboidal non-
ciliated epithelium.

The subepithelial loose connective tissue with its blood vessels,
nerves, and various connective tissue cells appears to undergo dynamic
changes under different physiological conditions. This subepithelial
tissue has been suggested to be involved in the pathogenesis of certain
middle ear diseases, such as otitis media (Paparella et al., personal
communication).

The tympanic membrane, which is suspended in air between the tympanic
cavity and the external auditory canal, consists of three layers - an
outer epidermal, an intermediate collagenous connective tissue layer,
and an inner lining of simple squamous epithelium. In the pars flaccida
the fibrous layer is replaced by loose connective tissue containing
both collagen and elastic fibers (Lim, 1970). In this connective tissue
stroma, mast cells are abundant (Widemar et al., 1984).

EMBRYOLOGY OF THE HUMAN MIDDLE EAR

The epithelial lining of the middle ear derives from the entoderm of the first pharyngeal pouch. As this pouch enlarges, four primary sacs bud off (Hammar, 1902). The entoderm of these sacs envelops each of the ossicles, in a manner similar to the peritoneal enclosure of the intestine. Where the sacs adhere to each other, mucosal folds are formed. Mesodermal remnants, including important vessels to the ossicles, are located in between the epithelial layers of these folds (Hamberger, Marcusson & Wersäll, 1963). The ossicles with the adjacent folds divide the tympanic cavity into small communicating compartments. The epitympanum becomes almost completely separated from the mesotympanum, except for two small openings, isthmus tympani anticus et posticus (Proctor, 1964). The entoderm of the first pharyngeal pouch continues to advance during infancy and childhood, and gradually forms the antral and mastoid air cells (Anson & Donaldson, 1973). The tensor tympani and stapedial muscles are derivatives of the mesoderm of the first and second pharyngeal arch, respectively.

The fetal stapedial artery originates from the internal carotid artery and penetrates the floor of the tympanic cavity (Altmann, 1947; Kelemen, 1958). It continues superiorly in the wall of the promontory. It then leaves the promontory and proceeds upwards between the crura of the stapes. It enters the facial canal which it leaves after a short distance, to proceed in the middle cranial fossa. Here the artery ends in three branches which accompany those of the trigeminal nerve. An anastomosing branch develops between the stapedial branches and the external carotid artery. From the third fetal month the main stapedial artery undergoes atrophy (Broman, 1899). Ultimately, all three remaining branches of the original stapedial artery (the medial meningeal, the infraorbital and the inferior alveolar artery) are supplied by the external carotid artery. In adults, none of the arteries in the region of the stapes can be definitely traced to the embryological stapedial artery (Nager & Nager, 1953). However, in some individuals the stapedial artery persists throughout life (Altmann, 1947; Kelemen, 1958; de Pinies, 1964).

ANATOMY OF THE RAT MIDDLE EAR

In order to facilitate a correlation between the anatomical features of the rat middle ear and those of man, the following positional words are used: anterior - posterior (man), indicating the direction from nose to tail (rat); and superior - inferior (man), which means the direction from the dorsal to the abdominal side (rat). When naming the structures of the skull and the middle ear of the rat, the terminology of Hebel & Stromberg (1976) and of Hellström et al (1982a), respectively, is used.

As in man, the rat middle ear is embedded in the temporal bone, which consists of the squamosal, petrosal, tympanic, and mastoid bones (Hebel & Stromberg, 1976).

Eustachian Tube

The rat Eustachian tube measures about 4.3 mm in length, and forms an angle with the horizontal plane of about 15° (Daniel III et al., 1982). A description of the nasopharyngeal part of the tube is hitherto lacking in the literature (see Paper I). The tympanal part of the tube is supported by bone and cartilage (van der Beek, 1981). The tympanal orifice of the tube is on the anterior portion of the medial wall of the tympanic cavity. Of the paratubal muscles, only the salpingopharyngeal has been described earlier. It originates from both the superior and inferior parts of the tube (House, 1953). Textbooks on the anatomy of the rat present very little data on the Eustachian tube (Hebel & Stromberg, 1976), if it is mentioned at all (Greene, 1963).

Tympanic cavity

The tympanic cavity can be divided into the epitympanum, mesotympanum, and hypotympanum (Hellström et al., 1982a). The greater part of the wall of the hypotympanum is made up of the tympanic bulla, which in the rat is an inferior protrusion of the typanic bone (Hebel & Stromberg, 1976). The following description of the tympanic cavity is mainly that of Hellström et al. (1982a). The superior part of the mesotympanum is partly separated from the epitympanum by the anterior and posterior tympanic spines. The major portion of the lateral wall of the tympanic

cavity consists of the tympanic membrane, which has quite a large pars
flaccida. Anterior to the promontory, which forms a major protrusion on
the medial wall, lies the opening of the Eustachian tube. Above this
opening there is a large shallow depression, the nasal fossa. Posterior
to the promontory the round and oval window niches are located. Ante-
rior to the round window niche, and through the crura of the stapes,
the stapedial artery passes along the medial wall. The major portions
of the ossicles are situated in the epitympanum, and together with ad-
jacent mucosal folds they divide the epitympanum into a medial and la-
teral compartment. Both compartments communicate inferiorly with the
mesotympanum, the lateral compartment through a narrow opening, the
tympanic isthmus. The rat middle ear lacks mastoid air cells.

Middle Ear Mucosa

According to van der Beek (1981), the rat Eustachian tube is lined with
a pseudostratified epithelium with ciliated and secretory cells, except
for a segment (roughly halfway between the openings) which is lined by
a simple non-ciliated epithelium. The ciliated and secretory cells of
the tube extend - on the medial wall of the tympanic cavity - as two
tracts, one anterior to, and the other inferior and posterior to the
promontory (Maeda et al., 1976; Tos, 1981; van der Beek, 1981). The re-
maining mucosa is lined by a single squamous epithelium. In contrast to
these reports, Daniel III et al.(1982) recently claimed that the tympa-
nal part of the tube as well as the tympanic cavity is lined with a
stratified, non-ciliated, squamous epithelium.

PHYSIOLOGICAL ASPECTS OF EUSTACHIAN TUBE IN MAN AND ANIMAL

There is general agreement that Eustachian tube dysfunction contributes
to the development and the course of middle ear disease, e.g. otitis
media with effusion (Miller, 1965; Holborow, 1975; Cantekin, Bluestone,
Saez & Bern, 1979; Sadé, 1979; Holmqvist & Olén, 1980; Magnuson, 1981;
Falk, 1982).

The functions normally attributed to the Eustachian tube are: (1) ven-
tilation of the middle ear; (2) drainage of the middle ear; and (3)
protection of the tympanic cavity from sound, nasopharyngeal pressure

19

variations and pathogenic agents (for references see Holmqvist & Olén, 1980; van Cauwenberge, 1982; Bylander, 1983). However, it is still not clear how the tube serves these functions.

Bartholomeo Eustachio, in the sixteenth century, declared that, in the normal state, the tube is open (quoted from Rich, 1920). This was generally accepted, until Toynbee (1853) observed in his classical investigation that, normally, the tube is closed and that it opens during the act of swallowing.

In studies on man and dog, Rich (1920) found that the nasopharyngeal orifice is normally closed and that it never opens independent of the swallowing, yawning or sneezing reflexes. The tubes exhibited no independent reflex dilations either periodically or irregularly. They were not opened by respiratory movements, either quiet or forced, and they were unaffected by mouth-breathing or by simple elevation of the soft palate. The mere existence of a disturbed pressure equilibrium will not bring about openening of the tubes, either independent or reflexwise by swallowing. At present it is commonly accepted that the Eustachian tube is normally closed (Cantekin, Bluestone, Saez & Bern, 1979; Honjo, Okazaki & Kumazawa, 1979; Holmquist & Olén, 1980; Bylander, Ivarsson & Tjernström, 1981; van Cauwenberge, 1982). If one accepts that the tube is normally closed in some part(s) and that it opens during deglutition, the question arises: What part(s) of the tube is (are) normally closed, and what mechanisms lie behind its opening and closing?

As the osseous part of the tube is a rigid canal, the cartilaginous portion including the nasopharyngeal orifice must be involved in the regulatory mechanisms (Aschan, 1955).

From nasopharyngoscopic observations it has been stated that the nasopharyngeal opening normally is closed (Bryant, 1907; Rich, 1920; Simkins, 1943; Graves & Edwards, 1944; Honjo et al., 1984). Regarding the cartilaginous tube, Proctor (1973) considered it to be closed throughout its entire length, whereas Bryant (1907) found the tympanal part of the cartilaginous tube normally to be open. Recently, Honjo, Okazaki & Kumazawa (1980) studied the opening process during swallowing. In a cineradiography study where contrast medium was injected into

human tubes via perforated ear drums, these authors found that the tube near the nasopharyngeal opening remained open at all times. However, during swallowing, this part increased its diameter. The remaining part of the cartilaginous tube was closed between the deglutitions. It is obvious that there is little agreement in the literature as to which part(s) of the tube is (are) normally closed.

Muscles are involved in the opening of the tube (Toynbee, 1853; Bryant, 1907; Perlman, 1939; Rich, 1920; Graves & Edwards, 1944; Proctor, 1973; Cantekin et al., 1979; Sadé, 1979; Honjo et al., 1980). In contrast, the closing of the tube is considered to be a passive process, aided by the elasticity of the cartilage (Yule, 1873; Bryant, 1907; Zöllner, 1937; McMyn, 1940; Aschan, 1955; Holborow, 1975; Seif & Dellon, 1978).

As shown in Table I, the tensor veli palatini muscle is generally considered to be the primary dilator of the tube. In 1920, Rich designed the classical experiment in which he studied movements of the nasopharyngeal opening in dog during stimulation of the paratubal muscles. The following muscles were stimulated: the internal pterygoid, the palatopharyngeal, the salpingopharyngeal, the pterygopharyngeal, the superior constrictor, the levator veli palatini, and the tensor veli palatini. Rich noted that the only muscle that affected the nasopharyngeal opening was the tensor veli palatini which opened the orifice. He then selectively transected each muscle and observed tubal opening during swallowing. The orifice was temporarily opened during each swallowing sequence until the tensor veli palatini muscle was severed, after which the tube no longer opened. However, Honjo et al., in a more recent clinical experiment (1980), found that the nasopharyngeal orifice is dilated by the levator veli palatini muscle, whereas the remaining part of the cartilaginous tube is opened by the tensor veli palatini muscle. Whether or not the salpingopharyngeal muscle is involved in the opening mechanisms is still not known (Table I). With respect to the tensor tympani muscle, which is active during vocalization and swallowing, for example (Djupesland, 1965; Salén & Zakrisson, 1978), this muscle has no opening effect on the tube (Honjo et al., 1983).

Some authors (Bryant, 1907; Proctor, 1973) have suggested that the tube is opened by some form of rotation or hingeing. However, according to Rood & Doyle (1982) this is not likely to be the case, since the carti-

TABLE I
THE PARATUBAL MUSCLES AND THEIR EFFECTS ON THE EUSTACHIAN TUBE

AUTHOR(S), YEAR, SPECIES	TTM	TVPM	LVPM	SPM
Valsalva, 1707, man		O	N?	
Toynbee, 1853, man		O	O	
Von Tröltsch, 1864, man		O	C	
Cleland, 1868, man		C	C	N
Yule, 1873, man		N	O?	O
Politzer, 1901, man – adult		O	O	
Bryant, 1907, man		N?	O	C
Rich, 1920, dog, man		O	N	N
Zöllner, 1937, man		O	O	O
McMyn, 1940, man		O	O	O
Simkins, 1943, man		C	O	C
Graves & Edwards, 1944, man – infant, adult	N	O	N	N
Aschan, 1955, man – adult		O	O	N
Ingelstedt & Jonson, 1967, man	O			
Proctor, 1973, man	N	O	O	O
Schuknecht, 1974, man		O	O	O
Dickson, 1975, man – adult	O	O		
Holborow, 1975, man – infant, child	O	O	O	N?
Rood & Doyle, 1978, man – fetal, adult	O			
Seif & Dellon, 1978, man – fetal, adult		O	O	
Honjo et al., 1979, dog		O	N	
Cantekin et al., 1979, monkey		O	C?	
Honjo et al., 1980, monkey, man – adult		O	O	
Guindi & Charia, 1980, man				C
Honjo et al., 1981, cat, dog, monkey		O	N	
Rood & Doyle, 1982, man – adult		O	O	
Kumazawa, 1982, man		O		
Honjo et al., 1983, cat	N			
Cantekin et al., 1983, monkey – juvenile		O	N	

TTM – Tensor tympani muscle   O – Opening function
TVPM – Tensor veli palatini muscle   O – Major opener
LVPM – Levator veli palatini muscle   C – Closing function
SPM – Salpingopharyngeal muscle   N – No effect

Question marks indicate uncertainty in interpretation of findings presented.

lage is firmly attached to the bony tube and to the sulcus of the skull base. This view gains further support from the finding that the tubal cartilage is not segmented.

CLEARANCE OF MIDDLE EAR IN MAN AND ANIMAL

The amount of middle ear fluid/mucous normally produced has not been established. In certain middle ear diseases, e.g. otitis media with effusion, an accumulation of fluid occurs. This must be due to an increased production of effusion material and/or a decreased clearance.

In the case of an increased production of fluid/mucous, in otitis media with effusion, both an exudation from blood vessels (Sadé, 1966) and an increased secretory activity of the mucosa (Tos, 1980) have been implicated.

In the process of clearanc of fluid from the middle ear, the effusion material could be removed by transport through the Eustachian tube (Holmgren, 1934; Terracol et al., 1949; Compere, 1958; Rogers et al., 1962; Sadé, 1967; Honjo et al., 1981; Nozoe et al., 1984), or by absorption through the mucosa (Ross & Rawson, 1935; Bortnick & Proud, 1965; Lim, 1974). The importance of one over the other elimination mechanism remains to be established.

The clearance of fluid through the Eustachian tube may be facilitated by a mucociliary system (Sadé, 1967; Lim, 1974), a pump-like function of the tube (Honjo et al., 1981), and/or hydrostatic forces (Duncan, 1960; Beauregard, 1971).

A mucociliary system of the meso- and hypotympanum is designed to work against gravity as the tympanal tubal orifice is situated well above the floor of the tympanic cavity. However, several factors affect the transport capacity of the mucociliary system. Such factors are the viscosity and the surface tension of the fluid (Sadé, 1967; Spungin & Silberberg, 1984).

From animal studies, Honjo et al. (1981) concluded that the tensor veli palatini muscle was able to pump fluid through the tube into the

23

pharynx. However, this clearance mechanism requires a satisfactory ven-
tilation of the tympanum (Nozoe et al., 1984).

Studies in the rat indicate that the production of fluid - in experi-
mentally induced otitis media with effusion - starts in the epitympanum
(Stenfors et al., 1981; Hellström et al., 1982b). Thus, in the rat,
clearance of the epitympanum is as important as clearance from meso-
and hypotympanum through the Eustachian tube.

VASCULARIZATION OF TYMPANIC MEMBRANE IN MAN AND RAT

The arteries of the human tympanic membrane are derived from branches
of the maxillary artery. Vessels from the deep auricular branch are lo-
cated beneath the epidermal layer, whereas the vessels of the tympanic
branch are distributed beneath the inner layer (for references see Ber-
endes, Link & Zöllner, 1979).

Under normal conditions the pars tensa of the tympanic membrane appears
to be only lightly vascularized, as a myringotomy can be performed
without causing any visible bleeding. Moreover, the major portion of
the rat tympanic membrane (pars tensa) is only about 4-5 µm thick,
which is less than the diameter of an ordinary capillary (5.0-10.0 µm).
Van der Beek (1981) described the presence of vessels solely in close
vicinity of the fibrocartilaginous annulus and along the handle of the
malleus. Thus, it would seem that a normal rat pars tensa is nourished
in some anomalous way.

MATERIALS AND METHODS

ANIMALS (I-V)

Healthy male Sprague Dawley rats (ALAB, Sweden) were used. They weighed
about 230-300 g, were maintained under normal laboratory conditions
with free access to water and were fed a standard pellet diet (Astra-
Ewos, Sweden).

VARIOUS EXPERIMENTAL PROCEDURES (III, IV, V)

For the dye-tracing study (III) the rats were anesthetized with pento-barbital (Mebumal[R]; ACO, Sweden) intraperitoneally. Under an operating microscope (Zeiss OpMi) the pars flaccida was perforated bilaterally with a myringotomy lancet. Through the perforation, rat serum (DAKO Labs., Denmark) - containing 0.01% Evans blue - was instilled until it filled the lateral epitympanic compartment. The rats were then kept either on their stomach, on their back, or tilted at an angle of roughly 45°, head up. After 15, 30 and 60 min a hole was made in the lateral wall of the tympanic bulla, through which the clearance of the serum from the epitympanum was studied and photographically registered.

In order to study the vascular supply of the tympanic membrane (IV), the ear drums of anesthetized animals were subjected to gentle repeated pressure from the outside on the handle of the malleus. This mechanical stimulation caused a marked dilation of the vessels. After 10 min the animals were decapitated, and the lateral wall of the tympanic cavity was dissected free and fixed by immersion in glutaraldehyde. The specimens were subjected to the benzidine technique (see below), and studied under a light microscope. Thereafter they were decalcified, embedded in Epon[R], and sectioned for further light microscopy studies.

To study the stapedial artery under normal conditions as well as in otitis media with effusion (V), rats were anesthetized by an intra-venous injection of sodium hexabarbital (Brietal[R]) through a tail vein. Under aseptic conditions the right tympanic bulla was opened and a po-lyethylene plug was placed in the tympanic orifice of the Eustachian tube. This procedure induced otitis media with effusion. At various in-tervals, ranging from 2 days to 2 weeks, the animals were reanesth-etized and killed by perfusion with glutaraldehyde. The stapedial arteries with adjacent mucosa were then dissected free and prepared for light and transmission electron microscopy.

Direct Inspection(I, III, IV)

To study the anatomy of the Eustachian tube (I) the tympanic bulla and the Eustachian tube of sacrificed animals were carefully dissected and photographed under an operating microscope (Zeiss OpMi). The soft tissue of some heads was removed after boiling in water, and the osseous structures were then studied and photographed.

In otheranesthetized animals the vascular supply of the tympanic membrane was studied under an operating microscope before and after vital staining with indian ink or Evans blue (IV).

Vascular Casts (IV)

Under pentobarbital anesthesia the circulatory system was flushed with 60 ml saline through the left heart ventricle. This procedure was followed by injection of an acrylic casting material (Mercox CL-2C, Ladd Research Industries Inc., USA). The following day the head and neck of the animals were dissected free and digested in 40% KOH at room temperature for 2 days. The vascular casts were subsequently rinsed in water, air-dried, and photographed under a stereomicroscope.

Benzidine Technique (IV)

Anesthetized animals were decapitated, and the lateral wall of the middle ear was dissected free and fixed overnight by immersion in glutaraldehyde. To study the vessels of the tympanic membrane, a benzidine solution was dropped onto the surfaces of the lateral wall. After 3-5 min, when the erythrocytes were stained, the specimens were transferred to ethanol, and studied and photographed under a stereomicroscope.

In Situ Staining and Whole-Mount Preparation (II)

In order to study the distribution of mast cells, the tympanic mucosa of sacrificed rats was fixed by instillation of formalin (40 formaldehyde) in ethanol (1:9) into the middle ear cavity. The middle ear was

divided into a lateral and a medial half, and the mucosa of both halves was stained with 0.5% toluidine blue (in 0.5% HCl). The specimens were analyzed under a stereomicroscope with respect to number and distribution of mast cells. Pieces of mucosa were also dissected free and examined and photographed as whole-mount preparations in a light microscope.

Decalcified Specimens (I, II, IV)

For the vascular study (IV), perfusion-fixed and decalcified animal heads were used. The lateral wall of the middle ear was dissected free, post-fixed in osmium tetroxide, and embedded in Epon[R]. These specimens were serially sectioned at 75-μm intervals, and the sections were stained with toluidine blue. For studies on the Eustachian tube (I) and the tympanic mucosa (II) the heads were embedded in Paraplast[R] (Kebo-Lab, Sweden). These specimens were serially sectioned at 100 μm intervals, and the sections were stained with hematoxylin-eosin, toluidine blue and periodic acid Schiff (PAS).

In a separate set of experiments in the mucosal study (II), animals were fixed by perfusion with glutaraldehyde through the left heart ventricle, followed by a rinse with saline. After clamping off the thoracic aorta, New Decalc[R] was perfused for 3 hours followed by a new saline rinse. The head was now soft and decalcifed, and could easily be divided with a scalpel into appropriate pieces. The specimens were processed for light microscopy.

Free Dissected Mucosal Specimens (II,III)

In perfusion-fixed but undecalcified skulls the middle ear mucosa of well defined areas was dissected free. The specimens were processed according to standard dehydration and embedding (Epon 812[R]) techniques. Semithin sections were cut and stained with toluidine blue (II,III) or with PAS (II).

ELECTRON MICROSCOPY

Scanning Electron Microscopy (II, III)

Temporal bones were divided, fixed by immersion in glutaraldehyde, and dried by critical point drying. The dried specimens were coated under continuous rotation and tilting with an approximately 20-nm thick layer of gold. The specimens were examined and photographed in a scanning electron microscope. For details, see the individual papers (II,III).

Some specimens studied in the scanning electron microscope were decalcified and embedded in Paraplast[R]. These specimens were then serially sectioned at 100-µm intervals. After staining with hematoxylin-eosin, toluidine blue or PAS, the sections were photographed in a light microscope.

Transmission Electron Microscopy (II, V)

Plastic-embedded specimens (see above) were cut in ultrathin sections in an ultramicrotome. The sections were contrasted with uranyl acetate and lead citrate, and then examined in a transmission electron microscope.

RADIOGRAPHY (IV)

Under pentobarbital anesthesia a polyethylene catheter was placed (1) immediately caudal to the carotid bifurcation, (2) in the external carotid artery, and (3) in the internal carotid artery. Serial angiography was performed in both ventrodorsal and lateral projection. Magnification microangiography was performed after injection of a contrast medium, and the X-ray plates were subjected to a photographic subtraction technique.

RESULTS

ANATOMY OF EUSTACHIAN TUBE IN RAT (I)

The Eustachian tube extends in a posterior, lateral and superior direc-
tion, from the nasopharynx to the tympanic cavity. It measures about
4.5 mm in length. The tube is angled at about 30º in relation to the
sagittal plane and about 10º to the horizontal plane. Its pharyngeal
one-third is soft and tractable, and forms the nasopharyngeal opening.
The tympanal two-thirds of the tube are supported by a cartilage and
are partly surrounded by bone.

Nasopharyngeal Orifice

The nasopharyngeal opening is located in the lateral wall of the naso-
pharynx at about half the distance between the posterior border of the
hard palate and the entrance of the larynx. The orifice consists of two
lip-like mucosa-covered swellings which form a groove, running in the
anteroposterior direction. In the sagittal plane the opening is framed
by bony structures - superiorly by the basisphenoid bone, anteriorly by
the medial pterygoid process, and inferiorly by the pterygoid hamulus.
Upon opening of the orifice, the entrance becomes funnel-shaped, with
its apex directed posterolaterally.

Cartilaginous and Bony Portion

The portion of the Eustachian tube running from the apex of the funnel-
shaped entrance to the tympanal opening is supported by a cartilage and
framed by bone. The cartilage is narrow at the apex, and forms the roof
of the mucosal tube. During its passage through the bony surroundings,
the cartilage grows in width and extension. Thus, at the tympanal open-
ing the cartilage, in cross-section shaped as a shepherd's crook, al-
most completely surrounds the mucosal tube. The cartilage is firmly
attached to the bony framing, which consists of the basisphenoid and
the tympanic bone.

Tympanal Orifice

The tympanal orifice of the tube is located in the anterior part of the tympanic medial wall, and at a distance above the floor of the bulla. The opening is partly surrounded by the tubal cartilage (medially, superiorly, and laterosuperiorly), and is completely framed by the tympanic bone. The floor of the tube passes over into the sulcus promontorialis occipitalis of the tympanic cavity, whereas the roof of the opening ends abruptly.

Paratubal Muscles

The tensor veli palatini muscle is thin and flat, and originates from (1) the medial pterygoid process (the lateral surface of its superior half), (2) the deep portion of the inferior lip of the nasopharyngeal tubal orifice, (3) the inferolateral membranous wall (part of tube not surrounded by cartilage), (4) the superolateral part of the tubal bone and cartilage, and (5) the dura covering the petrotympanic fissure. The muscle runs in an inferior, medial, and anterior direction over the crest of the medial pterygoid process where it inserts into the soft palate (into the superior layer of its posterior half).

The levator veli palatini muscle arises from (1) the inferior lip of the nasopharyngeal tubal orifice, (2) the pterygoid hamulus, and (3) the floor of the cartilaginous tube (its most pharyngeal part). The muscle runs inferiorly and medially, passes medial to the pterygoid hamulus, and inserts into the posterior part of the soft palate.

The salpingopharyngeal muscle originates from (1) the superior lip of the nasopharyngeal opening, and (2) the superomedial side of the cartilaginous tube. The muscle runs posteriorly and blends with the muscle fibers of the pharyngeal wall.

The tensor tympani muscle originates from (1) its entire bony canal, (2) the tubal cartilage in the roof of the tympanal orifice, and (3) the membrane covering the petrotympanic fissure. Thus, the muscle partly communicates, via this membrane, with fibers of the tensor veli palatini muscle. The tensor tympani muscle runs posteriorly in a deep

groove, located anterosuperiorly to the promontory. The muscle ends in a short round tendon which turns laterally at almost right angles, and inserts into the neck of the malleus.

MUCOSA OF THE MIDDLE EAR (I, II, III)

Middle Ear Epithelium (I,II,III)

The Eustachian tube is lined by a pseudostratified epithelium composed of ciliated cells, secretory cells, non-secretory/non-ciliated cells, and basal cells (I). The ciliated and columnar secretory cells are located chiefly in the inferomedial wall of the tube, and are more frequent in the pharyngeal than in the tympanal portion of the tube. In other areas the surface cells generally lack cilia and are considerably shorter than the tall, ciliated cells.

The tall ciliated and secretory cells of the Eustachian tube extend from the tubal opening as an anterior and an inferior tract along the medial wall of the tympanic cavity (II,III). The anterior tract extends superiorly into the nasal fossa, anterior to the promontory, whereas the inferior one extends posteriorly, inferior to the promontory, and then superiorly, posterior to the round window niche. The tracts arch via the tympanic spines over to the lateral wall where they end on the upper part of the pars tensa of the tympanic membrane. The anterior tract ends on the anterior part of the membrane and appears to be connected with the medial epitympanic compartment. The inferior tract, on the other hand, ends on the posterior part and appears to be connected - via the tympanic isthmus - to the lateral epitympanic compartment.

The epithelial lining of the tympanal opening of the tube is characterized by densely packed ciliated cells interspersed with protruding granulated secretory cells (II,III). These types of cells diminish in number along the tracts towards the epitympanum, and are replaced by non-ciliated cells that contain secretory granules. Along the borders of the tracts the epithelium changes rather abruptly into a simple squamous/cuboidal non-ciliated type.

The epithelium of the tympanic cavity outside the tracts described above is of a simple and squamous/cuboidal type. In the scanning electron microscope these cells are polygonal in shape, and their borders are clearly distinguished due to an increased occurrence of microvilli at the periphery of the cells. Many of these cells contain a small number of secretory granules.

The numerous secretory cells encountered in the middle ear epithelium may be classified according to the microscopic appearance of their secretory granules. The following types are recognized: (1) pale granules, (2) pale granules exhibiting a dark core, (3) dark granules, and (4) a mixed population of these different granule types. Pale granulated cells predominate within the tubal opening and are in all probability goblet cells. Those exhibiting dark-cored granules are common in the tracts. Cells with dark granules are frequent in areas of squamous/cuboidal epithelium in which areas the granules are minute and sparse. Microvilli are a common feature of all the secretory cells encountered as well as of the ciliated cells. Non-ciliated cells completely devoid of secretory granules are very rare.

Subepithelial Tissue (I, II)

The subepithelial layer of the Eustachian tube (I) consists of a loose connective tissue containing numerous vessels, nerves, and glands. Some fibers belonging to the paratubal muscles (with the exception of the tensor tympani muscle) intermingle between the glands. The latter are of both serous and mucous type. The glands are located in the lips of the nasopharyngeal opening, and in the inferomedial wall of the cartilaginous and bony portion of the tube.

The subepithelial loose connective tissue of the tympanic cavity in general varies greatly in thickness. There is no obvious correlation between the thickness of this layer and the height of the epithelium. The tympanic subepithelial layer contains both vessels and nerves, but glands have not been found.

Mast cells are a fairly common component of the subepithelial connective tissue of the middle ear (I,II). In the tubotympanic cavity they

are confined mainly to areas covered by ciliated epithelium, and to the floor of the tympanic bulla. These mast cells are observed mainly in areas that exhibit an extensive network of blood vessels. In addition, mast cells are found in the pars flaccida of the tympanic membrane, and along the manubrial vessels of the pars tensa.

MIDDLE EAR CLEARANCE (III)

A stained rat serum (Evans blue added) - resembling serous effusion material - was instilled into the lateral epitympanic compartment through a perforation in the pars flaccida. The fluid moved slowly towards the tubal orifice. It did not follow the tracts of ciliated epithelium, but, guided by the middle ear structures, it was transported by the forces of gravity. When the animal was kept in a supine position, no material was seen to leave the epitympanum. It is assumed that hydrostatic forces are important as far as middle ear clearance of a low-viscous fluid is concerned.

VASCULAR SUPPLY OF TYMPANIC MEMBRANE (IV)

Branches from the external carotid artery in the external auditory meatus extend to the pars flaccida, the manubrial region of the pars tensa, and the junction between the fibrocartilaginous ring and the tympanic bone. These ramifications within both pars tensa and pars flaccida are located immediately beneath the outer epidermal layer, and are closely associated with nerves and mast cells. The periphery of the pars tensa is supplied by minute blood vessels which originate from vessels of the tympanic cavity and which run just beneath the tympanal epithelium. These arterial branches of the tympanic cavity also seem to emanate from the external carotid artery. The major portion of the pars tensa, _viz._ that located between the manubrial part and the periphery, appears to be devoid of blood vessels.

After mechanical stimulation of the ear drum, the vessels of the tympanic membrane dilate. Thus, vessels normally detectable only in microscopic sections become clearly visible under the operating microscope. In spite of this, no vessels are to be noted in the major portion of the pars tensa mentioned above.

The structural attachment of the fibrocartilaginous ring is quite complex. The network of vessels, present between the fibrocartilaginous ring and the surrounding bone, appears to be connected mainly to meatal vessels, but also to tympanal ones.

THE STAPEDIAL ARTERY (V)

The stapedial artery is a branch of the internal carotid artery, and persists throughout life in the rat. The artery passes the middle ear along its medial wall, where it runs between the crura of the stapes. The lumen of the artery measures about 460 μm in diameter. The tunica intima of the wall consists of an endothelium, a very thin lamina propria intimae and a well developed internal elastic membrane. The tunica media is composed of 2-3 complete circular layers of smooth muscle cells. The tunica adventitia is about three times as thick as the media, and consists of collagen bundles oriented both longitudinally and tangentially. The surface of the artery facing the tympanic cavity is covered by the tympanic mucosa.

The stapedial artery exhibited no histological changes in the course of otitis media with effusion induced by blockage of the Eustachian tube.

DISCUSSION

THE EUSTACHIAN TUBE (I)

The rat Eustachian tube consists of a pharyngeal tractable portion, and
a tympanal cartilaginous portion, the latter partly surrounded by bone.
The pharyngeal portion forms the nasopharyngeal opening, which consists
of two lips arranged around a slit. This is also the case in man (Sim-
kins, 1943; Rood & Doyle, 1982). However, the opening slit is horizon-
tally oriented in the rat (I), whereas it runs vertically in man
(Bryant, 1907; Rich, 1920; Simkins, 1943; Graves & Edwards, 1944; Rood
& Doyle, 1982). When examined through an endoscope the nasopharyngeal
orifice in man is normally closed (Bryant, 1907; Rich, 1920; Simkins,
1943; Graves & Edwards, 1944; Honjo et al., 1984). In the rat, it has
not yet been possible to inspect the nasopharyngeal orifice without
cleaving the soft palate. When such an incision is made in an anesth-
etized animal the orifice is seen to be closed.

The tubal cartilage supports the rat tube, from its pharyngeal portion
to its opening in the tympanic cavity (I). In man there are somewhat
varying opinions as to the extent of a surrounding cartilaginous wall
into the osseous canal. Recently, Sadé et al. (1985) reported that car-
tilage supports the tube through the bony canal to its tympanal open-
ing. With respect to the tubal cartilage, it is not segmented either in
rat (I) or man (Rood & Doyle, 1982), and in both species it is firmly
attached to the skull base.

Graves & Edwards (1944) defined the tubal isthmus as the site where
bone completely surrounds the tube. Applying this definition to the
rat, the isthmus would correspond to the tympanal orifice, the widest
part of the tube. The term isthmus implies a narrowing and the tubal
isthmus should thus be the narrowest part along its extent. However, it
has been shown in man that the diameter of the tubal isthmus is only
slightly smaller than that of the tympanal opening (Graves & Edwards,
1944; Proctor, 1973).

The anatomy of the paratubal muscles of the rat shows several similari-
ties to that described for man. Thus, in man the tensor veli palatini

muscle is considered to be the primary dilator of the tube (Table I). This would also seem to be the case in the rat, as the tensor veli pa- latini muscle originates from the entire cartilaginous portion, and it may thus work as the main or sole dilator of this portion of the tube. With respect to the nasopharyngeal opening in man, it is not dilated by the tensor veli palatini muscle but by the levator muscle (Honjo et al. 1980), whereas in the rat this function appears to be carried out by the salpingopharyngeal muscle which retracts the superior lip. The levator veli palatini and the tensor veli palatini muscle appear to further dilate the nasopharyngeal lumen which is already opened by the salpingopharyngeal muscle. The importance of the palatal muscles for normal rat tubal function is obvious from experiments in which split- ting of the soft palate provoked otitis media with effusion (Hellström et al., 1983), probably by interfering with the opening mechanisms dis- cussed above. It is not clear if the salpingopharyngeal muscle in man acts in any way on the tubal wall (Table I). The tensor tympani muscle does not seem to have any tubal effect either in man (Honjo et al., 1983), or in the rat (I).

THE TYMPANIC CAVITY (II)

The architectures of the tympanic cavity appear to be alike in man and rat. However, in the rat, as in other rodents, the hypotympanum pro- trudes inferiorly, forming the tympanic bulla, which does not exist in man. Furthermore, the tympanic cavity in man is connected posteriorly with the tympanic antrum and mastoid cells. This is not the case in the rat. It is not clear if these structural differences are of any impor- tance as far as middle ear functions are concerned.

In both species the epitympanum is divided into smaller compartments. There are certain differences in the anatomy of the epitympanum, as described in the rat (Hellström et al., 1982a), and in man (Proctor, 1964). In man, however, the epitympanic compartment lateral to the os- sicles, including Prussak's space (medial to the pars flaccida), occa- sionally communicates inferiorly with the mesotympanum (Proctor, 1964). In these cases the anatomy of the epitympanum and its compartments shows a greater similarity between the two species.

THE MIDDLE EAR MUCOSA (I, II, III)

The middle ear epithelium of the rat consists of areas in which ciliated cells intermingle with secretory cells. These areas are confined to the floor and the medial wall of the Eustachian tube (I), and to the tracts along the medial wall of the tympanic cavity (II, III). One tract, anterior to the promontory, appears to be connected with the medial epitympanic compartment, whereas the other, the inferior one, communicates with the lateral compartment. The epithelium outside these tracts is non-ciliated. That these tracts appear to connect the epitympanum with the Eustachian tube has not been reported earlier. However, their course in the hypotympanum and parts of mesotympanum correlates fairly well with previous observations in the rat (Maeda et al., 1976; van der Beek, 1981; Tos, 1981). We did not observe ciliated and secretory columnar cells in the epitympanum as described by van der Beek (1981), nor could we confirm the findings by Daniel III et al.(1982) that the epithelium of the tympanic cavity consists solely of stratified squamous cells.

Tracts composed of ciliated and secretory cells, with a distribution roughly similar to that in the rat, appear to exist in man too (Sadé, 1966; Lim & Hussl, 1969; Shimada & Lim, 1972; Akaan-Penttilä, 1980).

The various epithelial cells present in the lining of the middle ear mucosa in man and animal undergo marked changes in pathological conditions. Thus, in otitis media, the squamous non-ciliated epithelium has been described as transforming into a ciliated and secretory epithelium (Paparella et al., 1970; Lim & Kleiner, 1971; Kuijpers et al., 1979; Zechner, 1980; Tos, 1981; van der Beek, 1981).

These observations emphasize the importance of improved knowledge concerning the distribution of various types of epithelial cells in the middle ear mucosa. If samples are taken at random, or from poorly defined areas, it may be difficult to ascertain whether the histological picture is normal or pathologic. Biopsies collected from carefully defined areas would also increase the possibility of comparing results from different research laboratories.

Several authors have attempted to correlate the structure of the secretory cells to various functional states. The varying appearance of the secretory granules within one and the same cell has been suggested to reflect various stages of granular maturation. Furthermore, due to similarities between different secretory cells, it has been suggested there is in fact only one kind of secretory cell in the middle ear mucosa which varies in appearance according to its functional activity (Hentzer, 1970b; Tos & Bak-Pedersen, 1976). The findings presented in Paper II could support this hypothesis, but conclusive evidence is still lacking.

Microvilli, present on all types of cells in the middle ear epithelium (II), must grossly increase the surface area of the cells and may thus be important for processes such as absorption or transepithelial transport. This is supported by a study by Lim (1974), who was able to show that all types of cells in the middle ear epithelium are capable of absorbing horseradish peroxidase macromolecules. It was also suggested that failure of the epithelium to resorb fluid may be a contributory factor in the pathogenesis of otitis media with effusion.

THE SUBEPITHELIAL TISSUE (I,II)

The subepithelial loose connective tissue of the middle ear is richly vascularized, carries an abundance of nerves, and contains the normal cellular constituents of this tissue, including mast cells.

Recently, middle ear mast cells with their capacity to release mediators of inflammation, e.g. histamine, have been considered to be involved in the pathogenesis of otitis media with effusion (Alm et al., 1982; Berger et al., 1984; Collins et al., 1984). Mast cells have been found in unusually large numbers in the pars flaccida of both rat (Alm et al., 1982), and man (Widemar et al., 1984). In several reports mast cells have been reported present in the subepithelial layer of the middle ear mucosa, without a closer definition of location (Hentzer, 1970a; Lim et al., 1973; van der Beek, 1981). In Paper II in the present thesis it is shown that the mast cells of the rat middle ear are confined to certain areas, namely those lined with a ciliated epithelium, and furthermore in the floor of the bulla, the pars flaccida,

and the manubrial region of the pars tensa. Further studies are necessary to establish the functional role of the middle ear mast cells, in both health and disease.

No glands whatsoever were observed in the subepithelial connective tissue of the rat tympanic cavity (II). These findings agree with those in other studies in the rat (Maeda et al., 1976; van der Beek, 1981; Tos, 1981; Daniel III et al., 1982). However, in the tympanic mucosa of adult humans, glands have been reported by several investigators (Sadé, 1966; Lim & Hussl, 1969; Hentzer, 1970a). It should be noted that in a large study on infants, Akaan-Penttilä (1980) did not observe any subepithelial glands. It has been suggested that glands observed in both the human and animal tympanic mucosa derive from invaginations in the mucosal epithelium under pathological conditions (Lim et al., 1973; Bak-Pedersen & Tos, 1973).

CLEARANCE OF FLUID FROM EPITYMPANUM (III)

In Paper III it was concluded that hydrostatic forces play an important role in clearing the epitympanum of a serum like fluid. It should be noted that the tracts of ciliated epithelium, which connect the epitympanum with the Eustachian tube, did not appear to transport this fluid, possibly because the instilled fluid had an unsuitable viscosity and/or surface tension, factors that are important for the optimal functioning of the mucociliary system (Sadé, 1967; Spungin & Silberberg, 1984). The fluid that reached the tympanal orifice of the tube accumulated in its close vicinity. The level of the fluid was lowered concomitant with swallowing. It would thus seem that hydrostatic forces play an important part in clearing the tympanic cavity, at least as far as serous secretions are concerned. A pumping function, as described by Honjo et al. (1981), may prevail in the Eustachian tube. It should be noted that several authors (Duncan, 1960; Rundcrantz, 1970; Beauregard, 1971) consider it of value to hold the head in a drainage position during otitis media with effusion.

VASCULAR SUPPLY TO TYMPANIC MEMBRANE (IV)

The vascular supply of the tympanic membrane of the rat (IV) is very similar to that in man (Berendes, Link & Zöllner, 1979). In vascularized areas of the ear drum the vessels, although barely visible to the naked eye or under the otomicroscope, are always detectable in histological sections. In large portions of the rat pars tensa, vessels were not detectable, either under the otomicroscope, or in histological sections. After mechanical stimulation of the external auditory canal in man (Lüscher, 1929) and of the ear drum in the rat (IV) the ear drum vessels dilated - even those earlier barely visible. The smallest vessels of the rat tympanic membrane are of capillary size in the light microscope. It is not known whether these vessels possess contractile properties as described for vessels in rabbit and frog mesenteries (Lübbers et al., 1979). It is tempting to speculate that at least part of the dilation may be due to release of vasoactive substances from abundant nerves and/or mast cells located close to the vessels (Alm et al., 1982; Alm et al., 1983; Widemar et al., 1985). Those areas of the pars tensa which apparently lack vessels are probably nourished by way of diffusion mechanisms.

The junction between the fibrocartilaginous ring and the tympanic sulcus is richly vascularized The attachment of the ring is medially firm and laterally loose. Such an arrangement could facilitate the compression or the dilation of the vessel network by tension variations in the tympanic membrane, e.g. brought about by the tensor tympani muscle.

It is interesting to note that the dilated vessels observed in acute otitis media (the vessels of the pars flaccida with adjacent skin, and the manubrial vessels) are derived from the meatal vessels. Those vessels mainly affected in otitis media with effusion (visible at the periphery of the pars tensa) originate in the tympanic cavity. Surprisingly enough, the two different disease entities appear to engage two different vascular beds. The reason for this remains obscure.

THE STAPEDIAL ARTERY (V)

A stapedial artery, which passes between the crura of the stapes, is
the normal finding in rodents (Bugge, 1970, 1974; Echeverria, 1960;
elemen, 1958). In man this artery normally undergoes atrophy during
the third fetal month (Broman, 1899), and its persistence throughout
life is a rare phenomenon (Altmann, 1947; Kelemen, 1958; de Pinies,
1964). The stapedial artery of the rat branches into the middle menin-
geal, infraorbital, and inferior alveolar arteries. This relatively
large artery has a thin wall, which over large areas is only covered by
the tympanic mucosa. It was somewhat surprising to find that, from a
histological point of view, the stapedial artery appeared completetly
normal in experimentally induced otitis media with effusion.

SUMMARY AND CONCLUSIONS

In the present thesis it has been shown that:

1.   The rat Eustachian tube consists of a nasopharyngeal, and a carti-
laginous and bony portion. The orifice of the nasopharyngeal portion is
formed by two soft tissue lips, which appear to open mainly by the ac-
tion of the salpingopharyngeal muscle, but also by the levator and ten-
sor veli palatini muscles. The cartilaginous portion appears to be
opened solely by the tensor veli palatini muscle. The tensor tympani
muscle seems to have no effect on the tube.

2.   A ciliated and secretory epithelium lines the inferomedial walls of
the tube throughout its length. In the tympanic cavity these epithelial
cell types extend as two tracts - one anterior and the other infero-
posterior to the promontory - which communicate with the epitympanic
compartments. Remaining parts of the middle ear are covered by a
squamous/cuboidal, non-ciliated epithelium. The subepithelial loose
connective tissue contains vessels, nerves, and connective tissue
cells, including mast cells. The mast cells are confined to areas cov-
ered by the ciliated epithelium, and in the floor of the bulla, in the
pars flaccida, and along the manubrial vessels. Glands are restricted
to the Eustachian tube.

3.   In the clearance/transport of serum-like material, from the epitym-
panum towards the tube, hydrostatic forces appear to be important.   The
tracts of ciliated epithelium, connecting the epitympanum with the Eus-
tachian tube, did not appear to transport this type of fluid.

4.   The tympanic membrane is vascularized from meatal and tympanal ves-
sels. Meatal vessels branch in the pars flaccida and along the handle
of the malleus, where they are located directly beneath the outer, ker-
atinizing, stratified, squamous epithelium. Furthermore, meatal vessels
form a vascular network at the junction between the fibrocartilaginous
ring and the tympanic sulcus. Tympanal vessels send branches to the
periphery of the pars tensa, where they run immediately beneath the
tympanal, simple, squamous epithelium. In the major portion of the pars
tensa, no blood vessels were found.

5. The rat stapedial artery is a thin-walled vessel with a wide lumen. Without branching it runs through the tympanic cavity to the extratympanal regions it supplies. In contrast to the corresponding artery in man, the rat stapedial artery persists throughout life. The artery does not seem to be affected by the fluid produced during experimentally induced otitis media with effusion.

6. The middle ear structure in the rat and in man show both similarities and differences. If the differences are kept in mind and considered, it would seem that the rat is indeed a suitable model for experimental middle ear research.

ACKNOWLEDGMENTS

REFERENCES

1. Akaan-Penttilä E. Middle ear mucosa in newborn infants. A topographical and microanatomical study. Thesis, University of Helsinki, 1980.

2. Altmann F. Anomalies of the internal carotid artery and its branches; their embryological and comparative anatomical significance. Report of a new case of persistent stapedial artery in man. Laryngoscope 57(1947)313-339.

3. Alm P.E., Bloom G.D., Hellström S., Salén B. & Stenfors L.-E. The release of histamine from the pars flaccida mast cells. One cause of otitis media with effusion? Acta Otolaryngol (Stockh) 94(1982)517-522.

4. Alm P.E., Bloom G.D., Hellström S., Stenfors L.-E. & Widemar L. Mast cells in the pars flaccida of the tympanic membrane. A quantitative morphological and biochemical study in the rat. Experentia 39(1983)287-289.

5. Anson B.J. & Donaldson J.A. Surgical Anatomy of the Temporal Bone and Ear. 2nd ed. W.B. Saunders Co., Philadelphia. 1973.

6. Aschan G. The anatomy of the Eustachian tube with regard to its function. Acta Soc Med Upsal 60(1955)131-149.

7. Bak-Pedersen K. & Tos M. Mucous glands in the middle ear and osseous Eustachian tube. Ann Otol Rhinol Laryngol 82(1973)80-88.

8. Beauregard W.G. Positional otitis media. J Pediat 79(1971)294-296.

9. Berendes Jr., Link R. & Zöllner F. Hals-Nasen-Ohren-heilkunde in Praxis und Klinik. Band 5, Ohr I, pp. 31-32. Thieme Verlag, Stuttgart.

10. Berger G, Hawke M, Proops DW, Ranadive NS & Wong D. Histamine levels in middle ear effusions. Acta Otolaryngol (Stockh) 98(1984)385-390.

11. Bortnick E. & Proud G.O. Experimental absorption of fluids from the middle ear. Arch Otolaryngol 81(1965)237-242.

12. Broman I. Die Entwicklungsgeschichte der Gehörknöchelchen beim Menschen. Anat Hefte 11(1899)507-566. Quoted from Kelemen, 1958.

13. Bryant W.S. The Eustachian tube, its anatomy and its movements: With a description of the cartilages, muscles, fasciae and the fossa of Rosenmüller. Med Rec 71(1907)931-934.

14. Buch N.H. & Jorgensen M.B. Eustachian tube and middle ear. Embryology and pathology. Arch Otolaryngol 79(1964)472-480.

15. Bugge J. The contribution of the stapedial artery to the cephalic arterial supply in muroid rodents. Acta Anat 76(1970)313-336.

16. Bugge J. The cephalic arterial system in insectivores, primates, rodents, an logomorphs, with special reference to the system classification. Acta Anat, Suppl. 62(1974)1-159.

17. Bylander A. Eustachian tube function in healthy children. A pressure equilibration study with an adult reference group. Thesis, Malmö, 1983.

18. Bylander A., Ivarsson A. & Tjernström Ö. Eustachian tube function in normal children and adults. Acta Otolaryngol (Stockh) 92(1981)481-491.

19. Candiollo L. Ricerche anatomo-comparative sul muscolo tensore del timpano, con riferimento alla innervazione propriocettiva. Z Zellforsch mikrosk Anat 67(1965)34-56.

20. Cantekin E.I., Bluestone C.D., Saez C.A. & Bern S.A. Airflow through the Eustachian tube. Ann Otol Rhinol Laryngol 88(1979)603-612.

21. Cantekin E.I., Doyle W.J. & Bluestone C.D. Effect of levator veli palatini muscle excision on Eustachian tube function. Arch Otolaryngol 109(1983)281-284.

22. Cleland . J Anat Physiol 3(1868)97-103.

23. Collins MP, Bakhshi K, Wareham CA & Church MK. Histamine and secretory otitis media. Clin Sci 67(1984)557-560.

24. Compere WE. Tympanic cavity clearance studies. Am Acad Ophthalmol Otolaryngol 62(1958)444-454.

25. Daniel III H.J., Brinn J.E., Fulghum R.S. & Barrett K.A. Comparative anatomy of Eustachian tube and middle ear cavity in animal models for otitis media. Ann Otol Rhinol Laryngol 91(1982)82-89.

26. de Pinies F. Persistence of stapedial artery. Arch Otolaryngol 80(1964)134-135.

27. Dickson D.R. Anatomy of normal velopharyngeal mechanism. Clinics in Plastic Surgery 2(1975)235-248.

28. Djupesland G. Electromyography of the tympanic muscles in man. Int Audiol 4(1965)34-41.

29. Doyle W.J. & Rood S.R. Comparison of the anatomy of the Eustachian tube in the rhesus monkey and man. Implications for physiologic modeling. Ann Otol Rhinol Laryngol 89(1980)49-57.

30. Duncan R.B. Positional otitis media. Arch Otolaryngol 72(1960)454-463.

31. Echeverria M. Note sur l'artère stapédienne. Ass Anat 46(1960)243-246.

32. Falk B. Sniff-induced negative middle ear pressure: Study of a consecutive series of children with otitis media with effusion. Am J Otolaryngol 3(1982)155-162.

33. Falloppio G. Observationes anatomicae. Coloniae, 1562.

34. Graves G.O. & Edwards L.F. The Eustachian tube. A review of its descriptive, microscopic, topographic and clinical anatomy. Arch Otolaryngol 39(1944)359-397.

35. Gray's Anatomy. Williams P.L. & Warwick E. (Eds.) 36th ed. Churchill Livingstone, Edinburgh, 1980.

36. Greene E.C. Anatomy of the Rat. Hafner Publishing Co, New York and London, 1963.

37. Guindi G.M. & Charia K.K.C. A reappraisal of the salpingopharyngeus muscle. Arch Otorhinolaryngol 229(1980)135-141.

38. Hamberger C.A., Marcusson G. & Wersäll J. Blood vessels of the ossicular chain. Acta Otolaryngol (Stockh), Suppl. 183(1963)66-70.

39. Hammar J.A. Studien über die Entwicklung des Vorderdarms und einiger angrenzenden Organe. Arch Mikr Anat 59(1902)471-628.

40. Hebel R. & Stromberg M.W. Anatomy of the Laboratory Rat. Williams & Wilkins Co., Baltimore, 1976.

41. Hellström S., Salén B. & Stenfors L.-E. Anatomy of the rat middle ear. A study under the dissection microscope. Acta Anat 112(1982a)346-352.

42. Hellström S, Salén B & Stenfors L-E. The site of initial production and transport of effusion material in otitis media serosa. A study on rat middle ear cavity. Acta Otolaryngol (Stockh) 93(1982b)435-440.

43. Hellström S, Salén B, Stenfors L-E & Söderberg O. Appearance of effusion material in the attic space correlated to an impaired Eustachian tube function. Int J Pediatr Otorhinolaryngol 6(1983)127-134.

44. Hellström S, Stenfors L-E, Alm PE, Bloom GD, Salén B & Widemar L. Provoking effusion in experimental otitis media with effusion. In Lim D.J., Bluestone C.D., Klein J.D. & Nelson J.D. (eds.) Recent Advances in Otitis Media with Effusion, pp.225-228. B.C. Decker Inc., Philadelphia, 1984.

45. Hentzer E. Histologic studies of the normal mucosa in the middle ear, mastoid cavities and Eustachian tube. Ann Otol Rhinol Laryngol 79(1970a)825-833.

46. Hentzer E. Ultrastructure of the normal mucosa in the human middle ear, mastoid cavities and Eustachian tube. Ann Otol Rhinol Laryngol 79(1970b)1143-1157.

47. Holborow C. Eustachian tubal function: Changes throughout childhood and neuro-muscular control. J Laryngol Otol 89(1975)47-55.

48. Holmgren G. Recherche experimentale sur les fonctions de la trompe d'Eustache. Communication préalable. Acta Otolaryngol (Stockh) 20(1934)381-387.

49. Holmquist J. & Olén L. Evaluation of Eustachian tube function. J Laryngol Otol 94(1980)15-23.

50. Honjo I., Okazaki N. & Kumazawa T. Experimental study of the Eustachian tube function with regard to its related muscles. Acta Otolaryngol (Stockh) 87(1979)84-89.

51. Honjo I., Okazaki N. & Kumazawa T. Opening mechanism of the Eustachian tube. A clinical and experimental study. Ann Otol Rhinol Laryngol 89(1980)25-27.

52. Honjo I., Okazaki N., Nozoe T., Ushiro K. & Kumazawa T. Experimental study of the pumping function of the Eustachian tube. Acta Otolaryngol (Stockh) 91(1981)85-89.

53. Honjo I., Ushiro K., Haji T., Nozone T. & Matsui H. Role of the tensor tym-
     pani muscle in Eustachian tube function. Acta Otolaryngol (Stockh)
     95(1983)329-332.

54. Honjo I., Ushiro K. & Mitoma T. Endoscopic observation of the Eustachian
     tube in otitis media with effusion. In Lim D.J., Bluestone C.D., Klein J.O.
     & Nelson J.D. (Eds.). Recent Advances in Otitis Media with Effusion, pp.
     238-240. B.C. Decker Inc., Philadelphia, 1984.

55. House EL. A myology of the pharyngeal region of the albino rat. Anat Rec
     116(1953)363-381.

56. Ibuki K., Matsuya T., Nishio J., Hamamura Y. & Miyazaki T. The course of
     facial nerve innervation for the levator veli palatini muscle. Cleft Palate
     J 15(1978)209-214.

57. Kelemen G. Arteria stapedia, in bilateral persistence. Arch Otolaryngol
     67(1958)668-677.

58. Keller J.T., Saunders M.C., Van Loveren H. & Shipley M.T. Neuroanatomical
     considerations of palatal muscles: Tensor and levator veli palatini. Cleft
     Palate J 21(1984)70-75.

59. Kobrak H.G. The Middle Ear. The University of Chicago Press, Chicago, 1959.

60. Kuijpers W., Van der Beek J.M.H. & Willart E.C.T. The effect of experimen-
     tal tubal obstruction on the middle ear. Preliminary report. Acta Otolaryn-
     gol (Stockh) 87(1979)345-352.

61. Kumazawa T. Die Tubo-Tympanoärodynamographie. Die Funktion der Tuba Eusta-
     chii aus der Sicht eigener Untersuchungen. Laryngol Rhinol Otol (Stuttgt)
     61(1982)146-149.

62. Lim D.J. Human tympanic membrane. An ultrastructural observation. Acta Oto-
     laryngol (Stockh) 70(1970)176-186.

63. Lim D.J. Functional morphology of the lining membrane of the middle ear and
     Eustachian tube. An overview. Ann Otol Rhinol Laryngol 83(1974)5-26.

64. Lim DJ & Hussl B. Human middle ear epithelium. An ultrastructural and cyto-
     chemical study. Arch Otolaryngol 89(1969)57-71.

65. Lim D.J. & Klainer A. Cellular reactions in acute otitis media - scanning
     and transmission electron microscopy. Laryngoscope 81(1971)1772-1786.

66. Lim D.J. & Shimada T. Secretory activity of normal middle ear epithelium.
     Scanning and transmission electron microscopic observation. Ann Otol Rhinol
     Laryngol 80(1971)319-329.

67. Lim D.J., Shimada T. & Yoder M. Distribution of mucus-secreting cells in
     normal middle ear mucosa. Arch Otolaryngol 98(1973)2-9.

68. Lübbers D.W., Hauck G., Weigelt H. & Addicks K. Contractile properties of
     frog capillaries tested by electrical stimulation. Bibl Anat 17(1979)3-10.

69. Lüscher E. Zur Topographie und Physiologie der cutanen oberflächlichen
     Trommelfellgefässe. Z Hals- Nas- Ohrenheilk 22(1929)13-52.

70. Maeda S., Mogi G. & Oh M. Fine structures of the normal mucosa in developing rat middle ear. Ann Otol Rhinol Laryngol Suppl 31(1976)1-19.

71. Magnuson B. On the origin of the high negative pressure in the middle ear space. Am J Otolaryngol 2(1981)1-12.

72. McMyn J.K. The anatomy of the salpingo-pharyngeus muscle. J Laryngol Otol 55(1940)1-22.

73. Miller G.F. Eustachian tubal function in normal and diseased ears. Arch Otolaryngol 81(1965)41-48.

74. Nager G.T. & Nager M. The arteries of the human middle ear, with particular regard to the blood supply of the auditory ossicles. Ann Otol Rhinol Laryngol 62(1953)923-949.

75. Nomina Anatomica. 5th ed. Excerpta Medica Foundation, London, 1983.

76. Nozoe T., Okazaki N., Koda Y., Ayani N. & Kumazawa T. Fluid clearance of the Eustachian tube. In Lim D.J., Bluestone C.D., Klein J.D. & Nelson J.D. (eds.). Recent Advances in Otitis Media with Effusion, pp. 52-55. B.C. Decker Inc., Philadelphia, 1984.

77. Paparella M.M., Hiraide F., Juhn S.K. & Kaneko Y. Cellular events involved in middle ear fluid production. Ann Otol Rhinol Laryngol 79(1970)766-779.

78. Paparella M.M., Sipilä P., Juhn S.K. & Jung T.T.K. The role of the subepithelial space in otitis media. Manuscript.

79. Perlman H.B. The Eustachian tube. Abnormal patency and normal physiologic state. Arch Otolaryngol 30(1939)212-238.

80. Politzer A. Lehrbuch der Ohrenheilkunde für practische Ärzte und Studierende. 4. Aufl. Verlag von Ferdinand Enke, Stuttgart, 1901, S.36-38.

81. Proctor B. The development of the middle ear spaces and their surgical significance. J Laryngol Otol 78(1964)631-648.

82. Proctor B. Embryology and anatomy of the Eustachian tube. Arch Otolaryngol 86(1967)503-514.

83. Proctor B. Anatomy of the Eustachian tube. Arch Otolaryngol 97(1973)2-8.

84. Rich A.R. A physiological study of the Eustachian tube and its related muscles. Johns Hopkins Hosp Bull 31(1920)206-214.

85. Rogers L, Kirschner F. & Proud G.O. The evaluation of Eustachian tube function by fluorescent dye studies. Laryngoscope 72(1962)456-467.

86. Rood S.R. & Doyle W.J. Morphology of tensor veli palatini, tensor tympani and dilatator tubae muscles. Ann Otol Rhinol Laryngol 87(1978)202-210.

87. Rood S.R. & Doyle W.J. The nasopharyngeal orifice of the auditory tube: Implications for tubal dynamics anatomy. Cleft Palate J 19(1982)119-128.

88. Ross E.L. & Rawson R.W. Absorption from middle ear. Arch Otolaryngol 22(1935)312.

89. Rundcrantz H. Posture and Eustachian tube function. Acta Otolaryngol (Stockh) 263(1970)15-17.

90. Sadé J. Middle ear mucosa. Arch Otolaryngol 84(1966)137-144.

91. Sadé J. Ciliary activity and middle ear clearance. Arch Otolaryngol 86(1967)128-135.

92. Sadé J. Inflammatory and non-inflammatory factors related to secretory otitis media. Int J Ped Otorhinolaryngol 1(1979)41-59.

93. Sadé J, Wolfson S, Sacks Z, Levit I, Yaniv E, Yarowitzka I & Abraham S. Eustachian tube isthmus lumen. Reported at the Extraordinary International Symposium on Recent Advances in Otitis Media with Effusion. Kyoto, Japan, Jan 12-15, 1985.

94. Salén B. & Zakrisson J.E. Electromyogram of the tensor tympani muscle in man during swallowing. Acta Otolaryngol (Stockh.) 85(1978)453-455.

95. Saunders W.H., Paparella M.M. & Miglets A.W. Atlas of Ear Surgery, p. 1. C.V. Mosby Co., St Louis, 1980.

96. Seif S. & Dellon A.L. Anatomic relationships between the human levator and tensor veli palatini and the Eustachian tube. Cleft Palate J 15(1978)329-336.

97. Shimada T. & Lim D.J. Distribution of ciliated cells in the human middle ear. Electron and light microscopic obserations. Ann Otol Rhinol Laryngol 81(1972)203-211.

98. Shrapnell H.J. On the form and structure of the membrana tympani. London Med Gazette 10(1832)120-124.

99. Simkins C.S. Functional anatomy of the Eustachian tube. Arch Otolaryngol 38(1943)476-484.

100. Singer C. Greek Biology & Greek Medicine, p. 82. Clarendon Press, Oxford, 1922.

101. Spungin B & Silberberg A. Stimulation of mucus secretion, ciliary activity, and transport in frog palate epithelium. Am J Physiol 247(1984)C299-C308.

102. Stenfors.L-E. Structure and Pattern of Motion of the Tympanic Membrane with Special Reference to Experimental Perforations and Subsequent Healing Processes. Thesis, Umeå, 1980.

103. Stenfors L-E, Carlsöö B & Winblad B. Structure and healing capacity of the rat tympanic membrane after Eustachian tube occlusion. Acta Otolaryngol (Stockh) 91(1981)75-83.

104. Terracol J., Carone A. & Guerrier Y. La trompe d'Eustache, pp. 122-128. Masson, Paris, 1949.

105. Tos M. Pathogenesis and pathology of chronic secretory otitis media. Ann Otol Rhinol Laryngol, Suppl. 68(1980)91-97.

106. Tos M. Experimental tubal obstruction. Changes in middle ear mucosa elucidated by quantitative histology. Acta Otolaryngol (Stockh) 92(1981)51-61.

107. Tos M. & Bak-Pedersen K. Goblet cell population in the normal middle ear and Eustachian tube of children and adults. Ann Otol Rhinol Laryngol 85(1976)44-50.

108. Toynbee J. On the muscles which open the Eustachian tube. Proc Royal Soc 5(1853)286-287.

109. Valsalva A.M. De aure humana tractatus. Trajecti ad Rhenum, 1707. Quoted from Rich, 1920.

110. Van Cauwenberge P. Otitis media with effusion. Functional morphology and physiopathology of the structures involved. Acta Oto-Rhino-Laryngol Belg 36(1982)5-240.

111. Van der Beek J.M.H. Experimental obstruction of the Eustachian tube - a contribution to knowledge of the pathogenesis of secretory otitis media. Thesis, Heerlen, 1981.

112. von Tröltsch A. Arch Ohrenheilk 15(1864). Quoted from Rich, 1920.

113. Widemar L., Bloom G.D., Hellström S. & Stenfors L.-E. Mast cells in the pars flaccida of the tympanic membrane. Acta Otolaryngol (Stockh), Suppl. 414(1984)115-117.

114. Widemar L, Hellström S, Schultzberg M & Stenfors L-E. Autonomic innervation of the tympanic membrane. Acta Otolaryngol (Stockh) in press.

115. Yule C.J.F. On the mechanism of opening and closing the Eustachian tube. J Anat Physiol 8(1873)127-132.

116. Zechner G. Auditory tube and middle ear mucosa in nonpurulent otitis media. Ann Otol Rhinol Laryngol, Suppl. 68(1980)87-90.

117. Zöllner F. Die klaffende Ohrtrumpete, Störungen dadurch und Vorschläge zu ihrer Behebung. Z Hals Nas Ohrenheilk 42(1937)287-298.