MIDDLE EAR STRUCTURE IN RELATION TO FUNCTION
The Rat in Middle Ear Research

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ABSTRACT
The present study was undertaken to evaluate the rat as a model for middle ear research. 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 independent 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 muscle, but also by the levator and tensor 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.

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 vessels 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 fibrocartilaginous 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 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.

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.

Key words: rats; Eustachian tube; ear, middle; tympanic membrane; palatal muscles; mucous membrane; blood vessels; mast cells; otitis media; microscopy, electron.
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by
NILS ALBIIN

Umeå university 1985
To Carina
Cover: Drawing by
Karin Nordin.
All animals are equal
but some animals are more equal than others

George Orwell
1903-1950
This thesis is based upon the following papers, which will be referred to by their Roman numerals.


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).


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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 interesting field of research in its connexion with the science of acoustics". Thus, in order to study the normal physiology and pathophysiology of the middle ear, a thorough knowledge of its structure 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 applicable 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.
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 (Simpkins, 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 superiorly, 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.

Tympnic 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 lining of the middle ear is continuous with the respiratory mucosa of the nasopharynx. Thus, the respiratory mucosa extends through the Eustachian tube into the tympanic cavity, which it lines completely. Posteriorly 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, respectively (Sadé, 1966; Lim & Shimada, 1971; Shimada & Lim, 1972; Lim et al., 1973; Tos & Bak-Pedersen, 1976; Akaan-Penttilä, 1980). The remaining 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 (Hamberg, 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 tympanic 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. Anterior 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 adjacent mucosal folds they divide the epitympanum into a medial and lateral 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 remaining mucosa is lined by a single squamous epithelium. In contrast to these reports, Daniel III et al. (1982) recently claimed that the tympanic 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) ventilation of the middle ear; (2) drainage of the middle ear; and (3) protection of the tympanic cavity from sound, nasopharyngeal pressure
variations and pathogenic agents (for references see Holmgvist & 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 opening 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>
<thead>
<tr>
<th>Author(s), Year, Species</th>
<th>TTM</th>
<th>TVPM</th>
<th>LVPM</th>
<th>SPM</th>
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<tr>
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<td>Cleland, 1868, man</td>
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<td>O</td>
<td>C?</td>
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<tr>
<td>Honjo et al., 1983, cat</td>
<td></td>
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<td>N</td>
<td></td>
</tr>
<tr>
<td>Cantekin et al., 1983, monkey - juvenile</td>
<td>O</td>
<td></td>
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</table>

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 clearance 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 & Silverberg, 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
pharynx. However, this clearance mechanism requires a satisfactory ventilation of the tympanum (Nozoe et al., 1984).

Studies in the rat indicate that the production of fluid - in experimentally 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 located beneath the epidermal layer, whereas the vessels of the tympanic branch are distributed beneath the inner layer (for references see Berendes, 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 pentobarbital (Mebumal®; 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®, 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 intravenous injection of sodium hexabarbital (Brietal®) through a tail vein. Under aseptic conditions the right tympanic bulla was opened and a polyethylene plug was placed in the tympanic orifice of the Eustachian tube. This procedure induced otitis media with effusion. At various intervals, ranging from 2 days to 2 weeks, the animals were reanesthetized and killed by perfusion with glutaraldehyde. The stapedial arteries with adjacent mucosa were then dissected free and prepared for light and transmission electron microscopy.
LIGHT 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 other anesthetized 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. 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 (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 was perfused for 3 hours followed by a new saline rinse. The head was now soft and decalcified, 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) techniques. Semithin sections were cut and stained with toluidine blue (II,III) or with PAS (II).
TEMPORAL BONES 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. 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.
ANATOMY OF EUSTACHIAN TUBE IN RAT (I)

The Eustachian tube extends in a posterior, lateral and superior direction, from the nasopharynx to the tympanic cavity. It measures about 4.5 mm in length. The tube is angled at about 30° in relation to the sagittal plane and about 10° 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 nasopharynx 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 opening the cartilage, in cross-section shaped as a shepherd's crook, almost 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 epi tympanic 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 epi tympanic 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 eptympanum. 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 tympanic 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 intima 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 tympanic 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 (Simkins, 1943; Rood & Doyle, 1982). However, the opening slit is horizontally 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 anesthetized 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 cartilage supports the tube through the bony canal to its tympanic opening. 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 tympanic 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 tympanic opening (Graves & Edwards, 1944; Proctor, 1973).

The anatomy of the paratubal muscles of the rat shows several similarities 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 palatini 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 splitting of the soft palate provoked otitis media with effusion (Hellström et al., 1983), probably by interfering with the opening mechanisms discussed 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 protrudes 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 importance 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 ossicles, including Prussak's space (medial to the pars flaccida), occasionally 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 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.
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.
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 meningeal, 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 completely 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 cartilaginous and bony portion. The orifice of the nasopharyngeal portion is formed by two soft tissue lips, which appear to open mainly by the action of the salpingopharyngeal muscle, but also by the levator and tensor 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 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.

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

4. The tympanic membrane is vascularized from meatal and tympanic vessels. Meatal vessels branch in the pars flaccida and along the handle of the malleus, where they are located directly beneath the outer, keratinizing, stratified, squamous epithelium. Furthermore, meatal vessels form a vascular network at the junction between the fibrocartilaginous ring and the tympanic sulcus. Tympanic vessels send branches to the periphery of the pars tensa, where they run immediately beneath the tympanic, 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 extratympanic 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.
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