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HISTOLOGIC STUDY

A histologic study on the temporal bone of guinea pigs

Guinea pig temporal kemik anatomisinin histopatolojik özellikleri

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Objectives: We examined histopathologic and anatomical features of the guinea pig temporal bone and evaluated the differences and similarities with respect to the human ear.

Study Design: Seventeen adult guinea pigs were deeply anesthetized with pentobarbital and then decapitated. Each temporal bone was removed and fixed in 10% formaldehyde for a week, then decalcified in 10% formic acid for three weeks. Paraffin-embedded specimens were serially and horizontally sectioned at 7-micron thickness. One of every five sections was stained with hematoxylin and eosin and studied under light microscopy.

Results: The temporal bone consisted of a mastoid-like process, a tympanic bulla, a tympanic ring, a petrosal segment, and a poorly developed squamosal bone. The tympanic bulla was a semispherical cavity surrounded by the tympanic ring. The head of the malleus and the body of the incus were fused, forming a malleoincudal complex. The diameters of the tympanic sulcus and the tympanic membrane were much greater than that of the tympanic ring, resulting in protrusion of the external ear-canal into the bulla. The Eustachian tube was J-shaped, lying in a bony hiatus at the anteromedial aspect of the bulla. The inner ear consisted of the cochlea, semicircular canals, and the vestibule. The cochlea made 3.5 turns and projected into the bulla. No internal auditory meatus was observed.

Conclusion: The guinea pig temporal bone was found to have histological similarities to that of humans, making it a good model for selected experimental studies in otology.

Key Words: Ear/anatomy & histology; ear, middle/anatomy & histology; guinea pigs/anatomy & histology; temporal bone/anatomy & histology.

Amaç: Bu çalışmada guinea pig temporal kemiğinin histolojik anatomik özellikleri incelendi ve bunların insan kulağı ile benzerlikleri ve farklılıkları değerlendirildi.

Çalışma Planı: Çalışmaya 17 erişkin guinea pig alındı. Derin anesteziden sonra hayvanların yaşamı sonlandırıldı. Çıkarılan temporal kemikler %10 formaldehidde bir hafta süreyle bekletildi; %10 formik asitte üç hafta süreyle dekalsifiye edildi. Parafine gömülen örneklerden seri olarak yatay planda 7 mikron kalınlığında kesitler alındı. Her beş kesitten biri hematoksilen-eosin ile boyandı ve ışık mikroskobunda incelendi.

Bulgular: Guinea pig temporal kemikleri mastoid benzeri bir çıkıntı, timpanik bulla, timpanik halka, petröz parça ve iyi gelişmemiş skuamöz kemikten oluşmaktaydı. Timpanik bulla, timpanik halka ile çevrili yarım daire şeklinde bir boşluk olarak gözlendi. Malleus başı ve inkus gövdesi kaynaşarak malleoinkudal kompleksi oluşturmaktaydı. Timpanik membran ve timpanik sulkusun çapı timpanik halkanın çapından büyüktü. Bu nedenle kemik dış kulak yolu kanalının bullaya doğru çıkıntı yaptığı görüldü. Östaki tübü J şeklinde idi ve bullanın anteromedialinde bir kemik yarık olarak gözlendi. İç kulak koklea, semisirküler kanallar ve vestibülden oluşmaktaydı. Kokleanın 3.5 dönüş yaptığı ve bullaya çıkıntı oluşturduğu görüldü. İnternal akustik kanal gözlenmedi.

Sonuç: Guinea pig temporal kemiği histolojik olarak insan temporal kemiğine benzer bulundu. Seçilmiş deneysel otoloji çalışmalarında guinea pig kulağı iyi bir model oluşturabilir.

Anahtar Sözcükler: Kulak/anatomi ve histoloji; kulak, orta/anatomi ve histoloji; guinea pig/anatomi ve histoloji; temporal kemik/anatomi ve histoloji.

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Guinea pigs have long been used for animal experimentation in otology, because the temporal bone and otologic structures provide a good model for the human ear and allow easy manipulation if differences and similarities are known. The surgical anatomy of guinea pig middle ear and its contents has been described by various studies,[1-3] but histologic data on the anatomy of the temporal bone is lacking.

The histologic characteristics of the inner ear and the mucosa, and architecture of the middle ear may provide further insight into the functioning of these structures. In this study, we investigated the histologic features of the Eustachian tube, middle ear, and inner ear of the guinea pig, in comparison with differences from, and similarities to, those of human ear.

MATERIALS AND METHODS

The study included 17 healthy guinea pigs weighing 500 to 900 grams. They were obtained from the Institute for Experimental Medical Research, Istanbul University (DETAE), Istanbul, Turkey. The animals were anesthetized with pentobarbital (60 mg/kg) and then decapitated. The temporal bones were exposed, and all soft tissues were removed. The temporal bones were then processed for study under light microscopy. Each temporal bone was fixed in 10% formalin for a week, and then decalcified in 10% formic acid for three weeks. They were embedded in paraffin blocks and then serially sectioned horizontally to obtain 7-micron thick sections. One of every five sections was stained with hematoxylin and eosin, mounted on a glass slide, and studied under light microscopy. Histologic findings and anatomical details were noted. The experimental protocol of the study was reviewed and approved by the Institutional Review Board for Experimental Medical Research of the University of Istanbul.

RESULTS

The temporal bone of the guinea pig lies at the posterior and inferior aspect of the squamosal bone. It consists of a tympanic bulla, a tympanic ring, a mastoid-like process, a petrosal segment, and a squamosal portion. The tympanic bulla of the guinea pig, which corresponds to the hypotympanum and mesotympanum in human temporal bone (Fig. 1), is a smooth-surfaced and semispherical cavity. The inner surface is covered with ciliated pseudostratified columnar epithelium (Fig. 2). It continues with air cells posterosuperiorly and anterosuperiorly. There is a tiny osseous bulla wall laterally. The bulla is separated from the epitympanum by the cochlea. The air-cell system of the guinea pig temporal bone can be divided into two groups, namely, epitympanic and retrotympanic cells. This area, which corresponds to the attic in the human ear, communicates with the bulla via a hiatus (Fig. 1). The retrotympanic cells and the inferior subspace communicate with the inferior bulla. The external ear-canal is opened into the middle ear via the tympanic membrane. Its surface is covered by a multilayered squamous epithelium which is rich in hair-follicles and sebaceous glands. The osseous canal continues into the bulla inferior where the tympanic ring becomes larger. The canal, which hangs over the bulla, is larger near the membrane. The diameter of the tympanic membrane is greater than that of the external ear canal (Fig. 3). The thin tympanic membrane is covered by one layer of squamous epithelium externally, and one layer of cubic epithelium internally. A peripheral collagenous tissue is seen between these two layers of epithelium.

The Eustachian tube of the guinea pig presents as a J-shaped structure, lying in a bony hiatus on the anteromedial side of the bulla. The tube is located between the nasopharyngeal opening and the bulla (Fig. 4). Its inner surface is covered by a pseudostratified columnar epithelium with ciliated mucus-secretory goblet cells. The hyalin chondrium that binds to the tube is of a semilunar shape and is connected to the surface epithelium and osseous tissue by a fibrous tissue.

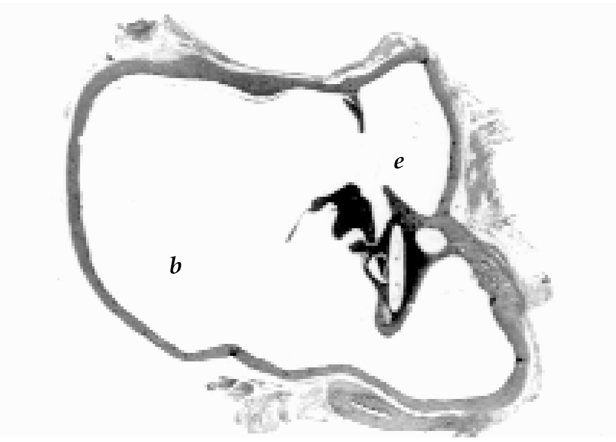

Fig. 1 - *The tympanic cavity in the guinea pig ear, the bulla (b), and the epitympanum (e) (H-E x 10).*

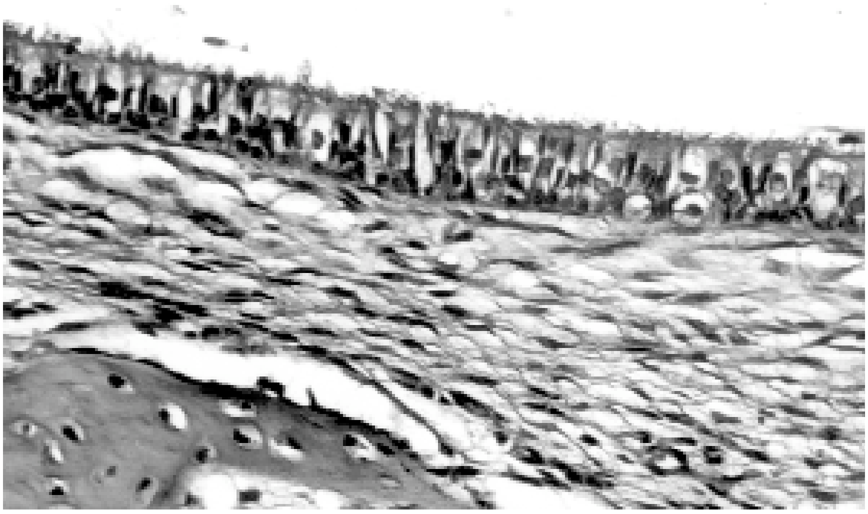

Fig. 2 - *The inner surface of the bulla covered with ciliated pseudostratified columnar epithelium (H-E x 310).*

In the guinea pig, the head of the malleus and the body of the incus are fused, forming a malleoincudal complex. The line of the fusion can be identified under light-microscopy as a chondrial tissue (Fig. 5). As in humans, the handle of the malleoincudal complex is located within the two layers of the tympanic membrane. The long process of the complex articulates with the stapes via the lenticular process. The stapes of the guinea pig is similar to that in humans. The stapedius muscle lies in a bony canal and is inserted into the neck of the stapes. The tensor tympani muscle and its tendon, which lie in a bony groove, are inserted into the neck of the handle of the malleoincudal complex. The oval window and round windows are located just above the basal turn of the cochlea, with a thin osseous lamella between them (Fig. 6). The stapedial footplate is occluded anterior to, and the tympanic sinus is situated posterior to, the oval window. The pyramidal eminence lies in a posterosuperior to anteroinferior plain with reference to the oval window. After leaving the pyramidal eminence, the tendon of the stapedius muscle extends into the neck of the stapes.

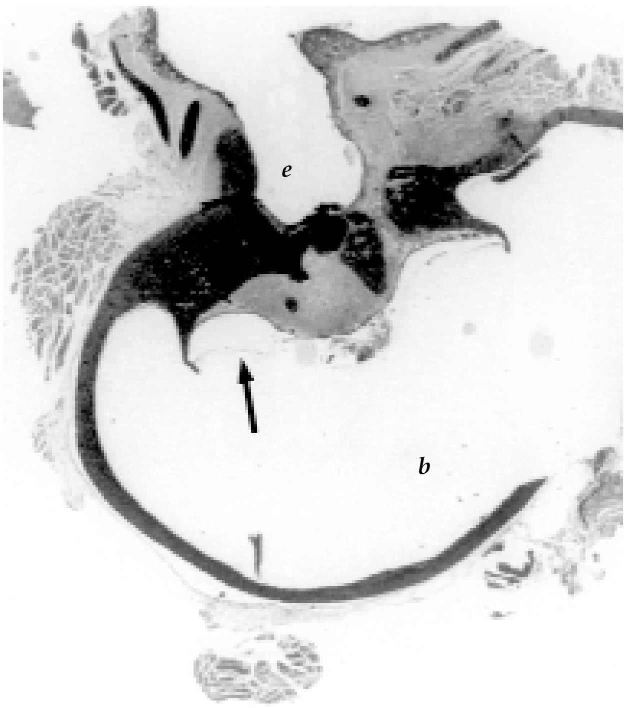

Fig. 3 - *The appearance of the bulla (b), external ear canal (e), and the tympanic membrane (arrow) (H-E x 10).*

Fig. 4 - *The Eustachian tube is a J-shaped structure lying in a bony hiatus. The basal cochlear turn is posterolateral to the Eustachian tube (H-E x 40).*

The facial nerve of the guinea pig enters the temporal bone through a foramen located superior to the entry of the cochlear nerve and anterior to the vestibular nerve. The acoustic-vestibular-facial nerve bundle enters the temporal bone directly, with no distinct internal meatus, but with readily discernable transverse and vertical bony crests. These nerves run into the temporal bone in separate canals. After a course of 1 to 2 mm in the petrous portion, the facial nerve shifts upwards at the medial aspect of the basal cochlear turn, and is connected with the portion of the nerve in the middle ear that courses horizontally and posteriorly. Here, it runs near the oval window and then inferiorly between the bulla and a mastoid-like process, and leaves the temporal bone via a foramen located posterosuperior to the tympanic ring.

The conical structure of the cochlea projects into the bulla, from a medial to an anteroinferior direction (Fig. 7). Its basal turn lies posterolateral to the Eustachian tube (Fig. 4). From its second turn to the final turn, the cochlea projects into the bulla. Microscopic structure of the cochlea shows a spiral cochlear canal, as in humans (Fig. 8). The modiolus is seen in the middle. Projecting out from the modiolus is a thin bony plate called the osseous spiral lamina. The cochlear canal is partially subdivided into an upper passage (scala vestibuli) and a lower passage (scala tympani). The two scalae join apically at the helicotrema. Within the bony cochlea and attached to its outer wall is the scala media, or cochlear duct, an epithelium-lined tube somewhat triangular in transverse section. The epithelium of the cochlear floor is greatly elevated and specialized, constituting the complicated spiral organ of Corti in which the fibers of the cochlear nerve terminate to make up the peripheral sense-organ of hearing. In the organ of Corti, there is a the basilar membrane with some cells lying on it. These are limbic cells, cochlear neurons, and spiral ganglial cells. They appeared to be damaged during the processing of the specimens (Fig. 9)

The external part of the membranous cochlear labyrinth of the guinea pig is formed by a spiral ligament of connective tissue. Its external surface lies firmly against the bony labyrinth, and its internal surface lines the cochlear duct and scala tympani. Forming the floor of the cochlear duct, is the Reiss-

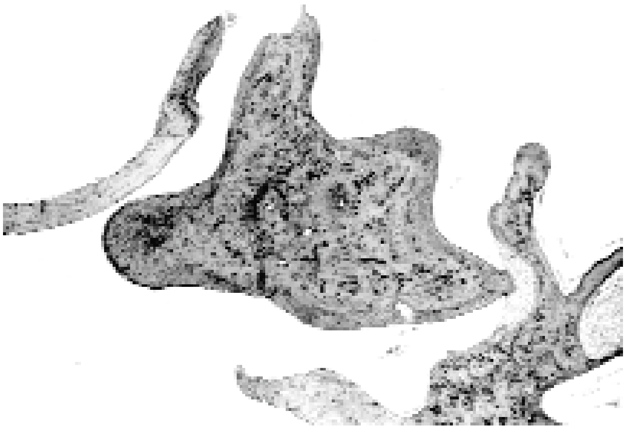

Fig. 5 - *Line of fusion in the malleoincudal complex is identified as a chondrial tissue.*

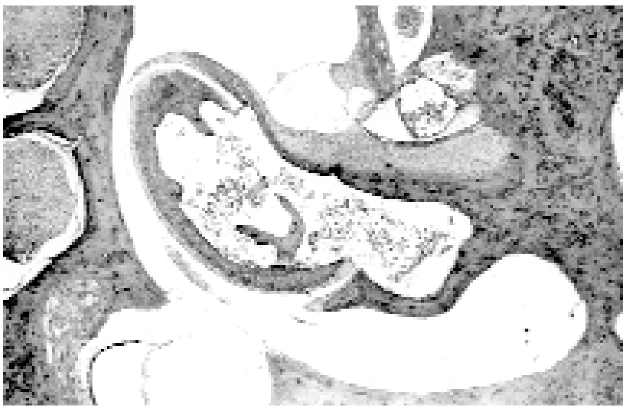

Fig. 6 - *The oval window and round windows are located just above the basal turn of the cochlea, with a thin osseous lamella between them (H-E x 40).*

Fig. 7 - *The cochlea projecting into the bulla (H-E x 10).*

ner's membrane lying from the spiral ligament to the osseous spiral lamina to form an angle of 45 degrees with the basilar membrane. The stria vascularis lies on the internal surface of the spiral prominence to the Reissner's membrane. It consists of a superficial specific tissue which is very dense with numerous capillaries.

The semicircular canals and the vestibule are important structures of the inner ear in guinea pig. The lateral semicircular canal lies above the facial canal, communicating anteriorly with the superior semicircular canal, which turns superiorly, and posteriorly to meet the posterior semicircular canal just behind the entry of the vestibular nerve. The diameter of the membranous canal is shorter than that of the bony canal. The membranous canal is covered by one layer of epithelium and is not firmly attached to the bony canal. Fibers of the vestibular nerve are distributed to the crista ampullaris of the lateral canal. Here there is a modified and elevated portion of the epithelium of the ampulla into which the terminal fibers of the ampullary nerve extend (Fig. 10). Posterior to the cochlea in the superoposterior region of the vestibule lies the utricle, which is slightly flattened. The internal carotid artery lies in the carotid canal, which is surrounded by a perivascular sheath in which the pericarotid nerve-plexus and the pericarotid venous plexus are embedded.

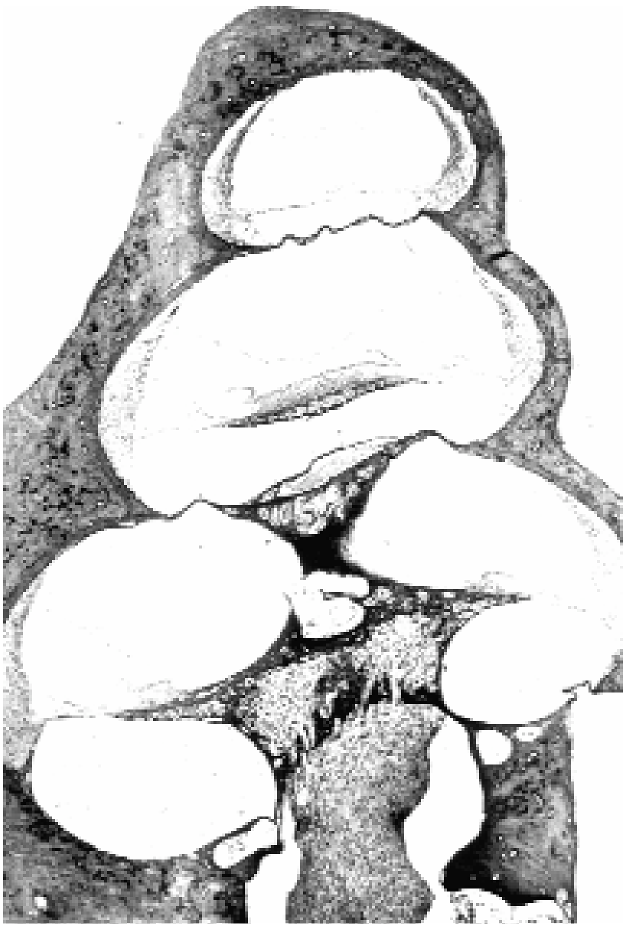

Fig. 8 - *Microscopic structure of the cochlea (H-E x 40).*

DISCUSSION

Selecting the type of animal for experimental protocols in otology is very important. Knowing the anatomy of the ear of a specific animal may help to make a more appropriate selection. Guinea pigs and chinchillas are frequently used in experimental otological studies.[4] Earlier studies on the surgical anatomy revealed both differences and similarities between guinea pig and human ears.[1-5] In our histopathologic study, we examined the guinea pig temporal bone and found it to be similar to human ear in microscopic structure. Anatomic differences from the human ear on gross surgical examination were confirmed in our microscopic study.

We found that mastoid cells and the temporal air-cell system in guinea pigs were different from those of the human ear.[1-4] The guinea pig ear seems to have a more developed air-cell system than that of the human ear. The temporal bone of the guinea pig has three large cavities with smooth surfaces: the bulla, epitympanum, and retrotympanum. In contrast, air cells in the human ear are smaller in volume, multiple in number, and divided by an osseous lamella. These differences suggest that the guinea pig ear is not an appropriate model for studies on the middle ear or mastoid aeration.

As seen macroscopically, the guinea pig has a malleoincudal complex in the tympanic cavity. Its line of fusion can be identified under microscopy as a cartilaginous tissue. We noted that the stapedius muscle was near the stapedial footplate and in the bony canal.

Göksu et al.[3] demonstrated that the tympanic membrane of guinea pigs is extremely thin and devoid of a fibrous layer. Our histopathologic sections of the tympanic membrane showed a collagenous connective tissue between the two epithelial layers, especially at the periphery. The guinea pig tympanic ring has a thin bony process toward the tympanic bulla. The diameter of the tympanic membrane is much greater than that of the tympanic ring, or of the external ear-canal. In studies of surgical dissection, a hidden space has been shown lying between the tympanic ring and the tympanic membrane. These findings may imply drawbacks for transtympanic approaches. Surgically, approach to the ossicules may present many difficulties. The surgical approach to the middle ear should be via the bulla, so that any damage to the tympanic membrane may be avoided. Thus, manipulation of the ossicules may be possible via the bulla, or after removal of the tympanic membrane and tympanic osseous crest.

Cochlear structures of the guinea pig ear, such as the modiolus, organ of Corti, spiral ligament, stria vascularis, Reissner's membrane, cochlear nerve, and ganglions were found to be similar to human ear under light-microscopy.[6] These similarities make the guinea pig cochlea, superior and posterior semicircular canals readily accessible, making the guinea pig ear a good model for inner ear studies.

In addition, the course of the facial nerve can easily be manipulated together with the intra- and extra-temporal bones, thus making guinea pig models more convenient for facial nerve studies.

In conclusion, both similar features and histologic and anatomical differences in the temporal bone in guinea pigs and humans should be considered. In this respect, while guinea pig models may not be convenient for otologic studies dealing with aera-

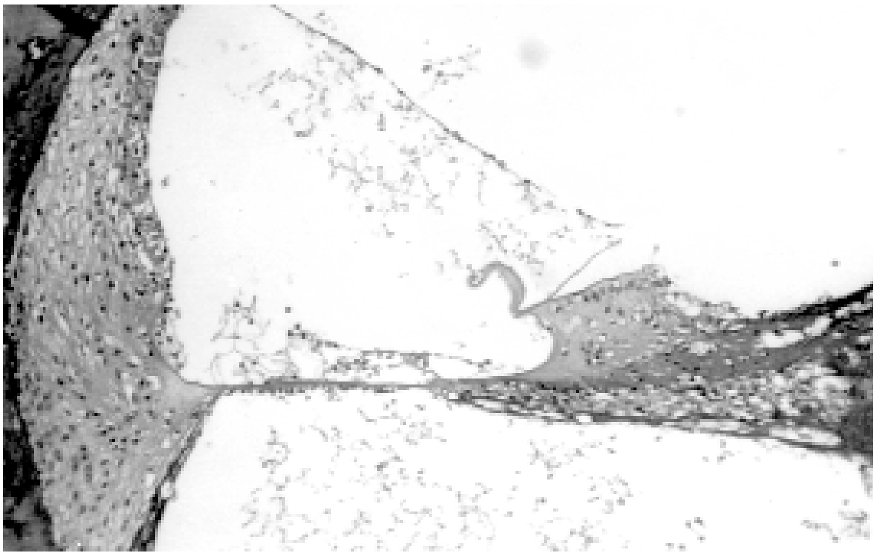

Fig. 9 - *Microscopic appearance of the organ of Corti (H-E x 125).*

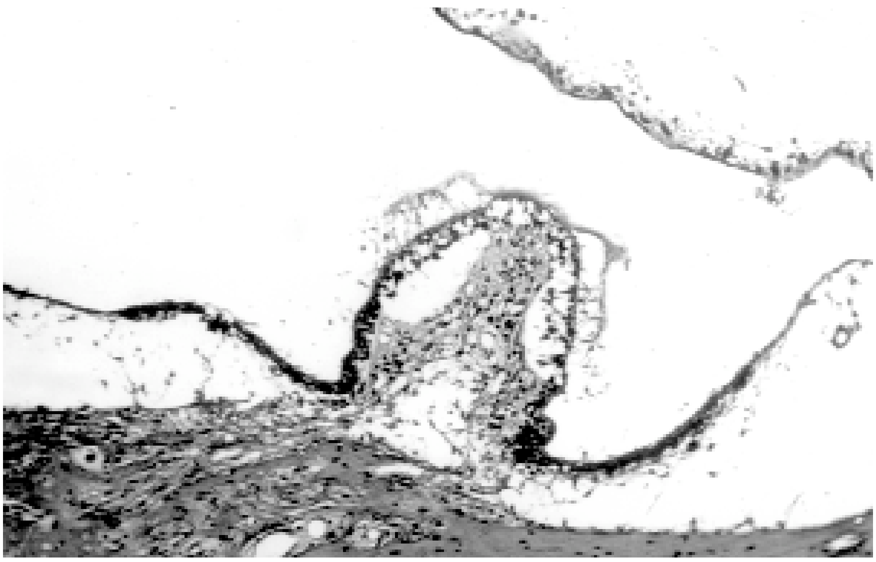

Fig. 10 - *Modified portion of the epithelium of the ampulla. (H-E x 125).*

tion, or transtympanic approaches to the middle ear, they seem to provide appropriate means to study inflammatory middle ear, autoimmune diseases, or structure and functions of the cochlea and the facial nerve.

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