Yonsei Medical Journal
Vol. 33, No. 2, 1992

Luminal Development of the Eustachian Tube and Middle Ear: Murine Model

Keehyun Park[1] and David J. Lim[2]

The purpose of this study was to describe the luminal development of the murine eustachian tube and middle ear. Thirty specimens, aging from gestational day 11 to postnatal day 21, were investigated through the light microscopic observations. The present study also used digitizer, computer, and serially sectioned temporal bone specimens for three-dimensional reconstruction to measure the volume of the eustachian tube and middle ear cavity at different gestational and postnatal ages. The first pharyngeal pouch elongated during gestational day 12 to form the tubotympanic recess. Between gestational day 13 and 14 this tubotympanic recess extended to the middle ear area. A rapid increment in the volume of the tubotympanic recess was noted between gestational day 15 and 16. At this age, a definite division of the tubotympanic recess into the eustachian tube and middle ear cavity was observed. During the postnatal period, the maximum change of the middle ear volume was noted on postnatal day 11 when the mesenchymal tissue in the middle ear cavity disappeared completely.

Key Words: Developmental study, eustachian tube, middle ear, mice

The mouse is an excellent animal model for studying developmental anatomy of the mammalian eustachian tube and middle ear cavity because of its relatively short gestational and rapid postnatal developmental periods. It is useful since it breeds rapidly and prolifically, allowing rapid development of inbred strains. Furthermore, it is consistently available and not expensive to purchase, ship, and maintain.

The properties of the eustachian tube and middle ear in the adult mouse have been reported (Ichimiya *et al.* 1988; Takahashi *et al.* 1989), and the developmental anatomy of the murine inner ear and some parts of the middle ear were described (Lim and Anniko 1985; Huangfu and Saunders 1983), but to date developmental description of its eustachian tube and middle ear cavity has been sketchy. In this report the luminal development of the murine eustachian tube and middle ear aging from gestational day 11 to postnatal day 21, was investigated through light microscopic (LM) observations including computer-aided three-dimensional (3D) reconstruction.

This study will be useful in understanding the development of the tubotympanal cavity, and will provide the anatomical basis for the future animal studies concerning the pathogenesis of otitis media in the earlier ages using the murine model.

MATERIALS AND METHODS

A total of thirty BALB/c mice ranging from gestational day 11 through postnatal day 21 were used in this investigation. Sexually mature female mice were placed with males overnight. Day one of gestation was regarded as the day when mice had vaginal plugs in the morning after fertilization.

For LM observations, the animals were decapitat-

Received May 7, 1992
Accepted June 22, 1992

Department of Otolaryngology[1], Yonsei Univeristy College of Medicine, Seoul, Korea

Otological Research Laboratories[2], Department of Otolaryngology, The Ohio State University College of Medicine, Columbus, Ohio, U.S.A.

Address reprint requests to Dr. K Park, Department of Otolaryngology, Yonsei University College of Medicine C.P.O. Box 8044, Seoul, Korea, 120-752

ed without perfusion of the fixative and immersed in 10% neutral buffered formalin for 7 days at 4°C. Postnatal specimens were decalcified with 10% ethylene diamine tetraacetic acid (EDTA) in 0.1 M Tris-buffer (pH 6.95). The end point of EDTA decalcification was checked by Seilly's chemical test (Seilly 1982). Specimens were dehydrated with graded ethyl alcohol and embedded in glycol methacrylate (JB-4: Polysciences, Warrington, Pennsylvania). Using glass knives, 5 micro-meter sections were made and stained with hematoxylin and eosin.

For 3D reconstruction, the reference points which are indispensable for a computer-aided study of serial sections were put into the JB-4 block at three points adjacent to the specimen. To make the reference points, three V-shaped cutting notches on the different walls of JB-4 block were made. The notches were parallel to each other and as close to the perpendicular to cutting surface as possible. The block was then re-embedded and cut into serial sections as 5 micrometer intervals. Unstained slides were used for 3D reconstruction because reference notches in the re-embedded block could not be identified after staining. These sections was imaged with a projection microscope. The outline of the eustachian tube and middle ear cavity with all of the reference points aligned was traced on a digitizer tablet and recorded in the image analysis computer system (PC 3D: Jandel Scientific, Corte Madera, California). Data entry simultaneously generated 3D images of the structures on the monitor as a wire-frame model displayed in perspective. The volume of the eustachian tube and middle ear cavity was measured using the same system with the different program. The sections for every 20 micrometer interval (sometimes 40 micro-meter or more according to the size of the specimens) were selected, and the area of cross-section of each eustachian tube and middle ear cavity was measured on the digitizer. Thus the volume of the eustachian tube and middle ear cavity was calculated, and by adding these values the volume of the whole eustachian tube and middle ear cavity was obtained.

RESULTS

The primitive pharynx, derived from the foregut, widens cranially where it joins the primitive mouth

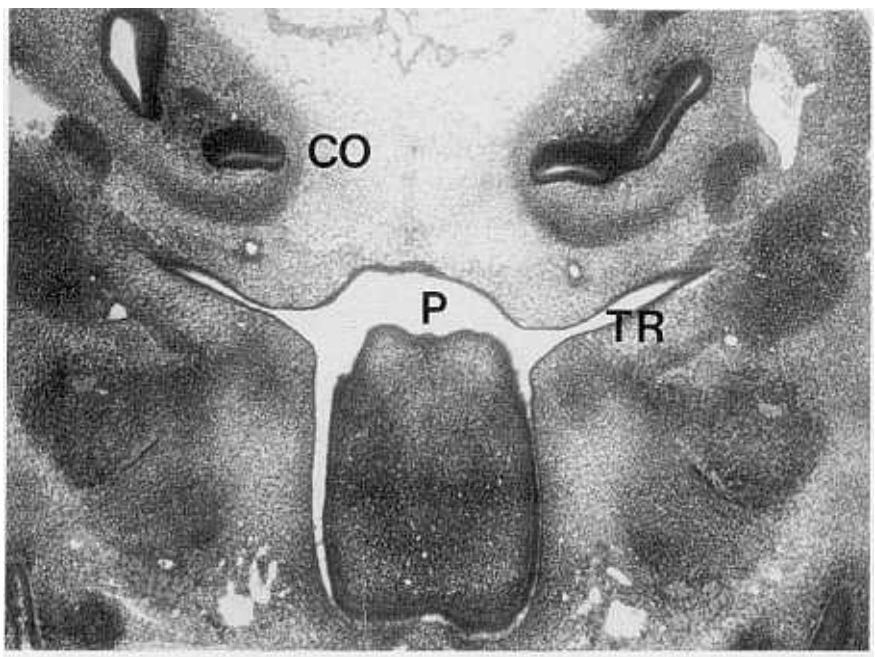

Fig. 1. The pharyngeal pouch (P) elongates to form the tubotympanic recess (TR) in a 12-day gestational mouse. Cochlea-CO (Axial, H & E, ×33).

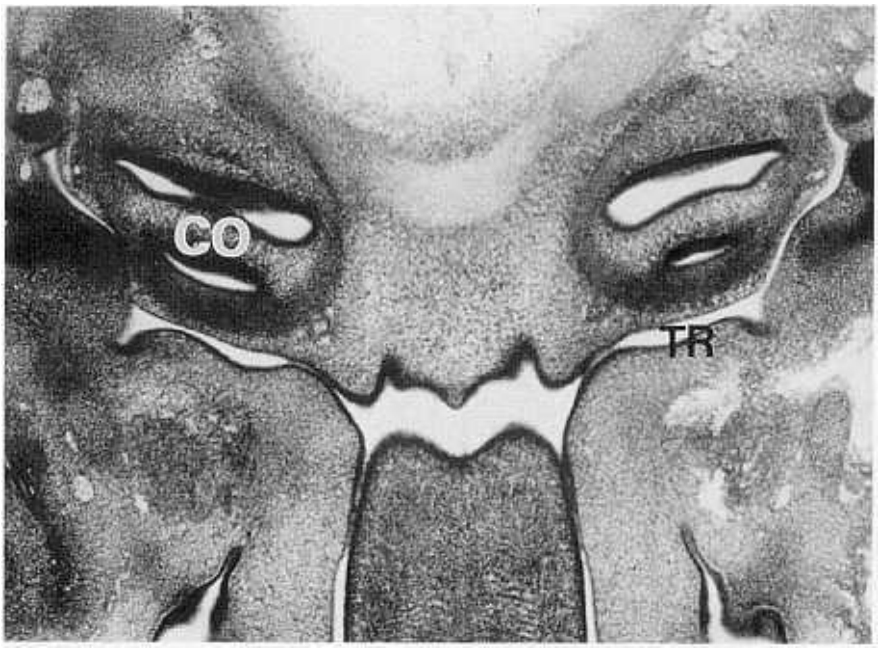

Fig. 2. The tubotympanic recess (TR) extends to the middle ear area and surrounds the cochlea (CO) in a 14-day gestational mouse (Axial, H & E, ×33).

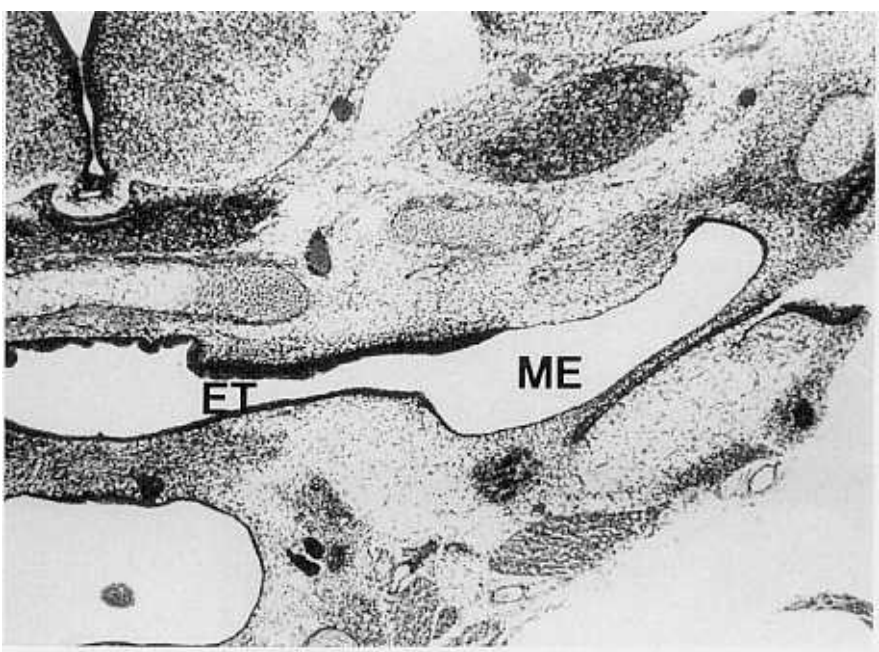

Fig. 3. The tubotympanic recess divides into the eustachian tube (ET) and middle ear cavity (ME) in a 16-day gestational mouse (Coronal, H & E, ×33).

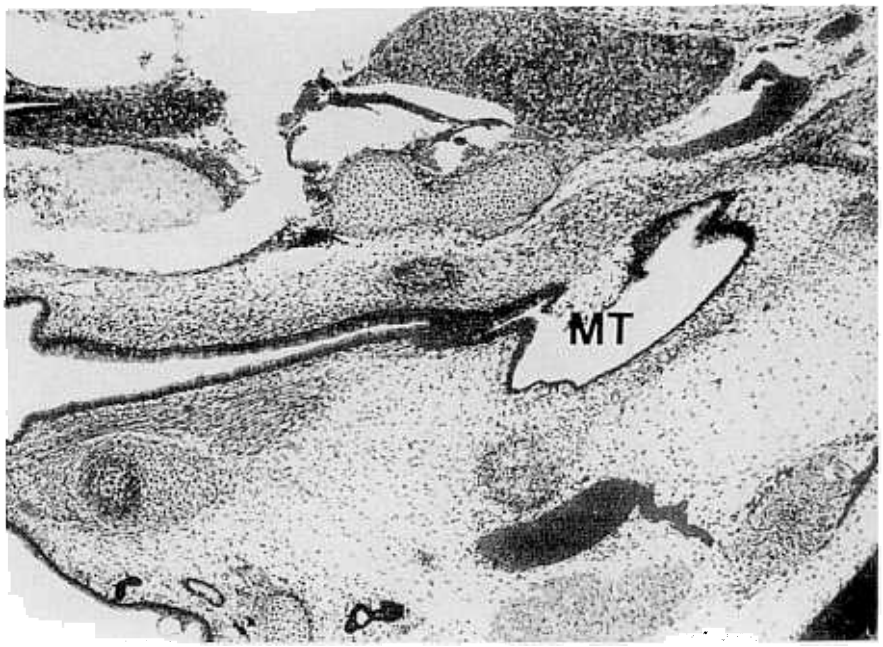

Fig. 4. The volume within the cavity of the middle ear starts to be occupied by the mesenchymal tissue (MT) in a 17-day gestational mouse (Coronal, H & E, ×33).

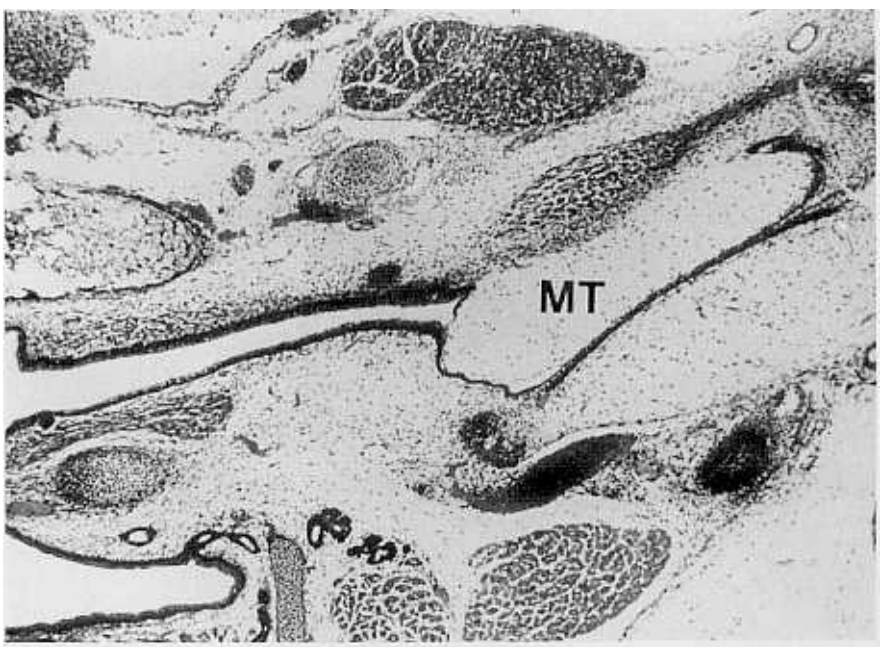

Fig. 5. Most of the future middle ear cavity is filled with the mesenchymal tissue (MT) in a 1-day postnatal mouse (Coronal, H & E, ×33).

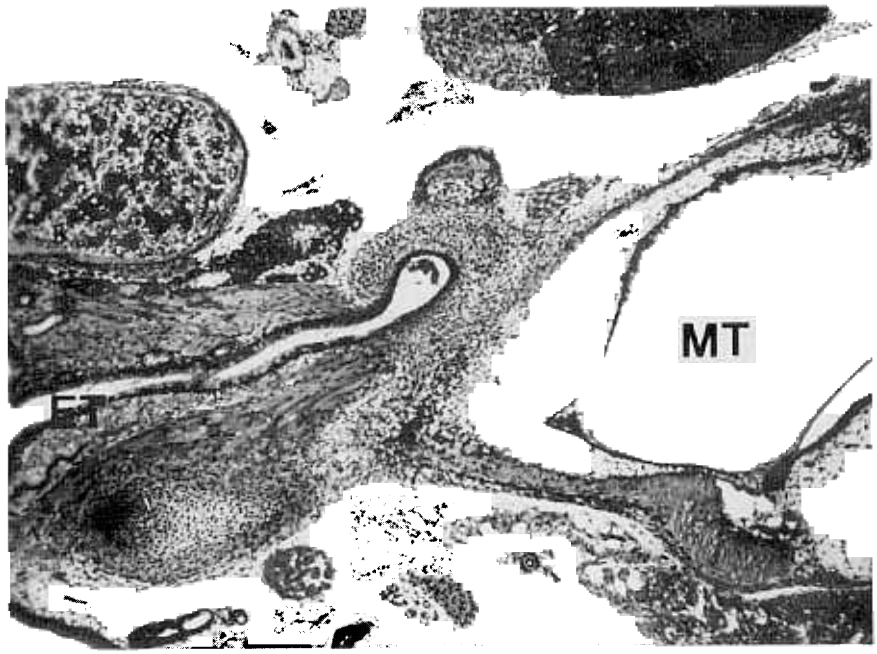

Fig. 6. The eustachin tube (ET) attains an adult form in a 9-day gestational mouse. The mesenchymal tissue (MT) is noted in the middle ear cavity (Coronal, H & E, ×33).

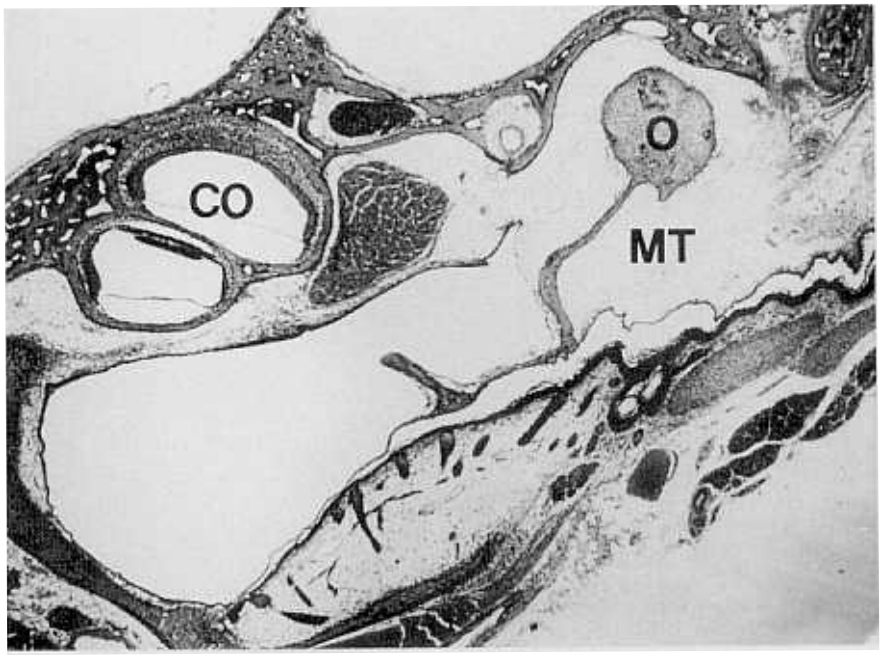

Fig. 7. The mesenchymal tissue (MT) has disappeared from the middle ear cavity in a 11-day postnatal mouse. The mesenchymal tissue is still around the ossicle (O). Cochlea-CO (Coronal, H & E, ×55).

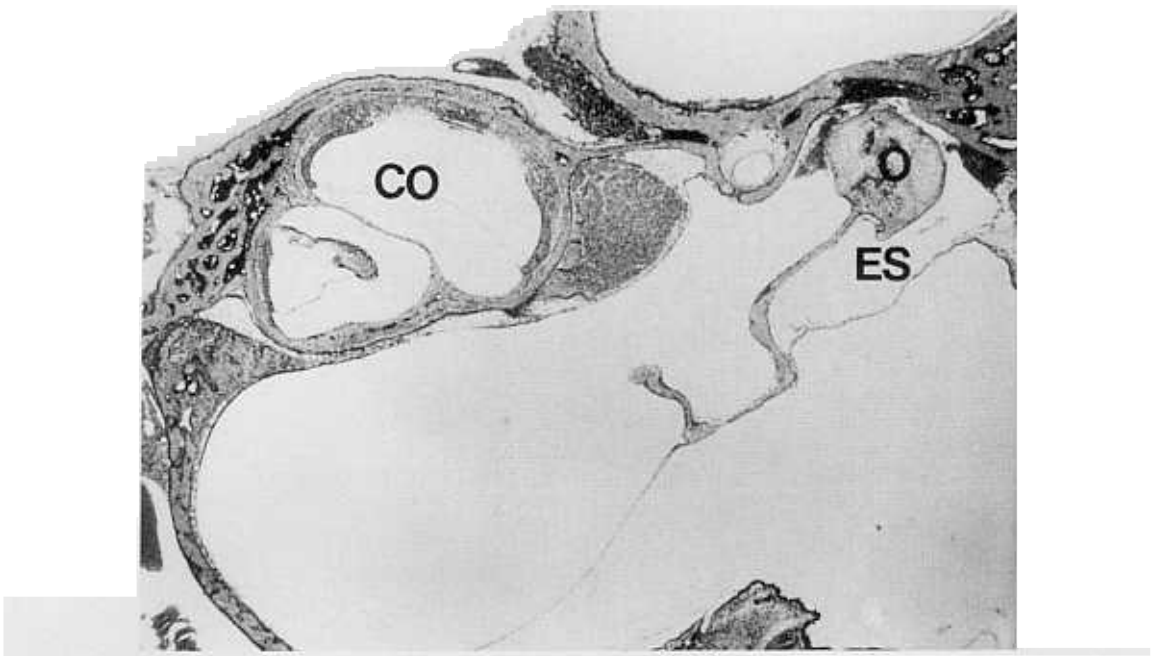

Fig. 8. The mesenchymal tissue around the ossicle (O) has disappeared and the epitympanic space (ES) attains its volume in a 15-day postnatal mouse. Cochlea-CO (Coronal, H & E, ×55).

after the rupturing of the oropharyngeal membrane, which has been already noted on 11-day gestational specimens. The lining epithelium of the first pharyngeal pouch was continuous with that of the primitive oral cavity. The tubal lumen, representing the persistence of the first pharyngeal pouch, is the only identifiable structure in the earlier embryo. In the youngest specimen studied (gestational day 11), we could not observe the tubal lumen which seemed like the first pharyngeal pouch. Between gestational day 12 and 13 the first pharyngeal pouch elongated to form the tubotympanic recess (Fig. 1), and its lumen showed a relatively smooth margin with unciliated low columnar epithelium. Between gestational day 13 and 14 the tubotympanic recess extended to the middle ear area where the stapedial primordium and the Reichert's cartilage were continuous with each other (Fig. 2). At gestational day 16 the eustachian tube and middle ear cavity became distinctly segregated (Fig. 3). As the future middle ear cavity enlarged after the segregation, its volume was occupied by loose connective tissue, which lay between the epithelium and the future bony structure. At gestational day 17, the mesenchymal tissue in the middle ear cavity began to increase (Fig. 4) and at birth most of the middle ear cavity was filled with mesenchyme (Fig. 5). At around postnatal day 9, the eustachian tube attained an adult shape (Fig. 6), and the middle ear cavity attained its adult shape near postnatal day 11 when the mesenchymal tissue in the middle ear disappeared, but it still remained around ossicle (Fig. 7). At postnatal day 15, the epitympanic space attained its adult volume by an absorption of the mesenchymal tissue (Fig. 8). By postnatal day 21, the eustachian tube and middle ear cavity attained their adult volume, which is about 0.007 mL.

Wire-frame models of the lumen of the eustachian tube and middle ear cavity at different gestational and postnatal ages were obtained through computer-aided 3D reconstruction from the histological sections (Fig. 9). The volume of the tubotympanic recess underwent a drastic change between gastational day 15 and 16, at which time the tubotympanic recess really divided into the eustachian tube and middle ear cavity (Fig. 10A). During the postnatal period, the maximum volume change occurred on postnatal day 11, when the mesenchymal tissue in the middle ear cavity disappeared completely (Fig. 10B).

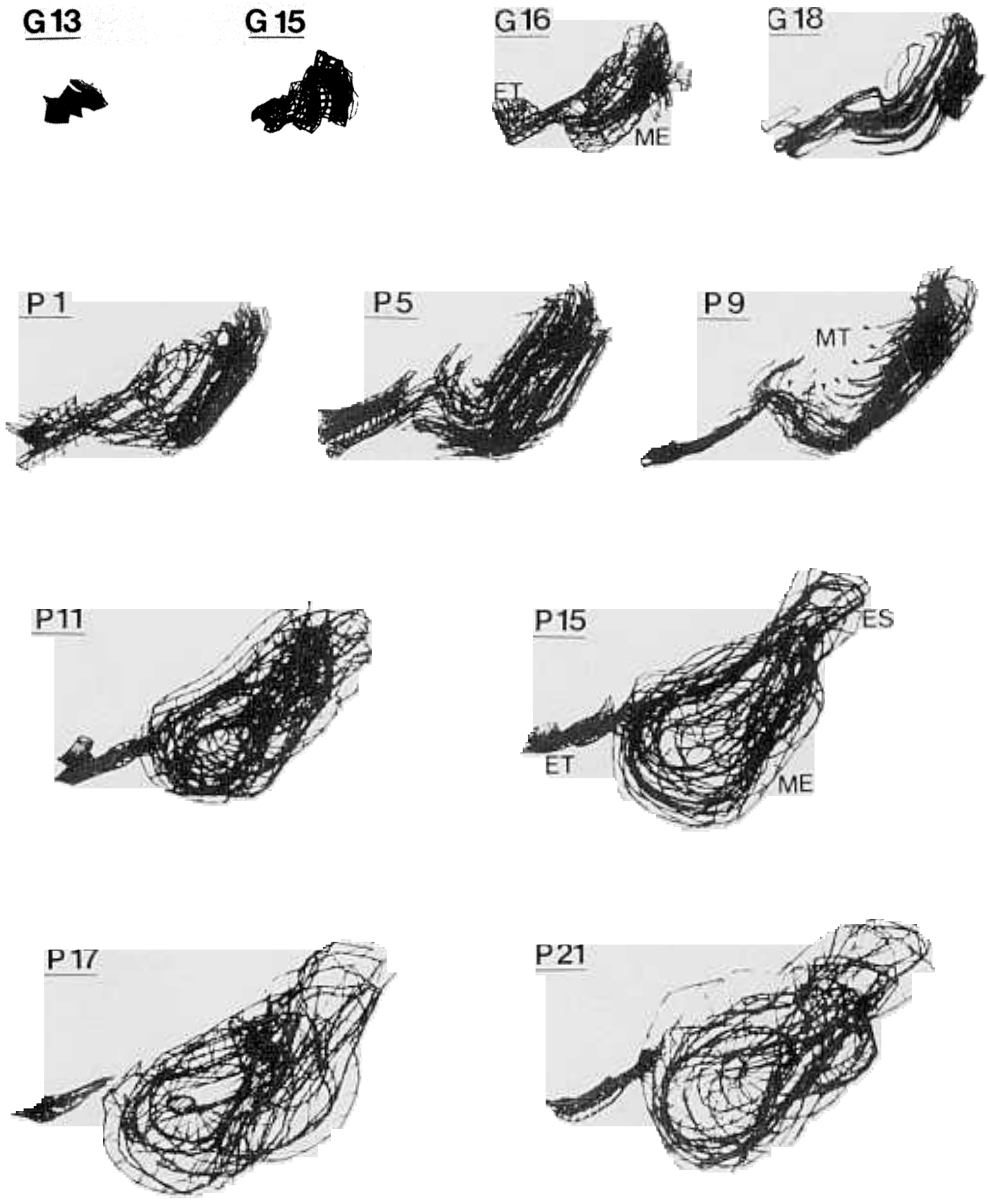

Fig. 9. Wire-frame 3D reconstruction of the lumen of the eustachian tube (ET) and middle ear cavity (ME) at different gestational (G13-18) and postnatal (P1-21) age. Mesenchymal tissue in the middle ear cavity-MT, Epitympanic space-ES (Coronal view of a right side, ×15).

DISCUSSION

The normal development of the eustachian tube and middle ear has received much attention in the field of otology. The middle ear cavity and eustachian tube are derived from the expanding terminal end of the endoderm lined the first pharyngeal pouch, which is an outpocketing of the foregut. The tubotympanic recess from the first pharyngeal

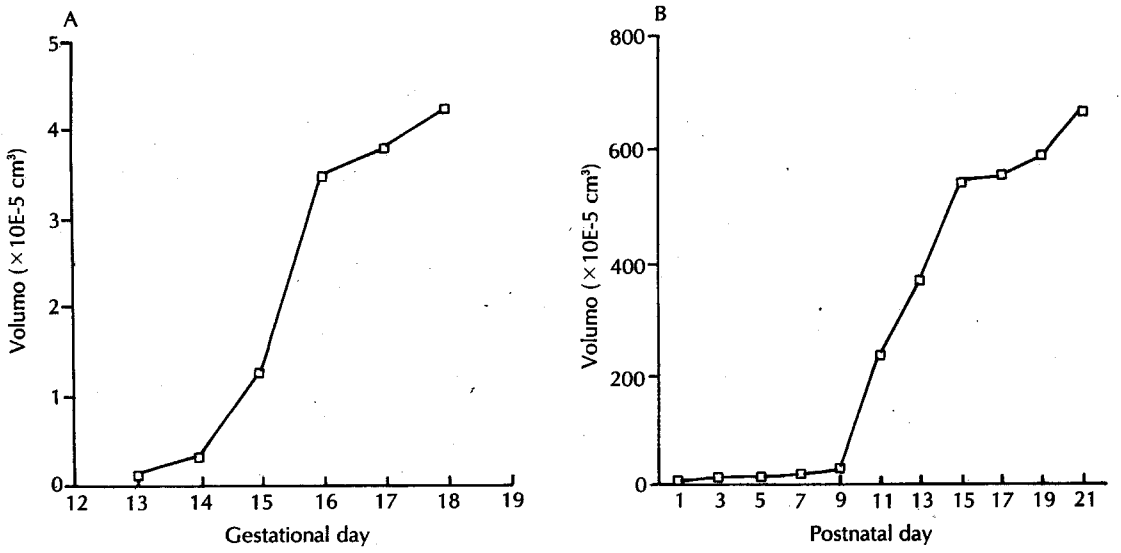

Fig. 10. *A) Graph of the incremental change of the tubotympanic recess volume during the gestation. The volume of the tubotympanic recess undergoes the maximum change before and after gestational day 15. So-called bottleneck constriction is made at gestational day 16 when the tubotympanic recess divides into the eustachian tube and middle ear cavity.*

B) Graph of the incremental change of the eustachian tube and middle ear cavity volume after birth. The maximum change of the volume is noted at postnatal day 11 when the mesenchymal tissue in the middle ear cavity disappears.

pouch undergoes a bottleneck constriction. The medial constricted portion lengthens and becomes the eustachian tube. The blind outer end of this tube expands into a flattened pouch to form the early middle ear cavity. On 12-day gestational specimens, we could confirm the tubotympanic recess which started to extend into the middle ear portion, although previous investigation had indicated that the first pharyngeal pouch in mice seemed to appear on the 11th day of gestational age (Masuda *et al.* 1986). Between gestational day 15 and 16, so-called bottleneck constriction, which meant the definite division of the tubotympanic recess into the eustachian tube and middle ear cavity, was noted, and drastic changes in the volume of the tubotympanic recess occurred at this age. Although the distal expanded part of the eustachian tube, which represents the primitive middle ear cavity, started to extend between gestational day 15 and 16, it was only a slit-like flattened space up to the 9th day after birth when the eustachian tube had already attained an adult form. Before this age the middle ear cavity is only a potential space as it is solidly filled with mesenchyme except along the course of the eustachian tube. Later at this age, the middle ear cavity radically increases in size due to absorption of the gelatinous tissue. By postnatal day 21 the eustachian tube and middle ear cavity attain their adult volume, which was already measured as about 0.006 mL (Vrettakos *et al.* 1988). Different from the pneumatization of the murine tubotympanal cavity, that of the human middle ear cavity is almost complete before birth. At 18 to 21 weeks of fetal life when the otic capsule has attained its maximum size, the middle ear cavity is becoming well defined by loosening mesenchymal tissue. By the 30 th week, the pneumatization of the middle ear cavity is complete, while the pneumatization of both middle ear cavity and epitympanic space is virtually completed during the last month of fetal life (Bast and Anson 1949).

The mechanisms involved in the luminal development during organogenesis of the eustachian tube and middle ear are not well understood. However, it has been commonly believed that the luminal development is formed by the invagination of the

tubotympanic recess and the resorption of the mesenchymal tissue in the tympanic cavity. Cell division might be accelerated and mesenchymal regression might be exhibited through connective tissue guidance in the lumen formation. The volume of the tubotympanal cavity increases in two distinct phases. The first increase occurs from gestation day 14 through 16, owing to the rapid development of the tympanal cavity. This cavity becomes prominent with the formation of bottleneck constriction of the tubotympanic recess. The later increase occurs from postnatal day 9 through 15, via the rapid resorption of the tympanal mesenchyme. It is most likely that the epithelial-mesenchymal interaction is dictated in these two distinct phases. Thus, the intercellular communication of the lumen formation in the developing stage also requires further study.

The second objective of this study was to expand our understanding of computer-aided 3D reconstruction in developmental anatomy. This 3D reconstruction has been already introduced into the otological field, and 3D computer graphic modelling of the temporal bone structures including the eustachian tube from histologic sections has been attempted (Harada *et al.* 1988; Mori *et al.* 1987; Hashimoto and Kimura 1988; Todhunter *et al.* 1984; Takai and Sando 1989). Takai *et al.* (1989) described the advantages of a 3D reconstruction technique which enables us to observe the real 3D shapes of the structures, to appreciate the 3D relationship between structures viewed from any direction and under any magnification by easy manipulation, and to do quantitative morphometry using temporal bone serial sections. Thus, the computer-aided 3D reconstruction technique gives us further valuable information about developmental anatomy, although it requires much time and more technical steps.

REFERENCES

Bast T, Anson A: *The temporal bone and the ear: The origin and development of the middle ear and related air space.* Springfield, Charles Thomas Company, *1949, 306-336*

Harada T, Ishii S, Tayama N: Three-dimensional reconstruction of the temporal bone from histologic sections. *Arch Otolaryngol Head Neck Surg 114: 1139-1142, 1988*

Hashimoto S, Kimura RS: Computer-aided three-dimensional reconstruction and morphometry of the outer hair cells of the guinea pig cochlea. *Acta Otolaryngol (Stockh) 105: 64-74, 1988*

Huangfu M, Saunders JC: Auditory development in the mouse: Structural maturation of the middle ear. *J Morphol 176: 249-259, 1983*

Ichimiya I, Ueyama S, Mogi G: Experimental otitis media in mice: Immunohistochemical observations. *Acta Otolaryngol (Stockh) [Suppl] 457: 148-153, 1988*

Lim DJ, Anniko M: Developmental morphology of the mouse inner ear: A scanning electron microscopic observation. *Acta Otolaryngol (Stockh) [Suppl] 422: 1-69, 1985*

Masuda Y, Honjo H, Naito M, Ogura Y: Normal development of the middle ear in the mouse: A light microscopic study of serial sections. *Acta Med Okayama 40: 201-207, 1986*

Mori K, Naito Y, Hirono Y, Honjo I: Three-dimensional computer graphics of the eustachian tube. *Am J Otolaryngol 8: 211-213, 1987*

Seilly DJ: A chemical test to determine the end point of EDTA decalcification. *Med Lab Sci 39: 71-73, 1982*

Takahashi M, Peppard J, Harris JP: Immunohistochemical study of murine middle ear and eustachian tube. *Acta Otolaryngol (Stockh) 107: 97-103, 1989*

Takai A, Sando I: Computer-aided three-dimensional reconstruction: A method of measuring temporal bone structures including the length of the cochlea. *Ann Otol Rhinol Laryngol 98: 515-522, 1989*

Todhunter JS, Siegel MI, Doyle WJ: *Computer-generated eustachian tube shape analysis.* In Lim DJ, Bluestone CD, Klein JO, Nelson JD, eds. *Recent advances in otitis media,* Toronto and Philadelphia, B.C.Decker Inc, *1984, 101-104*

Vrettakos PA, Dear SP, Saunders JC: Middle ear structure in chinchilla: A quantitative study. *Am J Otolaryngol 9: 58-67, 1988*