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Normal development of the middle ear in the mouse: a light microscopic study of serial sections.

Yu Masuda*
Masayuki Naito[‡]

Hiroki Honjo[†]
Yoshio Ogura**

*Okayama University,

[†]Okayama University,

[‡]Okayama University,

**Okayama University,

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Yu Masuda, Hiroki Honjo, Masayuki Naito, and Yoshio Ogura

Abstract

Development of the ear, especially the middle ear, was studied histologically in ddN and CF mice. Primordia of the 3 ossicles and the otic capsule appeared on day 12 of pregnancy. The stapelial primordium was observed as a mass of mesenchymal cells lateral to the primordium of the otic capsule, attaching to the medial part of the facial nerve. On day 13, the stapelial primordium continued to develop with the Reichert's cartilage. On day 14, the malleus and incus were differentiated. On day 15, the 3 ossicles were mostly completed in shape, and the stapelial footplate had a bilaminar structure at this stage. This structure appeared to correspond to the lamina stapelialis in the developing human stapes.

KEYWORDS: normal development, middle ear, mouse

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Normal Development of the Middle Ear in the Mouse: A Light Microscopic Study of Serial Sections

Yu Masuda, Hiroki Honjo, Masayuki Naito and Yoshio Ogura

Department of Otolaryngology, Okayama University Medical School, Okayama 700, Japan

Development of the ear, especially the middle ear, was studied histologically in ddN and CF mice. Primordia of the 3 ossicles and the otic capsule appeared on day 12 of pregnancy. The stapelial primordium was observed as a mass of mesenchymal cells lateral to the primordium of the otic capsule, attaching to the medial part of the facial nerve. On day 13, the stapelial primordium continued to develop with the Reichert's cartilage. On day 14, the malleus and incus were differentiated. On day 15, the 3 ossicles were mostly completed in shape, and the stapelial footplate had a bilaminar structure at this stage. This structure appeared to correspond to the lamina stapelialis in the developing human stapes.

Key words : normal development, middle ear, mouse

It is well-known that maternal hypervitaminosis A induces ear malformation in the fetus of some experimental animals (1-4). We have studied the histopathology of this malformation in the mouse (5), but have had some difficulties in evaluating the findings obtained since few reports on the normal development of the mouse ear have been published. To overcome these difficulties, the normal development of the mouse ear, especially the middle ear, was investigated in the present study by light microscopy of serial sections.

Materials and Methods

Fifty-four fetuses were removed from ddN and CF mice 7 to 18 days of pregnancy. All examined embryos and fetuses consisted of 8 ddN mice of 7 day of pregnancy, 3 of 8, 7 of 9, 2 of 11, 2 of 12, 2 of 13, 6 of 14, 6 of 15, 2 of 16 and 4 of 18, adding 4 CF mice in every 15, 16 and 18 day fe-

tuses; total number was 54, consisted of 42 ddN mice and 12 CF mice. Their heads were immersion-fixed with 10% formalin, embedded in paraffin, sectioned serially, stained with hematoxylin and eosin, and observed under a light microscope. Day 0 of pregnancy was regarded as the day when mice had vaginal plugs in the morning after fertilization.

Results

The otic vesicle developed from the otic pit, which appeared first in the ear region as the primordium of the membranous labyrinth on day 7 to 8.

On day 9 of pregnancy, the otic vesicle was observed as a closed sphere under the ectodermal epithelium. On day 11, the closed sphere appeared to be a vesicle, and the endolymphatic duct was observed to bud mediosuperiorly to this vesicle (Fig. 1). On day 12, a mesenchymal cellular condensa-

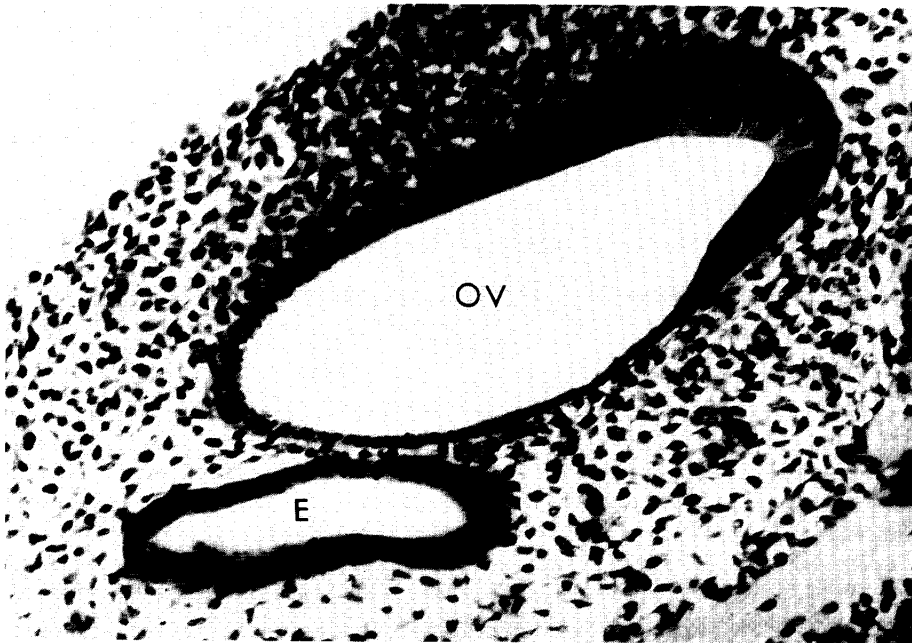


Fig. 1 Horizontal section of the inner ear of 11 day embryo. Endolymphatic duct (E) appears mediosuperiorly to the otic vesicle (OV). ddN mouse, Hematoxylin-eosin, $\times 66$.

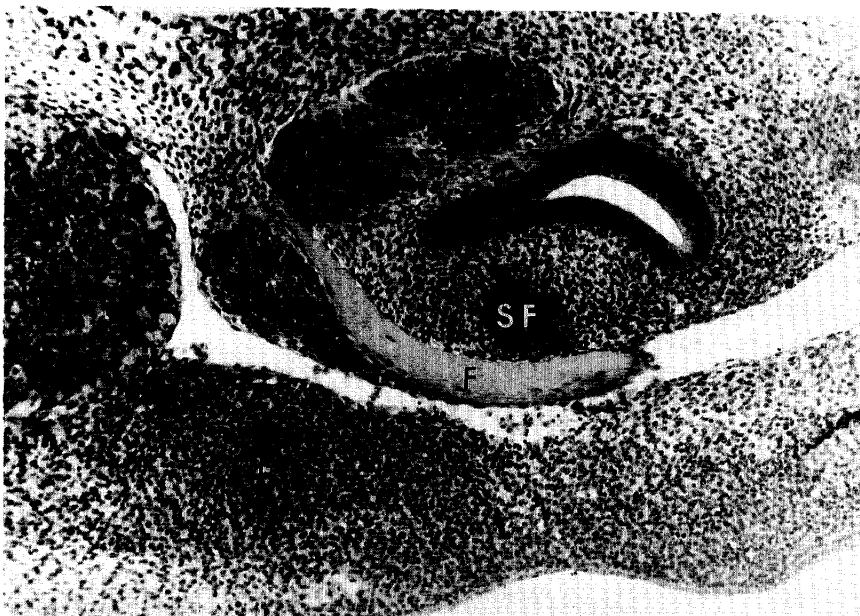


Fig. 2 Horizontal section of 12 day embryo, showing relations between the stapedial footplate, facial nerve and other ossicles. Stapes footplate (SF) appears medially to the facial nerve (F). Malleus and incus (MI) are seen laterally to the horizontal portion of the facial nerve (F) with the geniculate ganglion (GG). ddN mouse, Hematoxylin-eosin, $\times 33$.

tion, clearly recognized as the primordium of the otic capsule, occurred around the membranous semicircular canals. Differentiation of the border between the inner ear and the middle ear became clearer at this stage since the developing facial nerve transversely ran along the lateral side of the vestibular portion. Another mesenchymal cellular condensation, regarded as the primordia of the malleus and incus, was observed lateral to the anterior cardinal vein. The developing stapelial primordium was located in the area of the otic capsule adjacent to the facial nerve (Fig. 2).

On day 13, the first pharyngeal pouch (namely, the tubotympanic recess) extended to the middle ear region. The Reichert's cartilage was observed anterior to the descending portion of the facial nerve. A more differentiated stapelial primordium was

continuous with Reichert's cartilage. The Meckel's cartilage appeared in the mandibular arch.

Between day 12 and 13, the developing membranous labyrinth formed the vestibular portion. However, the malleus was still fused with the incus at this stage. On day 13, the membranous labyrinth had the semicircular canals, vestibule and cochlear duct. The malleus and incus were clearly differentiated at this stage. The tubotympanic recess extended to the middle ear area where the stapelial primordium and the Reichert's cartilage were continuous with each other (Fig. 3).

On day 14, the stapes separated from the Reichert's cartilage. The main portion of this cartilage appeared as a styloid process and had a position lateral to the vertical portion of the facial nerve (Fig. 4).



Fig. 3 Horizontal section of 14 day embryo, showing relations between primordia of 3 ossicles and Meckel's cartilage. Primordia of 3 ossicles (M malleus; I incus; S stapes) and Meckel's cartilage (MC) are histologically continuous with each other. Vertical portion of the facial nerve (F) and styloid process (SP) are together. The stapes is penetrated by the stapelial artery (SA). Periotic tissue is developing near the vestibule (V). ddN mouse, Hematoxylin-eosin, $\times 33$.

Fig. 4 Frontal section of 15 day embryo, showing appearance of the bilaminar structure in the stapedial footplate and cartilagification of adjacent area of the inner ear. Cartilaginous development of the otic capsule (OC) and incus is shown. The stapes (S) is still precartilaginous, and its footplate is seen as a bilaminar structure at the vestibular surface. The facial nerve (F) and stapedial artery (SA) are seen in cross section. CF mouse, Hematoxylin-eosin, $\times 33$.



Fig. 5 Horizontal section of 15 day embryo, showing the view of developing ear centering around the stapedial footplate. Precartilaginous stapes (S) has the laminar structure of the footplate and developing annular ligament (arrow) is seen clearly. The perilymphatic space (PS) arises at the vestibular portion. The vertical portion of the facial nerve (F) does not have an anterior canal wall yet. The tympanic cavity (TC) is expanding as an elongated tubotympanic recess. ddN mouse, Hematoxylin-eosin, $\times 33$.

Table 1 Development of the mouse ear

Day of pregnancy	External and middle ear	Inner ear
7	(1 st and 2 nd branchial arches are developing)	Otic pit
9	(Forelimbs)	Otic vesicle
11	(1 st pharyngeal pouch)	Endolymphatic duct Acoustic ganglion
12	Stapedial primordium appears with other ossicles developing in a mass Facial nerve running transversely at the vestibular portion Tubotympanic recess extending into the middle ear portion Reichert's cartilage continuous with the stapedial primordium	Membranous semicircular canals developing
13	Stapedial primordium clearer Malleus and incus fused together and continuous with Meckel's cartilage	
14	Malleus and incus not yet differentiated	Shapes of membranous semicircular canals almost complete
15	Malleus and incus separated Annular ligament developing Middle ear cavity extending Stapedial footplate having bilaminar structure	Cochlear duct twisting 2 turns Ampullar cristae developing
16	Tympanic ring clearer Ectodermal epithelial plate completely formed	Periotic tissue developing
18	Three ossicles having individual cartilaginous forms Ectodermal epithelial plate having a narrow space forming the external auditory canal Auricle seals orifice of external auditory canal	Cochlear duct shows 2 turns Periotic tissue developing

The malleus and incus were still continuous with the Meckel's cartilage.

On day 15, all the ossicles were observed as completely independent entities with individual shape. The stapes showed a bilaminar structure in the footplate (Fig. 5).

The above described developmental process of the mouse ear is summarized in Table 1.

Discussion

The developmental process of the main parts of the mouse ear is very similar to that of the human ear described in several reports (6-9). The formation of the otic vesicle was observed on day 9 of pregnancy

in the mouse and at the beginning of the 5 th week in man (6, 7). Comparisons of the developing steps of the ear in the mouse and man are shown in Table 2 composed of data from this study and previous reports.

Little ossification of the otic capsule was observed at birth in the mouse; all the ossicles were mostly cartilaginous. Organ of Corti was not formed and did not have ridges on the 18 th day of pregnancy in this study. The stapedial primordium appeared inside the transverse portion of the facial nerve on day 12 in the mouse, as in the man (8). In this stage, the footplate of the stapes was observed as a mass of cells in a group of mesenchymal cells gathered around the otic capsule. As a result of progress in developmental biology, development of mes-

Table 2 Differences in the embryonal ages of the mouse and man during the development of the ear

Findings of the developing ear	Embryonal ages	
	Mouse (Days)	Man (Weeks)
Formation of the otic vesicle	9 ^e	5 ^{a,b}
Stapedial primordium appears	12	5 ^a
Malleus and incus in the same mass	12	6 or more ^a
Reichert's cartilage continuous with stapedial primordium	12	8 ^c
External completion of membranous labyrinth	15 ^f	8 or more ^b
Stapes footplate has bilaminar structure	15	9 or more ^d
Malleus and incus separate histologically	15 ^f	12 ^c
Three ossicles have definite shape	18 ^f	16 ^c
Beginning of ossification of cartilaginous otic capsule	18 or more ^e	16 or more ^c

a: Bast and Anson (6). b: Patten (7).

c: Masuda (8). d: Masuda *et al.* (9).

e: Rugh (10). f: Masuda *et al.* (11).

enchymal parts in the head and neck region have been found to depend upon the migration of neural crest cells (12). In a recent experimental study using a quail-chick chimera technique, grafting quail cells from the neural crest into the neural crest portion of the chick revealed that neural crest cells migrated and differentiated into mesodermal cells and mesenchymal derivatives such as the otic capsule and ossicles (13). This theory has gained appreciation recently, and the ossicles are now thought to originate from several sources including neural crest cells in vertebrates. Thus, the development of the ossicles is very complicated and difficult to delineate.

The normal development of the stapedial footplate has received much attention in the field of otology. In this study, the bilaminar structure of the footplate consisted of an outer area including pale mesenchymal cells and an inner area of dark fibroblastic cells (Fig. 5). This structure is very similar to

that described by Cauldwell and Anson (14) in the lamina stapedialis observed in human embryos from 25 mm to 30 mm of the crown-rump length. Bast and Anson (6) and Masuda *et al.* (9) described the structure in terms of an inner layer and outer layer. The bilaminar structure of the footplate in the mouse and that in man seems to correspond to each other.

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Reprint requests to:

Yu Masuda

Department of Otolaryngology

Okayama University Medical School

2-5-1 Shikata-cho

Okayama 700, Japan