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Yu Masuda* Ryusuke Saito[†] Yoichi Endo[‡]
Yutaka Kondo** Yoshio Ogura^{††}

*Okayama University,

[†]Okayama University,

[‡]Okayama University,

**Okayama University,

^{††}Okayama University,

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Yu Masuda, Ryusuke Saito, Yoichi Endo, Yutaka Kondo, and Yoshio Ogura

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HISTOLOGICAL DEVELOPMENT OF STAPES FOOTPLATE IN HUMAN EMBRYOS

YU MASUDA, RYUSUKE SAITO, YOICHI ENDO,
YUTAKA KONDO and YOSHIO OGURA

*Department of Otolaryngology, Okayama University Medical School
Okayama 700, Japan (Director: Prof. Y. Ogura)*

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Abstract. Normal development of the human stapes footplate was investigated in serial sections by light microscopy. Materials were obtained from 35 Japanese embryos from the 6th to 32nd week of embryonal age. Eighteen embryos up to 16 weeks of age (3.5mm to 105mm in crown-rump length) were examined, focusing particularly on the lamina stapedialis of the otic capsule. The present study showed that primordial formation of the lamina stapedialis appeared in 16mm embryo and that the lamina was completely formed and fused to the base of the annular stapes in a 35mm embryo. In a 50mm embryo, the adult form of stapes was found with a rim and annular ligament. The results, therefore, seemed to essentially agree with the theory of dual origin and development of the footplate proposed by Cauldwell and Anson, and teratogenic agents might affect any stage of the process producing anomalies, including congenital footplate fixation, congenital absence of the oval window and calcification of the annular ligament.

Key words: development of stapes, stapes footplate, footplate

In the recent decade, surgical correction of middle ear anomalies has been performed to improve hearing (1-3). Severity of stapedia anomalies, including its absence, is especially important, because it directly influences the results of the operation (4-6). In a majority of the cases, fenestration operation or stapes surgery are usually indicated. The results and procedures of stapes surgery depend largely upon the condition of the stapes footplate.

In many cases of stapedia anomalies, such as congenital footplate fixation, congenital absence of stapes or the oval window, calcification of the annular ligament and bony fusion of the footplate to the otic capsule, favorable surgical results are difficult (7). In the occurrence of these anomalies, a histological understanding of the lamina stapedialis as advanced by Cauldwell and Anson (8) is important.

In the present study, investigations were conducted on the relationships between embryonic development and clinical abnormalities in stapes footplate.

MATERIALS AND METHODS

Materials used were 35 human embryos ranging from the 6th to the 32nd week of embryonal age (Table 1). The normal development of the stapes footplate was investigated in histological serial sections stained by hematoxylin and eosin by light microscopy, and occasionally by phase contrast microscopy.

TABLE 1. MATERIAL COLLECTED FROM 35 JAPANESE HUMAN EMBRYOS

Embryonal age (weeks)	Crown-rump length (mm)	Crown-heel length (mm)	Number
6	3.5		1
8	9.0		1
9	13.5-19.0		8
10	20.0-22.5		3
11	35.0		1
12	50.0-53.3		2
13-16	90.0-105.0		2
17-32		222.0-395.0	17
Total			35

Eighteen embryos of earlier stage measuring from 3.5mm to 105mm of crown-rump (C. R.) length were observed to examine the lamina stapediale of the otic capsule to clarify the relationship between the lamina stapediale and the annulus stapediale derived from the second branchial arch and formed main portion of the stapes (Fig. 1).



Fig. 1. Two sources of human stapes (From Cauldwell and Anson (8)).

- a Reichert's cartilage
annulus stapediale * greater mass of the stapes
- b the lateral capsular wall of otic capsule
lamina stapediale * basal perichondrium
* vestibular surface of the footplate
* stapediale rim
* annular ligament

RESULTS

In 3.5 mm embryo, the otocyst was already developing. However, no structures were evident in the middle ear region.

In 13.5 mm embryo, the primordium of the stapes was initially ring-shape with the stapediale artery adjacent to both the facial nerve and Broman's "interhyale". The stapes, stapediale artery and "interhyale" were derived from

Reichert's cartilage. In this stage, the mesenchymal cellular condensation, regarded as the primordium of the otic capsule, surrounded the footplate region of the stapedia primordium. Between these two tissues, a slight linear lamellous structures were partially seen (Fig. 2).

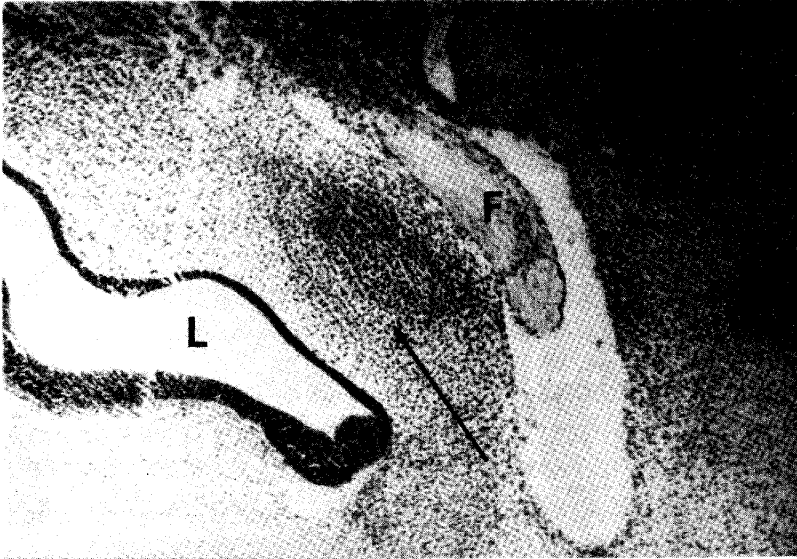


Fig. 2. First appearance of the primordium of lamina stapedialis (arrow). L, membranous labyrinth; F, facial nerve. From 13.5 mm embryo. $\times 100$.

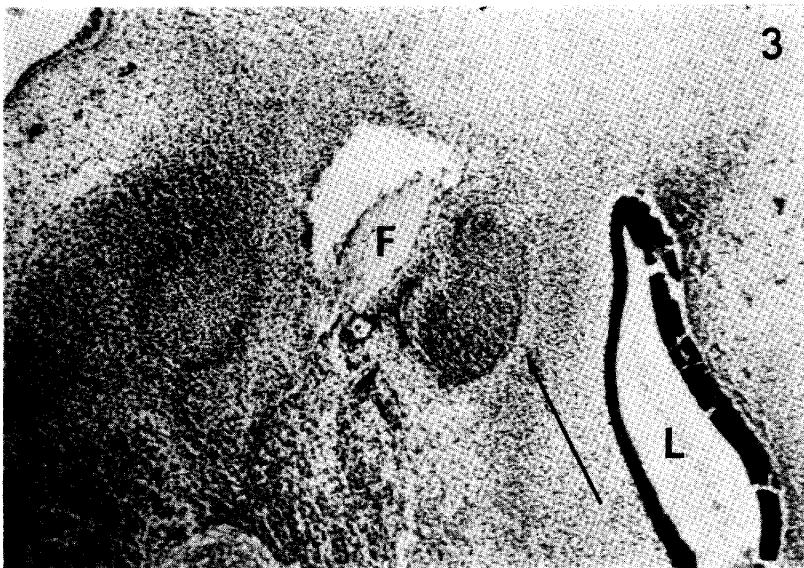


Fig. 3. Area of clearness in the primordium of lamina stapedialis (arrow). L, membranous labyrinth; F, facial nerve. From 16 mm embryo. $\times 100$.

In 16mm embryo, the lamellous structures were relatively clear and could be regarded as the primordium of the lamina stapediais. In this stage the stapediais artery persisted (Fig. 3). In 19mm embryo, the stapediais primordium was situated in the precartilaginous otic capsule. In 35mm embryo, the precartilaginous stapes had a completely cartilaginous lamina stapediais (Fig. 4).

In 50mm embryo, the general shape of the stapes attained the completed form (Fig. 5 a). The marginal portion of the lamina stapediais showed degeneration to the fibroblastic cell tissue. This tissue was differentiated in staining tendency from neighboring areas around it. This indicates initiation of the annular ligament. In the intermediate portion of the margin of the lamina, multiplying fibrous cells were forming the stapediais ring (Fig. 5 b). On the other hand, the innermost layer to the vestibule situated in the central portion of the footplate was changing to the vestibular perichondrium. This layer was still partially composed of fibroblastic cells with a deeply stained nuclei. Outward and close to that layer, a bright cartilaginous cell area was seen. It was probably derived from the lamina stapediais, and more externally, a very thin layer of fibroblastic cells was observed. It was most likely the changing primitive perichondrium of the base of the annulus stapediais. Above these layers, the cartilaginous mass of ringshaped stapes was seen (Fig. 5 c).

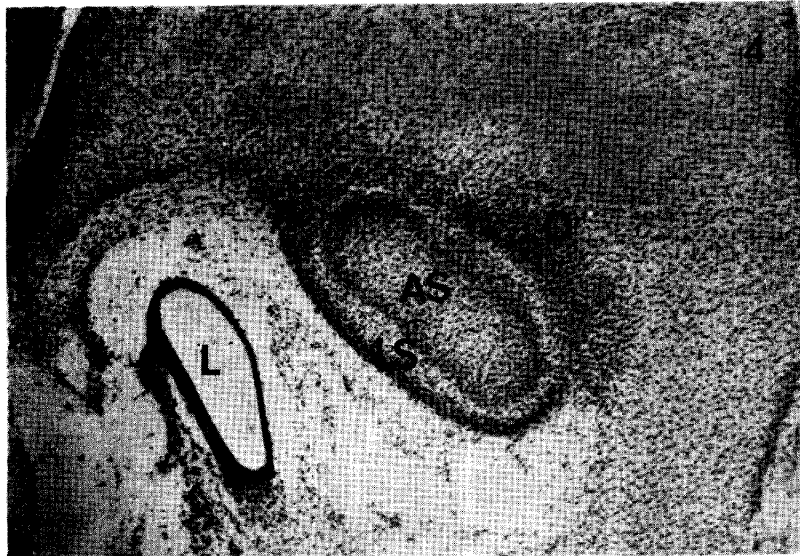


Fig. 4. Completion of cartilaginous lamina stapediais (LS) between the primordium of annulus stapediais (AS) and the otic capsule (O). From 35mm embryo, $\times 100$.



Fig. 5a. Completion of the cartilaginous stapedial footplate and crura. From 50mm embryo. $\times 100$.



Fig. 5b. Differentiation of the stapedial rim in the posterior margin of the footplate (arrow). From 50mm embryo. $\times 400$.

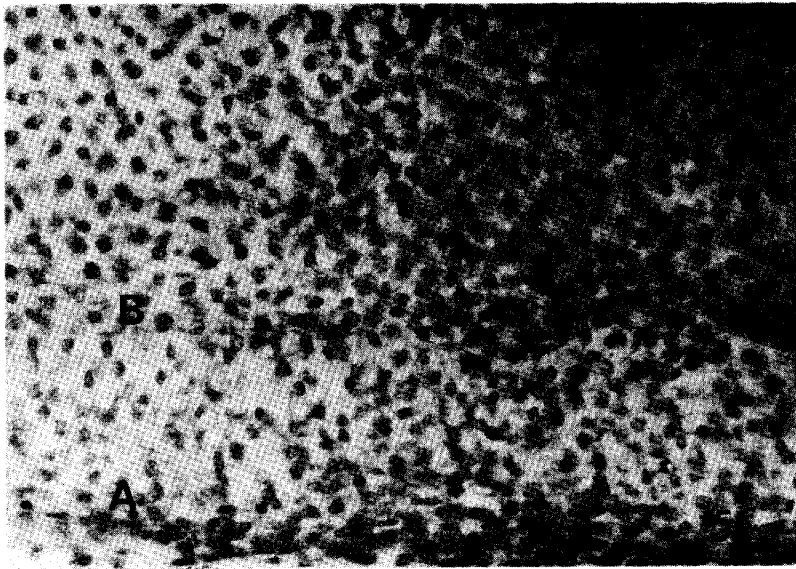


Fig. 5c. Developing stapes footplate. A; vestibular perichondral layer; B; basal perichondral layer. From 50 mm embryo. $\times 400$.

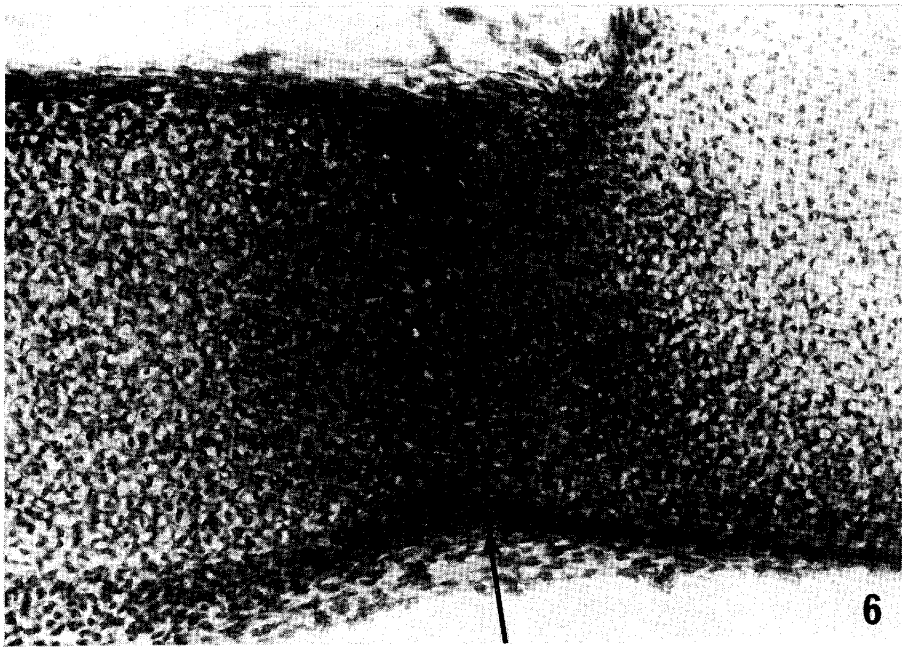


Fig. 6. Differentiation of the annular ligament in the posterior margin of the stapes footplate (arrow). From 105 mm embryo. $\times 200$.

At the 105 mm stage, it was difficult to histologically differentiate the annulus stapedialis and the lamina stapedialis. The stapes appeared as a complete homogenous structure. The marginal portion of the stapedial rim, however, was undergoing a process to become annular ligament according as degeneration (Fig. 6).

DISCUSSION

According to Cauldwell and Anson (8) and Bast and Anson (9), the lamina stapedialis histologically emerges in 30 mm embryos as a deeply stained compact layer though the primordial lamina stapedialis seems to appear in 25 mm embryos. These investigators have pointed out the bridge of precartilage extends between the cochlea and canalicular portion of the capsule and separates the stapes from the labyrinthine otic cavity. This was termed the primordial lamina stapedialis. In 30 mm embryos, this bridge becomes thinner and more deeply stained and appears to consist of two cellular zones. One is a pale-staining layer in apposition to the basal perichondrium of the primitive stapes. The other is a dense layer of fibroblastic tissue stretching across the future fenestra vestibuli. The former is called the outer layer and the latter the inner layer.

We observed the primordial lamina as a partial condensation of mesenchymal cells around the primordial stapes buried in the otic capsule (Fig. 2). At the 16 mm stage, the primordial lamina becomes clearer (Fig. 3). These areas were considered the same as the lamina stapedialis of Cauldwell and Anson, and Bast and Anson (8, 9).

Since the nineteenth century, many opinions have been reported on stapes development (6, 10, 11). In anatomy recently, the stapes is considered deriving from Reichert's cartilage, and that on the other hand, the malleus and incus are believed arising from Meckel's cartilage (12-14). Cauldwell and Anson and Bast and Anson, however, reported the validity of the nineteenth century opinion of the dual origin of the footplate (8, 9). Then, the thought of dual development of the stapes came out to the light.

According to Anson, the true fenestra vestibuli appears in the 43 mm embryo, and by this time, the lamina stapedialis is gradually differentiated to the annular ligament from the marginal portions and formed the fenestra. In this process, cartilaginous tissue underwent dedifferentiation from the annular ligament and stapedial rim. In 50 mm embryo, the fenestra vestibuli was completely formed with the ligament and rim. However, in all other areas of otic capsule cartilaginous growth was in progress.

Two possible developmental sources of the footplate are recognized: Reichert's cartilage from the second branchial arch and the otic capsule. The former derives the annulus stapedialis of mesenchymal tissue and develops and changes

from precartilaginous to cartilaginous, and the latter derives the lamina stapedialis of mesenchymal tissue and develops and changes from precartilaginous to cartilaginous, partially dedifferentiate to fibroblastic tissue. Thus, two developmental processes differentiated from each other fused to form the footplate and oval window. Fig. 7 schematically shows these relationships.

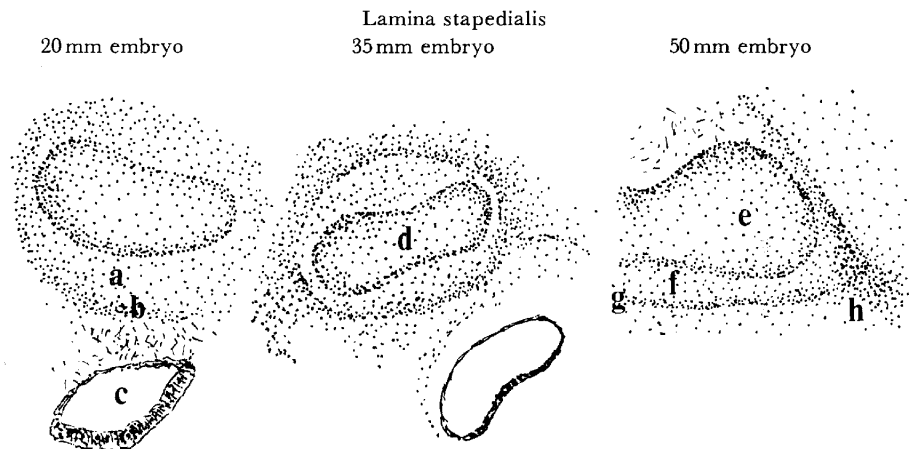


Fig. 7. Schematic drawing of the development of the stapes footplate. a, Pale staining outer layer; b, dense, fibroblastic inner layer; c, membranous labyrinth; d, primitive footplate; e, mature cartilage; f, perichondrium; g, young cartilage; h, annular ligament.

In conclusions, from our present results on Japanese embryos, we agree with the view of the dual origin of the stapes footplate.

In our embryonic observations, primordial lamina appears at the 3.5 mm stage and becomes clear at the 16 mm stage. This primordial area was considered the same as the lamina stapedialis of Cauldwell-Anson, and Bast and Anson.

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