The Development of the Ear of Rabbit Embryo

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Abstract : **The ear consists of three parts which are of different origin but function as one unit. The internal ear originates from the surface ectoderm covering the lateral sides of myelencephalon at the fourth week. This ectoderm thickens to form the otic placode and then invaginates to form the otocyst and splits from the surface ectoderm. The otocyst or otic vesicle divides into 2 parts, the ventral cochlear and the dorsal utricular portions. The cochlear portion gives rise to the saccule and the cochlear duct while the utricular portions gives rise to the utricle, semicircular ducts and endolymphatic duct. These epithelial structures so formed are known as the membranous labyrinth. The bony labyrinth and the perilymphatic space originate from the mesenchymal otic capsule. The middle ear, consisting of the tympanic cavity and the auditory tube, are lined with epithelium of the endodermal origin of the first pharyngeal pouch. The ear ossicles, the malleus and incus are derived from the first and the stapes from the second arch cartilages. The external auditory meatus develops from the first pharyngeal cleft, while the tympanic membrane originates from the ectoderm at the bottom of the first cleft with the endoderm of the first pouch and the mesenchyme between. In order to understand ear development, pig and chick embryos were used in the laboratory studies. Since the pig embryos are presently not available, this study compared the ear development of the pig and rabbit embryos, which indicate that the ear of the pig and rabbit develop in the same manner and the rabbit embryos can be used in the future instead of pig embryos for studying ear development.**

Key words : Embryo, Ear, Otocyst

เรื่องย่อ : การเจริญพัฒนาการของหู

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หู ประกอบด้วยส่วนสำคัญ 3 ส่วน ที่มีต้นกำเนิดที่ต่างกัน แต่ร่วมกันทำงานอย่างเป็นระบบ หูชั้นใน พัฒนามาจากเนื้อเยื่อผิว เอ็คโตเดิม บริเวณสมองส่วนปลาย เจริญหนาตัวขึ้น และหวำเข้าด้านในจนกลายเป็นถุง โอติก เวสิเคิล แล้วพัฒนาต่อไปเป็นส่วน เมมเบรนัส ลาบิริน ของหูชั้นใน ส่วนกระดูกที่ล้อมรอบและช่องว่างที่ล้อมรอบ เจริญมาจาก มีโซเดิม หูชั้นกลางเจริญมาจากเอ็นโดเดิม ที่บุฟารินเจียล เพาช์ที่หนึ่ง กระดูกหูเจริญมาจากกระดูกอ่อน ประจำ ฟารินเจียล อาร์ช ที่หนึ่งและสอง หูชั้นนอกส่วนรูหูเจริญมาจากเอ็คโตเดิมของ ฟารินเจียล กรูฟที่หนึ่ง และเยื่อแก้วหูเจริญมาจาก เอ็คโตเดิม ของกรูฟที่หนึ่งร่วมกับเอ็นโดเดิมของเพาช์ที่หนึ่ง และมีโซเดิมที่แทรกอยู่ตรงกลางเล็กน้อย ในการเรียนการสอนช่วงพัฒนาการของหูในห้องปฏิบัติการวิทยาเอมบริโอ ของภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล ใช้ตัวอ่อนหมูและไก่ ตัดสีเรียล เพื่อดูถุงโอติกเวสิเคิล และเนื้อเยื่อใกล้เคียง แต่ขณะนี้เราไม่สามารถหาตัวอ่อนหมูมาทำสีเรียลเซ็คชั่นได้อีก จึงจำเป็นต้องศึกษาการพัฒนาการของหูจากสัตว์อื่น จากการศึกษานี้พบว่าตัวอ่อนกระต่ายมีการพัฒนาของหูเหมือนกับที่เห็นในตัวอ่อนหมู จึงสามารถใช้แทนกันได้ เมื่อถึงเวลาที่สไลด์สีเรียลเซ็คชั่นตัวอ่อนหมูมีไม่เพียงพอ

INTRODUCTION

The ear consists of three anatomical parts : external, middle and internal. The external and middle parts are mainly concerned with transferring the sound waves while the internal ear, which contains the vestibulocochlear duct, is concerned with the equilibrium and hearing.

The internal ear is initially formed in the fourth week after conception, as a thickening of the surface ectoderm, the otic placode, which appears on each side of the myelencephalon. The otic placode soon invaginates and sinks into the underlying mesenchyme, forming the otic pit. The edge of the otic pit soon comes together and fuses to form an otic vesicle or otocyst, the primordium of the membranous labyrinth. The otic vesicle soon loses its connection with the surface ectoderm. A diverticulum forms medially at the otocyst and then elongates to form the endolymphatic duct and sac. Two regions of the otocyst soon become recognizable : the dorsal utricular portion from which the utricle, semicircular ducts and the endolymphatic duct arise, and the ventral saccular protion which gives rise to the saccule and cochlear duct. Three flat disc-like diverticula grow from the utricular portion. The central area of each diverticulum fuses and soon degenerates. The peripheral part of each diverticulum becomes the semicircular duct which opens into the utricle. The ampulla develops at one end of each semicircular duct. Sensory nerve endings differentiate in the ampullae at the crista ampullaris and in the utricle and saccule at the macula acousticae. The cochlear duct originates from the ventral saccular portion as an elongated and coiled tube to form the cochlea which connects to the saccule. The spiral organ (Organ of Corti) differentiates from cells in the wall of the cochlear duct. Ganglion cells of the eighth cranial nerve migrate along the coils of the cochlea to form the spiral ganglion and send nerve processes to terminate on the hair cells of the spiral organ.

The mesenchyme surrounds the otocyst aggregates to form the mesenchymal otic capsule which soon differentiates into the cartilaginous otic capsule. As the membranous labyrinth enlarges, vacuoles appear in the cartilaginous otic capsule and coalesce to form the perilymphatic space. The membranous labyrinth is now suspended in fluid, the perilymph, in the perilymphatic space. This space which relates to the cochlear duct develops in two divisions, the scala tympani and the scala vestibuli. The remaining cartilaginous otic capsule later ossifies to form the bony labyrinth of the internal ear.

The middle ear develops from the first pharyngeal pouch which expands at the distal end to become the tympanic cavity and constricts at the proximal end to become the eustachian tube. As the tympanic cavity expands, it gradually engulfs the middle ear bones, their tendons, ligaments and the chorda tympani nerve. The development of the middle ear bones are from the first and the second arch cartilages. The external ear, the external auditory meatus and the auricle, develops from the first

branchial groove, the hyomandibular groove. The tympanic membrane originates from the first branchial membrane which comprises the ectoderm of the first groove and the endoderm of the first pouch. The mesenchyme later invades between both layers to become the fibrous layer of the tympanic membrane. The ear auricle develops from the six swellings, six ear hillocks, arising around the margins of the first groove[1-3].

In the embryonic period, the formation of the organ of hearing and equilibrium, the otocyst, can be studied in the serial section of the 36 somites chick embryo and the 10-15 mm. pig embryos. The latter also reveals evidence of medial growth of the endolymphatic duct. The otocyst is associated with the ganglia of the seventh and eighth cranial nerves, the acousticofacial ganglion. The condensation of the mesenchyme around the otocyst is the mesenchymal otic capsule.

For the second-year medical students studying the development of organ systems at the Department of Anatomy, Faculty of Medicine Siriraj Hospital, serial sections of chick and pig embryos are used as comparative models of human development. However, there is a lack of pig embryos for preparing new slides to replace those which are lost every year. Moreover, the number of medical students is increasing, thereby creating a growing demand for such slides. In reality there are many ways to solve this problem. The easier way, as in other medical schools, is for student to study developmental biology using only textbooks and atlases instead of tracing serial section slides. However, at Siriraj, we still believe that the better way to generate an understanding of the way organs develop is by tracing serial sections of embryos. Therefore, it is important to study the normal development of mammal embryos, such as those of rats, mice[4-11] and rabbits which are easier to obtain for slide preparation. The objectives of this study are to find and demonstrate the suitable stage of the ear development in rabbit embryos and then compare them with the standard 10-15 mm. pig embryos. These findings can also serve as models for medical students in studying human development in the Laboratory of Embryology in the future.

MATERIALS AND METHODS

Maternal rabbits *(Oryctolagus cuniculus)* with their embryos were obtained from the Department of Animal Laboratory, AFRIMS. They were bred and then fed until their embryos reached 12 and 15 days following conception. At these respective stages, the maternal rabbit was injected with an overdose of an anesthetic drug. A low midline incision was done. The uterus, containing embryos at the proper stage was dissected from the abdominal cavity. Each embryonic mass was separated from the others and placed in Bouin's solution for fixation for at least 24 hours. The process of removing the excess fixative from the specimen was done by placing it in 70% ethyl alcohol. The solution was changed dialy until the fixative was entirely removed, which was determined by observing the color of the specimens which gradually changed from yellow to white. The embryos were dissected from the uteri and placed in 70% ethyl alcohol. They were then dehydrated, cleared, embedded and serially sectioned. The mounted sections were stained with hematoxylin. The 4-14 mm. rabbit embryos were carefully studied at the level of myelencephalon to observe the developing otocyst. Then they were compared with the ear development of the 10-15 mm. pig embryos.

RESULTS

1. The transverse section of 4-5 mm. rabbit embryos (sections cutting through the myelencephalon)

At this stage, the otocyst has already lost its connection with the ectoderm. The detached ovoid sac is situated midway between the surface ectoderm and the hindbrain. The wall of the otocyst is made up of a tall columnar epithelium, which is surrounded by the normal distribution of the head mesenchymal cells without condensation of the mesenchymal otic capsule. The anterior cardinal vein is prominent and takes over the venous drainage from the head region, situated between the otocyst and the surface ectoderm. On the rostral side of the otocyst is the mass of aggregated nerve cells that represent the temporary facial-acoustic ganglia, which will later

separate into geniculate and acoustic subdivisions. The acoustic portion of the common ganglionic mass is in contact with the otocyst wall (Figure 1,2).

2. The transverse section of 12-14 mm. rabbit embryos (sections cutting through the diencephalons ventrally and the myelencephalon dorsally).

The otocyst of this stage shows slightly advanced development. It is situated between the myelencephalon and the head ectoderm. The myelencephalon shows a thin-walled roof plate and the typical arrangement of the brain wall. The interval between the two portions of the brain, myelencephalon dorsally and diencephalon ventrally, contains the unpaired basilar artery and the infundibulum evaginates from the diencephalon. The otocyst wall comprises the unequal thickness of epithelial cells. The dorsal part or the utricular part of the otocyst is composed of somewhat shorter cells than the ventral part or the saccular part of which the cells appear more in the columnar epithelium. The medial aspect of the otocyst shows the endolymphatic duct, growing medially toward the brain wall. The acousticofacial ganglion is located ventrally to the otocyst, and yet still cannot be distinguished between the seventh and the eighth ganglia of the cranial nerve. The mesenchymal cells condense surrounding the otocyst to form the so-called mesenchymal otic capsule which will become the cartilaginous capsule in the older stage (Figure 3,4).

3. The transverse section of 10 mm. pig embryo.

The otocyst of the 10 mm. pig embryo is situated between the myelencephalon of the hindbrain and the head ectoderm. It is a large cavity surrounded by columnar epithelial cells of equal size. Associated ventrally is the acousticofacial ganglion of the eighth and seventh cranial nerves. More ventral to the acousticofacial ganglion is the larger trigeminal ganglion situated beside the rhombomere of the metencephalon. The otocyst is relatively larger and more advanced than that observed in the 4-5 mm. rabbit embryo. As yet there is no subdivision of the otocyst into the utricular or saccular portion. The surrounding mesenchyme is still loosely spread without condensation around the otocyst to form the mesenchymal otic capsule (Figure 5).

4. The transverse section of 15 mm. pig embryo.

The otocyst of the 15 mm. pig embryo shows far more advanced development than the 10 mm. It is situated beside the myelencephalon and is already surrounded by a dense aggregation of mesenchyme which is called the mesenchymal otic capsule. The otocyst can be divided into two subdivisions, the dorsal utricular and the ventral saccular portions. Figure 6 shows the utricular portion of the otocyst with the outgrowth of the three semicircular ducts, the posterior, the lateral and the anterior. There is also one outgrowth bud in the medial direction toward the brain wall, the endolymphatic duct. The epithelial cells of the utricular portion of the otocyst are not equal in height, as they are rather thick at the end of each sprout and rather thin elsewhere. The myelencephalon shows the typical three layers, the ependymal, the mantle and the marginal layer surrounded by the external limiting membrane. The mesenchyme around the brain is loosely arranged, with the ganglia of the seventh and the fifth cranial nerves lying close to the brain wall.

DISCUSSION

The ear consists of a sound-conducting apparatus and a receptive sense organ. The reception and transmission of sound waves are the functions of the external and middle ears. The end organ proper is the internal ear, with the auditory sensibility residing in the cochlear duct. The remainder of the internal ear, the semicircular ducts, utriculus and sacculus, serves as an organ of equilibrium.

The internal ear develops earlier than the other two parts. It originates as a thickening of the surface ectoderm covering the hindbrain to form the so-called otic placode. The otic pit and the otic vesicle or otocyst are formed as a consequence. Approximately at the point where the otocyst joins the ectoderm, a tubular recess, the endolymphatic duct, pushes out straightaway as a new growth and then shifts to the medial portion.

The development of the internal ear observed in the 10 mm. pig embryo can be compared with that of the 4-5 mm. rabbit embryo. In both the otocyst is already formed and detached from the head

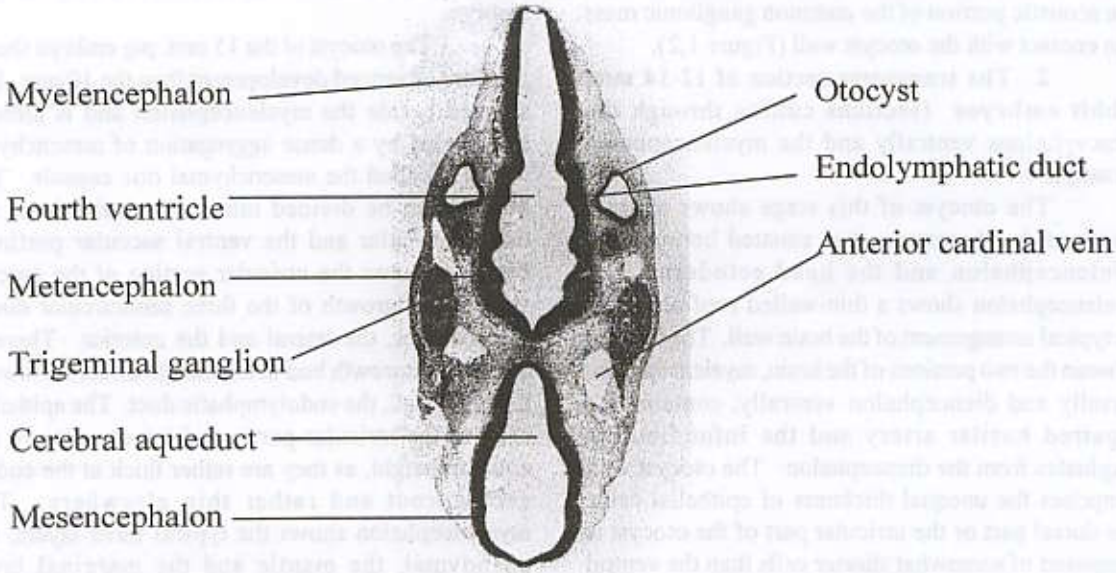

Figure 1. Transverse section of rabbit embryo 4-5 mm. through the internal ear (otocyst).

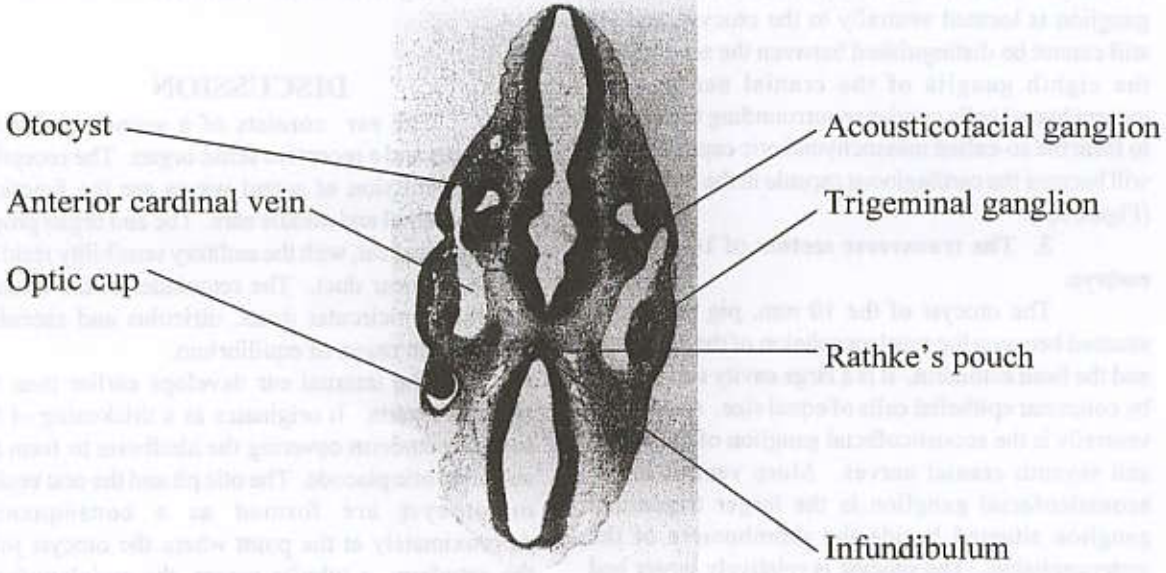

Figure 2. Transverse section of rabbit embryo 4-5 mm. through the internal ear (otocyst).

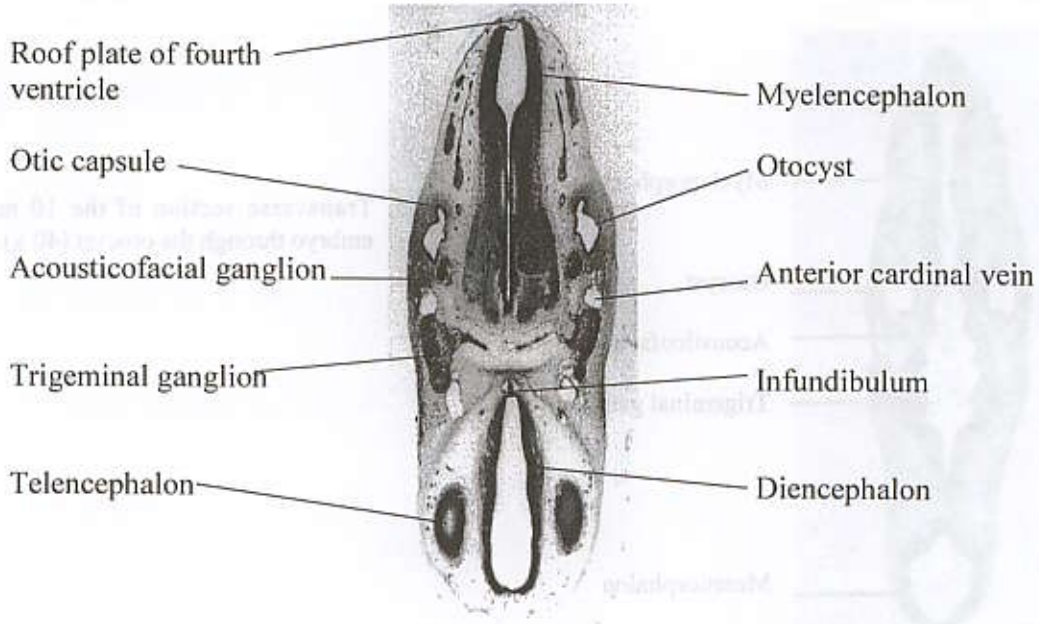

Figure 3. Transverse section of 12-14 mm. rabbit embryo through the otocyst and brain vesicle.

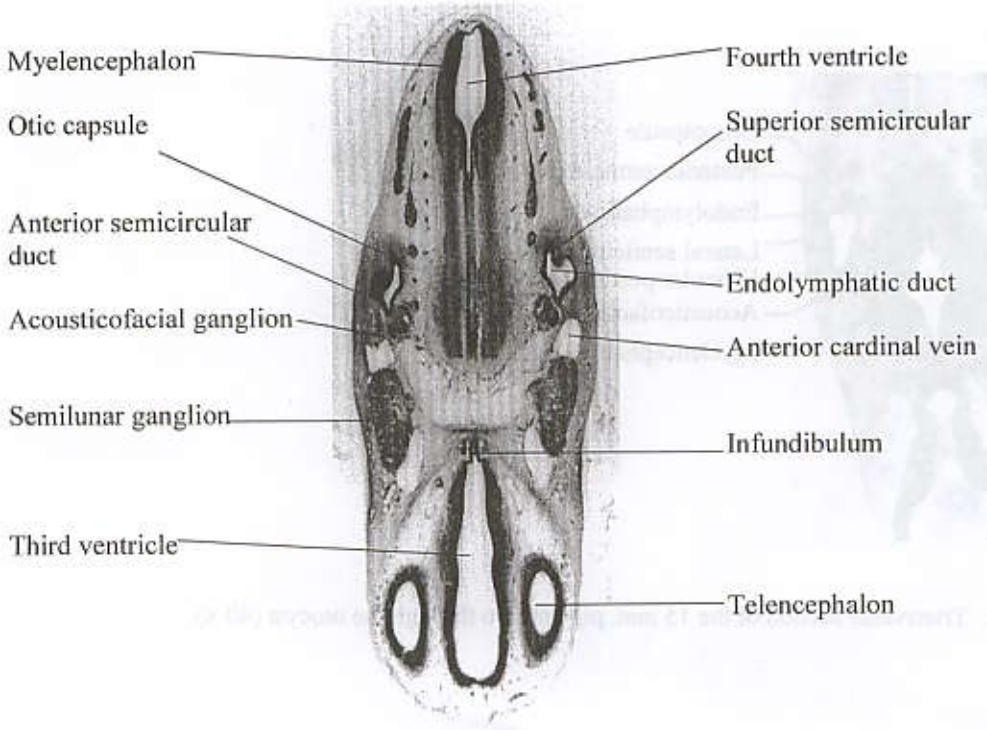

Figure 4. Transverse section of 12-14 mm. rabbit embryo through the superior semicircular canal.

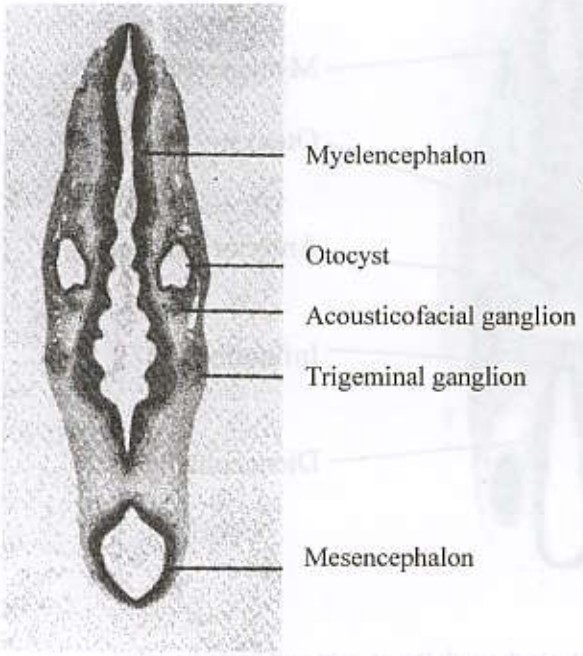

Figure 5. Transverse section of the 10 mm. pig embryo through the otocyst (40 x).

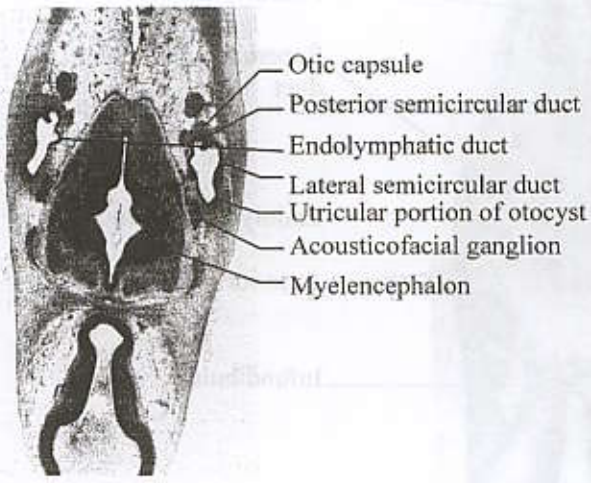

Figure 6. Transverse section of the 15 mm. pig embryo through the otocyst (40 x).

ectoderm by the double fusion of the otic pit. The epithelial cells of the otocyst are columnar in shape and equal in size, without evidence of any subdivision. The surrounding mesenchyme is not yet aggregated and is evenly distributed. The acousticofacial ganglion is closely associated with the otocyst at the ventral position and cannot be divided into the seventh and eighth cranial nerve. This stage of the ear development can be compared with the fourth and fifth week of human embryo development.

The 12-14 mm. rabbit embryos show further development of the otocyst and can be compared with the 15 mm. pig embryos. The otocyst can be divided into the dorsal utricular and the ventral saccular portions. The utricular portion gives the appearance of an outgrowth of the semicircular duct primordia, and one projects medially toward the brain wall, the endolymphatic duct. The mesenchyme around the otocyst is aggregated to form the mesenchymal otic capsule. The ganglion associated very closely to the saccular portion is the acoustic ganglion, which can be distinguished from the seventh ganglion. The 12-14 mm. rabbit embryos and the 15 mm. pig embryos can be compared with the human embryo at about the sixth week of development.

This study indicates the identical nature of the ear development of the rabbit, pig and human embryos. The rabbit embryos of about 4-14 mm. are suitable for use as models for studying ear development, since the pig embryos are not available for slide preparation.

CONCLUSION

The ear development of the 10 mm. pig embryo can be compared with the 4-5 mm. rabbit embryo. Both of them exhibit the otocyst beside the hindbrain. The otocyst at this stage cannot be divided into the utricular or saccular portion. The surrounding mesenchyme is not condensed to form the otic capsule. This stage can be compared with the fourth to fifth week of human ear development. The 12-14 mm. rabbit embryo can be compared with the 15 mm. pig embryo and the sixth week of the human embryo. At this stage the otocyst can be divided into the dorsal utricular and the ventral saccular portions. The endolymphatic duct grows medially toward the myelencephalon. The surrounding mesenchyme is aggregated to form the mesenchymal otic capsule which will eventually form the cartilage and the perilymphatic space. The rabbit embryo is very suitable to use as a model for medical education in the embryology lab since the pig embryo is not available.

REFERENCES

1. Langman J. Medical embryology. 4th ed. London : William and Wilkins, 1981: 199-227.
2. Moore KL. The Developing human. Clinically oriented embryology. 4th ed. London: WB Saunder, 1988: 286-332.
3. Arey LB. Developmental Anatomy. A textbook and laboratory manual of embryology. 7th ed. London : WB Saunders, 1966: 340-64.
4. Pilakasiri K. The heart of rat embryo. Siriraj Hosp Gaz 1988;**40:** 725-30.
5. Rojananin J. The observation of serial sections of rat embryo related with pig and chick embryos. Siriraj Hosp Gaz 1987; **39:** 617-23.
6. Rojananin J. The development of the mice embryo's heart. Siriraj Hosp Gaz 1992; **44:** 964-73.
7. Rojananin J. The development of the ear. Siriraj Hosp Gaz 1993; **45:** 233-9.
8. Rojananin J, Sangvichien S. Development of face and nasal cavities. Siriraj Hosp Gaz 1992; **44:** 430-5.
9. Rojananin J, Sangvichien S. Development of the branchial apparatus in rat, pig and chick embryos. Siriraj Hosp Gaz 1991; **43:** 847-51.
10. Rojananin J, Sangvichien S. Development of the respiratory system. Siriraj Hosp Gaz 1992; **44:** 21-7.
11. Rojananin J. Development of the urinary system. Siriraj Hosp Gaz 1993; **45:** 7-13.