The Development of the Rabbit Eye

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Abstract : **Serial sections of the rabbit embryos measuring 4-14 mm. were carefully studied to compare the developing eye with 36 somites chick embryos and 10-15 mm. pig embryos. The eyes of the 4-5 mm. rabbit embryos were at an earlier stage than those of the 36 somites chick embryos, but at about the same stage as the 10 mm. pig embryos. The eye development of the 4-5 mm. rabbit embryos were about the same as those of the fifth-week human embryos. At this stage, the optic cups had already formed but some of the lenses had incompleted double fusion. The eyes of the 12-14 mm. rabbit embryos were somewhat identical to the 15 mm. pig embryos and can be compared to the sixth week human embryos. At this stage the optic cups were divided into the outer pigment and inner nervous layers; the lens was characterized by a thinner anterior lens epithelium and the longer posterior lens fiber. The mesenchyme surrounding the optic cups of this stage showed a slight condensation to form the vascular and fibrous coats of the eyeball. The rabbit embryos of 4-14 mm. were more suitable for use as laboratory models in studying eye development than of the pig embryos since the latter were no longer available for slide preparation.**

Key words : Embryo, eye, optic cup, lens.

เรื่องย่อ : **การเจริญพัฒนาการของลูกตา**

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การเจริญพัฒนาการของลูกตา ศึกษาจากเล็คชั่นตัวอ่อนกระต่ายขนาด 4-14 มม. เปรียบเทียบกับตัวอ่อนไก่ขนาด 36 โสไมท์ และตัวอ่อนหมูขนาด 10 และ 15 มม. พบว่าพัฒนาการของลูกตากระต่ายขนาด 4-5 มม. อยู่ในขั้นที่อ่อนกว่าของตัวอ่อนไก่ 36 โสไมท์ และอยู่ในขั้นเดียวกับตัวอ่อนหมูขนาด 10 มม. ซึ่งเทียบได้กับตัวอ่อนมนุษย์อายุครรภ์ประมาณ 5 สัปดาห์ กล่าวคือ ออปติคคัพ มีผนัง 2 ชั้น ส่วนเลนส์ตาของบางตัวยังไม่มีการเชื่อมเป็นถุง ส่วนลูกตาของตัวอ่อนกระต่ายขนาด 12-14 มม. มีลักษณะเหมือนกับของตัวอ่อนหมูขนาด 15

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มม. และเทียบได้กับตัวอ่อนมนุษย์อายุครรภ์ ประมาณ 6 สัปดาห์ กล่าวคือผนังของออปติคคัพสามารถแยกเป็น 2 ชั้น คือ ผนังชั้นนอกบางมีเม็ดสี ส่วนผนังชั้นในหนา เป็นชั้นของเซลล์ประสาท และเซลล์รับความรู้สึก ส่วนเลนส์ตามีลักษณะเป็นถุง หุ้มด้วยเซลล์ด้านหน้าเป็นเซลล์สี่เหลี่ยมลูกบาศก์ ส่วนเซลล์ด้านหลังเป็นเซลล์ตัวยาว ยังคงมีช่องภายในเลนส์อยู่ ส่วนมีเซนไคม์ที่ล้อมรอบออปติคคัพ เริ่มรวมกลุ่มจนหนาตัว เพื่อเจริญเป็นชั้นหลอดเลือด และชั้นไฟบรัสหุ้มลูกตา จากการศึกษานี้พบว่าตัวอ่อนกระต่ายมีการพัฒนาการของลูกตาคล้ายตัวอ่อนหมู และตัวอ่อนมนุษย์ จึงสามารถใช้ตัวอ่อนกระต่ายแทนตัวอ่อนหมูในการเรียนการสอนวิชาวิทยาเอมบริโอได้ เมื่อเราไม่สามารถหาตัวอ่อนหมูมาทำสไลด์ได้อีกต่อไป

INTRODUCTION

The developing eye appears in day 21-22 of the human embryo as a pair of shallow grooves of neuroectoderm on each side of the developing forebrain, the optic sulcus. Then the groove forms the out-pocketing of the forebrain, the optic vesicle. The vesicle subsequently comes into contact with the surface ectoderm and induces changes in the ectoderm necessary for lens formation. Shortly thereafter, the optic vesicle begins to invaginate and forms the double-walled optic cup. The inner and outer layers of this cup are initially separated by a lumen, the intraretinal space, but soon this lumen disappears and the two layers are then apposed to each other. The invagination is not only restricted to the central portion of the cup but also involves a part of the inferior surface, the choroid fissure. Formation of this fissure enables the hyaloid artery to reach the inner chamber of the eye. During the seventh week, the lips of the choroid fissure fuse and the mouth of the optic cup become a round opening, the future pupil. While these events occur, the cells of the surface ectoderm, initially in contact with the optic vesicle, begins to elongate to form the lens placode. This placode subsequently invaginates and develops into the lens vesicle. During the fifth week, the lens loses its contact with the surface ectoderm and then locates itself in the mouth of the optic cup.

The outer layer of the optic cup is characterized by the appearance of small pigment granules and is known as the pigment layer of the retina. The inner nervous layer of the optic cup is more complicated. In the posterior part, known as the pars optic retinae, the cells bordering the intraretinal space differentiate into light - receptive elements, the rods and cones. Adjacent to the photoreceptive layer is the mantle layer which, as in the brain, gives rise to the neurons and supporting cells. The nerve fibers of the ganglion cells converge toward the optic stalk which develops gradually into the optic nerve. The anterior one-fifth of the inner layer, known as the pars caeca retinae, later divides into pars iridica retinae and pars ciliaris retinae.

Shortly after the formation of the lens vesicle, the cells of the posterior wall begin to elongate in an anterior direction and form long fibers which gradually fill the lumen of the vesicle. By the end of the seventh week these primary lens fibers reach the anterior wall of the lens vesicle. Growth of the lens is continuously added to the central core.

The cornea is formed from the outside by a layer of surface ectoderm and the underlying mesenchyme. The corneal stroma, formed from the mesenchyme, is continuous with the sclera and the endothelial layer bordering the anterior chamber.

There are many congenital ocular abnormalities, particularly the congenital cataract of the lens, the non-closure of the anterior chamber resulting in the coloboma iridis or retinae and the congenital glaucoma etc[1-3].

Second-year medical students study the development of the organ systems at the Department of Anatomy, Faculty of Medicine Siriraj Hospital, by using serial sections of chick and pig embryos as comparative models of human development. However, there is a problem in preparing new slides because pig embryos are presently not available. Moreover, the number of medical students is increasing, thereby

creating a growing demand for such slides. There are several ways to solve this problem. For example, in many other medical schools students study developmental biology by using textbooks and atlases instead of tracing serial section slides. But here, at Siriraj, we still believe that the best way to generate understanding of how organs develop is by tracing serial section slides. Therefore, it is important to study the normal development of other mammal embryos, such as those of rats, mice[4-11] and rabbits, which are easier to obtain for slide preparation. The objective of this study was to find the suitable stages of eye development in the rabbit embryos and compare them with those in the standard chick and pig embryos. These findings can also serve as a reference model for medical students in studying human eye development in the Laboratory of Embryology in the future.

MATERIALS AND METHODS

The maternal rabbits (*Oryctolagus cuniculus*) with their embryos were obtained from the Department of Animal Laboratory, AFRIMS. They were bred and later fed until their conceptions were 12 and 15 days, respectively. At each proper stage, the maternal rabbit was injected with an overdose of an anesthetic drug. A low midline incision was made. The uterus, at the proper embryonic stage, was dissected from the abdominal cavity. Each embryonic mass was separated from the other and placed in Bouin's solution for fixation for at least 24 hours. The process of removing the excess fixative was performed by placing it in 70% ethyl alcohol. The solution was changed daily until the fixative was entirely removed by observing the color of the specimen which gradually changed from yellow to white. The embryos were dissected from the uteri and placed in 70% ethyl alcohol. They were dehydrated, cleared, embedded and serially sectioned. Then the mounted sections were stained with hematoxylin. The optic cup at the region of developing diencephalons was observed under a light microscope. The eye development of the 4-14 mm. rabbit embryos were compared with that of the 10 mm. pig embryo and the chick embryo.

RESULTS

1. The transverse section of 4-5 mm. rabbit embryos. (sections cutting through the developing eye) (Figure 1, 2)

The optic region of the 4-5 mm. rabbit embryos in serially transverse section was located more caudally than the otic region. Because of the flexion of the head, the brain vesicle was cut twice, ventrally in the forebrain and dorsally in the hindbrain. The first two pairs of branchial arch are located between the forebrain and hindbrain. Further development of the optic vesicle is achieved when its proximal portion becomes constricted to form the optic stalk which connects to the brain while the distal portion invaginates to form the double walled optic cup. The outer wall of the optic cup is thin and loses its neuronal capacity, while the inner wall is thick, comprised mainly of nerve cells and photoreceptor cells. The cavity between the two layers, the intraretinal space, connects with the third ventricle of the diencephalon by the cavity in the optic stalk which is still broad and short. The thickened-surface ectoderm, the lens placode, invaginates into the cavity of the optic cup to form the lens pit. The lens pit is incompletely fused to form the lens vesicle. The epithelium of the lens vesicle is columnar-shaped and of equal size throughout the vesicle. The surface epithelium covering the lens is thin. There is a groove between the developing eye and the maxillary prominence which is called the nasolacrimal groove.

2. The transverse section of the 4-14 mm. rabbit embryos. (section cutting through the developing eye) (Figure 3, 4)

The developing eye at this stage is far more advanced and the flexion of the head becomes less obvious, bringing the optic and otic regions into view in the same transverse section. The optic cup joins the diencephalon with the optic stalk. It consists of double walls, the outer pigment and the inner nervous layers, which are separated by a cavity known as the intraretinal space. This space joins the third ventricle of the diencephalon by the cavity in the optic stalk which will eventually obliterate. The outer pigment layer of the optic cup is thin, comprising a thin layer of pigment cell which will soon elaborate

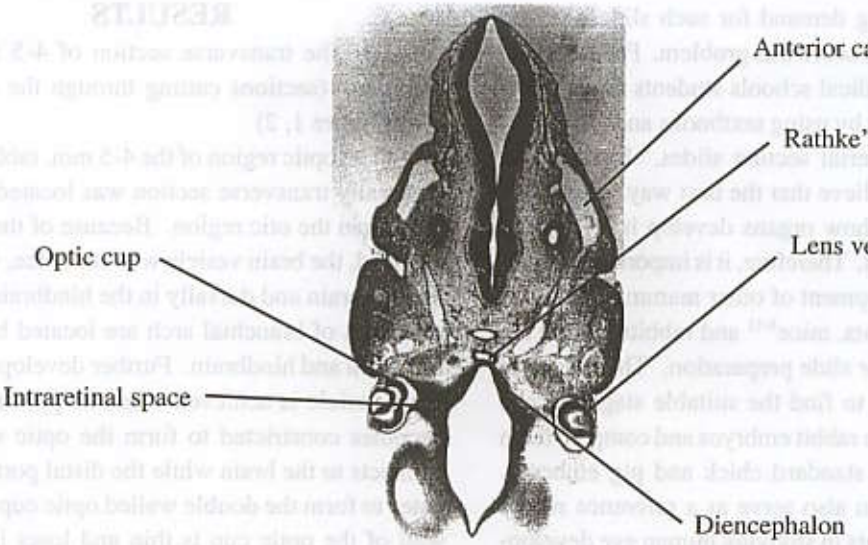

Figure 1. Transverse section of 4-5 mm. rabbit embryo through the eye.

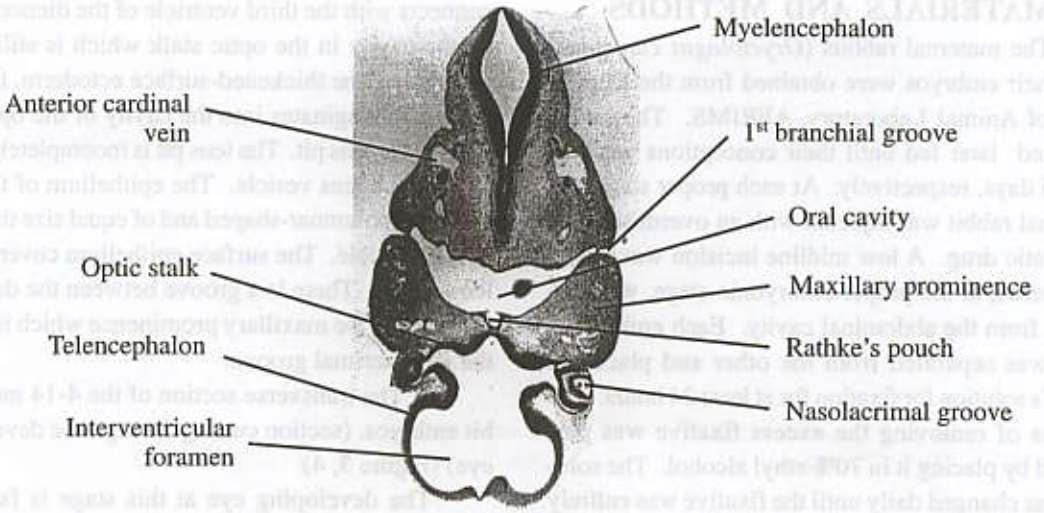

Figure 2. Transverse section of 4-5 mm. rabbit embryo through the eye.

on the pigment granules in the cytoplasm. The inner nervous layer is thick and forms several layers of neurons, which later become the photoreceptive cells and ganglion cells. The nerve fibers of the ganglion cells converge to form the optic nerve while the cavity in the optic stalk will soon obliterate. The lens vesicle, which is located in the cavity of optic cup, is composed of two distinct layers, the anterior lens epithelium and the posterior lens fibers, which are separated by the nearly obliterated lens cavity. These slender cells of the posterior lens fibers transform into the transparent lens fiber. The space between the optic cup and lens vesicle is filled by loose mesenchymal cells which will eventually differentiate into the vit-

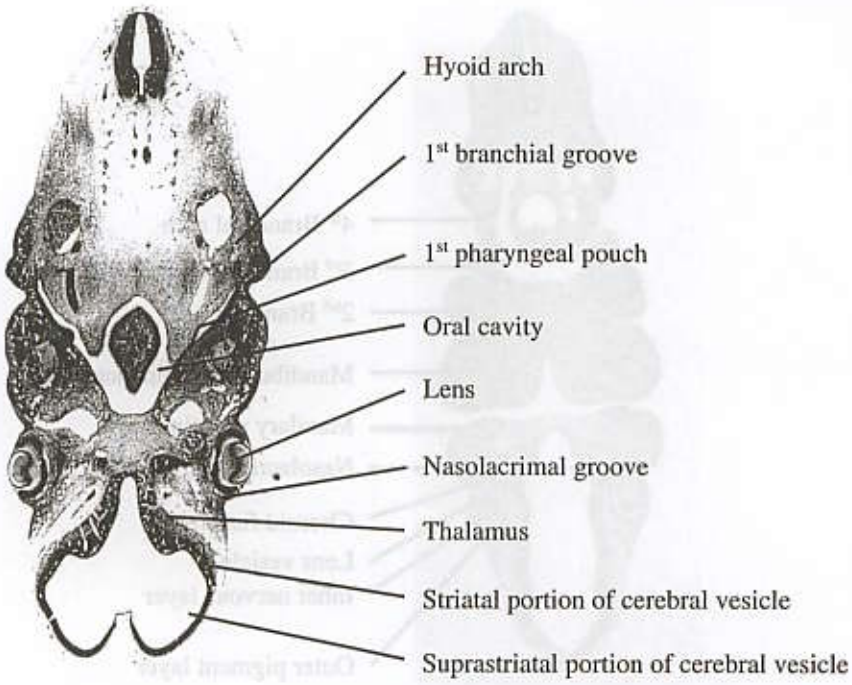

Figure 3. Transverse section of 12-14 mm. rabbit embryo through the 1st branchial arch.

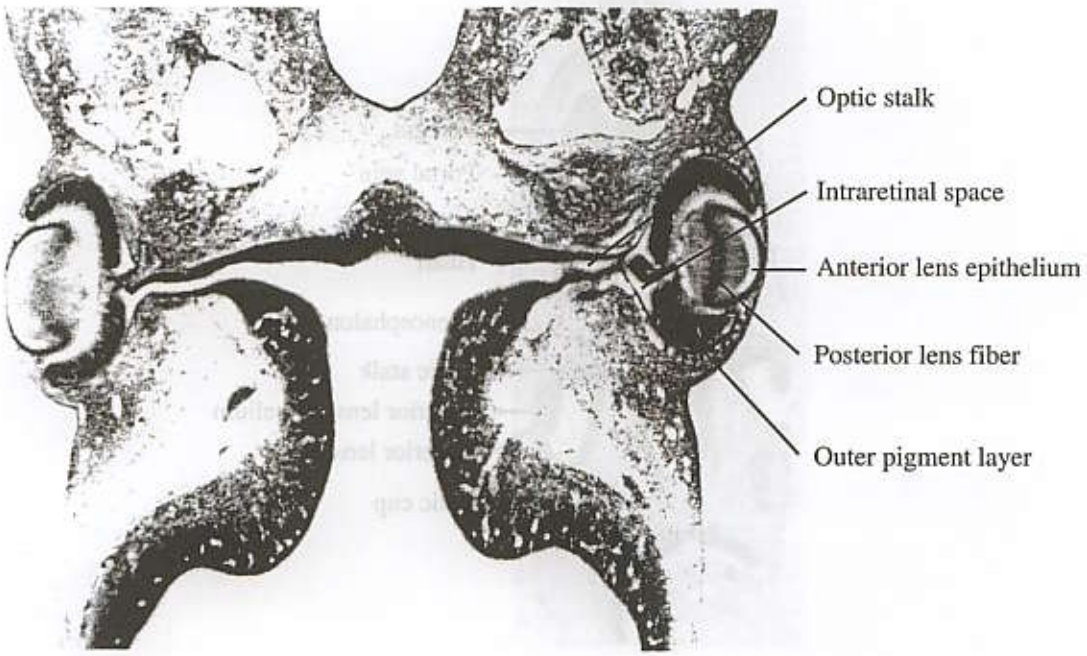

Figure 4. Transverse section of 12-14 mm. rabbit embryo through the 1st branchial arch.

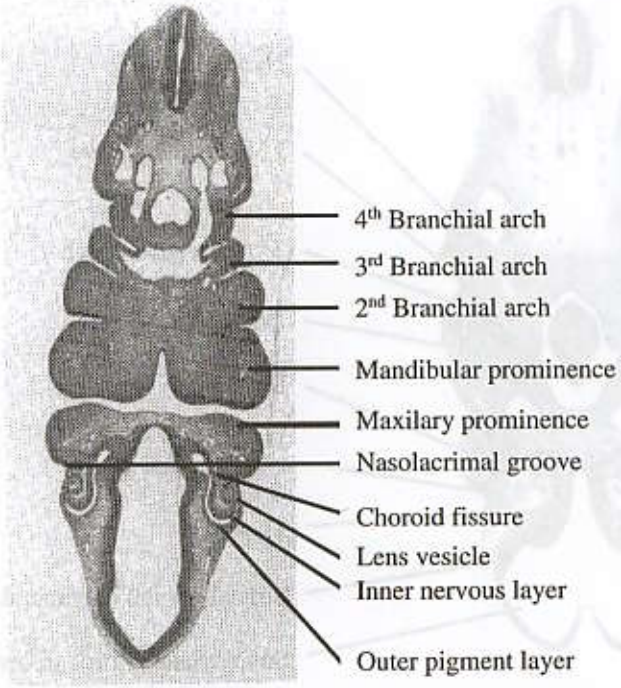

Figure 5. The optic stalk and the optic cup of 10 mm. pig embryo (× 40).

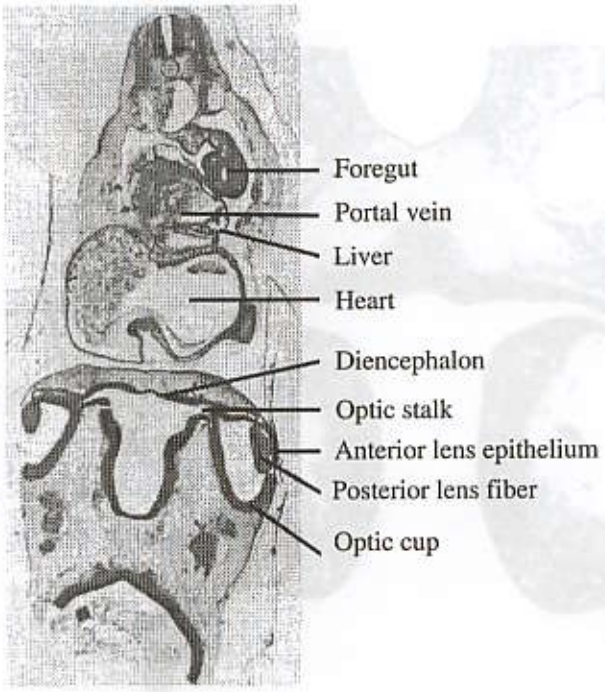

Figure 6. The optic stalk and the optic cup of 36 somites chick embryo (× 40).

reous body and the hyaloid vessels. The surface ectoderm covering the optic cup and lens vesicle is thin and together with the underlying mesenchyme will form the cornea. The mesenchyme surrounding the posterior 4/5 of the optic cup becomes slightly condensed to form the vascular and fibrous coat of the eyeball. No eyelid forms at this stage of development.

3. The transverse section of the 10 mm. pig embryo. (sections cutting through the developing eye) (Figure 5).

The optic stalk evaginates from the diencephalon. The end of the stalk forms a relatively small double-wall optic cup. The outer layer is thin while the inner layer is thicker; they are separated by the intraretinal space. The space between the two layers is the intraretinal space, communicating with the third ventricle of the diencephalon. In this figure inferior surface of the optic cup is cut through the choroid fissure which is penetrated by the mesenchyme primordium of the choroid vessels. The lens pit undergoes double fusion to form the lens vesicle. The epithelial lining of the lens is columnar-shaped both anteriorly and posteriorly.

4. The transverse section of the 36 somites chick embryo. (section cutting through the developing eye) (Figure 6).

The optic stalk evaginates from the diencephalon. The lateral end of the optic stalk carries a relatively large optic cup. The optic cup appears as a double-walled cup with the outer layer being thinner and the two layers are separated by a thin intraretinal space. The lens vesicle occupies the cavity of the optic cup. The posterior epithelium of the lens is longer to form the posterior lens fiber. The lens cavity is nearly obliterated.

5. The transverse section of the 15 mm. pig embryo. (section cutting through the eyeball) (Figure 7, 8).

The optic canal joins the intraretinal space and the third ventricle is nearly obliterated along with the intraretinal space. The inner layer of the optic cup is composed of nerve cells and photoreceptive cells and can be separated into two distinct layers. The outer nuclear layer comprises several layers of nerve cells and photoreceptor cells while the nerve fiber layer contains only the nerve fibers. The outer layer of the optic cup is marked by dense pigmentation. The lens

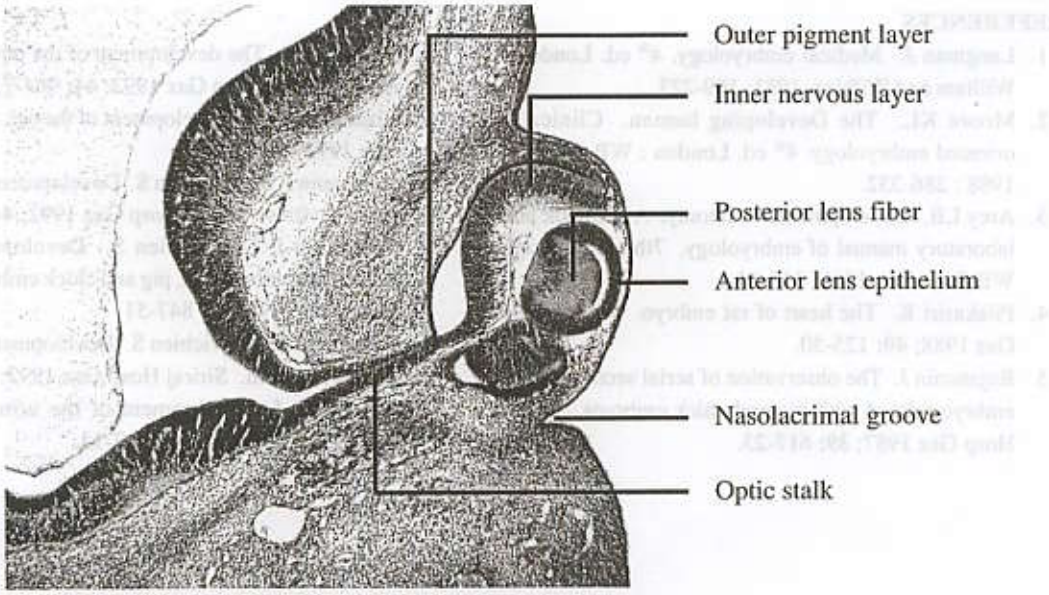

Figure 7. Transverse section of 15 mm. pig embryo (× 100).

is composed of anterior lens epithelium and posterior lens fiber while the lens cavity is thin or nearly obliterated. Posterior to the lens is the cavity of the optic cup or the vitreous chamber; anterior to the lens is the cornea, represented by the surface ectoderm and the underlying mesenchyme. Posterior to the optic cup is the denser mesenchyme of the choroid and sclera.

DISCUSSION

The eyes of the rabbit, pig and chick embryo develop in the same manner, except for slight differences in size. The optic cup of the chick is relatively larger than those of the 10 mm. pig and 4-5 mm. rabbit embryos. The developing eye of the rabbit embryo is highly similar to that of the pig embryo. The cup consists of two layers, the outer pigment layer and the inner nervous layers, separated by the intraretinal space. The developing lens undergoes a double fusion to form the lens vesicle. The posterior epithelium of the lens is lengthened as development proceeds. The choroid fissure can be identified in all three animals. The vitreous chamber of the chick is the largest of the three. The space shows a network of delicate mesenchyme which are arranged very loosely among the transparent gelatinous substance. The eyelids are not formed in all stages of the three animals.

As stated above, the eyes of the pig, chick and rabbit embryos develop in the same manner; therefore, any of them can be used as models for studying human eye development. It is not necessary to use pig embryos, which are now unavailable.

CONCLUSION

The eyes of the pig, chick and rabbit embryos develop in a very similar way. Starting from the optic vesicle evaginating from the diencephalon, the optic cup forms the retina and a part of the ciliary body and iris. The lens is formed from the surface ectoderm which is indicated firstly as lens placode, lens pit and lens vesicle. The mesenchyme covering the optic cup forms the choroid and sclera. The most suitable stages of the rabbit embryo that exhibit all the structures stated above are the 4-5 mm. and the 12-14 mm.; these can be compared to the 10 mm. pig embryo and 15 mm. pig embryo, respectively. In the future we can use the rabbit embryos as models for studying human development instead of the pig embryos as they are not available for slide preparation.

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:** 125-30.
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:** 233-39.
8. Rojananin J, Sangvichien S. Development of face and nasal cavities. Siriraj Hosp Gaz 1992; **44:** 430-35.
9. Rojananin J, Sangvichien S. Development of the branch 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:** 21-25.
11. Rojananin J. Development of the urinary system. Siriraj Hosp Gaz 1993; **45:** 7-13.