The Development of the Eye in the White Rat

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白鼠眼球의 初期發生

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摘 要

白鼠(*Rattus norvegicus var. albinus*) 50頭를 키우며 雌性白鼠의 發情을 膣膏塗末法으로 確認하여 姙娠케 한후 各 단계별로 80個의 胚를얻었다. 固定液은 Bouin's solution과 Carnoy's solution II였으며 標本은 5-7μ의 두께로 連續切片을 만들었다. 染色은 Delafield's hematoxylin과 eosin-Y의 二重染色法과 神經纖維를 觀察하기 爲하여 Ramóney Cajal's silver-nitrate method를 使用하였다. 實驗結果는 眼窩가 stage 17에서 나타났고 眼胞가 stage 18에서 形成되었다. 深眼胞가 stage 19에서 形成되었고 網膜板과 水晶體板이 stage 21에서 形成되었으며 體表外胚葉과 접촉하고 있는 眼胞의 部分은 stage 22(A)에서 眼盃를 形成하기 爲해 陷入되었다. stage 22(B)에서 水晶體孔은 아직 열려있고 stage 23(A)에서 色素粒이 網膜의 色素層에 나타났고 水晶體孔이 닫히졌다. 水晶體窩는 stage 23(B)에서 體表外胚葉으로부터 分離되었고 水晶體 內壁細胞의 伸長이 stage 23(C)에서 시작되었다. 水晶體腔은 stage 25에서 水晶體纖維에 依해서 완전히 없어졌고 stage 26에서 虹彩가 形成되기 시작하였다.

INTRODUCTION

The early development of the eye in the white rat was studied by several workers (Huber, 1915; Hiraiwa, 1941; Christe, 1964).

Hiraiwa (1941) described the development of each organ of the white rat. The optic pit appeared in the embryo of the 10th day and the optic vesicle was distinguished at $10\,^{3}/_{4}$ days. The retinal pigment layer, retinal sensory layer, lens vesicle and cornea were recognized at $12\,^{5}/_{8}$ days.

Grunnerberg (1943), in his report of normal staging of the mouse embryo, reported that the eye cup was visible in the embryo of 9 days, and the lens vesicle at 10 days.

In the report of Otis (1954) on equivalent age in mouse and humen embryos, the optic sulcus of the mouse embryo appeared at $8\,^{13}/_{24}$ days and retinal and lens disks were formed at $9\,^{3}/_{4}$ days. The choroid fissure was closed and the cavity of the lens vesicle was obliterated by primary lens fibers at 13 days.

Recently, Christe (1964) investigated the developmental stage in somite and post somite rat embryos. The optic pit appeared as depression in the anterior surface of the neural folds at $9\,^{3}/_{4}$—10 days. The lens placode began to investigate and form the deep vesicle at $11\,^{1}/_{2}$—12 days. The lens vesicle was closed at 13—$13\,^{1}/_{2}$ days. They, however, studied the developmental process of each organ in early embryo, and the eye was only discussed briefly as one organ. This research was designed to investigate the early development of the eye in detail based on the differentiation of the cells of the eye region at each developmental stage of the whole embryo.

The stages in this paper correspond to stages 17 to 26 of Christe (1964).

MATERIALS AND METHODS

The experiments reported here were performed on embryos of white rat (*Rattus norvegicus var albinus*). Fifty pairs of white rats were bred in the temperature of 15—20°C and after confirming the age of puberty by the vaginal plug smearing method, made to copulate between 21—23 o'clock.

The materials used were 80 embryos of the white rat at various stages, from the embryo of 9 3/4—10th day to 14 3/4—15 1/2 days. The embryos from pregnant rats were fixed immediately in Bouin's solution and Carnoy's solution II for about 12 hours, and embedded in pure paraffin and celloidin paraffin following the method of V. Apáthy. Embedded samples were sectioned serially in 5—7 μ thickness and stained by Delafield's hematoxylin and eosin-Y, or Ramóney Cajal's silver nitrate method for nerve fibers.

OBSERVATION

Stage 17. (9 3/4—10 days, 5 somites, 1.6 mm)

Since the neural fold has not yet fused, the neural groove has not become converted into the neural canal. The optic pit dimly appeared on the lateral aspect on the fore brain.

Stage 18. (10—10 1/2 days, 8 somites, 1.9 mm)

The neural groove becomes inverted into the neural canal. The optic pit was evaginated to form the optic vesicle and the wall of the optic vesicle which is 65 μ in thickness was consisted of about 5—6 layers of nuclei. The cells of the optic vesicle are 13.75 μ long and 5.5 μ width. The thickness of the optic vesicle and the size of cells are larger than those of the previous stage.

Stages 19 and 20. (10 1/2—10 3/4 days, 14 somites, 2.1 mm~10 3/4—11 1/2 days, 22 somites, 3 mm)

The part of the optic vesicle in contact with the surface ectoderm commenced to form the optic cup. The margin of the wall of the fore brain projected more than the previous stage and a deep optic vesicle was formed. The optic vesicle which was seen as an outgrowth of the fore brain, came into contact laterally with the surface ectoderm. The wall of the optic vesicle is 68μ thick and contained 5—6 nuclear layers. In part of the optic vesicle the cells were speedily multiplicated, so that they increased in number. The optic vesicle was forming the future retinal disk. On the surface ectoderm, cubical cells still stood in a row.

Stage 21. (11 1/2—12 days, 25—26 somites, 4.1 mm)

The optic vesicle in contact with the surface ectoderm was invaginated as if air were taken out off from a rubber ball. The optic vesicle had a crecentric slit and the optic cup commenced to be formed. The optic cup has a thickness of 60 μ. The cells of the optic cup region are 13.8 μ in length and 6.1 μ in width. While these changes are going on, the surface ectoderm opposite thickened to form the lens plate. Cells of the lens plate undergoing mitosis were observed in the vicinity of the optic vesicle.

On section the nuclei of the lens plate seemed to be arranged in two rows and the lens plate began to form the lens pit in a thickness of 30 μ. Cells were more densely distributed on the retinal disk than the mesenchyme near the eye ball and cells similar to the mesenchymal cells were observed between retinal disk and surface ectoderm.

Stage 22(A). (12—12 1/2 days, 30 somites, 4.9 mm)

The lens and retinal disk were invaginated to form lens pit and optic cup. The central part of the lens pit is 42 μ thick. Cells of this region are 13 μ long and 5 μ wide. Erythrocytes and something like cell were observed at the future vitreous chamber between the optic pit and the optic cup.

Stage 22(B). (12 1/2—13 days, 36 somites, 5.8 mm)

As the lens pit had considerably advanced since the previous stage, the deep lens vesicle was formed. The lens pit gradually deepened but the lens pore was not closed and the formed lens pit appeared like C-shape. The lens vesicle was 60 μ thick and composed of three layered nuclei. The cells in this region were arranged in a few rows and is 15 μ long and 4.5 μ wide. The optic cup was divided into two layers; the outer layer which is called the retinal pigment

Table 1. Summary of early development of the eye in the white rat based on present findings.

Feature \ Researchers / Materials / Division	Author				Christe (1964)				Hiraiwa (1941)		Otis (1954)		O'Rahilly(1959)		
	White rat				Royal wistar strain of rat				White rat		Mouse		Chick		
	Stage	Somite	Age(day)	length(mm)	Stage	Somite	Age(day)	length(mm)	Somite	Age(day)	Somite	Age(day)	Stage	Somite	Age(hour)
Optic pit	17	5	$9^3/_4$～10	1.6	17	4～6	$9^3/_4$～10		5	10					
Optic vesicle	18	8	10 ～$10^1/_2$	1.9	18	7～9	10 ～$10^1/_2$						9	7	29～33
Deep optic vesicle	19	14	$10^1/_2$～$10^3/_4$	2.1	19	10～14	$10^1/_2$～$10^3/_4$		15	$10^3/_4$	16	$8^{11}/_{12}$	13	19	48～52
Optic stalk	21	25～26	$11^1/_2$～12	4.1					25	$11^1/_2$	26	$9^3/_4$	11	13	40～45
Retinal disk	21	25～26	$11^1/_2$～12	4.1							26	$9^3/_4$	12	16	45～49
Beginning of optic cup	21	25～26	$11^1/_2$～12	4.1							26	$9^3/_4$	12	16	45～49
Optic cup	22(A)	30	12 ～$12^1/_2$	4.9								$10^{1/2}$	14	22	50～53
Retinal pigment layer	22(B)	36	$12^1/_2$～13	5.8					40	$12^5/_8$					
Retinal sensory layer	22(B)	36	$12^1/_2$～13	5.8					40	$12^5/_8$					
Choroid fissure still open	24		14 ～$14^1/_4$	9.5									14	22	50～53
First sign of pigment	24		14 ～$14^2/_4$	9.5								$11^1/_2$	21	43～44	
First sign of nerve fiber in optic stalk	25		$14^1/_4$～$14^3/_4$	10.5									18	30～36	65～69
Scleral condensation	26		$14^3/_4$～$15^1/_2$	12									14		96
Ectoderm thickened in region of lens	19	14	$10^1/_2$～$10^3/_4$	2.1								13.0	11	13	40～45
Lens disk	21	25～26	$11^1/_2$～12	4.1									12	16	45～49
Beginning of lens pit	21	25～26	$11^1/_2$～12	4.1	21	$^{23}/_{24}$～28	$11^1/_2$～2	3.0～4.1					12	16	45～49
Lens pit	22(A)	30	12 ～$12^1/_2$	4.9								$10^1/_2$	14	22	50～53
Lens vesicle still open	22(B)	36	$12^1/_2$～13	5.8	22(B)	33～37	$12^1/_2$～13	4.6～5.8					16	26～28	51～56
Lens pore close	23(A)	40	13 ～$13^1/_2$	6.8	23(A)	$^{38}/_{39}$～41	13 ～$13^1/_2$	5.8～7.1				11.0	17	29～32	52～64
Lens separated from ectoderm	23(B)	42	$13^1/_2$～$12^3/_4$	7.8					40	$12^5/_8$					
Cornea	23(B)	42	$13^1/_2$～$13^3/_4$	7.8					40	$12^5/_8$			17	29～32	52～64
Elongation of posterior epithelium of the lens	23(C)	46	$13^3/_4$～14	8.2								$11^1/_2$	18 19	30～36 37～40	65～69 68～72
Early lens fiber	23(C)	46	$13^3/_4$～14	8.2											
Lens cavity like crecentric slit	24		14 ～$14^1/_4$	9.5								$12^1/_2$			
eye lid	24		14 ～$14^1/_4$	9.5	24	48～	14 ～$14^1/_4$	9.4～10.3		$14^3/_4$		$31^1/_2$			
Cavity of lens beside sobliterated by primary lens fibers	25		$14^1/_4$～$14^3/_4$	10.5						$14^3/_4$		13.0			
Beginning of iris	26		$14^3/_4$～$15^1/_2$	12								$16^1/_2$			

layer, was suddenly reduced to one or two nuclear rows. The inner layer, the retinal sensory layer which suffered invagination became considerably thickened and underwent a very complex process of specialization to form ultimately the adult retina. The retinal sensory region which came in contact with the retinal pigment layer was undergoing the cell division.

Stage 23(A). (13—13$^1/_2$ days, 40 somites, 6.8 mm)

The lens pore had perfectly closed and the anterior epithelium of the cornea was seen. Superficial cells were observed in the lens cavity. The lens body is 190 μ long and 150 μ wide. The surface ectoderm came into contact with the lens vesicle which was composed of cuboidal and squamous cells and would form the cornea. As the continued differentiation of the two layers of the optic cup, the anterior layer of the optit cup was consisted of 5 or 6 rows of nuclei and the posterior layer of that was abruptly reduced to one or two rows of nuclei but this would be remained all through life as pigment layer.

Stage 23(B). (13$^1/_2$—13$^3/_4$ days,42 Somites, 7.8 mm)

A double layer of the optic cup at this stage resembled that of the previous stage. The lens was separated from the surface edtoderm, and located between the optic cup and the future cornea. The surface ectoderm contained 1 or 2 rows of nuclei. The nuclei of the future cornea are 7.5 μ long and 6.25 μ wide.

Stage 23(C). (13$^3/_4$—14 days, 46 somtes, 8.2 mm)

The posterior epithelium of the lens differentiated first: they elongated and become columnar. The posterior wall of the lens is 140 μ thick and consists of three rows of the nuclei. The lens is 280 μ long and 200 μ wide and its cavity became crecentric. The nuclei in the lens body are 12.5 μ long and 6.5 μ wide. The elongated cells of the posterior epithelium of the lens were regard as early lens fibers. The superficial cells which were observed in the lens vesicle of stage 23(A) were not seen at this stage. The choroid fissure was not closed, and the vitreous cavity contained many erythrocytes and mesodermal cells which penetrated through the choroid fissure or the space betweeen lens and optic cup.

Stage 24. (14—14$^1/_4$ days, 9.5 mm)

The choroid fissure which was not closed resembled to that of the previous stage. The lens cavity like crecentric slit appeared on section and the size of the lens is 260 μ long and 240 μ wide. For the first time the small pigment granules are found at the retinal pigment layer. In some specimens many fibers seemed to be enamated from the lens and the retina.

Stage 25. (14$^1/_4$—14$^3/_4$ days, 10.5 mm)

As the elongated cells of the posterior wall of the lens ultimately reached the anterior wall, these cells obliterated the lens cavity completely. The anterior wall of the lens contained a row of nucleus and the lens is 430 μ long and 350 μ wide. The nuclei of the lens were crowded at the region of the equator and their size is 20 μ long and 350 μ wide. The retina is 130 u in thickness, and the oval nuclei at the retina region are 7.5 μ long and 5 μ wide. Many mitoses were observed in the vicinity of the junction between theretinal pigment layer and the retinal sensory layer. The choroid fissure was closed. Nerve fibers from ganglion cell layer passed through the optic stalk to the brain.

Stage 26. (14$^3/_4$—15$^1/_2$ days, 12 mm)

The oval shaped lens is 600 μ long and 477.5 μ wide. The size of the nuclei is 25 μ long and 5 μ wide. The nuclei of the lens were crowed near the anterior wall and seemed to be pushed for the vitreous side with fibers. The anterior border of the optic cup grew forwards to form the ciliary region and the ectodermal portion of the iris. The mesodermal cells seemed to come into the space between the lens body and the future iris. The condensation of the mesenchyme to form the sclera is recognized in the ablenticular half of the eye and the eye lid was formed in front of the cornea.

DISCUSSION

According as the forebrain is developed, the optic pit has dimly appeared on the lateral aspect on the forebrain at stage 17 prior to the formation of the neural canal. The optic pit resembled that seen in the white rat embryo at

the 10th day of Hiraiwa(1941). Christe(1964) observed an optic pit at stage 17 and Otis(1954) found the optic vesicle in the mouse embryo at 18 days and 13 hours. The optic vesicle was more projected, so that the optic vesicle came into contact with the surface ectoderm and a spherical optic vesicle connected with the forebrain by a relatively narrow optic stalk. Then this optic stalk is similar to that appeared in a $11^1/_2$ day embryo of Hiraiwa (1941).

The part of the optic vesicle which came into contact with the surface ectoderm invaginated to form the retinal disk. While these changes are going on, the surface ectoderm was continuously thickend and differentiated to form the lens disk. Finally the lens disk was invaginated to form the optic pit. The lens pit of the mouse was formed at 10 days and in the chick it was formed at 50—53 hours (O'Rahilly, 1959).

According to Hiraiwa (1941) the lens pit was invaginated to form the optic vesicle in a $12\,^5/_8$ day embryo and Christe (1964) observed the optic vesicle at stage 21. Because the lens pit gradually deepens, the lens pit was formed at stage 21 and the lens pore was closed at stage 23(A) (Christe, 1964). The lens pore, however, was closed in the 11th day mouse embryo (Otis, 1954) and in the 31—32th day human embryo (Streeter, 1948). Immediately the lens vesicle was separated from the surface ectoderm. The superficial cells which was found in the lens vesicle at this stage were shut up inside when the lens pit was closed to form a vesicle, but they soon was disappeared by degeneration. They took no share in the development of the lens (Duke-Elder, 1942).

After lens was seperated from the surface ectoderm, the remainder of surface ectoderm was differentiated to form the cornea and the posterior wall of the lens underwent the remarked cell division; they were elongated and ultimately reached the anterior wall. Therefore, the lens cavity was obliterated by them. The lens in white rat was completely formed at $14\,^3/_4$ days (Hiraiwa, 1941). The cells of the posterior wall of the lens were elongated at the $11\,^1/_2$th days of the mouse embryo (Otis, 1954) and at 33± days of human embryo (Streeter 1948). These early lens fibers remain unchanged throughout life, ultimately occupying the central area of the lens.

On the other hand, as soon as the bulged optic vesicle had reached the surface ectoderm, a change occured in the ectoderm. This change was presumably determined by some chemical stimulus from the vesicle itself (Duke-Elder, 1942). A double layered eye cup at stage 21 resembled to that illustrated by Hiraiwa (1941) in the $12\,^5/_8$ days embryo which was formed retinal pigment layer from the posterior wall and retinal sensory layer from the anterior wall of the eye cup. The pigment granules first appeared at the cells of edge of eye cup. Otis (1954) found pigment granules at the outer layer of retina in the $11^1/_2$ day mouse embryo. O'Rahilly (1959) found them at the $3^1/_2$ days chick embryo.

The retinal sensory layer of the eye cup was divided into primitive-neuro epithelium and marginal zone. This marginal zone was increased in width and some of cells invaded into the marginal zone from primitive layer. This area was vascularized by penetration of fine capillaries. Mann (1928) stressed that the capillaries were seen only in mammalia. The developed retina consists of 7 layers; nerve fiber layer, ganglion cell layer, inner fiber layer, outer fiber layer, inner nuclear layer, outer nuclear layer and rods. Because the dells on the ventricle part of the optic stalk were behind the other part in development, the choroid fissure was formed at this area. Many erythrocytes and mesodermal cells penetrated into the vitreous cavity through this fissure. The choroid fissure was closed at stage 25 in white rat, at 11 mm stage in human (Mann, 1922) and at the 12 day embryo in mouse (Otis, 1954).

The development of the vitreous was studied by many researchers. Considering that fibers in some specimens seemed to diffuse from the outer part of the retina and lens, these fibers and mesenchyme which is penetrated between eye cup and lens, seemed to form the vitreous body.

Nerve fibers were growing from the ganglion cells of the retina; entering the optic stalk, they invaded the invaginating layer and ran towards the brain on its ventral aspect. Otis (1954) found the early lens fibers at the 13 days mouse embryo and Mann (1928) at stage 16 in human. The anterior border of the optic cup grew fowards to form the ciliary region and the ectodermal portion of the iris.

The chief findings of the present investigation are summarized in Table 1 to compare with other vertebrates, especially with mammalia.

SUMMARY

1. The optic pit was found in the white rat embryo of the stage 17 and became invaginated to form the optic vesicle at stage 18. The deep optic vesicle appeared at stage 19 and the optic vesicle was in contact laterally with the surface ectoderm at this stage.
2. The retinal disk and lens plate were formed, each plate was formed, and each optic vesicle had an optic stalk at stage 21.
3. The part of the optic vesicle in contact with the surface ectoderm was invaginated to form the optic cup and lens plate invaginated to form the lens pit at stage 22(A). The lens pore was still opened at stage 22(B), and the future cornea and the lens body were recognized at this stage.
4. Pigment granules were seen in the retina and the lens pore was closed at stage 23(A). The lens pit which ultimately invaginated was separated from the ectoderm at stage 23(B). The elongation of the posterior lens cells was commenced and the hyaloid blood system appeared in the eye at stage 23(C).
5. The cavity of lens vesicle was obliterated by primary lens fibers at stage 25, and the neural ectoderm of the optic stalk gave rise to the optic nerve.
6. The anterior border of the optic cup grew forward to form the ciliary region and the ectodermal portion of the iris by stage 26.

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Explanation of Figures

Fig. 1. Stage 18. The optic vesicles have expanded laterally. The neural folds have fused. ×100

Fig. 2. Stage 19—20. Deep lens vesicles were in contact with the surface ectoderm. ×100

Fig. 3. Stage 21. Optic cup and optic pit are commencing to form. ×100

Fig. 4. Stage 22(B). Optic cup was divided into retinal pigment layer and retinal sensory layer. Lens pore has not yet closed. ×60

Fig. 5. Stage 23(A). Lens pore has closed, so that an anterior epithelium of the cornea is present. ×60

Fig. 6. Stage 23(C). Formation of early lens fibers from cells of posterior wall of lens vesicle is commencing. The hyaloid blood system appeared in the eye. ×60

Fig. 7. Stage 24. The lens cavity is reduced to a crecentric slit and choroid fissure is still opened. Pigment granules were first seen in the cells closed to the margin of the optic cup. ×60

Fig. 8. Stage 25. Nerve fibers from ganglion cell layer passed through the optic stalk to the brain. ×40


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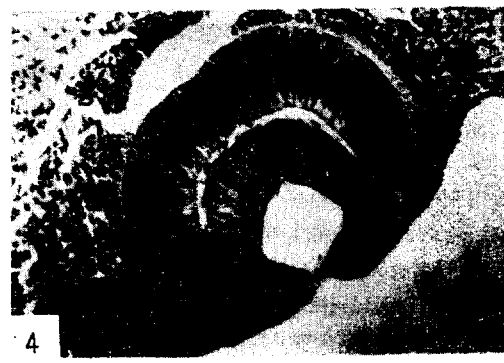
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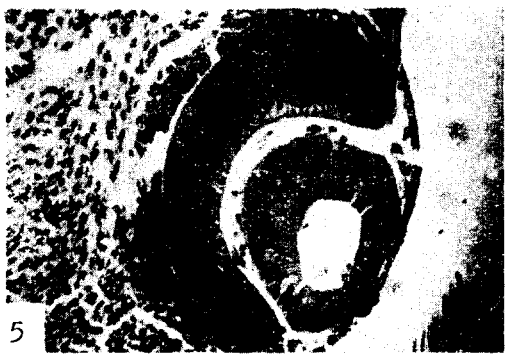
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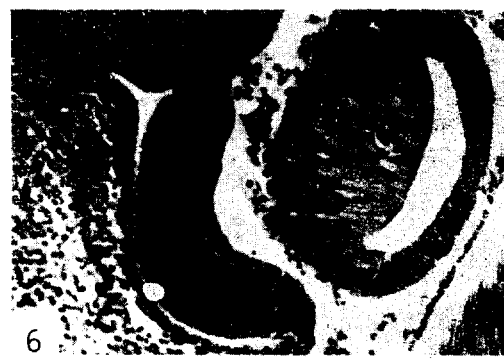
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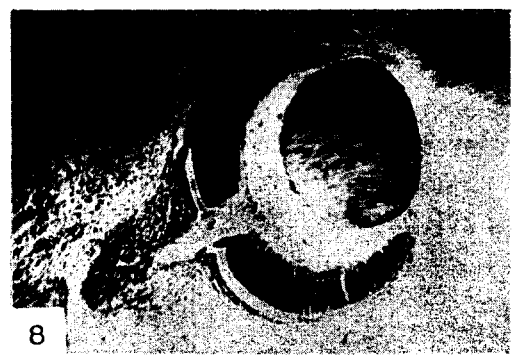
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