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SEQUENTIAL SCANNING ELECTRON MICROSCOPIC ANALYSES OF NORMAL AND SPONTANEOUSLY OCCURRING ABNORMAL OCULAR DEVELOPMENT IN C57Bl/6J MICE

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Abstract

Embryos of C57Bl/6J mice were examined grossly, and by light and scanning electron microscopy on days 8 through 19 of gestation. Adult eyes were examined by slit lamp biomicroscopy and light microscopy. A spontaneous incidence of eye malformations including microphthalmia, microphakia, corneal opacity and anterior segment dysgenesis was observed at a rate of 13.2% in the adults and 10.8% in the day 14 embryos. Scanning electron microscopy demonstrates the complex series of coordinated changes in shape and tissue interrelationships observed in normal ocular development. Possible routes of abnormal ocular morphogenesis beginning as early as the time of optic vesicle formation are discussed.

KEY WORDS: optic sulcus, optic cup, lens placode, lens vesicle, coloboma, anterior segment dysgenesis, persistent pupillary membrane.

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Introduction

Ocular development has been described in several species, most notably the human (O'Rahilly, 1975; Müller and O'Rahilly, 1985; Mann, 1964; Duke-Elder and Cook, 1963), amphibians (Jacobson, 1963) and the chick (Hilfer, 1983; Johnston, et al., 1979). Previous descriptions of ocular development in the mouse have emphasized light microscopy (Pei and Rhodin, 1970) although scanning electron microscopy has been used to illustrate early stages of ocular development (Kaufman, 1979). Scanning electron microscopy (SEM) offers a unique, three dimensional perspective ideally suited to demonstration of the intricate and rapid series of tissue outgrowth, folding, and vesicle formation characteristic of ocular morphogenesis. A recent description of ocular development in the chick clearly demonstrated these advantages of scanning electron microscopy (Hilfer, 1983).

In addition to enhancing visualization of the events involved in normal ocular morphogenesis, SEM can be of value in the early identification of abnormalities in ocular development. Alterations in size of the optic primordia and relationships between the optic cup and surface ectoderm are examples of aberrations observable early in gestation. Lens development from placode induction through detachment from the surface ectoderm to formation of the secondary lens fibers is easily followed by SEM, allowing detection of abnormalities. Identification of a persistent choroid fissure is readily accomplished with SEM. Scanning electron microscopy has obvious advantages over serial light microscopic sectioning and computerized reconstruction for evaluation of such tissue defects and interrelationships.

Congenital ocular abnormalities have been previously described in mice, including the C57Bl strain (Pierro and Spiggle, 1967, 1969). An embryologic malformation was speculated upon as the etiology of corneal and lenticular opacities identified in these mice. Our investigations further substantiate this original hypothesis as well as providing an illustrated review of normal ocular morphogenesis through SEM.

Materials and Methods

C57Bl/6J mice were obtained from the Jackson Laboratory and placed with males for one hour between 7 and 8 AM. The presence of a copulation plug was noted and this time considered 0 days of gestation. Females were sacrificed by cervical dislocation on days 8 through 19 of gestation. Embryos were removed from the uterus and those of gestational day 14 or less fixed in 2.5% glutaraldehyde in phosphate buffer. Fetuses older than day 14 were fixed in a combination of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer. Following fixation for a minimum of 24 hours, tissues to be prepared for SEM were rinsed in phosphate buffer. The amnion was removed at this time and notation made of the somite count or morphological rating (for embryos older than 12 days) (Trasler, 1965). Embryos of 12 days of gestation or less were fractured through the optic primordia in a frontal plane using a scalpel. Older fetuses were fractured horizontally using a razor blade. Tissues were postfixed in 2% osmium in phosphate buffer for one hour. Following a phosphate buffer rinse, specimens were dehydrated in a graded ethanol series and critical point dried from CO_2. The specimens were mounted on aluminum stubs with double sided tape and silver paint when necessary to provide adequate adhesion, and then sputter coated with gold palladium. SEM analyses were performed at 15 kV on a JEOL SM 35 electron microscope.

The eyes of the adult mice were examined with a table-mounted slit lamp biomicroscope (American Optical, Buffalo, NY) following induction of general anesthesia with intraperitoneal tribromoethanol (0.13 mg/g body weight). Following cervical dislocation, eyes were removed and fixed in a combination of 4% paraformaldehyde and 2.5% glutaraldehyde. Tissues prepared for light microscopy were infiltrated and embedded according to the protocol for the JB-4 plastic kit for light microscopy (Polysciences, Inc., Warrington, PA). Sections 3.0 µm in thickness were obtained with a glass knife, mounted on glass slides and stained with methylene blue and acid fuchsin or with periodic acid Schiff's reagent and counterstained with hematoxylin. Light micrographs were obtained with a Zeiss Photomicroscope II.

Observations and Discussion

Normal Ocular Development

The optic vesicle primordia are apparent as outgrowths of the developing neural tube very early in organogenesis, at approximately 8.5 days of gestation (6-10 somite pairs) (Figure 1). The optic sulcus gradually becomes deeper and, approximately coincident with closure of the cranial neural tube on day 9 (14-20 somite pairs), is identified as an optic vesicle (Figure 2 a, b). As the distal optic vesicle enlarges, the connection with the forebrain cavity appears relatively narrower and is called the optic stalk. The optic vesicle continues to enlarge and approach the surface ectoderm, gradually eliminating the interposed layer of mesenchyme and extracellular matrix (Figure 2c). Although there are species variations, induction by the optic vesicle (van Doorenmaalen, et al., 1982; Sirlin and Brahma, 1959; Karkinen-Jaaskelainen, 1978; van der Starre, 1977; Silver and Hughes, 1974) with possible contribution by the adjacent mesenchyme and extracellular matrix (Muthukkaruppan, 1965) results in thickening of the surface ectoderm to form a lens placode which subsequently invaginates forming a lens pit during day 10 (28-33 somite pairs) (Figure 3a). The initially columnar cells of the outer layer of the optic cup become cuboidal while the cells of the inner layer elongate further in preparation for their ultimate differentiation into the multilayered neural retina. The optic vesicle undergoes differential growth and invaginates eccentrically to form a two-layered optic cup meeting ventrally at the choroidal fissure (Figure 3b). There is evidence that optic cup invagination is dependent on extracellular calcium (Brady and Hilfer, 1982). The embryonic choroid fissure fuses initially along its center, then progresses anteriorly and posteriorly. Anterior fusion is normally complete by 12 days of gestation in the mouse.

The lens pit progressively enlarges and, surrounded by the basement membrane of the surface ectoderm, forms a hollow vesicle lined by a single layer of cuboidal epithelium. This process of invagination occurs through a combination of elongation of the placodal cells and contraction of their terminal bars and apical cytoskeleton (Wrenn and Wessels, 1969). The posterior epithelial cells gradually elongate, forming the primary lens fibers and eliminating the space within the lens vesicle (Figure 3c) (Beebe et al., 1982). At this stage (day 11, 40 - 50 somite pairs), pigmentation of the outer layer of the optic cup is first observed.

The vascular supply to the embryonic eye, the hyaloid artery, enters the optic cup via the choroidal fissure, ramifies within the future vitreous cavity and surrounds the lens vesicle to form the tunica vasculosa lentis (Figure 3d). The hyaloid artery and its associated extracellular matrix form the primary vitreous (Mutlu and Leopold, 1964) with the secondary, or definitive vitreous gel developing around the hyaloid artery remnant late in gestation or early postnatally.

The external connection to the lens vesicle becomes smaller and gradually closes during the 10th day of gestation. The lens remains attached to the surface ectoderm which becomes continuous over the optic cup. Following detachment of the lens on day 12, adjacent mesenchymal cells consisting primarily of neural crest cells (Johnston et al., 1979) migrate in front of (lateral to) the optic cup. As the margin of the optic cup advances in front of the lens, it is accompanied by this mesenchymal tissue. Inductive influences of extracellular matrix components on corneal development have been demonstrated (Hay, 1977, 1980). The single layered corneal endothelium is the first to differentiate from this mesenchyme and in turn, secretes its basement membrane, Descemet's membrane. The primary corneal stroma is formed next, interposed between the endothelium and

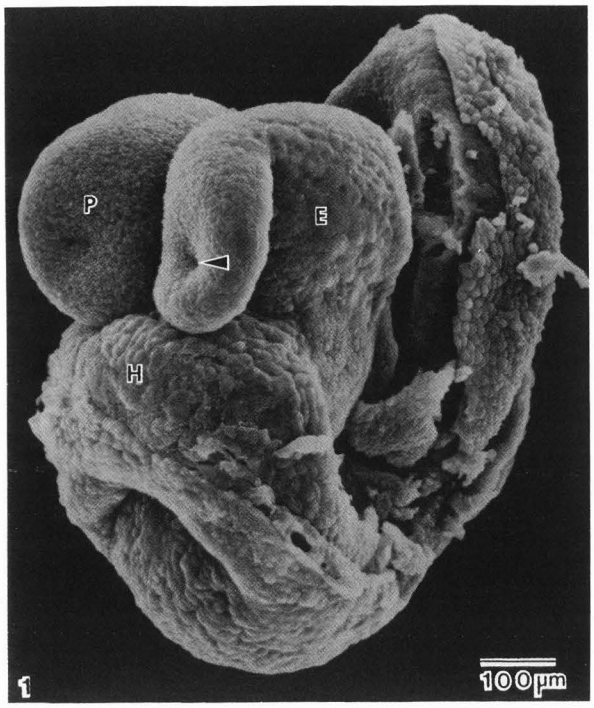
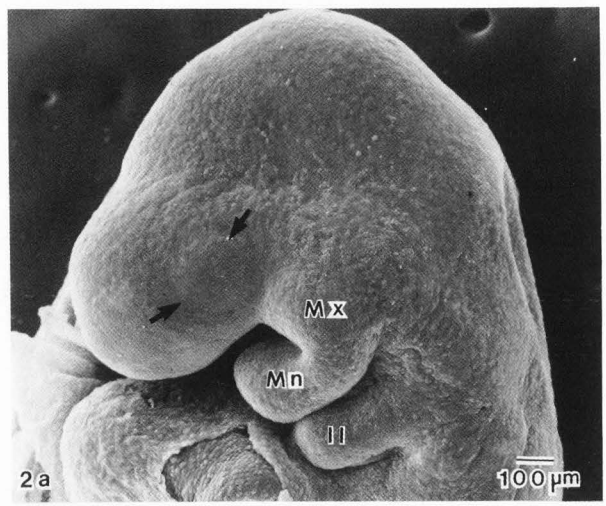


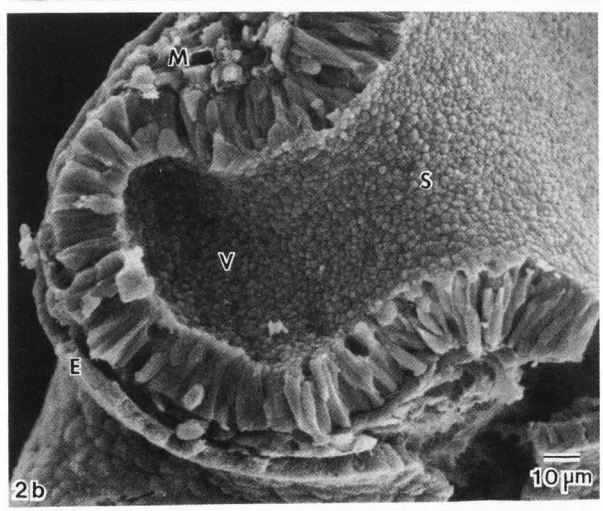
Figure 1: Mouse embryo on day 8 of gestation (5 somite pairs). Arrow indicates optic sulcus developing within the prosencephalon (P). (H = heart; E = surface ectoderm) (Reprinted with permission from the American Journal of Medical Genetics).

surface epithelium (Figure 4). Lastly, mesenchyme which will form the anterior iris and corneal stroma joins the margin of the optic cup. Remodeling of the mesenchyme of the anterior segment forms the anterior chamber and the aqueous drainage pathways of the iridocorneal angle. The anterior extension of the bilayered optic cup will give rise to the pupillary sphincter and dilator muscles as well as the iridial pigment epithelium. The corneal epithelium assumes its characteristic flattened, geometric array similar to the surface ectoderm by day 16.

Gross examination of a day 15 embryo reveals the anterior margin of the pigmented optic cup, the future pupil (Figure 5). Light microscopy and SEM illustrate the secondary lens fibers which are formed by elongation of the epithelial cells at the equator (Figure 6 a, b). These fibers pass circumferentially around the primary lens fibers (embryonal nucleus) to form the fetal lens nucleus in the adult.

The inner layer of the optic cup, the anlage of the neurosensory retina, initially





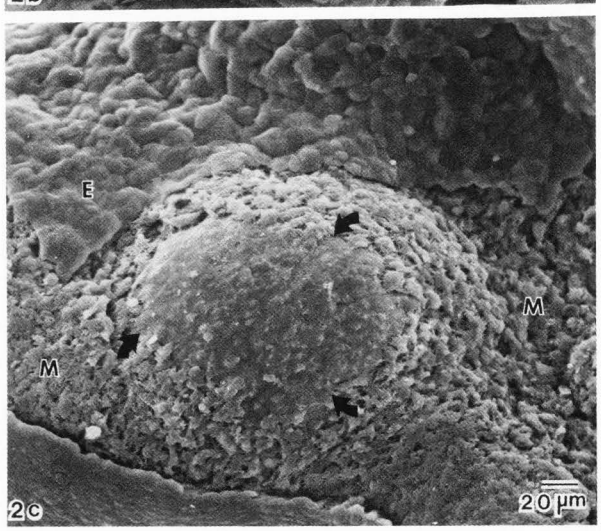


Figure 2: Normal embryo on day 9 of gestation (25 somite pairs). [a] A bulge indicating the developing optic vesicle can be seen externally (arrows). (Mx = maxillary prominence of first pharyngeal arch; Mn = mandibular prominence of first arch; II = second pharyngeal arch). [b] Frontal fracture at the level of the optic vesicle (V). The optic stalk (S) is continuous with the cavity of the forebrain. (M = primitive mesenchyme; E = surface ectoderm). [c] Removal of the surface ectoderm (E) from an embryo with 25 somite pairs reveals the basement membrane of the optic vesicle (arrows) as well as adjacent mesenchymal cells (M).

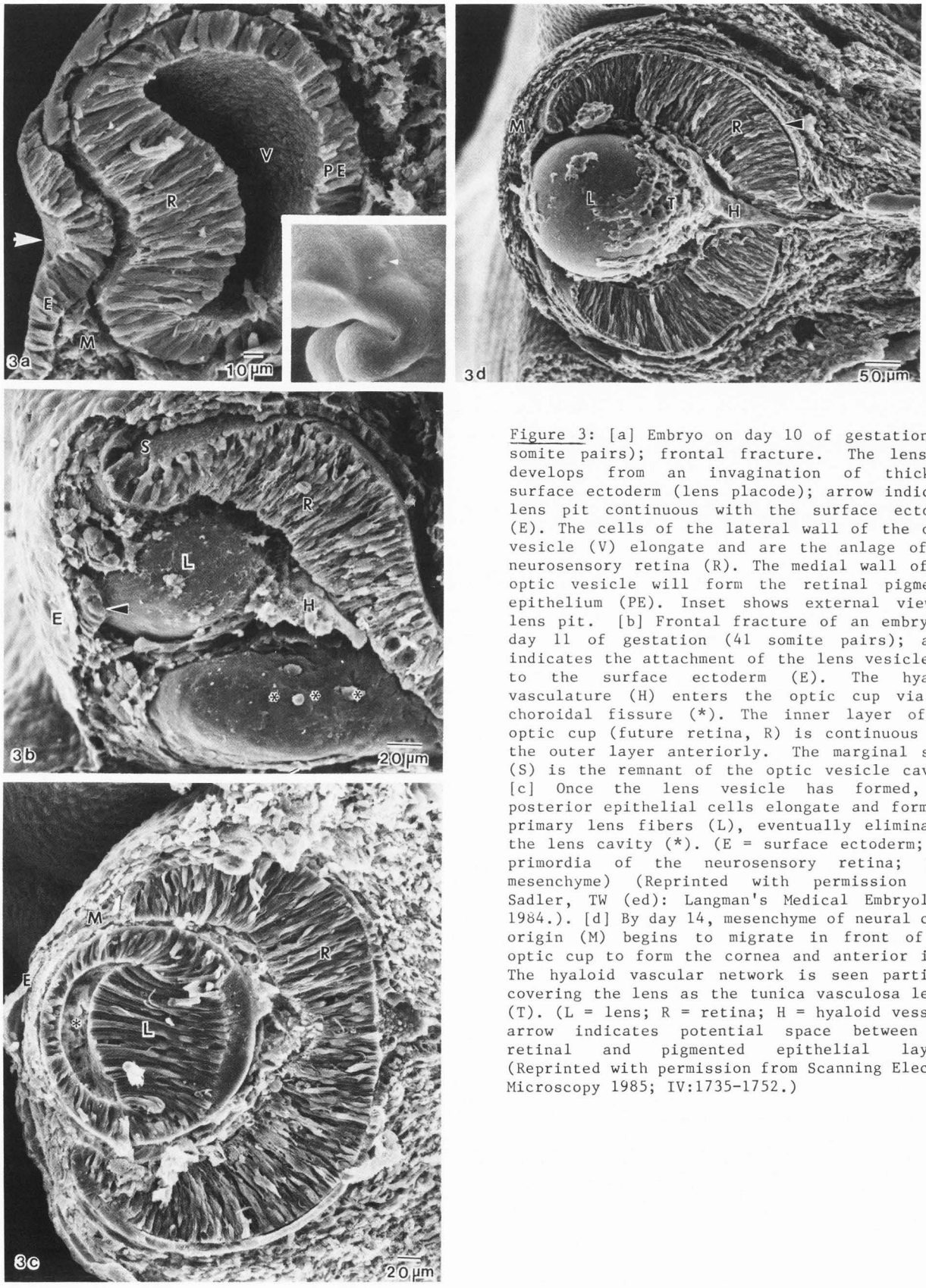


Figure 3: [a] Embryo on day 10 of gestation (29 somite pairs); frontal fracture. The lens pit develops from an invagination of thickened surface ectoderm (lens placode); arrow indicates lens pit continuous with the surface ectoderm (E). The cells of the lateral wall of the optic vesicle (V) elongate and are the anlage of the neurosensory retina (R). The medial wall of the optic vesicle will form the retinal pigmented epithelium (PE). Inset shows external view of lens pit. [b] Frontal fracture of an embryo on day 11 of gestation (41 somite pairs); arrow indicates the attachment of the lens vesicle (L) to the surface ectoderm (E). The hyaloid vasculature (H) enters the optic cup via the choroidal fissure (*). The inner layer of the optic cup (future retina, R) is continuous with the outer layer anteriorly. The marginal sinus (S) is the remnant of the optic vesicle cavity. [c] Once the lens vesicle has formed, the posterior epithelial cells elongate and form the primary lens fibers (L), eventually eliminating the lens cavity (*). (E = surface ectoderm; R = primordia of the neurosensory retina; M = mesenchyme) (Reprinted with permission from Sadler, TW (ed): Langman's Medical Embryology, 1984.). [d] By day 14, mesenchyme of neural crest origin (M) begins to migrate in front of the optic cup to form the cornea and anterior iris. The hyaloid vascular network is seen partially covering the lens as the tunica vasculosa lentis (T). (L = lens; R = retina; H = hyaloid vessels; arrow indicates potential space between the retinal and pigmented epithelial layers) (Reprinted with permission from Scanning Electron Microscopy 1985; IV:1735-1752.)

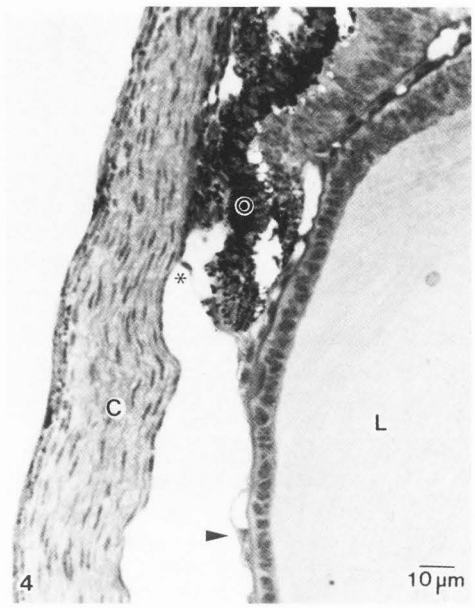


Figure 4: By day 19, close to parturition, the corneal endothelium and stroma are completely formed (C) but the anterior iris stroma and iridocorneal angle structures are not yet mature. The outer, pigmented layer of the optic cup (O), which will form the pupillary sphincter and dilator muscles, is in apposition to the cornea in the area of the future aqueous outflow pathways (*). An arrow indicates the presence of the capillaries of the anterior tunica vasculosa lentis. (L = lens).

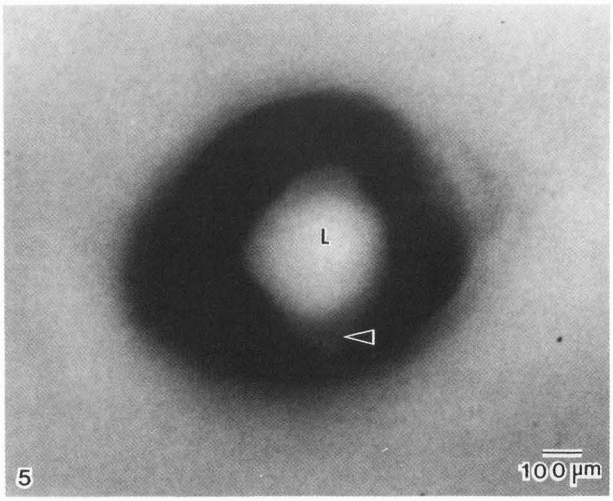
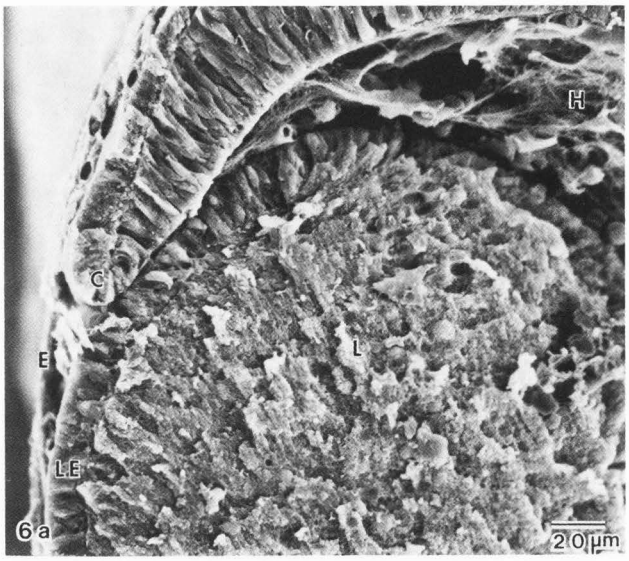


Figure 5: On day 15 the anterior margin of the pigmented optic cup (future pupil) is irregular and the anterior choroid fissure is nearly closed (arrow). (L = lens)



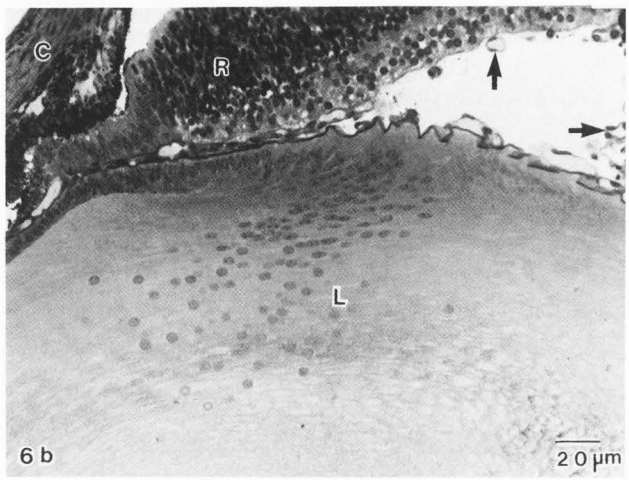
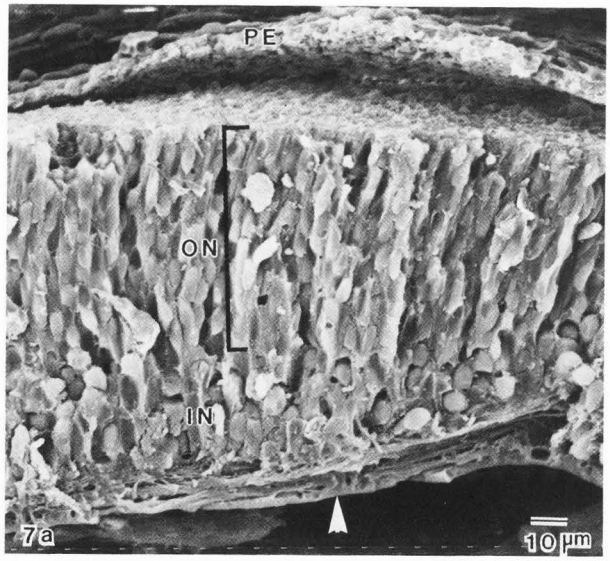
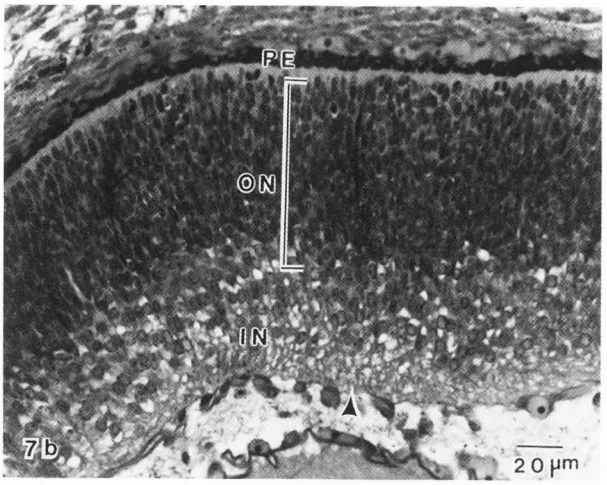


Figure 6, gestational day 13: [a] The anterior optic cup (C) advances between the surface ectoderm (E) and the anterior lens epithelium (LE). (L = lens; H = hyaloid vasculature). [b] Elongation of lens fibers at the equator form the secondary lens fibers surrounding the primary fibers. The pattern of distribution of the nuclei form the lens bow. (L = lens; R = retina; C = cornea; arrows indicate capillaries of the hyaloid vasculature)

differentiates into inner marginal and outer nuclear zones by gestational day 12. Maturation of the retina occurs in the mouse, as in other species, in a wave from central to peripheral (Aguirre et al., 1972). The ganglion cells are the first to become distinct, migrating into the inner marginal layer from the outer nuclear zone to form the inner neuroblastic layer. Ganglion cell axons become identifiable as retinal nerve fibers, the innermost layer of the optic cup (Figure 7 a, b). The remaining outer nuclear zone becomes known as the outer neuroblastic layer separated from the inner neuroblastic layer by the transient fiber layer of Chievitz. This





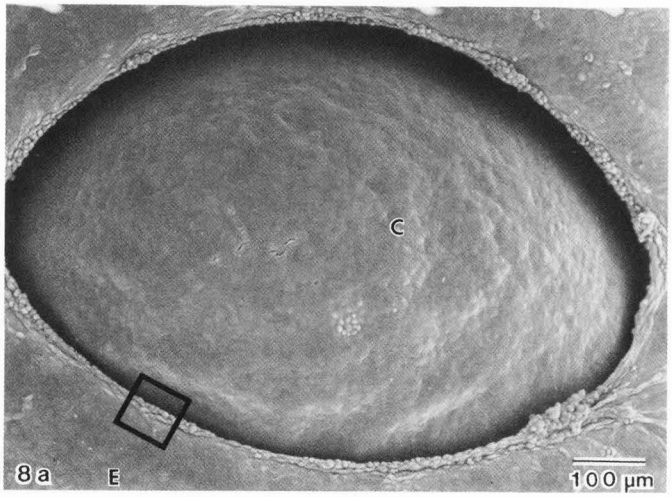
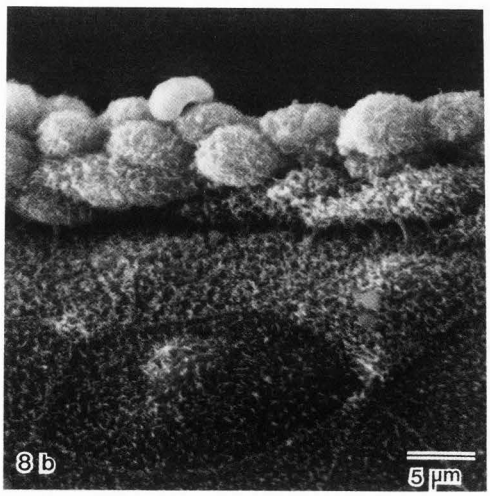
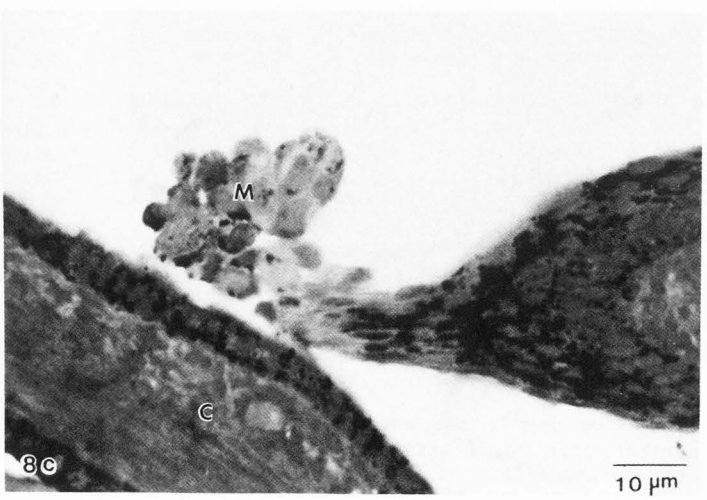


Figure 7: [a], [b] By day 15, the retina has segregated into an inner neuroblastic layer (IN) containing the primitive ganglion cells whose axons form the nerve fiber layer (arrow), and an outer neuroblastic layer (ON) containing the primordia of the photoreceptors and retinal interneurons and glial cells. (PE = retinal pigmented epithelium).

Figure 8: Eyelid closure begins on day 15 of gestation. [a] (E = surface ectoderm; C = cornea). [b] Enlargement of area indicated in [a]. The actively migrating marginal epithelium assumes a characteristically round appearance while the adjacent surface epithelium is hexagonal and flattened, similar to the corneal epithelium. [c] Light micrograph of the eyelid marginal epithelium (M) and adjacent cornea (C).





temporary layer is created by the cell process of the migrating ganglion cells. The outer neuroblastic layer can be further differentiated with the Muller cells and future amacrine cells located in the inner portion. Bipolar cells develop from the middle of the outer neuroblastic layer. Photoreceptors and horizontal cells differentiate postnatally from the outer region. The photoreceptors do not mature or become functional until several weeks postnatally, characteristic of species born with fused eyelids. Thus, at birth, the mouse retina is at a stage of maturity equivalent to the human retina at 12 weeks of gestation.

Closure of the eyelids begins on day 15 and is completed by day 17 of gestation. The eyelid margin is characteristically irregular during this time (Figure 8 a, b, c).

Abnormal Ocular Development

Spontaneously occurring ocular malformations have been described previously in several strains of mice including the C57Black strain (Pierro and Spiggle, 1967, 1969). Abnormalities in ocular development may be identified by gross examination as early as 11 days of gestation when optic cup pigmentation is first evident, and are clearly visible by day 14. Microphthalmus and coloboma (notching) of the inferior optic cup are the defects visible with the dissecting microscope, occurring in our study with an incidence of 10.8% of the embryos (n = 92) (Figures 9 a, b). The right eye is affected with nine times the frequency of the left. By SEM, reduction in size of the optic cup and lens vesicle are readily apparent (Figure 10 a). Often the reduction in size of the lens (microphakia) is more pronounced than that of the globe as a whole, resulting in a larger than normal vitreous cavity, filled with an apparently exaggerated proliferation of hyaloid vessels. In most microphthalmic eyes observed by SEM, the lens remained attached to the surface ectoderm (Figure 10 b). This prolonged attachment was observed as late as day 16 in the C57Bl/6J of this study and has also been documented to occur in the Microphthalmic White mouse strain both in vivo and in vitro (Berman and Pierro, 1969). The inferior coloboma of the optic cup results from incomplete closure of the choroidal fissure (Figure 11 a, b). Often, severe microphthalmus was accompanied by a small palpebral fissure (Figure 12).

Spontaneous congenital ocular malformations are observed with a similar incidence in the adult mice (13.2%; n = 53) and are characterized by microphthalmos, corneal opacities, anterior uveal coloboma, and persistent pupillary membranes (Figure 13 a, b). Light microscopy demonstrates focal to diffuse defects in Descemet's membrane, persistent pupillary membranes, dysgenesis of the anterior chamber, and disorganizaton and vascularization of the corneal stroma associated with the areas of corneal opacity (Figure 14 a, b). Occasional attachments may be observed between the anterior lens and posterior cornea (Figure 15 a). It is likely that delayed detachment of the lens vesicle from the surface ectoderm interferes with the migration of neural crest cells which are the primordia of the corneal endothelium and secondary stroma. The resultant defects in the corneal endothelium and irregular arrangement of the stromal cells lead to persistent corneal edema, opacification, and vascularization. The anterior iridial stroma, corneal stroma and endothelium originate from neural crest. Cleavage and remodeling of this anterior segment mesenchyme separates the cornea from the iris and forms the iridocorneal angle. Persistent attachments between the anterior stroma and the cornea are seen in areas associated with corneal endothelial defects and represent abnormal cleavage, possibly related to persistent lenticular attachment.

Posterior segment abnormalities including persistent hyperplastic primary vitreous and retinal dysplasia occur less frequently but, when present, may be accompanied by a defect in the posterior lens capsule and associated cataract (Figure 15 b, c).

Conclusions

Scanning electron microscopic studies bring

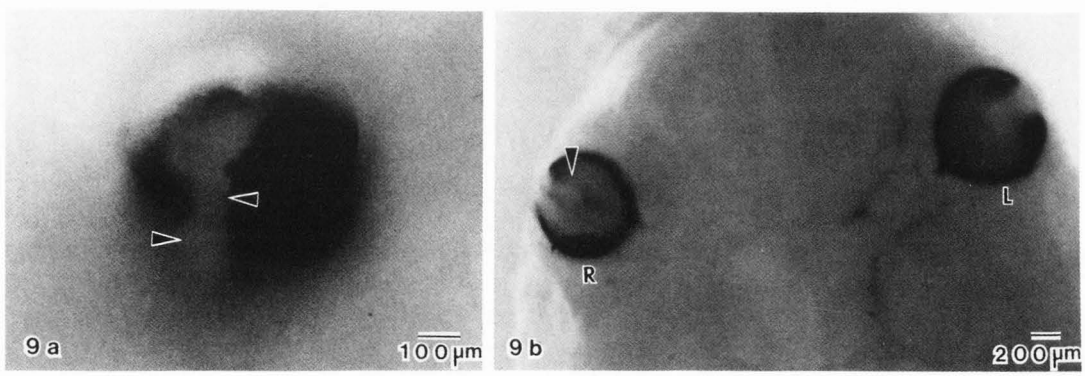


Figure 9: Same embryo as that shown in Fig. 5 (day 15). [a] The opposite eye is smaller and the choroidal fissure is widely open (arrows). [b] Superior half of head after horizontal fracture. The lens in the smaller right eye is correspondingly deficient and is elongated anterio-posteriorly (arrow). (L indicates left eye; R indicates right eye).

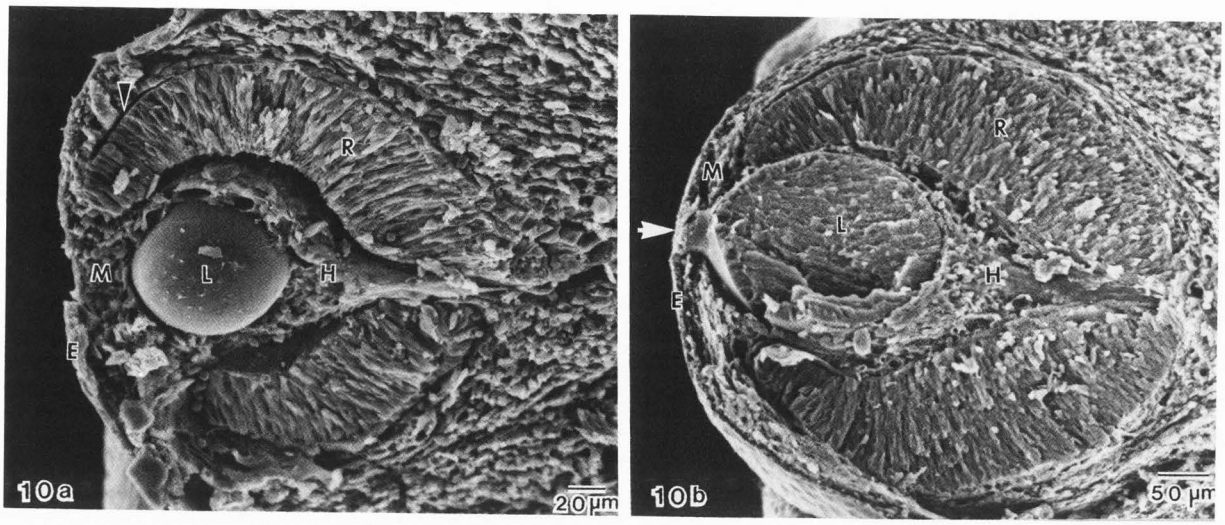


Figure 10: Abnormal ocular development: microphthalmia. [a] Eye of day 14 embryo illustrating microphakia (L = lens), extensive hyaloid vasculature (H) posterior to lens and mesenchyme (M) anterior to the lens. (R = retina; E = surface ectoderm; arrow indicates potential space between the two layers of the optic cup). [b] Eye of day 13 embryo; arrow indicates persistent attachment of the small lens (L) to the surface ectoderm (E) preventing migration of mesenchyme (M) to form the axial corneal stroma and endothelium. (R = retina; H = hyaloid vasculature).

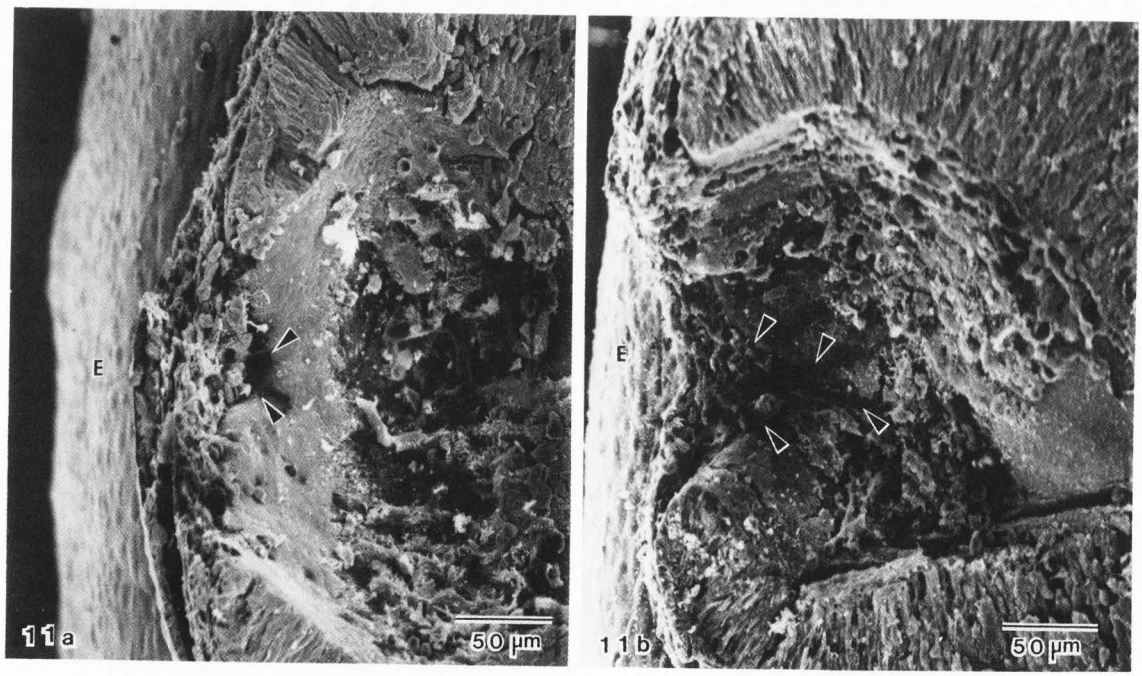


Figure 11: Persistence of the choroid fissure. [a] Arrows indicate area of normal closed anterior choroid fissure of left eye shown in figures 5 and 9b. The lens and most of the hyaloid vasculature have been removed. (E = surface ectoderm). [b] Arrows indicate abnormally open choroidal fissure of microphthalmic right eye shown in Figs. 9a and b. The lens and most of the hyaloid vasculature have been removed. (E = surface ectoderm).

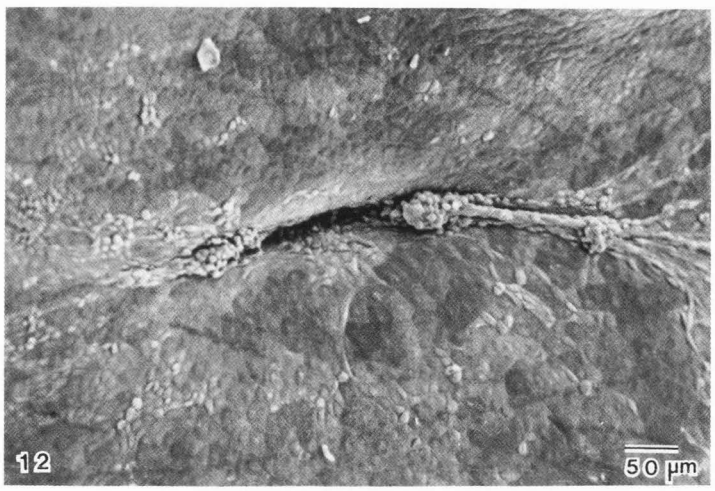


Figure 12: Small palpebral fissure of opposite eye of day 16 fetus seen in Fig. 8a.

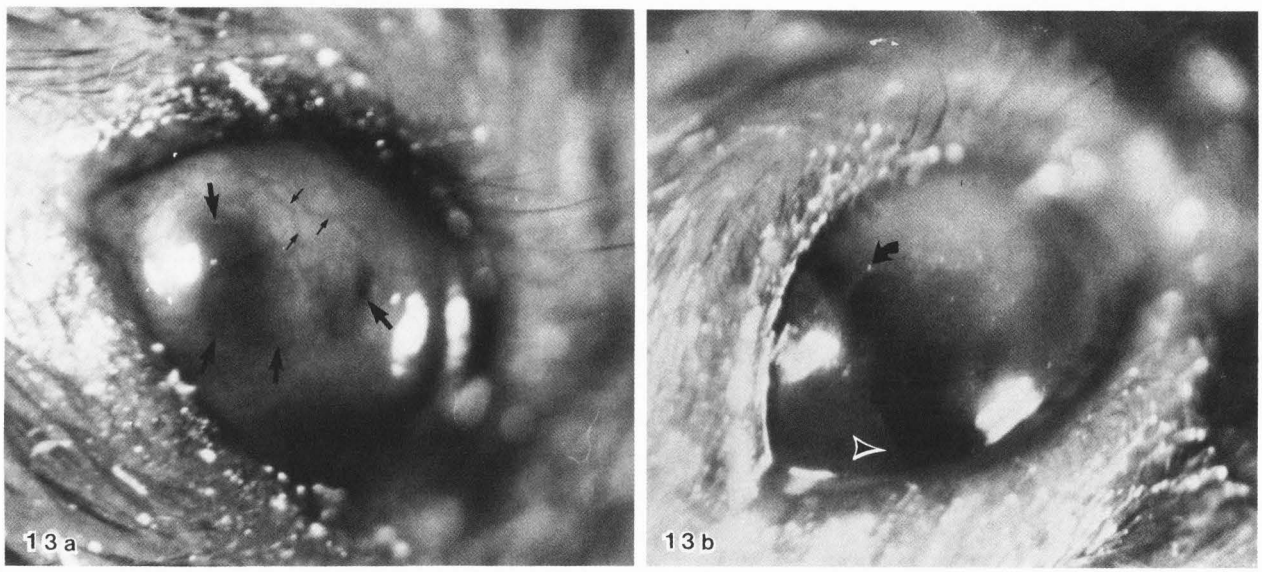


Figure 13: Ocular malformations clinically visible in adult mice. [a] Corneal edema, opacification, and vascularization (small arrows). The pupil is elliptical and irregular (large arrows) and the anterior chamber collapsed nasally. [b] Corneal edema and opacification associated with areas of attachment of persistent pupillary membranes (upper arrow). The choroidal fissure has persisted forming an anterior uveal coloboma (lower arrow).

a unique perspective to the field of ocular embryology. Previous reports on development of the mouse eye have utilized light microscopic sections which provide a limited, one dimensional view of a three dimensional process. For example, evaluation of the relationship between the developing optic cup and invaginating lens vesicle in their normal or abnormal conformation requires serial light microscopic sectioning but is easily observed by SEM. Similarly, the perception of the volume of the optic vesicle or cup or that of the lens vesicle requires a three dimensional image. This review of normal ocular embryology thus augments the existing literature and provides a foundation for future investigations of abnormal ocular morphogenesis.

Microphthalmia and anophthalmia occur with regularity in strains of inbred mice (Silver and Hughes, 1974; Horch et al., 1978; Zwaan and Silver, 1983; Robb et al., 1978; Oda et al., 1980), hamsters (Jackson, 1981) and rats (Kobayashi and Otani, 1981; Kinney et al., 1982). Proposed origins of microphthalmia include deficiency in the optic vesicle or arrested growth of the optic cup related to abnormal lens induction or persistence of the choroid fissure allowing loss of vitreous (Packer, 1967). Deficiency in size of the optic vesicle may

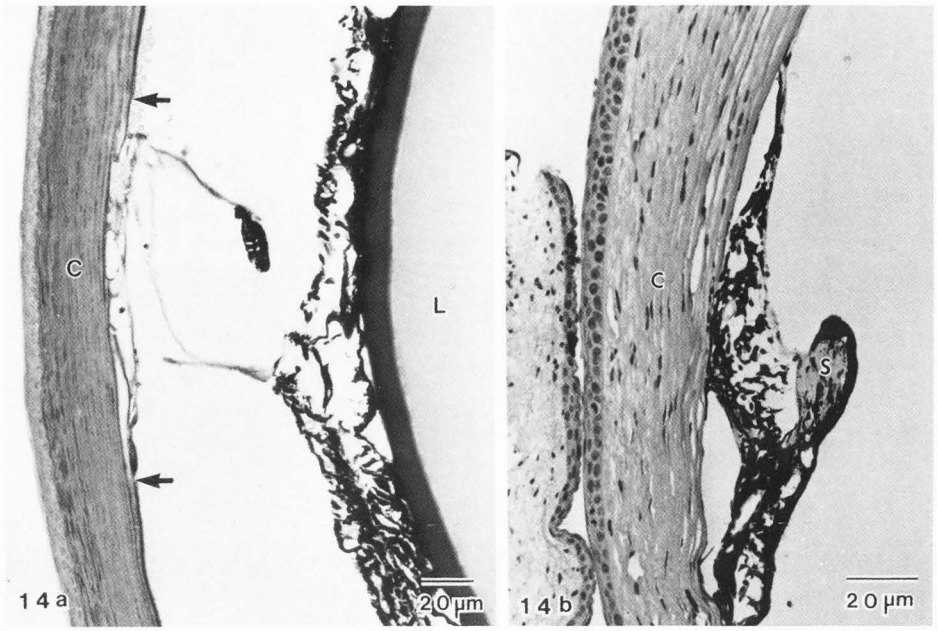


Figure 14: Histology of iridocorneal attachments. [a] Persistent pupillary membranes bridge the anterior chamber between the lens (L) and cornea (C). Descemet's membrane and corneal endothelium are absent between the arrows. [b] A more severe example of anterior segment dysgenesis with large areas of attachment of the hypoplastic iris to the cornea (C). A small remnant of pupillary sphincter (S) muscle can be seen.

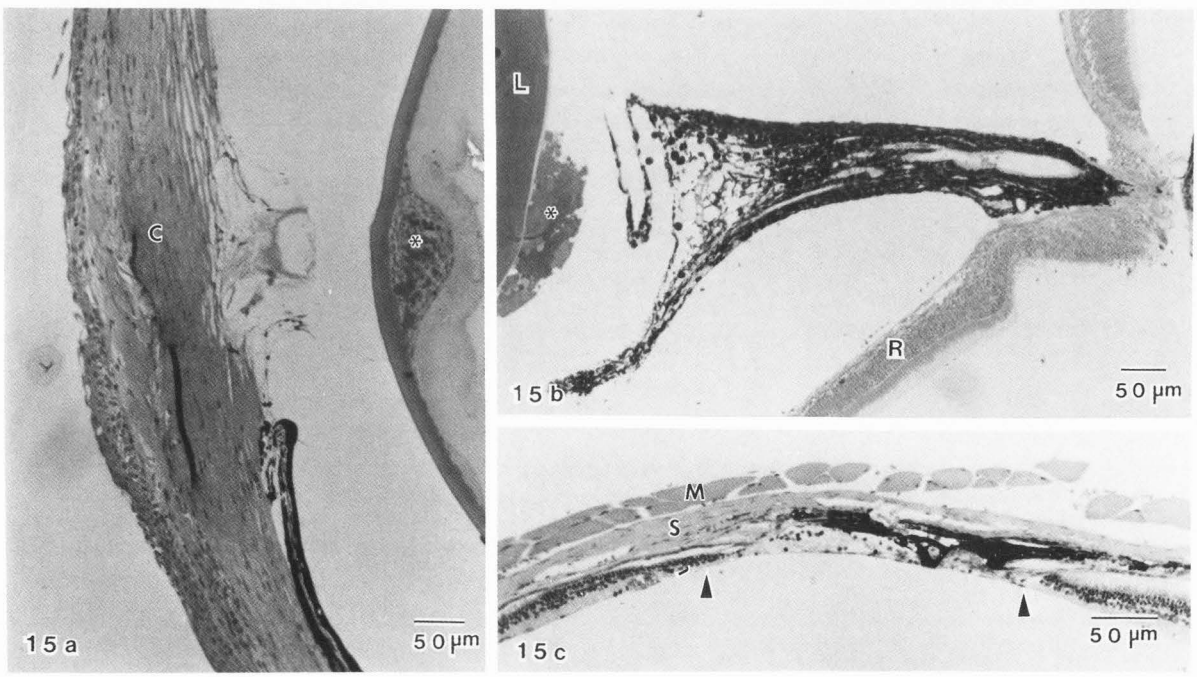


Figure 15: [a] Persistent attachment between the anterior lens and axial cornea (C) in an adult mouse eye. The attachment is associated with a cataract (*) and metaplasia of lens epithelium and corneal fibrosis and vascularization. [b] Persistent hyperplastic primary vitreous forming a pigmented retrolenticular membrane and associated cataract (*) in an adult eye. (R = retina; L = lens). [c] Retinal hypoplasia and dysplasia in an adult eye (arrows). The adjacent pigmented choroid and sclera (S) are also hypoplastic, forming a posterior segment coloboma. (M = extraocular muscles).

result in an abnormal relationship with the surface ectoderm. A corresponding reduction in the inductive influences of the optic vesicle and associated extracellular matrix in combination with misalignment of the optic vesicle may be responsible for the lens abnormalities observed secondarily (Horch et al., 1978). Failure of mesenchymal tissue between the optic vesicle and surface ectoderm to undergo normal spontaneous necrosis has been proposed as a contribution to this abnormal relationship in a primary or secondary fashion (Zwaan and Silver, 1983; Silver and Hughes, 1974).

A variable degree of persistent attachment between the lens and the surface ectoderm is a frequent finding among the microphthalmic eyes examined between 12 and 15 days of gestation in this study. Neural crest abnormalities may result in the corneal opacities, and irido-corneo-lenticular adhesions identified in adult mice which are identical to those described in the human Peters' anomaly (Kivlin et al., 1986). This anomaly has been generally considered to result from a primary deficiency of neural crest cells (Bahn et al., 1984). However our studies, including preliminary analyses of teratogen-induced ocular defects comparable to Peters' anomaly, indicate that in C57Bl/6J mice the primary deficiency is related to reduction in the size of the optic vesicles. Subsequent delayed lens detachment appears to result in mechanical interference with neural crest cell migration. Studies in progress are designed to further clarify this issue.

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Discussion with Reviewers

G.R. Beauchamp: What do the authors speculate is the primary event? Are defects in the lens and its separation due to a global problem manifesting extremely early in development, i.e., the formation of the optic vesicle and cup? Is this a problem in adequate proliferation of cells? Are there alterations in the appropriate signals to certain developmental events, e.g., induction of the lens vesicle? Or, stated another way, is microphthalmia the problem, and all other events associated? Or is microphthalmia a manifestation of a more globally disturbed process?

Authors: It appears that spontaneous microphthalmia in this mouse strain occurs because of a generalized deficiency of the forebrain leading to formation of a small optic vesicle. All subsequent events, including microphakia and delayed detachment of the lens from the surface ectoderm occur secondarily. Ethanol exposure during gastrulation dramatically increases the incidence of these malformations and allows early identification of their morphogenesis. Cellular proliferation is probably impaired and it is likely that inductive influences are similarly affected.

R.G. Higbee: Brown (Ciba Foundation Symposium 19, The Human Lens in Relation to Cataract, 1973) demonstrated by slit-lamp photography that there is no significant change in the diameter of the embryonic lens nucleus with regards to age, however, continual growth of the cortex was observed. A large variability in cortical diameter (up to 1 mm) was seen at some age groups. You state that many of the eyes were of normal size but with a small lens. Do you feel that the observed differences in overall lens size could be due to variability (within the norm) of lens development? Were measurements qualitative or quantitative?

Authors: Measurements in all cases were qualitative, however, comparisons with normal eyes matched for stage of development, revealed dramatic differences in size of the eye and lens (when involved). Although the deficiency in the lens occasionally exceeded that of the eye as a whole, microphakia was not observed to occur in an eye which was otherwise normal. The work referred to by Brown was an investigation documenting the increase in depth of the lens cortex which occurs postnatally. Measurements of the nucleus in that study remained constant and demonstrated very little variability. The events leading to microphakia in the C57Bl/6J mouse is preceded by formation of a deficient lens vesicle and thus a small embryonic lens nucleus.

G.R. Beauchamp: Have the authors considered the possibility of a vascular insufficiency as a pathophysiologic mechanism? For example, occlusion of the stapedial artery has been implicated in certain anomalies of development in

the human eye.

R.G. Higbee: In the literature, speculation concerning the origin of microphakia tends toward malnutrition, which in turn leads to dysfunction in the tunica vasculosa lentis rather than heredity. Do you have any comments on this theory?

Authors: Origin of these ocular malformations during optic vesicle formation would preclude a vascular mechanism for the microphthalmia and microphakia observed in this strain of mouse.

G.R. Beauchamp: Why is the right eye affected nine times more frequently than the left? What does this tell about mechanisms of development and the influences upon them?

Authors: The prevalence for right eye involvement was noted previously by Pierro and Spiggle in their original description of this condition. Unfortunately, reasons for such laterality remain obscure although it is maintained in the ethanol-induced ocular malformations we have observed. As impaired cellular proliferation may be responsible for the teratogen-induced effects, it is possible that there is a difference in the number of cells which form the parent populations of the right and left optic primordia.

R.G. Higbee: Were any of the fetuses smaller than normal and did you record crown-rump lengths to determine possible runting?

Authors: Crown-rump lengths were not recorded; the developmental age of fetuses older than 12 days was estimated using a morphological rating system determined by the stage of development of the feet, ears and hair follicles. Some of the more severely affected individuals were observed to be smaller and less mature than their normal littermates.