Ocular retardation (or) in the mouse. RICHARD M. ROBB, JERRY SILVER, AND RICHARD T. SULLIVAN.

The ocular retardation (or) mutation in mice has been studied morphologically in serial 1 μ sections. This recessively inherited, fully penetrant mutation is characterized by an early arrest of retinal development, aplasia of the optic nerve, cataractous degeneration of the lens, and microphthalmia. We describe early alterations of normally occurring morphogenetic cell death in the optic cup and aberrations of optic fissure formation which appear to precede the arrest of retinal and optic nerve development. The subsequent disappearance of central retinal vessels and cataract formation are interpreted as secondary phenomena.

Ocular retardation (or) is a recessive gene mutation in the mouse which causes microphthalmia associated with a progressive dissolution of the retina, aplasia of the optic nerve, and cataractous degeneration of the lens. The mutation, which has complete penetrance in the homozygous state, was initially described in 1962 by Truslove,1 who suggested that failure of development of the retinal blood supply led to the eventual retrogressive changes in the globe. More recently at the Jackson Laboratory a mutation with similar pathological features arose spontaneously in the 129/Sv-SV/CP strain of mice. Tests for allelism revealed that this mutation was allelic with ocular retardation, and the new mutant was assigned the symbol or1.2 Theiler et al.3 in 1976 noted in the or1 embryo an absence of normally occurring cell death in the eye cup stage of development. Silver and Hughes4 previously had proposed such an absence of morphogenetic cell death as a causative factor in the production of anophthalmia in a different strain of mice. Because of our interest in the early morphogenesis of the eye, we undertook a further morphological study of the or1 mutation in order to define as precisely as possible the earliest aberrations of ocular development.

Materials and methods. Mutant mice of the 129/Sv-SV/CP strain, homozygous for the ocular retardation gene, were compared to normal animals from the same strain. Timed embryos were obtained by the vaginal plug method, the time of conception being taken as midnight preceding the morning on which a plug was found. Pregnant animals were killed on various gestational days with pentobarbital. The embryos were removed from the uterus in 0.15M phosphate buffer (pH 7.2), and the heads were fixed overnight at room temperature in a combination of 0.5% glutaraldehyde and 2% formaldehyde solution. The material was postfixed in 1% OsO4 at 4°C for 2 hr and was then processed through graded alcohols and propylene oxide to be embedded in Epon. The eyes of postnatal animals were fixed and embedded in similar fashion. For each gestational and postnatal stage, 1 μ serial sections were cut through the eye in each of three planes: sagittal, coronal, and frontal. The sections were stained with toluidine blue for light microscopy.

Results. At 10.5 days of gestation the optic vesicle of normal mice is well into the initial stages of invagination to form the optic cup. This cup surrounds the lens pit from the surface ectoderm on all sides except the ventral side, where the most distal or retinal portion of the optic fissure is located. This fissure allows mesenchymal cells access to the interior of the optic cup, where a vascular network is eventually established. The over-all appearance of mutant eyes at 10.5 days was similar to that of normal eyes. The volume of the eye and the extent of invagination to form the retinal fissure were comparable. In one respect, however, mutant eyes differed from normal eyes at 10.5 days. In the mutant retina there was no evidence of cell death, whereas zones of cell necrosis were present in the normal retina and in both mutant and normal retinal pigmented epithelium and lens. The same striking absence of necrotic foci in the developing retina of mutant animals was apparent at 11.0 and 11.5 days of gestation (Fig. 1, A). This was in contrast to the increasing numbers of necrotic cells in normal animals, located especially within the base of the retina just dorsal to the area of the retinal fissure (Fig. 1, B). The normal pattern of cell death during these and other stages of eye development has been described more fully by Silver and Hughes5 in 1973.

At day 11 abundant vascularization of the interior of the optic cup by way of the retinal portion of the optic fissure was present in normal and mutant eyes (Fig. 1). The more proximal extent of the optic fissure at the base of the retina and in the
optic stalk was just becoming apparent at 11 days, but by 11.5 days it was well established in the optic stalk of normal animals (Fig. 2, A). In mutant animals this optic stalk fissure was delayed in its formation and remained abnormal throughout development (Fig. 2, B). At 11.5 days only a small intrusion of neuroectodermal cells into the stalk lumen was evident. Vascularized mesenchyme was only sparsely represented in this ventral intrusion in the mutants, whereas in normal animals the vessels which had originally entered the eye through the retinal portion of the optic fissure eventually came to course well back into the optic stalk fissure (Fig. 2).

By 12.5 days of gestation other major differences were apparent between normal and mutant eyes. Retinal ganglion cells had differentiated and had sent axons back along the optic stalk in normal animals, whereas in or1 animals no evidence of ganglion cell differentiation was apparent. In the mutant animals there now were large numbers of degenerating cells in the retina and optic stalk, especially in the ventral region adjacent to the optic fissure (Fig. 3). These necrotic foci were in excess of the number seen at this time in normal animals. The edges of the fissure were partially overlapping and redundant, but they were closely approximated and appeared to be closing. Some blood vessels still entered the eye through the posterior extent of the optic fissure. The mutant eye appeared slightly smaller than its normal control, especially in its more ventral portions.

By 14.5 days of gestation, the eye and optic nerve of normal animals had steadily increased in volume as greater numbers of ganglion cells differentiated and sent axons back toward the brain (Fig. 4, A). In the or1 mutant by day 15 all remaining fissures in the retina and optic stalk had closed completely. A substantial retinal neuroblastic layer was present, but it was difficult to distinguish ganglion cells. Although there was some indication of nerve fiber formation at the inner surface of the retina, its development was meager, and no axons exited from the eye (Fig. 4, B). In the absence of an optic nerve, the optic stalk regressed and became a slender cord of cells. The opening in the posterior retina for passage of the hyaloid vessels was completely closed at this stage. Blood vessels,
Fig. 2. Eyes at 11.5 days' gestation. A, Normal eye. The optic stalk fissure is readily apparent in this coronal section (arrow). Vascularized mesenchyme is enclosed in the cleft of the optic fissure. (×165.) Inset: Cross-section of optic stalk at level indicated by black line. (×210.) B, Eye of or^+^ mutant. The optic stalk fissure is not apparent in this coronal section. (×165.) Inset: Cross-section of mutant optic stalk. Only a slight intrusion of neuroectodermal cells has formed on the ventral surface of the stalk where the optic fissure would normally exist. (×135.)

nevertheless, persisted within the secondary eye chamber, in part due to branches from the annular vessels which entered over the anterior rim of the optic cup. At this time the amount of cell death was much diminished in both normal and mutant retinas.

At 16.5 and 18.5 days of gestation further retrogressive changes were apparent in the mutant eye. The retina failed to differentiate into recognizable layers and gradually became thinner. Vitreous did not develop, although blood vessels remained on the adjacent surfaces of retina and lens. The lens itself, which had formed primitive lens fibers and a capsule, now developed vacuoles and lost its ordered fibrillar appearance. Although a clear cornea developed, the anterior margin of the optic cup did not mature into a recognizable iris and ciliary body. Postnatally the lens became cataractous, liquefied, and shrunken. The retina further degenerated into a layer 2 to 4 cells thick. The optic stalk disappeared except for a few cells at the posterior pole of the globe. The eye remained smaller than normal, shrunken behind closed lids, and apparently sightless.

Discussion. The presence of early abnormalities of cell death in the eye rudiment of or^+^ mice suggests that the pathophysiology of the mutation is different from the retinal vascular insufficiency proposed by Truslove. Our own observations indicate that the optic cup is well vascularized...
Fig. 3. Eye of or\textsuperscript{2} mutant at 12.5 days' gestation. A. Sagittal section. No axons are apparent in the developing retina, but zones of necrosis have appeared, especially in the ventral region of the optic cup. (×260.) B, Cross-section of optic stalk just behind globe, showing fissure (arrows) which is closed and curvilinear in shape. There are mitotic activity near the ventricular surface and cell necrosis adjacent to the fissure. (×220.) C. Frontal section of retinal fissure at level indicated by black line in A, showing overlap of the edges of the optic cup. (×220.)

Beyond the time of the early morphological aberrations of the mutant. It seems likely that the eventual absence of a central retinal vessel is a secondary phenomenon, as is the absence of an optic nerve.

Theiler et al.\textsuperscript{3} recognized the early absence of morphogenetic cell death but interpreted the abnormalities of optic fissure formation as a "plug" which might mechanically obstruct egress of optic nerve fibers or prevent access of a chemotactic factor for the outgrowth of nerve fibers. We are more inclined to interpret the abnormalities of optic fissure formation as a reflection of an underlying cellular defect which first expresses itself as an aberration in the timing of morphogenetic cell death in the optic cup and later becomes manifest as a failure of the retinal precursors to differentiate beyond a rudimentary level. It is not clear what role the optic fissure plays in facilitating the passage of retinal ganglion cell axons back to the central nervous system. Mann\textsuperscript{6} and Kuwabara\textsuperscript{7} have noted that the optic nerve fibers do not pass through the cleft of the optic stalk fissure itself on their way to the chiasm but rather travel between elongated stalk cells which eventually become the glia of the optic nerve. Therefore a simple plug-
Fig. 4. Sagittal sections of eyes at 14.5 days’ gestation. A, Normal eye. Axons of ganglion cells exit from the eye in increasing numbers. The hyaloid vessels can be seen reaching forward to the lens. (×160.) B, Eye of or1 mutant. The retinal fissure has closed, and hyaloid vessels are not apparent. The optic cup remains heavily vascularized, however, and there is a substantial retinal neuroblastic layer. No retinal ganglion cell axons exit from the eye. The optic stalk is reduced in size. (×160.)

The increase in zones of necrosis in the mutant retina and optic stalk on embryonic day 12.5 is as remarkable as is the earlier lack of morphogenetic cell death in these same areas. The burst of cell death coincides with closure of the retinal portion of the optic fissure anteriorly. Posteriorly it occurs along the swollen, overlapping edges of the optic stalk fissure. It may be that this late cell necrosis, occurring in areas which are actively changing shape, represents an effort of the eye to recover from its already aberrant pattern of development.

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Key words: microphthalmia, aplasia of optic nerve, genetic mutation, morphogenetic cell death, optic fissure formation, abnormal retinal development

REFERENCES
Iontophoresis of vidarabine monophosphate
into rabbit eyes. JAMES M. HILL, NO-HEE PARK,* LOUIS P. GANGAROSA, DAVID S. HULL, CAROL L. TUGGLE, KAREN BOWMAN, AND KEITH GREEN.

In order to investigate the efficacy of iontophoresis for increasing the penetration of vidarabine monophosphate into the eye, tritium-labeled vidarabine monophosphate was applied to rabbit eyes by topical and iontophoretic application, and the penetration of the compound into the eye, and its subsequent metabolism, were studied. At 20 min after treatment, the ratios of radioactivity for corneal, aqueous humor, and iris were compared, and the penetration of vidarabine monophosphate was found to be significantly greater for the iontophoretically treated group than for the topically treated group. In addition, the acid-soluble components extracted from the cornea and aqueous humor were found to be higher for the iontophoretically treated group than for the topically treated group. Vidarabine monophosphate, vidarabine, hypoxanthine arabinoside, adenosine, hypoxanthine, and adenine from the acid-soluble fraction were separated by thin-layer chromatography. The amount of vidarabine monophosphate and vidarabine in the cornea and aqueous humor from the iontophoretically treated group was six to 15 times higher than from the group that received topical application of the drug. It was concluded that iontophoresis resulted in significantly increased penetration of the antiviral drug vidarabine monophosphate into the anterior chamber of the eye. The effects of iontophoresis on vidarabine monophosphate on corneal epithelium, as observed by scanning electron microscopy, were equal to or less than those seen with the topical application of widely used preservatives in ophthalmic preparations.

5-Iodo-2'-deoxyuridine (IDU, idoxuridine) and 9-β-D-arabinofuranosyladenine (ARA-A, vidarabine) are two drugs which can be used topically for the treatment of herpes simplex keratitis. Vidarabine and IDU have several disadvantages as antiviral agents. (1) The water solubility of both drugs is extremely low. (2) They are rapidly metabolized to less effective or inactive compounds. (3) When applied topically, there is only limited penetration of the drug into the aqueous humor. The use of vidarabine monophosphate (9-β-D-arabinofuranosyl-adenine-5'-monophosphate, Ara-AMP), the phosphorylated form of vidarabine, may eliminate certain of the disadvantages of IDU and vidarabine, since vidarabine monophosphate is a highly charged molecule and its water solubility is high. Since Ara-AMP has a charged phosphate group, its transport across the cell membrane is limited. Iontophoresis was used in an attempt to enhance the penetration of this charged molecule into the anterior chamber of the eye.

Methods. Albino rabbits (2.5 kg body weight) were given intravenous urethane (1 to 2 gm/kg body weight) anesthesia. Prior to use in the experiments, the tritiated vidarabine monophosphate (spec. act. 5.0 Ci/mmol) was chromatographed with two different solvent systems, and all the radioactivity present was found to be associated with the vidarabine monophosphate.

An eye cup was inserted with its periphery applied within the limits of the corneal limbus, and 0.7 ml of a 0.1% solution of vidarabine monophosphate (containing 5 μCi of tritiated vidarabine monophosphate) was applied inside the cup for 4 min of topical application. For cathodal (+) iontophoresis, the cathode was in contact with the drug solution, the return electrode (anode) was connected to the shaved right forelimb of the rabbit, and 0.5 mAMP of current was applied for 4 min. Immediately after completion of topical or iontophoretic administration of vidarabine monophosphate, the eyes were washed with Ringer’s solution. After either 20 or 60 min, an anterior chamber paracentesis was performed to obtain 0.15 ml of aqueous humor. After rewashings of the corneal surface, the cornea, iris, and lens were removed. These eye tissues were weighed and homogenized with a polytron in 0.5N HClO₄ (PCA) for preparation of the acid-soluble fraction. The aqueous humor was treated with 0.5N PCA. After centrifugation, the supernatant, designated as acid-soluble fraction, was collected, neutralized with KOH, and lyophilized. Total radioactivity was determined from an aliquot of each tissue sample. Also the radioactivities of the contralateral eye and blood were determined. From the neutralized and lyophilized acid-soluble fraction, vidarabine monophosphate, vidarabine, hypoxanthine arabinoside (ARA-Hx, 9-β-D-arabinofuranosylhypoxanthine), adenine, hypoxanthine (Hx), and adenosine (Ado) were separated by thin-layer chromatography. An aliquot of the lyophilized sample was spotted on a 0.5 mm silica gel GF 254 (Brinkman chromatographic glass plate). Since the amounts of labeled compounds were so small, nonlabeled vidarabine monophosphate, vidarabine, adenine, ARA-Hx, Ado, and Hx were applied at the origin. The plates were developed with the lower phase of a chloroform-containing solvent prepared.