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Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 12/5/89
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I. 12/6/89
Section I, Toxicology Branch II (H7509C)

Review of the Registrant's Response to the Previous Review Comments Concerning the Rat Teratology Study with Prodiamine (Toxicology Branch Memorandum of January 12, 1987, Winnie Teeters)

Registrant's Response: "EPA has concluded that a NOEL for developmental toxicity has not been established based on the incidence of ocular abnormalities observed at the lowest dose tested, 100 mg/kg. The Agency's conclusions were primarily (if not solely) based on historical control data provided by the testing laboratory. While we believe such data are useful, it should not preclude statistical or other evidence which does not support this conclusion." "The Agency also determined that microphthalmia and/or anophthalmia exceeded overall historical control incidence for these ocular abnormalities, thus demonstrating compound relationship. As with the finding of omphalocele, there was no dose-response relationship. In this case, however, these observations were noted at the low and high dose levels, but not the mid-dose. As we previously indicated, these malformations are reported to occur in this strain as congenital anomaly which is inherited as an autosomal recessive trait (Appendix 2 attached). Furthermore, the incidence of these malformations occurring spontaneously is quite variable and recent historical control data clearly show these lesions to be increasing in occurrence (Appendix 1 attached)."

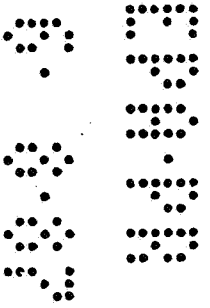
"Finally, malformations reported in this study would readily be demonstrated in a proper reproductive effects study if conducted at adequately high levels. The in-life portion of a two-generation reproduction study in rats with prodiamine will be completed shortly. This study is being conducted at levels up to 2000 ppm prodiamine in the diet (roughly equivalent to 200 mg/kg). No ocular abnormalities attributable to prodiamine and no evidence of omphalocele, microphthalmia or anophthalmia have been observed in any of the treatment or control groups (Appendix 3 attached). This observation further leads us to believe the abnormalities are random and laboratory and/or population specific." "A NOEL for developmental toxicity has been demonstrated for this study because (a) No dose-response for any reported malformations was observed, (b) Microphthalmia and anophthalmia occur in this strain as congenital anomaly, (c) Recent historical control data show these malformations are variable and spontaneously increasing in occurrence and (d) No evidence of these malformations has been observed in a rat reproduction study conducted at level higher than presumed effect levels in the teratology study."

Reviewer's Comments: The submitted addendum with the most recent historical control data for Charles River COBS CD rats (Appendix 1) and a copy of the manuscript by Kinney et al. concerning ocular defects in the Charles River CD rats (Appendix 2) provide adequate information for the spontaneous occurrences of microphthalmia and amophthalmia in the Charles River COBS CD rats. The incidences of ocular malformations at the 100 mg/kg level were found within the range for the historical control data recently submitted. The Registrant's explanations for the unusual incidences of such ocular abnormalities found at the 100 mg/kg dose group are considered to be reasonable. Since these incidences of omphalocele and ocular malformations cannot be confirmed in a rat reproduction study (Hungtington Research Center No. VCL 73/871075, February 22, 1988; Appendix 3 attached) at levels up to 2000 ppm prodiamine in the diet (equivalent to 100 mg/kg), we agree that the NOEL for developmental toxicity should be 100 mg/kg.

Recommendation: Registrant's response to the deficiencies cited in the previous Toxicology Branch review of this study is considered adequate and acceptable. The study is upgraded from Core Minimum to Core Guideline.

Developmental Toxicity NOEL = 100 mg/kg
Developmental Toxicity LEL = 300 mg/kg (based on increased incidences of omphalocele)

APPENDIX 1



February 9, 1987

Ms. Mildred Root
Toxicologist
Sandoz Crop Protection Corporation
341 East Ohio Street
Chicago, Illinois 60611-3371

Ref: WIL-15150
WIL-15153

Dear Ms. Root:

I have enclosed copies of our most recent historical information for Charles River COBS® CD® rats as well as New Zealand White rabbits as you requested. I have also enclosed a copy of the manuscript by Kinney et. al. concerning ocular defects in the Charles River CD® rat.

I hope this information proves beneficial. Please contact me if I can be of additional service.

Sincerely,

Mark D. Nemec, B.S.
Senior Toxicologist

MDN/tah

Enclosures

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WIL HISTURICAL CONTROL DATA — SUMMARY OF MATERNAL AND FETAL OBSERVATIONS AT CESAREAN SECTION — 14:01 9-FEB-87 PAGE 1
CHARLES RIVER COBS CD RATS

NO. OF ANIMALS IN THE HISTORICAL CONTROL:	1078
NO. OF ANIMALS THAT DIED:	0
NO. OF ANIMALS THAT DELIVERED:	0
NO. OF ANIMALS EXAMINED AT CESAREAN SECTION:	1078
NO. NONGRAVID:	112
NO. GRAVID:	966
NO.OF DAMS WITH ONLY RESORPTIONS:	2
NO.OF DAMS With LIVE FETUSES:	964
NO. OF LIVE FETUSES/DAM:	14.1 (11.9 - 16.1)
NO. OF POSTIMPLANTATION LOSSES/DAM:	0.7 (0.1 - 1.2)
NO. OF IMPLANTATION/DAM:	14.8 (12.8 - 16.7)
NO. OF CORPORA LUTEA/DAM:	16.4 (14.4 - 18.8)
FETAL SEX RATIO: MALE:FEMALE	6690:6893
MEAN FETAL BODY WEIGHT (G):	3.5 (3.3 - 3.9)

( ) - RANGE OF MEANS

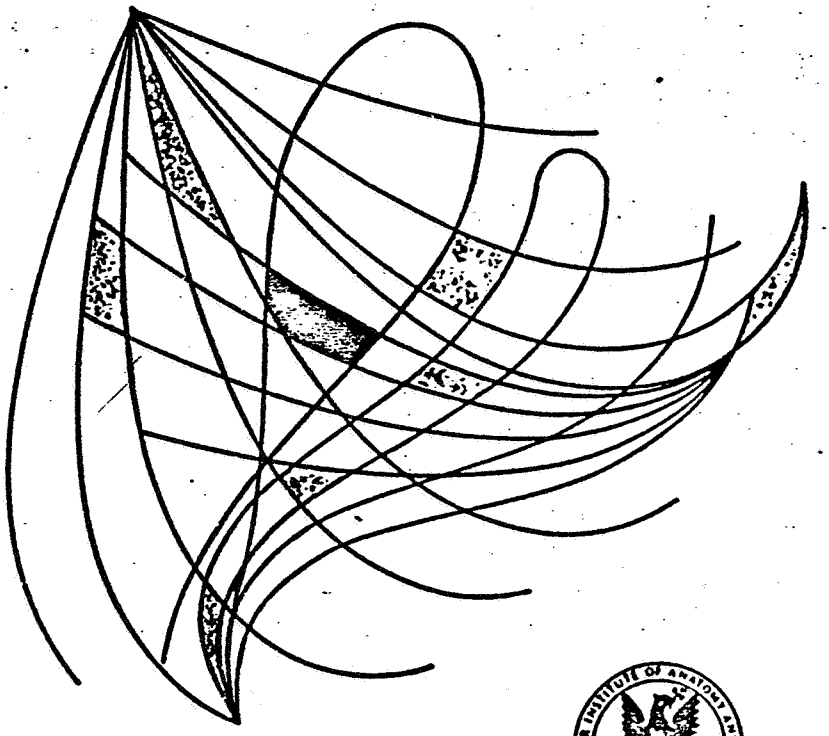
TOTAL NUMBER OF LITTERS EXAMINED	964
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	13583
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	7847
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	8456

	NUMBER FETUSES	NUMBER LITTERS	PERCENT (RANGE) FETUSES	PERCENT (RANGE) LITTERS
EXTERNAL MALFORMATIONS				
AGNATHIA	2	2	(0.0- 0.4)	(0.0- 5.0)
ASYMMETRICAL SKULL	1	1	(0.0- 0.5)	(0.0- 7.1)
CARPAL AND/OR TARSAL FLEXURE	1	1	(0.0- 0.3)	(0.0- 4.0)
GASTROSCHISIS	2	2	(0.0- 0.3)	(0.0- 4.2)
OMPHALOCELE	8	8	(0.0- 0.4)	(0.0- 5.0)
EXENCEPHALY WITH OR WITHOUT OPEN EYE LID	1	1	(0.0- 0.3)	(0.0- 4.2)
MULTIPLE ANOMALIES	2	2	(0.0- 0.3)	(0.0- 5.0)
TAIL ANOMALY WITH OR WITHOUT ANAL ATRESIA	2	2	(0.0- 0.3)	(0.0- 5.0)
FETAL ANASARCA	3	3	(0.0- 0.3)	(0.0- 4.5)
MICROPHTHALMIA AND/OR ANOPHTHALMIA	4	3	(0.0- 0.6)	(0.0- 4.3)
CLEFT PALATE	1	1	(0.0- 0.3)	(0.0- 4.5)
BRACHYDACTYLY	1	1	(0.0- 0.3)	(0.0- 4.3)
VERTEBRAL AGENESIS	1	1	(0.0- 0.3)	(0.0- 4.2)
TOTAL NUMBER OF FETUSES WITH EXTERNAL MALFORMATIONS	26	25		

APPENDIX 2

TERATOLOGY

THE INTERNATIONAL JOURNAL OF ABNORMAL DEVELOPMENT



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TERATOLOGY 26:203–212 (1982)

Congenital Cystic Microphthalmia and Consequent Anophthalmia in the Rat: A Study in Abnormal Ocular Morphogenesis

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Departments of Pathology (H.C.K., G.K.K.), Ophthalmology (G.K.K.) and Medicine (L.A.G., B.W.), Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT An otherwise normal adult Charles River rat (CD strain) was observed to have no recognizable eyes. Breeding and morphological studies were undertaken to determine the nature of the ocular defect, as well as its cause and pathogenesis. The anomaly was found to be inherited as an autosomal recessive trait with variable expressivity. It was characterized by unilateral or bilateral congenital microphthalmia with multiple associated ocular abnormalities including a neuroepithelial cyst, optic nerve aplasia, and cataract. In several elderly rats, no eye was found histologically in the orbit, suggesting reabsorption of malformed tissues as the basis of the anophthalmia. Study of the prenatal morphogenesis of the microphthalmia suggested that the primary disorder reflects a disturbance of the neuroepithelium of the retinal anlage and results in defective early formation of the optic cup. The abnormalities in other ocular structures, particularly in the lens, are considered secondary. This ocular malformation emphasizes the early interactions and interdependence of the lens and retina in normal morphogenesis and provides an animal model for study of lens-retinal relationships in abnormal morphogenesis. It is particularly relevant in understanding the pathogenesis of microphthalmia with cysts in the human eye.

The final structure of the normal mammalian eye is achieved by a precise sequence of complex, genetically determined interactions between different developing ocular tissues (Coulombre, '64). Because each ocular tissue is in turn a source and target of influence in normal morphogenesis, an aberration in one developing tissue may significantly alter the maturation of surrounding tissues. Consequently, the morphological study of an end-stage human malformation usually reveals multiple anomalies and prevents identification of the primary defect and its separation from secondary abnormalities. On the other hand, prenatal studies of similar spontaneously occurring malformations in animals allow the examination of evolving defects at sequential stages, which often allows distinction between primary and secondary abnormalities. This report describes observations on the prenatal morphogenesis of a congenital, inherited microphthalmia with an associated cyst in the Charles River rat that was found by chance during unrelated experiments. It further discusses the relevance of these observations to the pathogenesis of similar human entities, specifically, congenital cystic eyeball and microphthalmia with orbital cysts.

MATERIALS AND METHODS

An adult rat of the Sprague-Dawley substrain CD (Charles River Breeding Laboratories, Wilmington, Massachusetts) was discovered to have no recognizable eyes (Fig. 1). This defect appeared in a litter resulting from random matings for unrelated experiments within a limited breeding colony of less than 20 rats. Subsequent breeding experiments were conducted to investigate a possible genetic basis for the ocular malformation. The male propositus was back-crossed to his dam and the offspring of this mating was designated the F_1 "inbred" generation with subsequent generations designated $F_2 \ldots F_n$ according to the standard genetic convention. The inbred population was expanded by further back-crossing

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and then maintained by intercousin crossings of affected animals. Outbreeding experiments were conducted in the following manner: affected animals in the F_1 and F_2 "inbred" generations were mated with normal rats (CD strain) obtained outside the breeding colony and the offspring of these matings (F_1 "outbred") were mated to produce the F_2 "outbred" generation. The offspring were examined for clinical evidence of an ocular defect at the time of weaning, i.e., 21–25 days after birth. Affected rats had either no recognizable eyes and closed eyelids (clinical anophthalmia) or markedly small eyes with open eyelids (clinical microphthalmia).

The affected pre- and postnatal offspring of the above breedings formed the subjects of the morphologic study. Controls were obtained from breedings of unrelated, phenotypically normal parents of the CD strain. All rats were fed standard Purina Laboratory Rodent Chow and water ad libitum and maintained on a 12 hour light/12 hour dark cycle. Female rats determined to be in estrus by observation of cornified epithelial cells in vaginal smears were paired with male rats for timed periods; the females were then examined every 4 hours for vaginal plugs. Vaginal plug formation occurs 4–8 hours postcopulation and remains stable for 8–20 hours. As fertilization occurs approximately 24 hours after plug formation, gestation (day 1) was assumed to begin 24 hours after visualizing the vaginal plug (Nicholas, '42). Pregnant females were killed by ether inhalation at sequential gestational periods and the embryos and fetuses were dissected free of the uterus. These offspring were fixed in formalin-acetic acid-methyl alcohol (1 part 37% formaldehyde, 1 part glacial acetic acid, and 8 parts methyl alcohol) for 24–72 hours and then processed for microscopic examination. Serial sections (8 μm thick) of paraplast-embedded tissue were stained with hematoxylin and eosin. Eyes were examined microscopically in controls and mutants at sequential stages from day 10 of gestation until birth (approximately day 22). The number of affected eyes studied at different gestational ages were day 10—eight; day 12—seven; day 13—eighteen; day 14—ten; day 16—six; days 18–19—four; day 22—one. Control eyes were examined at each gestational age; after day 12, unaffected eyes in unilateral mutants were also used as controls.

The orbits and ocular tissues of 17 postnatal rats (aged 3 days to 17 months) with 22 eyes having the anophthalmia/microphthalmia phenotype were examined microscopically, together with normal controls. After killing of the postnatal rats with ether inhalation, the heads were fixed in 10% formalin, decalcified for 6–24 hours with rapid bone decalcifier (Dupage Kinetic Laboratories, Inc., Downer's Group, Illinois), and embedded in paraplast. Multiple step sections (8 μm thick) were obtained. Representative portions of all organs were examined microscopically.

Fig. 1. Adult mutant rat with no recognizable eyes and closed eyelids.

RESULTS

Genetic studies

The mating of affected rats with normal ones from outside the colony resulted in offspring with phenotypically normal eyes. Thus, an autosomal dominant pattern of inheritance was excluded. Males and females from these matings when mated to each other produced 105 offspring of which 82 were clinically normal and 23 affected. The defect was both unilateral and bilateral. Males and females were equally affected. By the chi-square test, the ratio of unaffected to affected animals was not significantly different from the predicted 3:1 ratio for an autosomal recessive trait. After nine and ten generations of inbreeding of affected rats, such matings produced 95% (n=54) and 100% (n=15) clinically affected offspring, respectively. These data support the conclusion that the ocular anomaly is transmitted as a single autosomal recessive gene.

Histopathologic studies

In the mutants malformations were limited to the eye and abnormalities were not detected in other parts of the body.

Day 10. In both the normal and mutant rat at day 10 of gestation, the optic vesicles formed lateral evaginations of the diencephalon with which they communicated by optic stalks (Figs. 2,3). At this time the surface ectoderm was one cell thick and had not yet formed a lens placode. Scattered mesenchymal cells between the future lens and optic vesicle appeared necrotic. The mutants could be distinguished from the controls at this stage because the tip of their optic vesicles appeared to have fewer neuroepithelial cells than normal and consequently made contact with smaller surface areas of the overlying surface ectoderm.

Day 12. By day 12 of gestation, the lens placode and optic vesicle had invaginated to form the lens vesicle and optic cup in the controls, and the inferior wall of the optic cup became infolded, forming the choroidal fissure through which mesenchyme entered to establish a vascular network (Fig. 4). Compared to day 10, the lens vesicle had detached from the overlying surface ectoderm, the primary lens fibers had begun to elongate, and the optic stalk had lengthened. At this stage of development in the mutant, however, the lens and optic vesicle were both smaller than normal, resulting in an overall small eye (Fig. 5). Unlike the control, the optic vesicle of the mutant was incompletely invaginated, and while the outer layer consisted of a normal, simple, cuboidal epithelium, the inner layer was much thinner (one to two cells thick) than normal (six to eight cells thick). Moreover, the inner and outer layers failed to appose one another as in the controls. The choroidal fissue had not yet formed and zones of necrotic cells, as described by Silver and Hughes (73), were not evident in the retinal primordium or optic stalk. As in the control, blood vessels were situated at the rim of the optic cup. In some mutants the lens vesicle was incompletely separated from the overlying surface ectoderm and the posterior lens fibers had not begun to elongate. In the mu-

Fig. 2. In the control rat at day 10 of gestation, the optic vesicle is in close contact with the overlying presumptive lens ectoderm. Hematoxylin and eosin. × 440.

Fig. 3. In the mutant rat at day 10 of gestation, the area of surface contact between the optic vesicle and presumptive lens ectoderm is smaller due to a decrease in the number of primitive neuroepithelial cells. Hematoxylin and eosin. × 440.

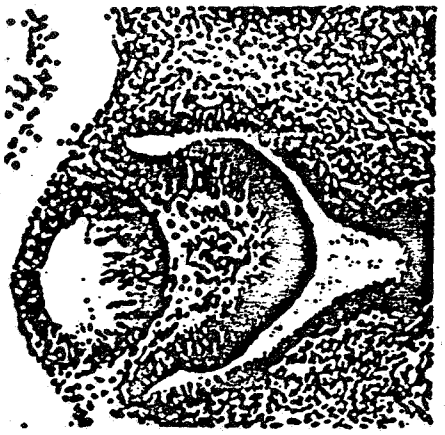

Fig. 4. Horizontal section through control eye at day 12 of gestation. Hematoxylin and eosin. × 170.

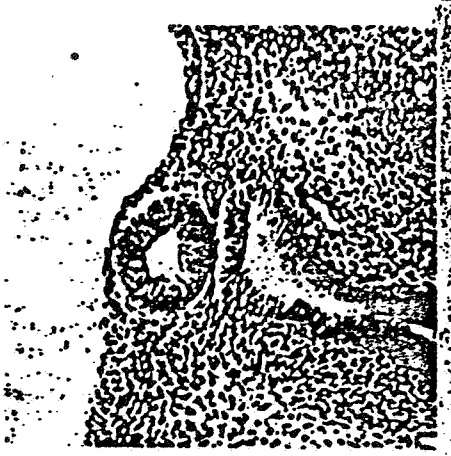

Fig. 5. In the day 12 mutant, the overall size of the eye primordia is smaller than the control. Invagination of the optic vesicle is incomplete, choroidal fissure formation is absent, and increased thickness of the inner layer of the optic cup is lacking. Horizontal section, hematoxylin and eosin. × 170.

Fig. 6. Control eye at day 13 of gestation. Horizontal section, hematoxylin and eosin. × 120.

Fig. 7. Day 13 mutant eye showing its overall small size with failure of choroidal fissure and hyaloid artery formation and lack of opposition of the inner and outer layers of the optic cup. The inner layer of the optic cup is still one cell thick. Mesenchyme extends between the lens and optic cup. Horizontal section, hematoxylin and eosin. × 120.

tants, mesenchyme surrounded the lens vesicle except where contact was made with the optic vesicle.

Days 13 and 14. In the normal rat at day 13, the inner and outer layers of the optic cup closely approached each other and the eight- to ten-cell thick sensory retina consisted of undifferentiated neuroblasts (Fig. 6). The choroidal fissure was closed and the hyaloid artery terminated in a tunica vasculosa that surrounded the lens. By day 14, axons of the retinal ganglion cells extended into the optic stalk, and the eyelids and ocular muscles became apparent. On the other hand, by days 13 and 14 in the mutants, the optic cup was imperfectly formed with incompletely apposed outer and inner layers and the latter was still only one cell thick (Fig. 7). Neither the choroidal fissure nor the hyaloid artery formed. In some instances a three- to four-cell thick inner layer of the optic cup buckled outward at the site where the choroidal fissure normally forms (Fig. 8). In the mutants the optic cup did not encompass the lens and vascularized mesenchyme extended between the lens and optic cup. Delicate blood vessels surrounded the incompletely closed lens.

Day 15–birth. Normally from day 15 until birth the inner layer of the optic cup differentiated into the various layers of the sensory retina and the lumen of the optic stalk progressively obliterated to form the optic nerve. By day 16 the epithelial layers of the ciliary body and iris could be identified and the cornea, lens, eyelids, and ocular muscles were well formed. During this period of gestation, the mutant eyes remained markedly smaller than the controls and each still lacked a choroidal fissure, hyaloid artery, and optic stalk (Figs. 9,10). The layers of the optic cup which had not apposed one another in younger specimens now formed the walls of a cyst located behind the lens. Thus, the cyst was lined by a single layer of undifferentiated neuroepithelium. Mesenchyme extended between it and the lens. By day 16 the ciliary body and iris had not formed. In the mutant eyes, the primary lens fibers began to degenerate and secondary lens fibers failed to

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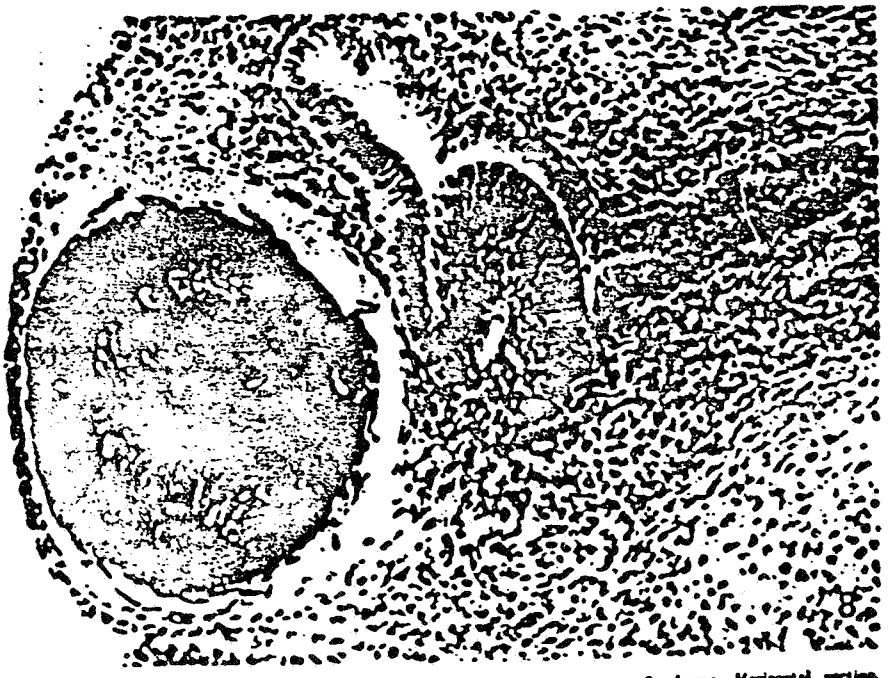

Fig. 8. In some day 13 mutants a three- to four-cell-thick layer of the optic cup buckles outwards at the site where the choroidal fissure normally forms. Horizontal section, hematoxylin and eosin. × 200.

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develop. At days 17–18, eyes of occasional mutants contained small foci of differentiated sensory retina in the posterior wall of the neuroepithelial cyst (Fig. 10), possibly representing differentiation of the buckled inner layer noted at day 13 (Fig. 8). As animals with malformed eyes became older, the lens became increasingly fragmented. At birth, the ocular defect consisted of a microphthalmic eye with a cataractous lens and a neuroepithelial cyst lined by a simple cuboidal epithelium, sometimes with a focus of partially differentiated sensory retina in the posterior wall (Fig. 11, 12). Such malformed eyes lacked a vitreous, optic nerve, ciliary body, iris, and hyaloid artery.

Postnatal. Postnatal mutant rats with both "anophthalmic" and "microphthalmic" clinical phenotypes were usually found to have varying degrees of microphthalmia on microscopic examination. In affected animals under 6 months of age the orbits contained a neuroepithelial cyst lined by a single layer of cuboidal epithelium that occasionally appeared ciliated (Fig. 13). In such cases foci of differentiated sensory retina with bipolar neurons, ganglion cells, photoreceptors, and occasional dysplastic areas of retina with rosettes or tubular structures were located in the posterior wall of the cysts. Despite serial sections of the globe, the vitreous, ciliary body, iris, and optic nerve were not found in such eyes. The cataractous lenses were often completely surrounded by a cuboidal epithelium. In mutant rats, the basic histologic features of the cornea, sclera, and extraocular muscles appeared unremarkable by light microscopy. In mutants older than 1 year, the defective eye usually consisted almost entirely of a cataractous, and often calcified, lens with variably shaped nucleated fibers surrounded by a duplicated capsule. In these animals, the retina was often replaced by a fibroglial mass. In four rats, 13 months of age or older, eyes were not found despite multiple histologic sections through the orbits.

DISCUSSION

The congenital microphthalmia found in the Charles River rat was characterized postnatally by a neuroepithelial cyst associated with a cataractous lens and aplasia of the optic nerve, iris, and ciliary body. Study of its prenatal morphogenesis was instructive in defining the pathogenesis of this end-stage malformation.

Fig. 9. In the day 16 mutant, the inner and outer layers of the optic cup (arrows) do not oppose one another and subsequently form the walls of a cyst lined by primitive neuroepithelium. Mesenchyme extends between the cyst and lens. Horizontal section, hematoxylin and eosin. × 140.

Fig. 10. In occasional eyes of mutants 17–18 days of gestation, a small focus of differentiated sensory retina (R) is present in the posterior wall of the neuroepithelial cyst (C). The primary fibers of the lens (L) are fragmented. Horizontal section rotated 90° relative to Figure 9. Hematoxylin and eosin. × 200.

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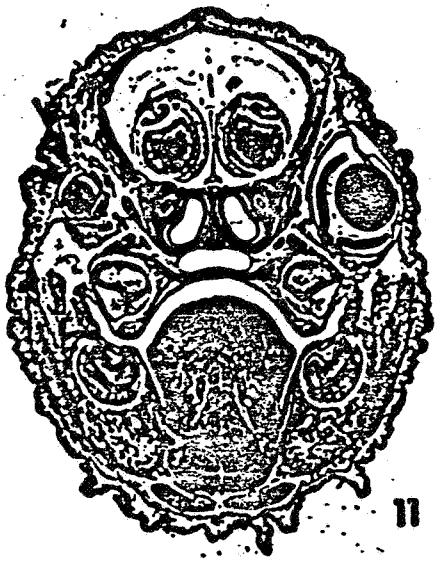

Fig. 11. A coronal section through the head of a mutant newborn with unilateral left-sided microphthalmia (arrow). Note the normal right eye. Hematoxylin and eosin. × 9.5.

Fig. 12. Higher magnification of microphthalmic eye shown in Figure 11. Note the neuroepithelial cyst (C) with focal differentiation of the sensory retina (R), fragmented lens (L), and the absence of the optic nerve, ciliary body, iris, and vitreous. Mesenchyme extends between the lens and the neuroepithelial cyst. Hematoxylin and eosin. × 50.

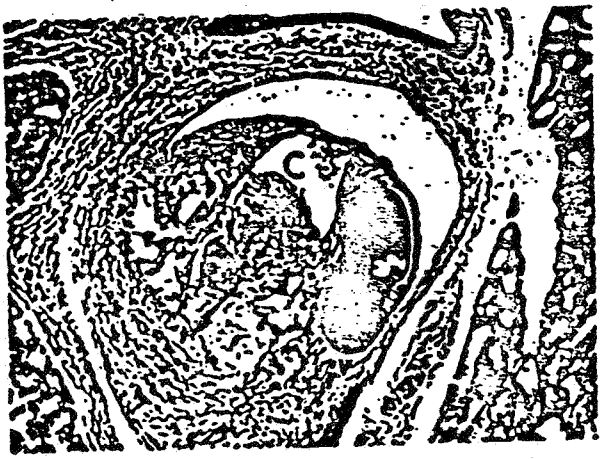

Fig. 13. Horizontal section of a postnatal microphthalmic eye consisting of a markedly cataractous lens and a neuroepithelial cyst (C) with focal differentiation of the sensory retina (R) into rods and cones, ganglion cells, and bipolar neurons. Hematoxylin and eosin. × 65.

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Examination of the mutant and control eyes at sequential ages demonstrated the initial morphologic abnormality in the mutants at day 10 of gestation, when the tip of the optic vesicle appeared smaller than normal due to fewer neuroepithelial cells. In addition the retinal anlage also manifested defective migration, differentiation, and degeneration, as judged by the improper and incomplete formation of the optic cup and failure of development of the choroidal fissure, sensory retina, ciliary body, iris, and optic nerve. Determination of the ocular anomaly as an autosomal recessive trait suggests a primary defect in the genetic control of the morphogenesis of the primitive neuroepithelium of the retinal anlage. This animal model provides insight into several aspects of normal and abnormal morphogenesis, specifically, the relationships between the developing lens and retina, the determinants of ultimate eye size, the formation of cysts in human microphthalmic eyes, and the pathogenesis of consecutive anophthalmia.

The prenatal morphogenesis of the ocular malformation in the Charles River rat underscores the relationships between developing ocular tissues, particularly of the lens and retina. Normally the optic vesicle induces lens formation in the overlying surface ectoderm by diffusible mediators and the number of cells in the lens placode is determined largely by the area of contact that the tip of the optic vesicle makes with the overlying ectoderm (Coulombre, '64). In the mutant reported in this paper, the microphthalmia presumably results from the abnormally small area of contact between the optic vesicle and the surface ectoderm. After lens induction the neural retinal normally continues to govern lens development by influencing (1) the differentiation of lens epithelium into fibers, (2) lens size, shape, and position, and the direction of lens fiber growth, and (3) the orientation of the lens to the retina (Coulombre, '64). Sequential histologic studies in the mutant indicate that the cataractous lens is preceded by abnormal lens development. The primary lens fibers began to degenerate late in gestation and secondary lens fibers failed to form, perhaps, at least in part, because of the lack of the normal retinal influence on the lens. The normal optic cup has the inherent capability to invaginate and differentiate into neural retina in the absence of other tissues as demonstrated in organ culture or heterotopic grafts in the embryo (Coulombre, '64; Mann, '64). The cyst formation in the mutant resulting from improper invagination of the optic cup hence probably reflects a primary disturbance in the retinal primordium.

The eye of the mutant Charles River rat underscores the influence that the size of the optic vesicle has in determining the ultimate size of the mammalian eye. Other animal models of congenital microphthalmia have provided insight into several determinants of eye size. These include, for the mouse, the size of the optic vesicle tip (Chase and Chase, '41; Konyukhov and Vakhrusheva, '69), patterns of morphogenetic cell death (Truslove, '62; Silver and Hughes, '74; Robb et al., '78), and, for the rat, the blood supply of the developing eye (Browman and Ramsey, '43; Browman, '61). The effect of optic vesicle size on the eventual ocular dimensions was previously emphasized in the studies of abnormal eyes in mice homogeneous for the fidget gene (Konyukhov and Vakhrusheva, '69). From those investigations, Konyukhov and Vakhrusheva ('69) postulated that the reduced growth rate of the optic cup led to its delayed contact with the ectoderm and hence prevented normal lens induction. Silver and Hughes ('74) stressed the relationship of morphogenetic cell death to the ultimate dimensions of the globe in a study of anophthalmic and microphthalmic mice. In these animals they noted failure of degeneration and reabsorption of the normally transient mesenchymal cells entrapped within the retina-lens interface following evagination of the optic vesicle. Silver and Hughes ('74) postulated that the viable mesenchyme intervening between the optic vesicle and prospective lens ectoderm interferes with the inductive process and diminishes the normal area of influence between the ectoderm and optic vesicle, thereby possibly causing the eventual small size of both lens and retinal rudiments. This mechanism did not seem to be important in the Charles River mutants as the degree of necrosis of mesenchymal cells between the presumptive lens ectoderm and optic vesicle did not differ significantly from the controls. Zones of cell death, however, were not appreciated in the retinal primordium or optic stalk; their absence may reflect further a primary defect in the genetic control of the primitive neuroepithelium of the retinal anlage. Proper formation of the choroidal fissure in the Charles River mutants is perhaps in part hindered by this failure of appropriately timed morphogenetic cell death.

In studies of inherited microphthalmia in the albino rat Browman and Ramsey ('43) and Browman ('61) observed that growth impairment of the eyes coincided with the formation

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of the ocular blood supply at about day 12 of gestation, and they suggested that agenesis of the central (hyaloid) artery was responsible for improper and incomplete optic cup and vitreous development and hence the subsequent microphthalmia. Lesser degrees of microphthalmia were explained by partial vascularization of the developing eye from the anastomosing annular vascular channels that encircle the rim of the optic cup. The descriptions and photographs of the abnormal eyes in the mutant rats studied by Browman and Ramsey ('43) and Browman ('61) bear such a striking similarity to those which are found in the mutant rats which we studied that we suspect the abnormalities may be identical. Browman and Ramsey postulated a primary defect in the developing blood supply of the eye because they detected initial morphologic changes when the ocular blood supply normally forms, about day 12 of gestation, and they did not observe the formation of the central artery. However, Browman ('61) hinted at earlier abnormalities at day 11 by stating that the "eye of the microphthalmic strain was already giving evidence of differentiating more slowly than in the normal colony." In the Charles River mutants, we interpret the initial morphologic abnormality, however, to reside in the primary neuroepithelium of the retinal analge as fewer neuroepithelial cells in the optic vesicle tip appeared to contact the overlying presumptive lens ectoderm at day 10 than normal. It seems more likely that the impaired vascular development is secondary to a failure of ocular development rather than vice versa.

Despite their significance with regard to human eyes with microphthalmia and cysts, there are very few animal models of microphthalmia with cysts which have been studied in an attempt to understand their pathogenesis (Fulton et al., '71; Koyanagi, '21; Mann, '57; Wyse and Hollenberg, '77). In humans, two distinct types of microphthalmia are associated with cysts, namely microphthalmia with orbital cysts (Alphen et al., '73; Arstikaitis, '69; Mann, '57; Meyer et al., '77; Waring and Roth, '78) and congenital cystic eyeball (Dollfus et al., '68; Helveston et al., '70; Mann, '57; Morton, '50). Both of these congenital anomalies are thought to begin early in embryogenesis but at different stages. In microphthalmia with orbital cysts, the eye, although malformed, is invariably present; the anomaly is believed to result from defective closure of the choroidal fissure after invagination of the optic vesicle with its cyst walls forming by herniation of the retina through the nonclosed clefts at the end of the sixth gestational week. This concept is based primarily on studies of animal mutants rather than on histologic observations of end-stage human affected eyes (Mann, '57). In congenital cystic eyeball, on the other hand, the entire eye is replaced by a cyst and this is thought to represent a sequel of improper optic vesicle invagination occurring by the end of the fourth gestational week. In the most severe cases of human congenital cystic eyeball, the orbit contains a cyst lined by incompletely differentiated neuroectoderm and a rudimentary lens or no lens at all. In less severe cases, the anterior cyst wall consists of a malformed retina with occasional foci of differentiation while the posterior wall is composed of a single layer of cells. The morphogenesis of human congenital eyeball is inadequately understood as sequential studies in an animal model have not been previously reported to our knowledge. The present mutant seems to be analogous to the human condition and our findings support the hypothesis that improper optic cup formation is a fundamental morphologic defect in congenital cystic eyeball.

Finally the present study not only underscores the observation that clinical anophthalmia frequently reflects severe microphthalmia but that microphthalmia may precede anophthalmia. While many adult mutant rats appeared to have no eyes their orbits usually contained extremely small eyes on microscopic examination. The fact that eyes were not detected in four of the elderly mutants despite extensive histologic sectioning presumably reflects complete absorption of a malformed eye, an interpretation impossible without detailed sequential studies in younger animals with the same inherited disorder. This indicates that at least in some situations the clinical distinction between severe microphthalmia and anophthalmia is academic and does not necessarily relate to entities of different cause or pathogenesis.

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APPENDIX 3

RECEIVED
TOXICOLOGY DEPT.

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THIS IS A FACSIMILE MESSAGE FROM
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OUR FACSIMILE TELEPHONE NUMBER IS:

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COMPANY SANDOZ CROP PROTE

CHICAGO

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FROM SJ BARTON

DATE 11-3-87

THERE ARE THREE PAGES BEING SENT TO YOU.

IF YOU RECEIVE LESS THAN THIS PLEASE CONTACT US ON
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MESSAGE FOLLOWS: RE: PRODIAMINE

PLEASE FIND ATTACHED PROVISIONAL LITTER FOR THE SECOND MATE OF THE F1B GENERATION

I HAVE CHECKED ALL AUTOPSY SHEETS OF PUP AT 2000 PPM PRIOR TO OR AT WEANING. THERE IS NO INDICATION OF ANY OBVIOUS ABNORMALITY THAT APPEARS TO BE ASSOCIATED WITH TREATMENT

REGARDS

S J Barton

BEST AVAILABLE COPY !

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