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Embryology of microphthalmus in Rattus norvegicus albinus the albino rat

A. Frank Ramsey
The University of Montana

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THE EMBRYOLOGY OF MICROPHTHALMIUS IN RATTUS NORVEGICUS
ALBICUS, THE ALBINO RAT

by

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1940

Approved:

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THE EMBRYOLOGY OF MICROPHTHALMUS IN RATTUS NORVEGICUS
ALBINUS, THE ALBINO RAT

INTRODUCTION

Several investigators (Hofmann '12, King '31, Bain '36, Browman '38) noted the sporadic appearance of microphtalmus in various strains of white rats. The causes, mode of transmission, and manifestation of this anomalous condition in various degrees are not thoroughly understood. Therefore it was thought that a study of the embryology of microphtalmus in the white rat would broaden the understanding of the occurrence and development of this anomaly.

It affords me pleasure to express my sincere appreciation to Dr. L. G. Browman, to whom I am indebted for the suggestion of the problem and for helpful advice, criticism, and assistance.

REVIEW OF LITERATURE

Eye anomalies of various kinds in the vertebrates have been reported. These can be placed in two categories: the experimental and the congenital. The artificial production of eye defects by various methods has contributed much to the understanding of the vertebrate eye.

Experimental. For many years the pathologic conditions of the adult eye induced by vitamin A deficiency were not understood. This disorder associated with the shortage of vitamin A is the ocular condition known as
xerophthalmia. The vitamin deficiency causes the failure of the normal secretions of the eyelids and lacrimal glands (Yudkin '35). The failure results in a condition of dry eyes which favors bacterial infection. Many animals have sore, pus-filled eyes, and a conjunctivitis that eventually causes total, permanent blindness. The condition is cured by supplying vitamin A to the animal’s diet in time. Yudkin ('35) and other investigators reported that a rat ration lacking vitamin B₂ produces an ocular disturbance in the form of cataract and keratitis. Male ('35), using pigs as experimental animals with diets deficient in vitamin A, reported eye anomalies in the offspring. In his experiments the gilts were placed on a ration low in vitamin A for 160 days before and for 30 days after breeding. Forty-four pigs were born without eyeballs as determined by macroscopic examination. Cannon ('40) stated that other workers who investigated reproduction in vitamin-A-deficient hogs reported abortion, resorption or the birth of dead fetuses, but they mentioned no anomalies. He cites that Hart and Miller failed to record any anatomical abnormalities among lambs from ewes kept on vitamin-A-low rations for nearly a year and night-blind at the time of lambing. Browman ('33) found no eye anomalies in 200 young rats born to females on low levels of vitamin A supplement. Likewise, Cannon ('40)
found that lack of vitamin A in the diets of female rats failed to induce anomalies in the young rats.

Adelmann (1930a) reported that Baresco, in 1877, was the first to undertake systematically the experimental production of developmental anomalies. He was able to produce monstrous chicks (among which were some exhibiting various degrees of cyclopia) by incubating eggs in a vertical position, using abnormal temperatures, varnishing the shell with substances impermeable to air, and warming the eggs unequally.

The production of cyclopia by the use of chemicals has been highly successful. Stockard (1907) first accomplished this among fishes in his now classical experiments on Fundulus embryos. He treated them with varied strengths of sea water solutions of magnesium chloride. In 1909 he further states that the cyclopean fish is exactly comparable to the monstrous cyclops of man and other mammals. McClendon (1912) in a similar manner produced cyclopia in variable numbers in fish embryos by treating them with varying strengths of sodium chloride, lithium chloride, sodium hydroxide, amyl alcohol, and acetone. Adelmann (1934) produced cyclopean amphibian embryos (Amblystoma) by treating the eggs in early stages of gastrulation with lithium chloride, ethyl alcohol, and chloral-hydrate.
Inhibition of the normal development of the fetus may result in various anomalies. Adelmann ('33a) stated that Spemann from 1901 to 1904 carried on a number of interesting experiments which enabled him to cause cyclopia in Triton by mechanical interference. He accomplished this by constricting the egg with a hair loop in stages ranging from the two cell to early gastrulation. By prick­ing the anterior end of the embryonic shield of Fundulus, Lewis ('09) obtained some typical cyclopean specimens. He believed that neither Spemann's nor Stockard's cyclopean monsters could be looked upon as germinal in origin but rather that they were truly due to environmental conditions.

Jones ('25), by a different type of experiment, probably caused the appearance of heritable eye defects in the young of albino rats. During the fourth day, without sev­ering any of the connections, he lifted the ovary, Fallopian tube and part of the uterus out of the body cavity, held them with forceps, and exposed them to the air for five minutes during which they were cooled and dried by a gentle fanning. The organs were then replaced and the cut sewed. Some of the young born after such operations had abnormal eyes. He suggested the possibility that the treatment caused the abnormality.

Guyer and Smith ('24) reported that they induced eye
anomalies in the fetal young of rabbits by injecting into
the mother a foreign serum immunized to rabbit lens. In
another strain the same investigators secured similar de-
fects in the unborn young by direct injection of pulped
lens into the pregnant mother. They further stated that
this character appeared in the offspring from these defec-
tive eyed animals without further treatment. In an exper-
iment on precipitin production through lens injury, Guyer
(’25) first tested all the serums of the rabbits used and
found without exception that lens antibodies were absent.
Then the lenses of the eyes were needled and from seven to
ten days after the operation, the serum of each rabbit was
again tested for lens precipitins. He found that in more
than 50 per cent of the cases, when the lens was injured,
a normal rabbit developed lens antibodies in its own blood
serum. Some of the offspring had defective eyes. Guyer
and Smith believed that germinal constitution could be
altered by immunologic influences and suggested this as
the best working hypothesis to account for their results.
However, Ibsen and Bushnell (’31) reported that they em-
ployed two of the methods used by Guyer for the production
of eye defects in the offspring of pregnant rabbits. They
were: (1) injecting rabbit-lens material into lens in order
to develop lens antibody in the blood serum of the latter,
later introducing this serum into the blood system of pregnant rabbits, and (2) needling the eyes of the rabbits to produce lens antibody in their blood directly. From their results they were unable to obtain any conclusive evidence that the treatment of the mothers had any effect on the offspring.

Begg and Little ('24) reported that germinal modification was produced in mice that had been x-ray irradiated. The eyes were defective in the young from these animals and this condition was transmitted to succeeding generations without further treatment.

From the above experiments it would seem reasonable to assume that any inhibitor of the eye primordia or eye "organizer" in vertebrates would result in the arrested development of the optic cup and associated parts.

Congenital. Many monstrosities of the human eyes have been recorded such as absence of the eye (anophthalmia), reduction in its size (microphthalmia), and virtual absence of the lens (aphakia). Portions of the pupillary membrane are often retained. This may cause more or less of an occlusion of the iris which interferes with vision. Congenital glaucoma results when the canal of Schlemm fails to develop and furnish normal drainage to the intraocular fluid. Coloboma is the absence of a sector or any local
area of the iris, ciliary body or choroid tunic. Cyclopia
is a single median eye replacing the paired condition.

Hofmann ('12) is one of the first workers to have carried on breeding experiments to determine whether microphthalmus in rats is inherited. King ('31) reported anophthalmus and microphthalmus had occurred in the rat colony at the Wistar Institute. Hain ('36) reported microphthalmia and other eye defects in a strain of rats obtained from the Wistar Institute. These workers have carried on extensive breeding programs in an attempt to enable them to work out the genetics of microphthalmus. However, the inheritance of this defect is still not understood.

MATERIALS AND METHODS

In February of 1937 at the Hull Zoological Laboratories at the University of Chicago, a unilaterally microphthalmic female rat, number 52, appeared in the experimental colony of albino rats of Dr. L. G. Browman, now at Montana State University. The anomaly appeared spontaneously in another individual from entirely different parents in the same colony. This female, number 51, was bilaterally microphthalmic. Members of this colony had not been subjected to any experimental treatment that would produce this modification, as far as is known.

A brother, number 50, of female 51 was mated to female
52. From this mating a litter resulted with only one uni-
laterally microphthalmic female, number 72. The microph-
thalmic strain with which we are concerned was started from
this source. Brother-sister matings of this strain with
eye anomalies were continued to increase the proportion of
small-eyed individuals per litter. The average percentage
of microphthalmic rats per litter increased from less than
5 per cent to about 45 per cent in six generations. It is
to be noted that in this strain no litter of 100 per cent
microphthalmus has been observed.

A total of twenty-two microphthalmic females were
used in the present investigation to obtain embryos and
fetuses. An exact history of each male and female was
known and their ancestry can be readily traced back to
female 52, first microphthalmic female to occur in this
strain. All females had had at least one litter previous
to operative recovery of embryos. This is significant
because it indicates the microphthalmic potentialities of
each female. After studying the ancestry and litters of
each female, a breeding and operating program was carried
out. Previous work indicated that the critical period for
the appearance and rapid development of the eye is between
the tenth and fifteenth days. On this assumption the
females that had had the highest percentage of litters with
microphthalmus were used for recovery of embryos on the above days.

The following information was compiled systematically on the data sheet for each female: a record of previous litters, record of mating, with the date and hour of copulation and identification of male used, record of daily weight during pregnancy, date and hour of operation, weight and crown rump measurement of each embryo, and a diagram of the uterine horns. The diagram was so numbered that the number assigned to each embryo was specific as to location in the uterus of any animal; that is embryo 1 of female A and embryo 1 of female B would be located in the distal end of the left uterine horn.

The females were placed in separate cages. A well balanced ration\(^1\) plus lettuce was supplied daily to each animal. During pregnancy this diet was supplemented every other day with bread and milk. Their weights were recorded daily. The oestrous cycle was followed by taking vaginal smears at 9 A.M. and 6:30 P.M. daily. If possible brother-

\(^1\) dry mixture: ground wholeswheat 40, ground whole yellow corn 15, skim milk 17, lacto casein powder 5, meat meal 5, wheat germ meal 5, alfalfa meal 5, dried pea meal 5, poultry yeast 5, bone meal 2, table salt 1, Benson (132) salt mixture. Formula for rat ration (by weight every 2 weeks): 94 parts dry mixture, 2 parts cotton seed oil, 1 part cod liver oil, 3 parts lard; 1/3 part wheat germ oil added once a month.
sister matings were made and if not some were mated with mothers as it was thought this would aid in securing a higher percentage of microphthalmus in each litter. Either the presence of a vaginal plug or the finding of spermatozoa in the morning smear was considered evidence of insemination. The pregnancy was dated from the midnight previous to this morning smear (Long and Burlingham '33) and the male was immediately removed.

At the appropriate stages of pregnancy the animals were anaesthetized with ether and the uterine horns were exposed while the animal was still alive. Each placental site was quickly sketched in the diagram on the data sheet. The uterine horn and embryos were removed and placed in warm Ringer's solution. Each embryo was immediately examined under the dissecting microscope to determine whether or not it was microphthalmic. The crown-rump measurements were taken in the fresh condition with a micrometer caliper and recorded in millimeters. The embryos were fixed in warm Bouin's solution. About five hours later the embryos were removed from Bouin's solution, rolled over twice on filter paper so that most of the moisture was absorbed, and then weighed with a torsion balance scale. The weights were recorded in milligrams. The early embryos were so delicate that hardening by fixation was necessary before they could
be weighed. The embryos were again placed in fresh fixing fluid for about twenty-four hours. If the fetus was large the head was severed from the body to insure better fixation.

The embryos were carefully oriented when embedded in paraffin and serially sectioned at 10 micra. Most of the sections were stained with Ehrlich's hematoxylin and eosin, but Mallory's triple stain was used for some of the older embryos.

NORMAL DEVELOPMENT OF THE EYE

In a series of normal embryos from microthalmic females, the normal development of the eye was followed from the optic vesicle stage (11 days) to the stage of development reached just before delivery (21 days of age).

The writer used the Long and Burlingame ('33) method for determining the chronological ages of embryos. In studying histological preparations of eyes of embryos, all sections of the wall of the optic cup were examined and counted. Since each section was 10 micra, an approximate transverse measurement of the optic cup or eyeball was easily computed. As shown in the tables (I, II, III, IV), the measurements of the optic cup, lens, and retina were taken at the greatest dimensions. Generally the middle section of the optic cup, as ascertained by count, had the greatest dimensions. Figure 1 illustrates the measurements taken of the eye.
The optic fovea, which formed on the inner surface of the anterior end of the neural fold, represented the incipience of the optic vesicle. It could be followed as a deepening pit until the neural tube closed. Long and Burlingame ('38) stated that at approximately 16½ days the optic pit was seen as a deepening depression in the lateral wall of the forebrain slightly toward its ventral aspect.

Closure of the neural tube in an embryo 11½ days old from female 804 was seen in the region of the diencephalon. The anterior part of the forebrain, the midbrain, and the anterior part of the hind brain were still open. Outpocketing of the lateral wall of the diencephalon had been completed and a well developed optic vesicle was present. The initial thickening of surface ectoderm covering the optic vesicle was apparent. The optic stalk was widely open.

Embryo 2 (11 days 21 hours) from female 634 clearly showed the inauguration of invagination of the optic vesicle, a very important phase in the development of the eye.
The cup formed is sometimes called the secondary optic vesicle, but the writer will speak of this invagination as the beginning of the optic cup.

Figure 2, Plate I, shows the extent of invagination of the optic vesicle. A thickening of the surface ectoderm, the lens placode, covering the optic cup was apparent. A slight depression in it marked the inception of invagination of the surface ectoderm to form the lens pit. The lens placode adhered to the inner layer of the optic cup. Blood vessels could be perceived in the mesoderm surrounding the optic cup. The region of greatest vascularity was at the lower part of the cup.

In embryos 12½ days old from female 805, invagination of the optic vesicle had almost been completed. There remained only a small crescent-shaped cavity between the primary layers of the cup. An increase in thickness was noted in the inner over the outer layer. As the optic cup had deepened, the basal layer of the lens placode had become curved and was adhering to the nervous retina with the convexity facing the cup. This foreshadowed the formation of a lens with a cavity. A plexus of fine blood vessels surrounded the cup.

Growth of the Eye from 12½ to 17 days (the lens vesicle stage)
A. Lens

By 12½ days the lens primordium had been pinched off from the surface ectoderm and had sunk into the optic cup. The form of the lens was an approximate hollow sphere as distinctly shown by Figure 3, Plate I. The nuclei of the anterior wall were more nearly spherical than those of the posterior wall. The nuclei of the cells and the cells themselves of the posterior wall were elongating and encroaching upon the lens cavity. At this stage the lens was entirely free of the walls of the optic cup.

By 13½ days the cells of the posterior wall had increased in length, and the lumen of the vesicle had become crescentic instead of circular (Figure 6, Plate II), as a result of the rapid growth of the epithelial cells. Lens fibers were taking shape from these cells.

The lens had increased considerably in size at 14½ days (Table III). Marked differentiation was seen in the posterior wall but not in the anterior wall. The nuclei of the cells of the posterior wall (epithelial) appeared to be migrating from the posterior to the anterior end of the cell (Figure 8, Plate II). The epithelial cells in the center near the anterior-posterior axis were the longest and straightest. They decreased regularly in length and increased in curvature, being more concave toward the sides
of the lens. The lens cavity was crescent shaped. The anterior wall possessed two to three layers of nuclei similar to the condition found at 13½ days.

At 15½ days the lens lay in the cup with its borders close to the pars caeca (Figure 10, Plate III). It was entirely free from the surface ectoderm. A thin sheet of mesoderm lay between it and the surface ectoderm. The cells of the posterior wall had increased to such an extent that the cavity of the lens had been obliterated (Figure 10, Plate III). Fewer nuclei remained in the vicinity of the anterior-posterior axis, and they appeared to have migrated toward the anterior wall of the lens. These nuclei were in the process of transformation as shown by the fact that they were only faintly stained in contrast to those of the anterior wall. The nuclei were narrow and about twice as long as in the 14½-day normal lens. Along the periphery, the nuclei were more heavily stained, broader, and shorter. Lens fibers were present in the posterior wall. The anterior wall had changed very little except that proportionately it was very thin. The nuclei still were stained heavily and were about the same shape as previously described in 14½-day lens.

B. Optic Cup and Eye Accessories

Figure 3, Plate I shows that the invagination of the
optic vesicle had been completed by 12½ days. The two primary retinal layers, nervous and pigment, had been established. The superficial or outer wall of the optic vesicle had become the inner wall of the optic cup. As development proceeded, this inner wall thickened considerably, underwent a complex process of specialization, and formed a definitive retina from the outer limbs of rods and cones to the internal limiting membrane. The inner wall (called the pars optica retinae except at the rim of the cup) already showed a separation into two zones; (1) the superficial or marginal zone more or less free from nuclei, and (2) the deeper zone of six to seven layers of elongated nuclei. The thickness of the pigment layer had been reduced to one cell layer. Near the optic stalk the nuclei were more spherical in form; toward the pars caeca they were elongated.

At 12½ days the choroid fissure was well developed. It was seen as a depression on the underside of the cup and optic stalk and continued almost to the forebrain. The hyaloid artery lay in the fissure and was supplying blood to the base of the cup and posterior surface of the lens. There was an optic stalk but no optic nerve.

Only slight differences were noted in the optic cup of the 13½-day embryo as compared with the 12½-day embryo. The pars optica of the nervous retina had a definite internal
limiting membrane. The marginal layer (free from nuclei) had increased in thickness. The deeper zone had seven to nine layers of elongated nuclei. The nuclei of the pigment retina near the stalk were oval, but nearer the pars ciliae they were more elongated.

Embryos 14½ days old showed the annular vessel (Kahn '63), called the ring artery by Rach and Soefelder ('14), at the rim of the cup. The lips of the choroid fissure had almost closed. The optic stalk had elongated and was very narrow but still patent throughout. Nerve fibers had developed from the cells of the inner layer of the cup and were migrating along the optic stalk, but had not reached the brain and could only be recognized in the distal end of the optic stalk. The primordium of the lower eyelid was present.

The pars optica was approximately 110 micra thick. Three zones could be discerned: (1) a marginal zone free from nuclei, (2) the inner neuroblastic zone with four to five layers of rounded nuclei, and (3) an outer neuroblastic zone with four to six layers of more elongated nuclei. The demarcation line between the latter two was rather indistinct. There was a definite internal limiting membrane. The pars optica and pigment layer were in contact with each other, but a basement membrane made the demarcation line distinct.
The pigment epithelium had a well defined layer of spherically shaped nuclei.

In 15½-day embryos the foetal or choroid fissure had closed except at the base of the cup where the hyaloid artery entered. A marked change had been brought about by the growth of the lower part of the cup, namely those areas bounding the foetal fissure. This growth below the insertion of the optic stalk had elevated the stalk nearer the center of the base of the optic cup. The optic stalk was very narrow in proportion to the rest of the eye. It was surrounded by the optic nerve and its lumen had been closed by the growth of the nerve. The optic nerve had contacted the brain. According to Mann (1'23) the optic nerve arises in the following way:

The optic nerve is developed in the substance of the optic stalk. The cells of the stalk itself, however, merely form the neuroglial supporting tissue of the optic nerve, the actual nerve fibers being developed from the ganglion cells of the retina (i.e. cells of the inner layer of the optic cup).

A well defined blood system was present. The hyaloid artery had broken up into branches and formed the foetal intracellular blood system. As the hyaloid artery emerged from the base of the cup it gave off a number of branches. Some supplied the inner layers of the cup with blood. One branch passed along the anterior-posterior axis to the back of the
lens. Surrounding the pigment layer of the cup was the rudiment of the choroid capillaries. Tributaries of these fine branches could be followed around the rim of the cup between the lens and the cup until they anastomosed with the intraocular blood supply. Both upper and lower lid folds were present.

Growth of the Eye from 17 to 21 days.

A. Lens

The lens of embryos approximately 17 days of age differed very little from the normal lens of 15½-day embryos, except in being larger. The lens lay farther from the mesoderm which adhered to the surface ectoderm. Lens fibers were more pronounced and the nuclear membranes of the posterior wall were only faintly delimited. The anterior wall had narrowed considerably in proportion to the size of the lens. Figure 13, Plate III definitely showed large vacuoles in the lens. This was not characteristic of a normal lens, but the rest of the eye did appear normal.

At 18½ days of age the anterior-posterior measurement of the lens was less than that from side to side (i.e. 509 micra; 825 micra). Each fiber could be seen to consist of a single epithelial cell with a nucleus. Each passed from back to front of the lens or to the anterior wall. The cells in the center were straight and long while those near the
sides were shorter and showed a concave curve at the side. By this stage the anterior wall of the lens had narrowed to a single row of cuboidal epithelial cells and the nucleus of each was spherical. The lens was free of the mesoderm which was associated with the surface ectoderm.

Measurements of the lenses of embryos approximately 21 days of age, (Table IV), revealed a considerable increase in size over the lenses of embryos 18½ days old. The single stratum of nucleated cells of the anterior border was still present. The lens consisted of a homogeneous mass of lens fibers except at the sides where transformation of epithelial cells into lens fibers had not entirely taken place.

B. Optic Cup and Eye Accessories

The optic cup in embryos approximately 17 days old was larger than in the younger embryos. The pars optica and pigment layers of the retina were separated, but this is an artifact probably caused by fixation and sectioning. The optic nerve appeared to be larger and completely encompassed the optic stalk. Remnants of the optic stalk could be traced from the optic cup to the brain. The foetal intraocular blood system from the hyaloid artery still persisted. Vitreous humor was present in the optic cup.

Upper and lower eyelids had grown until they were approaching one another, but they had not fused. The incep-
tion of the extrinsic eye muscles could be seen as massed
condensations of mesoderm around the optic cup.

The following structures could be seen in the inner
wall of the optic cup from its outer margin inward: (1) base-
ment membrane, (2) outer neuroblastic layer, (3) inner neuro-
blastic layer, (4) narrow marginal nonnucleated layer, and
(5) internal limiting membrane. The outer neuroblastic
layer consisted of six to seven layers of cells with elongat-
ed nuclei. The cells of the inner neuroblastic layer,
which were more differentiated, had migrated into the
marginal layer. They were rounded and more sparsely
scattered.

In the pigment layer of the retina the only change
taking place seemed to be that the nuclei in the region of
the pars caeca of the cup were more nearly round.

In the embryos 18½ days old the space between the
lens and the retina was filled with a peculiar hyaline
fibrillar tissue which is known as the vitreous body. The
space existing between the lens and the stroma of the cornea
associated with the surface ectoderm was the rudiment of the
anterior chamber.

Vascular mesenchymal tissue had gained entrance
around the edge of the cup and was in association with the
lens. As the hyaloid artery emerged from the optic nerve
at the base of the cup it gave off branches, some of which ran along the inside of the internal limiting membrane giving off numerous fine branches which formed a network on the inner surface of the cup. One branch from the main artery followed along the anterior-posterior axis to the lens, where it subdivided and vascularized the back surface of the lens. The optic nerve could easily be traced from the pars optica through the optic foramen to the forebrain. Strands and cells of the optic stalk persisted.

The marked advancement in the nervous retina was the presence of distinct nerve fibers running parallel with the internal limiting membrane until they passed from the eyeball as the optic nerve.

In the pigment epithelium the only change noted with the advance in age was that the nuclei occupied the complete width of the cells. This layer appeared to have been "stretched", as the nuclei were farther apart and the height of the cells was less.

The pars caeca was just starting to differentiate into the pars ciliaris and pars iridica. Vascularization in the choroid layer was very evident. Surrounding this the initial stages of sclera formation were noted.

The eyelids were fused. The lacrimal gland lay dorsally near the external angle of the eye. The plica semi-
lunaris, remnant of the nictitating membrane, was at the inner angle of the eye. From the inner angle the naso-lacrimal duct could be discerned. The extrinsic ocular muscles were recognized as distinct muscles.

In the optic cup of embryos approximately 21 days old, the basal layers (as found in the adult eye) were present. From the outer part of the cup inward the layers were: (1) pigment layer, (2) external limiting membrane, (3) layer of dense elongated nuclei approximately the width of all other strata combined, (4) narrow nonnucleated layer, (5) narrow layer containing large rounded nuclei sparsely scattered, (6) nerve fiber layer, and (7) internal limiting membrane.

Sections through the lens (Figure 16, Plate IV) foreshadowed the formation of the iris. From the equator of the lens, forward growth of the rim of the cup in close proximity with the lens had occurred. Slight folds were apparent in this growth, and marked the beginning of the pars ciliaris and pars iridica. A layer of mesoderm known as the iris stroma covered this growth.

The gap separating the cornea from the lens had increased in size and was easily recognized as the anterior chamber of the eye. The sclera was a well defined layer surrounding the choroid layer of the optic cup. A mesodermal layer, the cornea, continuous with the sclera, was
directly anterior to the anterior chamber. The surface ectoderm composed the external epithelium of the cornea. Close to the margin of the anterior chamber, at the junction of cornea and sclera, was the canal of Schlemm.

Retrogression of the foetal intracocular blood system was in its initial stages. The inner set of vasa hyaloidea propria were disappearing, but the outer set of vasa hyaloidea propria persisted. The writer found no evidence of the arteriosus centralis retina.

The lacrimal gland and nasolacrinal duct were well developed. Primordia of eyelashes, sweat glands, and oil glands were present in the eyelids.

THE DEVELOPMENT OF MICROPHTHALMUS

Abnormalities in the development of the eye were not evident during the formation of the optic vesicle. Embryos approximately 11½ days old (Table I) exhibited only small variations in measurements. The optic vesicles in these embryos were well formed and no aberrations of growth were detected.

A few hours after the above stage, initiation of invagination of the optic vesicle at its lateral bulge must have occurred because embryos of approximately 12 and 12½ days showed advance stages in this invagination. The coaptation and adhering of the lens placode to the developing
cup indicated that the retinal layer may have exerted a specific action on the surface ectoderm to influence the formation of the lens placode. Measurements of the right eye of embryo 5 from female 805 (Table I) indicated arrested development, but no aberrations in tissue could be discerned by histological examination. However, at this stage little differentiation in the retinal layers had occurred.

Severe Cases of Microphthalmia

Embryo 2, approximately 12½ days old, from female 641 illustrated clearly arrested development of the eye. The aberration of growth had occurred in the retinal layer which had retarded invagination of the optic vesicle. Spaces or gaps at the rim of the cup separated the nervous layer from the pigment layer which was not seen in normal eyes of litter mates. The choroid fissure had developed normally, but no evidence of a hyaloid artery could be found in it. The lens was smaller than that of a normal litter mate.

Numerous defects were apparent in the eye of 13-day embryo 3 from female 699 (Figure 5, Plate I). Compare with normal eye (Figure 4, Plate I). Apparently the arrest in development had occurred during the stage described for the normal eye of an approximate 12-day embryo. This arrest was more localized in the upper part of the cup than in the
lower, as differentiation and organization were more evident in the lower. The process of invagination of the whole optic vesicle had been retarded but a wider gap separated the nervous retina from the pigment retina in the upper part of the cup.

The lens of the eye had developed approximately one-half as compared with lenses of normal litter mates (Table II). Its size and location in the lower half of the optic cup indicated that only the nervous retina of the lower part of the cup had been able to evoke or stimulate the formation of the lens from the surface ectoderm. The location of the lumen of the lens in the center of the spherical lens was abnormal in that the cells of the posterior wall had not elongated. Persistence of mesenchyme from the regions lateral to the eye in the space between the lens and the retina was evident. The hyaloid artery was absent.

Another case of early arrested development was found in a 13½-day embryo, number 1, from female 65 4. Invagination of the optic vesicle had been completed and the chorioid fissure was present on the lower side of the cup and optic stalk. Yet the cup was deformed (Figure 7, Plate II).

The lens primordium appeared not to have developed specifically from the surface ectoderm covering the optic cup but more from the surface ectoderm covering the rim of
the lower part of the cup. Consequently the intimate association between the lens and optic cup as viewed in normal development was lacking and the lens was not lying in the cup proper. The lens was flattened anterior-posteriorly and differentiation and development in its posterior wall had been retarded as evidenced by the failure of migration of the nuclei of the cells toward the lens cavity and by failure of elongation of the cells themselves.

Abnormalities of histological structure in the retina were obvious. Sections in the region of the greatest diameter of the lens revealed that the nervous retina was highly disorganized. Here only one zone was seen. It had only three layers of nuclei which were mostly elongated. Sections having the lens absent had the two zones present as previously described for the normal eye of this age.

The hyaloid artery was absent and further lateral mesenchymal infiltration had occurred. Since this condition was not apparent in the optic cups of normal embryos of this age, it seems reasonable to assume that in some way the defective retina was not controlling the development of tissue within the optic cup as in normal development.

In embryo 3, 14½ days, from female 412, it was obvious that the lens, as well as the optic cup, were defective. The lens was not lying in the optic cup, but it was free from
the surface ectoderm. In the previous description of a microphthalmic eye, (Figure 7, Plate II) the possibility of the lens primordium having developed from an area of surface ectoderm not specifically bounded by the rim of the optic cup was suggested. A similar condition was noted here. As the lens developed, its growth appeared to have flattened the upper lip of the cup so that the upper lip was touching the lower side of the cup as shown in Figure 9, Plate II. It was noted again that the lens was smaller, differentiation had not taken place normally, and development of the lens had been retarded. The nuclei of the posterior wall appeared to have started to migrate toward the lens cavity. The posterior wall of the lens projected into the lens cavity in the form of a cone, and its point was topped by a giant cell not shown in Figure 9. The cells of the posterior wall had not elongated to the extent previously described for normal litter mate's eye (Figure 8). Lens fibers were apparent but not well defined. The anterior wall resembled that of the normal eyed litter mate.

Normal stages of development in the invagination of the optic vesicle had not taken place. The optic cup had developed in a haphazard manner seemingly without any controlling center. Elaboration of the nervous retina into specialized cells and layers was not occurring. A choroid
fissure had not formed and consequently no hyaloid artery was present. The annular blood vessel or a plexus of blood vessels had been formed around the rim of the cup. The presence of mesenchyme was noted in the space between the lens and retina while in normal litter mates it appeared that something in the optic cup had prevented this persistence of tissue from the lateral regions.

The microphthalmic eye of embryo 11, 15\frac{1}{2} days, from female 651, had developed very little, if any, beyond the 14\frac{1}{2}-day defective eye described above, as seen by comparison of dimensions found in Table III. The dimensions of the bilaterally microphthalmic eyes of embryo 7 (Figure 12, Plate III), litter mate of embryo 11, were less than those of the anomalous eye of the 14\frac{1}{2}-day embryo. These measurements and histological examinations indicate that severe arrest of development had occurred. The dimensions of defective eyes of embryos from female 651 closely approximate the measurements of the normal eyes of embryos from females 650 and 641 (Table II), showing that very little growth had occurred in the 15\frac{1}{2}-day defective eyes beyond the 12\frac{1}{2}-day stage.

The lens of embryo 11 from female 651 was almost a perfect sphere lying in a very shallow cup (Figure 11, Plate III). A sheath of mesoderm completely encircled the lens
and filled the rest of the cup. Dimensions of the lens revealed again that it was only about one-half as large as the normal lens of a litter mate. The lens cavity probably occupied half the volume of the lens and was located approximately in the center of the lens. Sloughed off lens material was in the cavity. Very little difference in thickness and type of cells could be discerned between the anterior and posterior walls of the lens. Although the lenses of the bilaterally microphthalmic embryo 7 varied somewhat in size, the same disorganization in the lens just described was noted.

The space between the lens and retina of embryo 11 was very shallow and had been entirely invaded by lateral mesenchyme. It was evident that development and invagination had been greatly retarded. All sections showed that the walls of the cup were semicircular. The semicircle consisted of a par optica and of pigment layer that were almost identical in thickness and type of cells. Each was composed of two to three elongated layers of nuclei, and initial stages of cytolysis were obvious. The lack of a definitive blood system in the optic cup causing insufficient nourishment for these cells is probably the most responsible factor for this condition. No hyaloid artery or annular vessel was present.

The optic cups of embryo 7 differed little from that of embryo 11. In both embryos, no evidence was found of an
optic nerve while in the normal eyed litter mate's eye, the optic nerve had already made contact with the brain. Remnants of the optic stalk were present in embryo 7.

Embryo 7 represented a severe type of microphthalmus. Associated with it was a brain anomaly. Well developed ventricles of the brain and a lumen in the optic stalk were absent. Mall ('36) reported that hydrocephaly was associated with microphthalmus, but of much rarer occurrence. Only two cases of associated brain anomalies with this eye defect were noted in the present investigation.

Both eyes of embryo 11, approximately 17 days, from female 654 were microphthalmic and showed different degrees of arrested development. One eye represented a severe case of microphthalmus. Measurements and examination showed that this eye was almost an exact duplicate of the microphthalmic eye of embryo 11 from female 651 which was approximately 15½ days old.

Vitreous humor was not present, but in normal litter mates eyes it was very evident. One readily deduces that the nervous retina is defective since it is generally believed that the vitreous body is primarily a product of the retina. The eyelids had entirely fused (Figure 14, Plate IV), indicating that the arrest in development of the optic cup had not affected their growth.
At 18½ days another severe case of microphthalmus was found in embryo 12 from female 645. A separate description is not necessary as the appearance of the eye conforms very nearly with the condition found in embryo 12 from female 640 (Figure 17, Plate IV). King ('31) reported that the optic nerves and eyeballs were absent in the adults of two unrelated albino female rats as found by autopsy. She states that bilateral anophthalmia is one of the rarer forms of eye anomalies in rats. However, she fails to specify whether it was a true condition of anophthalmia. In true anophthalmia the primary optic vesicles fail to bud out from the forebrain. It is highly probable that if the embryos described in this section had developed to mature adults, they would have been classed as anophthalmic rats, whereas actually they would only exhibit severe degrees of microphthalmia. Already marked degeneration in the lens and layers of the cup had taken place and at maturity the detection of an eyeball by autopsy would probably be most difficult.

It was evident that all severe conditions of early arrest in development of the eye of the embryo would result in atrophy and degeneration of tissue of the optic cup and lens by 19 days. As would be expected, the choroid, sclera, cornea, and iris were not found in the 18½ or 21-day microphthalmic individuals. Lacrimal glands and extrinsic eye
muscles were present in the orbit but the defective condition of the eye had distorted their growth and relationship with the eyeball.

**Mild Cases of Microphthalmia**

Eyes exhibiting lesser degrees of this anomaly were harder to detect in the earlier stages. Slight variations in size, if the cup was normal, could not be used as good criteria because small variations in the size of eyes from different embryos of the same litter are known to exist. Many of these abnormalities do not manifest themselves until a certain time when a cell or tissue fails to differentiate or develop. In other cases the malformation of the cup was very slight.

At 14½ days embryo 4 from female 618 showed a slight bilaterally microphthalmic condition. Both of the optic cups were smaller than those of a normal litter mate, and slightly malformed. Their layers appeared normal. Differentiation and development of the cells of the posterior wall of the lens had been retarded. A hyaloid artery could not be found in either eye and the optic cup appeared to be receiving its blood supply through the annular blood system.

The eyes of embryo 8 from female 651 exhibited minor defects. The shape of the cup appeared normal. It was
obvious that the lenses were defective as each had large vacuoles in the lens material. Stimulation of the normal elongation of the epithelial cells of the lens had failed to occur. A lens cavity was still present whereas in the normal lenses of litter mates, these cavities had been obliterated by the growth of the cells of the posterior wall. Nerve fibers from the cells of the inner layer of the retina had failed to develop in the left eye. In the right eye nerve fibers had arisen from the ganglion cells of the retina, but these fibers had pierced the nervous retina near the rim at the lower part of the cup instead of emerging from the base of the cup at the optic stalk. The controlling factor appeared to have been restrained as shown by the aimless wandering of these nerve fibers. The hyaloid arteries were absent. The annular blood plexus was highly developed and apparently the cup was receiving plenty of blood from this source.

At approximately 17 days of age, embryo 11 from female 664 demonstrated another slightly more severe case of this defect. Development and differentiation had not ceased. The lens presented a peculiar picture. The anterior wall was normal and was almost identical with that in a normal eye of the same age. The cells at the base of the posterior wall were long, but instead of running parallel
to the anterior-posterior axis some showed to the side. The lens cavity was entirely filled with sloughed off lens material, but its initial demarcation line was conspicuous (Figure 15, Plate IV). The material in this cavity was highly disorganized with nuclei scattered throughout. Large vacuoles were present.

The optic cup was smaller than normal and showed that development had been slightly retarded. The pars caeca of the cup had the form of a wide Y. Lateral mesenchyme had partially filled the space between the lens and retina. The formation of vitreous humor appeared to be lacking, which suggests defective retinal stimulation. The hyaloid artery was not found. Concentration of mesodermal tissue around the orbit showed the beginning of the extrinsic eye muscles. There was indication of sclera or choroid formation. The upper and lower eyelids were fused in some sections (Figure 15) but not in all.

The nervous retina was complicated. Near the pars caeca of the cup, starting from the outer margin to the inner, the following could be seen: (1) basement membrane, (2) neuroblastic layer with no separation of outer or inner layer, (3) an irregular marginal zone free of nuclei, (4) internal limiting membrane. In the center, differentiation had occurred spasmodically in small localized areas. In these
areas, outer and inner neuroblastic layers with their characteristic nuclei occurred as previously described. Near them were small areas free of nuclei scattered through the pars optica. It varied in thickness from 112 to 186 micra in the same section. At the greatest thickness or bulge, the marginal zone of nonnucleated fibers ran parallel to the internal limiting membrane. The fibers were definitely different from those previously described for marginal zones. These fibers or strands were compact and resembled those of the optic nerve. It was the optic nerve folded in the cup. However, the strands did not pierce the pars optica to any great depth.

Associated with this microphthalmic condition was a brain anomaly. The ventricles of the brain were slightly reduced in size. No trace of an optic nerve or stalk could be found.

**DISCUSSION**

Indications are in normal embryology: (1) that the lens primordium develops from the lens placode up to an early stage and (2) that the optic vesicle invaginates and early development of the optic cup is initiated. The retina must develop normally in order to produce continued normal development of the lens, optic cup, and vitreous humor.
Elaboration of the retinal layers into specific types of cells, and the formation of an optic nerve from the inner layers of the retina occur only in a normal eye. The retina apparently acts as an "organizer" following the initial vegetative processes of the lens and optic cup formations.

Severe cases of microphthalmia result when this "organizer" is lacking in the retina. The lack of normal development was noted early in the lens primordium stage (12½ days) when cells of the posterior wall failed to elongate and differentiate. The malformed optic cups suggested further that the development of the cup had been unplanned because of the lack of a directing influence.

The absence of a hyaloid artery in all of these defective eyes signifies its importance as a cause for the arrested development of the eye. In early development the optic cup and lens depend chiefly on receiving their intraocular blood supply by way of the hyaloid artery. Nourishment and vital materials must be constantly supplied to these tissues for normal development. The absence of the hyaloid artery may be explained in three ways: (1) an inherited defect, (2) the loss of the inherent quality of the nervous retina to induce the growth of a vessel (hyaloid artery) from the internal carotid artery into the choroid fissure, or (3) the malformation of the optic cup in which a choroid fissure
was not formed.

In embryos showing severe arrests of development of the eye mesenchyme from the regions lateral to the eye was always found in the space between the lens and retina, while in normal and mild cases of microphthalmia this condition was not persistent. At approximately 10½ days vitreous humor was present in the optic cup of the normal embryo but not in an embryo showing a severe degree of arrest in development of the eye. Since the formation of the vitreous humor is generally attributed to the retina, the presence of persistent mesenchyme in the cup and the failure of the retina to produce vitreous humor in these defective eyes is more substantial evidence that the cause of microphthalmia is the lack or partial loss of the "organizer" in the optic cup.

In mild degrees of microphthalmia the partial loss of the organizer in the retina was seen. The growth of the optic nerve fibers was haphazard and did not follow the tendency of the normal retina to stimulate the growth of the fibers along the optic stalk to the brain. Only two cases of brain anomaly were noted in microphthalmic embryos. In one the optic stalk was lacking and in the other only vestiges remained. The condition of the optic stalk may indicate inadequate intra-cerebral fluid, thus preventing
maintenance of lumina of the stalk, with consequent adhesion of walls of the optic stalk, degeneration and dispersion of cells of stalk and later of the optic cup.

**SUMMARY**

The development of the normal and microphthalmic eyes was followed in a series of litter mate embryos from the optic vesicle stage (11 days) to the stage of development reached just before delivery (21 days of age).

At 11½ days outpocketing of the lateral wall of the diencephalon had been completed and a well developed optic vesicle was present. The initial thickening of surface ectoderm and lens placode covering the optic vesicle was apparent. At this stage no defects were noted in the eye primordia.

The inauguration of invagination of the optic vesicle was noted at approximately 12 days, and this process had been completed by 12½ days in normal eyes but not in the microphthalmic eyes. At 12 days in normal eyes the lens placode adhered to the inner layer of the optic cup, and by 12½ days the lens vesicle had completely formed and separated from the surface ectoderm. Microphthalmia was obvious at 12½ days; the lens vesicle was smaller. The hyaloid artery lay in the choroid fissure in the normal eye, but its presence was not detected in the defective eyes.
By 13½ days in the development of the normal lens marked differentiation and growth of the cells of the posterior wall was taking place as compared with the anterior wall. The lens cavity had been obliterated by the elongation of the cells of the posterior wall at 15½ days. By 13 days, severe degrees of arrested development of the eye were very evident.

Differentiation and development in the posterior wall of the lens had been retarded as evidenced by the failure of the nuclei of the cells to migrate toward the lens cavity and by failure of the cells themselves to elongate. The lens cavity was only partially filled with sloughed off lens material at 15½ days.

Continued differentiation and growth were distinguishable in the walls of the optic cups of normal embryos 13½, 14½, and 15½ days old, but retardation of development and malformation of the layers of the optic cup were noted in microphthalmic eyes. In the normal eye at 14½ days nerve fibers were discerned developing from the cells of the inner layer of the nervous retina which foreshadowed the formation of the optic nerve, and by 15½ days the optic nerve had made contact with the brain, but no evidence was found of nerve fibers or optic nerve in embryos exhibiting this eye anomaly.
In severe cases of arrested development of the eye, the lack of a hyaloid artery was apparent while in the normal eye a definitive intraocular blood system was always present. Invariably the persistence of mesenchyme from the regions lateral to the eye in the space between the lens and retina was observed at 14½ and 15½ days in the defective but not in the normal development of the eye. The primordium of the lower eyelid was present in the normal and microphthalmic 14½-day embryos and the upper and lower lid folds were apparent at 15½ days, indicating that arrest in development of the eye proper had not affected this tissue.

At approximately 17 days of age vitreous humor was present in the optic cups of normal and slightly microphthalmic embryos but it was not found in severe cases of arrested development. Instead the space between the lens and retina was filled with persistent mesenchyme.

Eyes that exhibited a lesser degree of arrest in development were smaller, but development and differentiation had not ceased. In every case the posterior wall of the lens was found to have been retarded in development. The retinal layers, nervous and pigment, generally appeared normal, and defects were not discerned until the optic nerve failed to develop from the inner layer of the nervous retina. If nerve fibers did develop from this layer, their
growth failed to follow the normal path to the brain. The hyaloid artery was not observed, but the annular blood vessels were well developed and may have been the chief source for the intraocular blood supply.

In the optic cups of normal embryos approximately 21 days old, the basal layers, typical of the adult eye, were present. Significant changes were noted in the pars caeca, and the formation of the iris was foreshadowed. The cornea and anterior chamber were easily identified. The sclera was a well-defined layer surrounding the choroid of the optic cup. In eyes showing severe arrest in development at approximately the same age, it was not only evident that the structures described for the 21 day normal eye had failed to develop, but also that the layers of the optic cup were indistinguishable as a result of the advanced stage of degeneration.

At approximately 21 days of age a single stratum of nucleated cells of the anterior border of the normal lens persisted. The rest of the lens consisted of a homogeneous mass of lens fibers except at the sides where transformation of epithelial cells into lens fibers had not entirely taken place. In severe cases of microphthalmia the lens cavity was partially or entirely filled with sloughed off lens material. Large vacuoles in the lens always characterized mild
degrees of this condition.

Extrinsic ocular muscles were present in all microphthalmic eyes but had not developed to the same degree as those of normal eyed litter mates. Eye accessories such as eyelids, lacrimal gland, and naso-lacrimal duct did develop, but their normal relationship with the eyeball was distorted by the disorganization of the optic cup. Similar development of primordia of eyelashes, sweat glands and oil glands were present in all embryos studied.
TABLE I

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<th>Female Embryo</th>
<th>Days</th>
<th>Hours</th>
<th>Crown-rump length in mm.</th>
<th>Approximately sections thru eye ball</th>
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<th>Lens longitudinal</th>
<th>Cup transverse</th>
<th>Lens and cup longitudinal</th>
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NOTE: Measurements of the eyes in the last five columns are in micra. The first are for the left eye. Under the heading (Lens and cup longitudinal) the measurements of embryos from 804 represent the transverse of the optic vesicle.
TABLE I

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<th>Crown-Rump Length in mm</th>
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**Note:** Measurements of the eyes in the last five columns are approximate.
### Table III

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**TABLE 1**

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<th>Cup transverse</th>
<th>Lens longitudinal</th>
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<th>Growth from retina to lens length in mm</th>
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**Note:** Measurements of the eyes in the last five columns are mean microphthalmic age. The first age for the last eye.

**Male**
LITERATURE CITED


PLATE I

Explanation of Figures

2 Photomicrograph of longitudinal section of normal eye from embryo 2, female 634, showing invagination of the optic vesicle and formation of the lens placode from surface ectoderm. Approximate age, 11 days, 21 hours. X160.

3 Photomicrograph of longitudinal section of normal eye from embryo 11, female 641, showing completion of invagination of optic vesicle and development of the eye. Approximate age, 12 days, 16 hours. X160.

4 Photomicrograph of longitudinal section of normal eye from embryo 1, female 699, showing development of the eye. Approximate age, 13 days. X160.

5 Photomicrograph of longitudinal section of slightly microphthalmic eye from embryo 3, female 699, showing an early arrest in the development of the eye. Approximate age, 13 days. X160.
PLATE II

Explanation of Figures

6 Photomicrograph of longitudinal section of normal eye from embryo 2, female 654, showing development of the optic cup and lens. Approximate age, 13 days, 13 hours. X160.

7 Photomicrograph of longitudinal section of microphthalmic eye from embryo 1, female 654, showing a severe degree of arrested development. Approximate age, 13 days, 13 hours. X160.

8 Photomicrograph of longitudinal section of normal eye from embryo 8, female 618, showing further development of optic cup and lens. Note the migration of nuclei in posterior wall of the lens toward the lens cavity. Approximate age, 14 days, 12 hours. X160.

9 Photomicrograph of longitudinal section of microphthalmic eye from embryo 8, female 618, showing a severe degree of arrest in growth of the optic cup and lens. Note malformation of optic cup and failure of migration of nuclei and elongation of cells in the posterior wall of the lens. Approximate age, 14 days, 12 hours. X160.
PLATE III

Explanation of Figures

10 Photomicrograph of longitudinal section of normal eye from embryo 11, female 651. Note elongation of epitrichial cells of lens has obliterated the lens cavity. Approximate age, 15 days, 12 hours. X92.

11 Photomicrograph of longitudinal section of microphthalmic eye from embryo 11, female 651, showing a severe case of arrested development. Note invasion of mesoderm in optic cup, and unorganized gelatinous material in the lens cavity. Approximate age, 15 days, 12 hours. X92.

12 Photomicrograph of longitudinal section of microphthalmic eye from embryo 7, female 651, showing a severe case of arrested development. Note primordium of upper and lower eyelids. Approximate age, 15 days, 12 hours. X92.

13 Photomicrograph of longitudinal section of a slightly microphthalmic eye from embryo 2, female 664, showing a mild degree of arrested development. Approximate age, 16 days, 20 hours. X92.
Explanation of Figures

14 Photomicrograph of longitudinal section of microophthalmic eye from embryo 11, female 664, showing a severe case of arrested development. Note fusion of upper and lower eyelids. Approximate age, 16 days, 20 hours. X92.

15 Photomicrograph of longitudinal section of a slightly microophthalmic eye from embryo 11, female 664, showing an arrest of development more severe than in Figure 13, and less than in Figure 14. Approximate age, 16 days, 20 hours. X92.

16 Photomicrograph of longitudinal section of normal eye from embryo 10, female 640, showing development of the eye. Note the formation of cornea, anterior chamber, extrinsic eye muscle, lacrimal glands, vitreous humor, and primordium of iris. Approximate age, 20 days, 21 hours. X41.5.

17 Photomicrograph of longitudinal section of microophthalmic eye from embryo 12, female 640, showing a severe case of arrested development. Note degeneration of tissue of lens and optic cup. Approximate age, 20 days, 21 hours. X41.5.