1958

Study in the developmental anatomy of the eye in a selected series of postnatal microphthalmic white rats

George A. Schultz

The University of Montana

Let us know how access to this document benefits you.
Follow this and additional works at: https://scholarworks.umt.edu/etd

Recommended Citation
https://scholarworks.umt.edu/etd/6262

This Thesis is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mso.umt.edu.
A STUDY IN THE DEVELOPMENTAL ANATOMY OF THE EYE IN A SELECTED SERIES OF POSTNATAL MICROPHTHALMIC WHITE RATS

by

GEORGE A. SCHULTZ

B. A. University of Chicago, 1953

Presented in partial fulfillment of the requirements for the degree of Master of Arts

MONTANA STATE UNIVERSITY

1958

Approved by:

Chairman, Board of Examiners

Dean, Graduate School

Date
ACKNOWLEDGMENT

I am deeply indebted to Dr. Ludvig G. Browman whose patience, understanding and criticisms have been freely given and whose guidance was instructive. I also wish to thank Dr. G. W. Bartelmez who gave me useful advice especially in regards to the tissue stain that was used in the study. I wish to thank Dr. O. L. Stein for his criticism of the English and grammar. I further wish to thank the staff of the Department of Zoology for all the aid that they have given to me.

PLEASE NOTE: This dissertation is not a publication.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>A REVIEW OF THE LITERATURE</td>
<td>2</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>6</td>
</tr>
<tr>
<td>Materials</td>
<td>6</td>
</tr>
<tr>
<td>Methods</td>
<td>6</td>
</tr>
<tr>
<td>NORMAL EYE DEVELOPMENT</td>
<td>8</td>
</tr>
<tr>
<td>Embryonic Development of the Normal Eye</td>
<td>8</td>
</tr>
<tr>
<td>Postnatal Development of the Normal Eye</td>
<td>11</td>
</tr>
<tr>
<td>THE MICROPHTHALMIC EYES</td>
<td>15</td>
</tr>
<tr>
<td>The Explanation of the Micophthalmic Categories</td>
<td>15</td>
</tr>
<tr>
<td>Embryonic Development</td>
<td>15</td>
</tr>
<tr>
<td>Postnatal Development - The Seemingly Normal Eyes</td>
<td>17</td>
</tr>
<tr>
<td>Seemingly normal with an optic nerve</td>
<td>17</td>
</tr>
<tr>
<td>Seemingly normal with no optic nerve</td>
<td>19</td>
</tr>
<tr>
<td>100 day anomalies</td>
<td>20</td>
</tr>
<tr>
<td>Postnatal Development - The Grossly Micophthalmic Eyes</td>
<td>23</td>
</tr>
<tr>
<td>Grossly micophthalmic with eye tissue</td>
<td>24</td>
</tr>
<tr>
<td>The anophthalmic eyes</td>
<td>30</td>
</tr>
<tr>
<td>Eye Anomalies</td>
<td>31</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>32</td>
</tr>
<tr>
<td>I</td>
<td>32</td>
</tr>
<tr>
<td>II</td>
<td>40</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSION</td>
<td>55</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>58</td>
</tr>
<tr>
<td>PLATES</td>
<td>63</td>
</tr>
</tbody>
</table>

iii
Microphthalmia is a developmental eye defect which is known to occur in most groups of vertebrates. In its broadest definition microphthalmia means small eyes, and the small eyes occur both through natural causes and by experimental induction. According to Gruenwald ("47, p. 517), "there is no known type of teratogenic agent which will not under certain conditions produce ocular malformations." Most developmental ocular malformations produce small eyes in the adult so microphthalmia broadly defined is not uncommon. It has been estimated that 0.1 of 1% of human blindness is a type of microphthalmia.

Natural microphthalmia occurs both as a heritable and a nonheritable defect. No truly heritable microphthalmia has been produced experimentally except by high energy radiation (Bragg and Little, '24). The emphasis of the present study is placed upon a type of heritable microphthalmia which was discovered in a rat colony, and which has been maintained by selective breeding for twenty-one years. Apparently the microphthalmia was initiated as a natural mutation.

The work which has been done to date on the microphthalmia of this strain of animals has been directed towards an understanding of the embryology (Ramsey, "40;
Browman and Ramsey, '43) and the genetics (Browman, '54). It was therefore thought that a more complete picture of the over-all problem could be obtained if a histological study of the development of a selected series of the post-natal eyes was made.

A REVIEW OF THE LITERATURE

For many years isolated cases of microphthalmia have been reported in the literature, and to review all of the incomplete reports would be impractical. The literature reviewed here will be of the few papers which report instances of microphthalmia that have either been studied histologically, genetically, or both.

One of the more important general sources for the study of eye development is Willier et al. ('55). Another general source, Gruenwald ('47), is a summary of much of the work that has been done in the field of abnormal development. As an example of a specific teratological problem Gruenwald discusses the work that has been done on cyclopia. For an excellent review of the earlier papers related to the problem of cyclopia Adeleman ('36) should be consulted; for more recent papers Rogers ('52) is a good source.

Papers which discuss experiments with the eye are very numerous in the literature, particularly those which are concerned with experiments upon the lens or lens induction. No summary paper of lens experiments exists, but
Reyer ('48, '50) can be cited as a lead to the early literature. An often cited earlier work which contains a thorough study of lens induction is Lewis ('04).

Some of the earlier papers which discuss experiments on the development of the eye are: Stockard ('07), a classical study of the chemical and physical production of cyclopia; Werber ('16), a review and experimental study of monsters, especially of defects of the head and the eye; Guyer and Smith ('24), a controversial paper on the experimental production of a heritable defect; Ibsen and Bushnell ('31), a critical experiment that was unable to confirm the experiments of Guyer and Smith; Eaton ('37), a short review of genetic eye defects that cites authors who had the same difficulty as Ibsen and Bushnell; and Gruenwald ('47), a paper already cited, but which cites Margold as a summary-review paper of experimental work on eye until 1931.

The first of the papers concerned with the over-all problem of the development of the eye and the growth relationships of the eye structures and which reviews the early literature is Ballard ('39). Two papers by Twitty ('30, '32) describe the growth of the eye and its associated structures, and a third paper, Twitty ('40), has a review of growth controlling factors of development with the eye used as a special example.

Heritable microphthalmia is frequently reported in the
literature, but no complete studies of the phenomenon exist. A simple recessive gene is responsible for some types of heritable microphthalmia, but for the majority of cases which have been studied the pattern of inheritance is not clearly understood. The histological picture is equally obscure.

King ('31, p. 258) discusses a heritable microphthalmic defect that "was inherited as a mendelian character, and that was due to the interaction of several genetic factors, one or more of which might be carried by individuals (overlaps) that did not exhibit the defect." Degenerative changes were present in the retina, lens and iris. No mention was made of the optic nerve, and nine generations comprising 1884 rats were studied.

Eaton ('37) discusses a heritable type of microphthalmia in guinea pigs which was similar to the type that is studied in this paper. His conclusion was that "there must be a close interaction between genetic and physiological forces during the development period." (p. 358)

Jeffery ('41) gives a gross picture of some heritable microphthalmic eyes that he observed in chicks. The eyeball was reduced to $\frac{1}{2}$ the normal size and there was a pleiotropic effect on the comb. The character was inherited as a single Mendelian recessive gene and the retina contained retinal rosettes.
Chase and Chase ('41) make an extensive report on a secondary anaphthalmia which they found in mice. They were convinced that the causative agent of the eye condition acts about 10 days after fertilization. Chase ('45) refers to the set of publications that further describe the mice that were studied. Quissenberry and Brown ('42) studied a heritable defect in rats which closely fits the description of microphthalmia studied in the present paper.

Gruenwald ('44) did a histological analysis of the chick eyes that were genetically described by Jeffery ('41). The chick microphthalmia was initiated late in development, and the principal defect was found in the retina. In another paper Gruenwald ('44b) studied nonheritable, sporadic types of microphthalmia found in chicks. Gruenwald ('45) extends his discussion of eye anomalies to a study of early brain anomalies.

Hollander ('48) reports microphthalmia in pigeons which was due to a single Mendelian recessive. Roberts ('48) studied a microphthalmia in swine in which the character was inherited as a dominant of low penetrance. Butler and Robertson ('53) made a genetic study of some eye defects including microphthalmia in mice, but they did not make a full histological study. Browman ('54) discusses the heredity of microphthalmia of the ancestors of the rats that are used in this study. In one of the latest papers which
describe microphthalmia, Gubay ('56) discusses the normal small eyes of a mole, a natural microphthalmia.

MATERIALS AND METHODS

Materials

The rats used in this study were obtained from two different strains that are part of the Montana State University Zoology Department's rat colony. The microphthalmic animals are from a strain developed by Dr. Ludvig G. Browman. Microphthalmia first appeared in this strain in 1937, and it has been maintained and increased in frequency by brother-sister matings for 27 generations and subsequently by strain inbreeding. The microphthalmia is inherited as a multiple factor, maternally influenced character which is described by Browman ('54).

A total of 51 rats were used in this problem; 38 were microphthalmic and 13 were normal. For this study 73 eyes were described; 60 from the microphthalmic strain and 13 from the normal strain. Normal prenatal development was studied from slides which were borrowed from Dr. Browman.

Methods

The age groups used in this problem are: 1, 2, 3, 5, 7, 10, 13, 16, 20, 25, 35, 50 and 100 days. A day for this series is defined as "day of life" so that a rat of age one day was killed on the afternoon of the day of birth.
The eyes were prepared for sectioning in the following manner: The rats were killed by decapitation, and the skin around the eyes was trimmed away to expose the eyeballs. In the older, larger eyes the fascia around the Harderian glands was pierced and torn so better penetration of the fixative into the orbit would occur. The lenses were removed from the normal and the seemingly normal eyes, and the eyes were dehydrated in ethanol solutions. They were embedded in colloidin, cleared in cedarwood oil, and re-embedded in rubber paraffin. The ten micron thick serial sections were arranged on two slides so that two comparable areas of the eye could be stained differently. Each of the eyes then was stained with two different stains, Ehrlich's hematoxylin and eosin, and Heidenhain's modification of Mallory's connective tissue stain (Bensley and Bensley, '38). Heidenhain's stain proved to be the most useful.

After the eyes were sectioned and stained they were classified, and the data obtained from each eye was recorded on a separate mimeographed form. The eyes were classified by external appearance into a seemingly normal and a grossly microphthalmic. For each of the age groups mentioned above one normal, and at least one seemingly normal, and one to several grossly microphthalmic eyes were described. After the slides were studied the two categories of eyes were subdivided into two subcategories each.
Embryonic Development of the Normal Eye

The study of the embryonic development of the normal rat's eye was begun at 11 days post fertilization. The neural tube was almost completely closed at this time, and the optic bulb had developed. The blood vascular system and the blood has begun to appear, and the heart was still a tube.

At 11 days the proximal part of the optic bulb was smaller in diameter where the optic stalk would form, and the skin ectoderm had thickened; that is, the lens was being induced by the optic bulb. The cells of the stalk, which were several layers thick, were very uniform; mitotic activity was confined to the inside layer close to the neurocoel. There was little difference between the eye tissue and the brain tissue at this time. Early signs of vascularization were present near the optic stalk, and the mesenchyme which borders the stalk was beginning to thicken.

A definite indentation, or invagination, of the optic placode was present at 11½ days, and the two layers of the retina were forming. The pigment layer was three cell layers thick and the sensory layer was slightly thicker. There was a well defined lens placode in the ectoderm, and there were indications of protoplasmic connections between the sensory layer and the placode. Blood cells were found proximal to
the developing eye.

A definite lens pit was present in the ectoderm by 12 days, and the blood supply was becoming more definite. Rudimentary vessels were present near the edge of the optic cup and in the chorioid fissure. Apposition between the sensory and pigment layers was complete except for the marginal canal. The sensory layer itself, the primordial retina, was beginning to show the first signs of differentiation. The lens pit was present at 12½ days, and it was in the shape of a thimble.

Three layers of the brain were distinct by 13 days, and the retina had two different layers. The blood system and the chorioid fissure were better defined. There were no indications of extrinsic muscles, and the lens pit had changed to a lens vesicle. By 13½ days the epitrichial lens cells were differentiated, and the optic stalk had narrowed considerably, and it was still hollow throughout its length. The chorioid fissure was still noticeable.

Most of the cranial nerves had differentiated by 14 days. The primordia of the eyelids were found, and there were indications of a cornea. Indications of muscle formation in the mesenchyme were present. The size of the eyes was noticeably larger. Both the epitrichial and the epithelial cells of the lens were well differentiated, and the epithelial cells composed most of the bulk of the lens which was still hollow. Extensive mitotic activity was present in the
ependymal layer of the brain.

A reduction to one cell thickness had taken place in the pigment layer, and the retina had two distinct layers of cells although they were not yet separate. Mitotic activity was very evident in the basal layers of the retina. The ganglionic cells sent processes, axons, to the area of the chorioid fissure near the optic stalk which at this age was not patent throughout. The mesoderm around the optic stalk had thickened considerably.

A slight cavity was present in the 15 day old lens and the lens showed signs of being histologically hard; it was displaced from the optic cup in some sections. There were clearly two different types of nuclei in the ganglionic layers of the retina. The basal layer with many mitotic figures was composed of spheroid nuclei, and the outer ganglionic layer was composed of spherical nuclei. Optic nerve fibers from the retina reached the brain in some of the rats.

The lens and the vitreous humor occupied most of the volume of the optic cup at 16 days. The retina had three distinctly staining layers, and the extraocular muscles were well defined. At 17 days the eyelids had not completely fused. Innervation of the developing muscles was now complete, and a thick, well defined mesodermal sheath had formed around the optic nerve. In the retina there were indications of
Mullerian fibers which had undoubtedly formed earlier.

Harderian glands were beginning to form from conjunctival ectoderm, and the eyelids were fused by 18 days. The sclera had become more definite. The inner nuclear layer of the retina was apparent at 19 days. In fact, all the basic structures of the adult eye were present at 19 days, and the anterior chamber had formed. The beginnings of the ciliary folds were indicated, but the pupil had not differentiated. The anterior epithelial layer of the lens was several layers thick, and the outer epithelial layer of the cornea was only one layer thick. Indications of the solid or semi-solid nature of the vitreous humor was present; it was pulled away from the sclera. This is comparable to what was pictured by Mann (28, p. 167).

At 20 to 21 days a thinning of the pupillary membrane was taking place in the area of the future pupil, and the adjacent iris mesoderm was well vascularized. The anterior layer of the lens was now one distinct layer. Muscles were developed in the eyelids, and the layers of the retina were well defined. The canal of Schlemm was present, and there were the beginnings of a regression in the intraocular vascularization. At this age the rats are born.

Postnatal Development of the Normal Eye

Since all of the basic structures of the adult eye were present at birth, it is only necessary to elaborate upon their
development. At 1 day of age the retina had two distinct nuclear layers. Many mitotic figures were present in the inner edge of the inner nuclear layer. The eyeball was well defined and well vascularized, and a large blood vessel entered it near the optic nerve, branched into the chorioid and in part continued into the intraocular cavity. At birth the blood vessel into the intraocular cavity was degenerating. In the vitreous humor capillaries were plentiful and distinct.

The sclera was thin and compact, but the nuclei were still round and definite, not flattened and obscure as in the older eyes. The cornea was present, but there were still two different layers of connective tissue -- the developing cornea and the connective tissue from which it was developing. Mitotic figures were present in the corneal epithelium and the corneal mesoendothelium were only one layer thick. There was still a slight corneal vascularization. The thin iris mesoderm was not penetrated by the pupil.

There was a slight indication of the inner nuclear layer differentiating into the inner and the middle layers by two days. Aside from this there were no new developments in the 2 day old eye. By 3 days the pupil had formed in the iris which was well defined. The ciliary folds were also well defined. No signs of vascularization were found in the cornea. At 5 days the outer plexiform layer of the retina was
beginning to form. This was the beginning of the three adult nuclear layers. The inner ganglionic layer was well stratified, and the mitotic activity was reduced in the retinal basal layer. Intraocular vascularization was still present.

Only one mitotic figure was found in the cornea of the 5 day old eye, and the nuclei and the cells of the cornea were flattened, but distinct. The cornea continued into the thinner more compact sclera. The Harderian glands were compact, and the amount of connective tissue and vascularization in the glands was reduced because the gland acini were enlarging. In the 7 day old eye the only new development was an increase in definition of the inner plexiform layer.

All of the mitotic activity in the retina had ceased by 10 days, except near the ora serrata; and a complete separation of the middle and the inner ganglionic layers had taken place. The lens of the 10 day old eye was complete, and the nuclei of the epitrichial cells were most abundant in the equatorial region.

Mitotic activity in the retina of the 13 day old eye was very scarce or absent, and the layers were fully defined. The corneal epithelium was beginning to thicken and develop a second layer of cells. Incipient fat bodies were found around the base of the orbit at the exit of the optic nerve.
At 16 days the bacillary layer of the retina was beginning to be well defined. The cells in the middle ganglionic layer stained as if there were two different layers present. Vascularization was present in the vitreous humor, but it was considerably reduced in extent.

There were still small vessels in the vitreous humor at 20 days. The corneal epithelium was thickening, and this was the first eye of the series to show Harderian gland secretion. The intraocular vascularization of the 25 day old eye was considerably reduced, but otherwise it was not different from the 20 day old eye.

No major changes were present in the 35 and the 50 day old eyes, except that they were larger and the corneal epithelium was thickening. At 100 days the only difference, except in size, was that the corneal epithelium had the approximate 5 cell layers which is the adult number. According to Donaldson and King ('37) the eye of the rat continues to grow very slowly even after the animal is over 550 days old.

In all of the eyes the optic nerve was well developed. Much vascularization was present in the chorioid; and, in the very young eyes, vessels penetrated into the intraocular cavity. The attachments of the extraocular muscles were plainly seen, and the muscles were clearly innervated before birth. The smooth muscle of the extraocular muscles, the orbitalis muscle of Muller, was seen in all of the normal postnatal eyes. (Pl. III, fig. 11).
THE MICROPHTHALMIC EYES

The Explanation of the Microphthalmic Categories

Two major categories of microphthalmic eyes were recognized – Seemingly Normal (SN) and Grossly Microphthalmic (GM). The first of these two major categories, SN, was subdivided into Seemingly Normal with an optic nerve Present (SNP) and Seemingly Normal with an optic nerve Absent (SNA). The second of the major categories, GM, was subdivided into, Grossly Microphthalmic with much Tissue present (GMT) and Grossly Microphthalmic with little tissue present, or, in effect, Anophthalmic (GMA).

Seemingly normal means that the eye appeared normal in its gross anatomical features. In the majority of cases, however, the SN eyes were not histologically normal. Aside from the lack of the optic nerves in the SNA eyes, retinal defects were present in all of the eyes except three. Retinal rosettes, as pictured by Mann ('37, p. 209), were the most prevalent retinal defect. Grossly microphthalmic means that the eye was completely defective and nonfunctional. The GM eyes did not have an optic nerve nor a mature retina, and the eyes were always very small, or nonexistent.

Embryonic Development

Ramsey ('40) studied the embryonic development of this strain of rats, so it is only necessary to review his findings
and correlate them with the findings of the present study. Ramsey divided the embryonic eyes into normal eyes and two cases of microphthalmic eyes - severe and mild. Ramsey obtained his normal rat's eyes from "normal embryos from microphthalmic females." (p. 11). The eyes were obtained from the strain before the percentage of microphthalmic eyes was very high so many normal eyes were present.

Ramsey does not mention any eyes that would fit into the SN category which is used in the present study. Since the large percentage of normal eyes was present at the time that Ramsey made his study, and since a seemingly normal eye with an optic nerve would undoubtedly have looked normal at a prenatal age, a confusion could have existed. However, Ramsey does not mention a seemingly normal eye without an optic nerve, and this omission is more difficult to explain. All of the eyes that Ramsey called microphthalmic in his study can be classified in the grossly microphthalmic category of the present study. Each of the lenses of the eyes in Ramsey's categories contained vacuoles, and would probably never have grown to eyes of normal size.

The paper by Browman and Ramsey (1943) is essentially the publication of Ramsey's study, but includes additional data concerning this microphthalmic strain of animals. Additional adult rat eyes were autopsied, and from the autopsies it was found that 19 of 48 apparently normal eyes
were normal but lacked an optic nerve. It was suggested that lack of a posterior hyaloid artery and the presence of an annular circulation were responsible for this condition. These eyes are the SNP and the SNA eyes of the Browman and Ramsey study.

Browman and Ramsey conclude that the development of the microphthalmic eye is normal until it becomes necessary for the developing tissue to obtain its nutrients from a blood supply. The failure of the blood supply which is always associated with the maldeveloped eyes is suggested as the cause of the microphthalmia.

**Postnatal Development - The Seemingly Normal Eyes**

The eyes of the SN rat, while in the live animal, have the same gross appearance as the eye of the normal rat; however, some were slightly smaller than normal eyes. Two subcategories, SNP and SNA, were distinguished after sectioning 24 SN eyes taken from 17 animals. There was one bilateral SNP rat; two bilateral SNA rats; four rats with representatives in both categories; and ten unilateral SN rats.

Seemingly normal with an optic nerve. The eight eyes in this subcategory represent seven animals since one was bilaterally SNP. The retinalae of the SNP eyes corresponded closely to the retina of the eye in a normal rat of the same age. Only one exception was found and since this
exception will be described later, only seven of the eight SNP eyes will be described in this section. Of the seven eyes, three were histologically as normal as the normal eyes. Of the four eyes that were not quite normal, two had retinal irregularities, and two had regular but immature retinae. One that had retinal irregularities had ganglionic cells in the optic nerve; and the other had, not only ganglionic cells, but also many retinal rosettes.

Only one eye, a 100 day old eye, was smaller than a normal eye; and in all of the eyes vascularization was present and comparable to normal. There was no reason to believe that the eyes were not photosensitive and image forming, since the optic nerve was continuous throughout the orbit, and undoubtedly continued to the brain. In the eyes the fat body appeared at the normal time. Five representative eyes will now be described in detail.

5734-42 (1 day old) This eye was from a unilateral SNP rat, and the eye was normal in every way.

5794-22 (7 days old) This normal sized eye was from an unilateral SNP rat. The retina, which was mature as normal, had retinal folds and rosettes which continued far into the otherwise normal optic nerve. Mitotic figures were present in the retina. The other 7 day old eye was normal in every way.

5692-42 (10 days old) This eye from a bilateral SNP rat was as large as the normal eye, but it had a retina that was slightly immature and the outer plexiform layer had not separated to form the inner and outer nuclear layers as in a normal 10 day old eye. In the optic nerve of the eye was a small amount of ganglionic nuclei. The other eye of the rat was similar to this eye, but lacked the ganglionic nuclei
in the optic stalk.

5638-21 (100 days old) This eye was from a bilateral SN rat which had a normal optic nerve. The chief difference from a 100 day old normal eye was that the retina was not as developed as normal. The retina as a whole was thin and the layers within it were thin. Within each of the nuclear layers the number of nuclei was reduced. The layer of optic nerve fibers was also thin.

Seemingly normal with no optic nerve. In spite of the lack of the optic nerve all the eyes in this group except two 100 day anomalies had well developed retinas. In seven of the fourteen eyes which are described here, the retina was equal to that of a similar aged normal eye. In seven remaining eyes the retina was less developed and thinner than normal. Eight of the fourteen eyes had retinal folds or rosettes; two eyes had both retinal folds and a thin retina; and only one eye had neither folds nor a thin retina. .

In all of the SNA eyes a large blood vessel, the ophthalmic, penetrated the scleral wall. The vessel always transversed the fat body near where the optic nerve normally would exit from the sclera. A lack of blood supply in the chorioid layer was not apparent. Most of the eyes equalled the normal eye in size; five eyes were smaller, and ten eyes were equal to normal in size, and no correlation between eye size and retinal development was present. In all other characteristics no difference between the SNA eyes and the respective normal eyes was found. Of the total of sixteen eyes in the SNA category there were only two pairs
of bilateral SNA eyes. Several of the eyes will now be described in detail.

5734-22 (1 day old) This eye was from an unilateral SN rat, and it was very similar to 5734-41 (1 day old). In both of the eyes the retina was essentially normal in development, but there were retinal irregularities that can neither be called folds or rosettes in both of the eyes. Several large round pieces of tissue, probably accumulations of optic nerve fibers, were present in the two eyes the nerve ganglionic processes might have accumulated. As the eye grows the bundles of nerves might spread in the normal manner.

5794-31 (7 days old) and 5654-51 (2 days old) Both eyes were from unilateral SN rats. Except for the lack of the optic nerve and presence of retinal rosettes the eyes were normal.

5740-11 (20 days old) This smaller than normal eye was from an unilateral SN rat. The eye had a thin retina with retinal rosettes. 5658-42 (16 days old) had a retina comparable to normal, but otherwise the eye was essentially like this 20 day old eye.

5720-21 (35 days old) This eye of normal size was from a bilateral SN rat. There was an immature retina in which fewer nuclei with less stratification were present within each nuclear layer. The ganglionic layer also contained fewer nuclei, but the layer of the optic nerve fibers was not thin when compared to a normal eye. No retinal folds or rosettes were present. What was true for this eye was essentially true for the 25 day old eye, 5712-41.

All of the SNA eyes were well vascularized, and all the accessory structures were normal. Aside from the retinal irregularities the only essential difference from normal in the SNA eyes was the lack of an optic nerve.

100 day anomalies. In addition to the eyes described above three SN eyes were unique enough to warrant a separate description. Two of the eyes were from one rat; both eyes had grossly immature retinas. The third eye was from a rat
which had a SNP eye in the other orbit.

One of the eyes from the pair that had the grossly immature retinae had an optic nerve; the other one did not. The eye without the nerve had the more immature retina. The pigment layer was fairly well defined, but it was not always continuous, and it was not always covered by the sensory layer. The sensory layer was no more developed than in the GMT eyes that are described later. The immature retina was an irregular mass of unstratified cells which lined the scleral cavity. There was an unique blood supply to this eye. The ophthalmic artery came to the sclera to one side of the fat body and paralleled the scleral wall for a short distance, then branched. One branch entered the chorioid; the other branch continued along the sclera for a short distance and it too entered the chorioid. However, even with this blood supply, the chorioid layer was irregularly vascularized and not normally developed.

The iris was quite well defined in the eye in spite of the lack of growth of the retina. The growth of a relatively mature iris from such an immature retina indicates, either that the "immaturity" of the iris was really degeneration of the retina, or that the differentiation of the iris was not dependent upon a mature retina. Lens epitrichial cells, or cells that were epitrichial-like, were present in a projection of tissue from the region of the iris which is
evidence of Wolffian type regeneration, but a large normal appearing lens was removed from the eye prior to sectioning.

The second eye of the pair of eyes with the grossly immature retinae had a retina that was poorly developed, but an optic nerve was present. The retina contained no retinal folds or rosettes, but was very thin and at an immature stage of development. Three conventional nuclear layers were present near the ora serrata. However, as the distance from the ora serrata increased the number of ganglionic cell nuclei in the ganglionic layer decreased. Near the bottom of the optic cup only two layers could be distinguished because the ganglionic cells merged into the middle nuclear layer. The optic nerve fiber layer was correspondingly decreased in thickness until it became ill-defined. The resulting optic nerve was very thin.

The chorioid layer had a close resemblance to the normal chorioid layer, and it was vascularized by the ophthalmic artery in the usual manner. The iris that was present was comparable to the normal 100 day old iris in both size and structure. The eye was comparable to normal in every other respect. It is of interest to note that from each of the 100 day old eyes with the grossly immature retina a lens was removed which was macroscopically comparable to the normal 100 day old lens.

The third eye in the 100 day old abnormal group had
no optic nerve. But what was more unusual about the rat from which the eye was obtained was that it was recorded as a bilateral SN animal (independently, by two people) several weeks before it reached 100 days of age. At 100 days of age the rat was rechecked and one SN eye was missing. The eye could have been removed because of a fight with a cage mate or because it was diseased. No disease was obvious, but the circulation to the eye was not as well developed as it was in the other SN eyes. Perhaps as a result of defective circulation the eye was loosely attached to the orbit. The other eye in the rat was a normal SNP eye.

Postnatal Development - The Grossly Microphthalmic Eyes

Two subcategories were present in the GM eyes. The first of the subcategories, GMT, contained all of the eyes in which the tissue was arranged so there was a semblance of an eye. The eyes fell within a narrow size range; that is, the large 100 day old eyes were only slightly larger than the 1 day old eyes.

The second subcategory, GMA, was a somewhat artificial division because an unequivocal GMA, or anophthalmic, eye did not occur. In all the GMA a minute amount of eye tissue was present, or there were indications that tissue had been present. The separation was made because a large gap with no intermediate types was present between the GMT and the GMA eyes. A total of 38 eyes, 34 GMT and 4 GMA, were described
for the GM category.

**Grossly microphthalmic eyes with eye tissue.** Most of the eyes that were placed in this subcategory were found buried deep in the Harderian gland, and were composed of arrested and degenerating optic tissue in a scleral "cyst" which was always surrounded by muscles that were attached to the scleral wall. (Pl. I, fig. 1, 2, and 3; Pl. II, fig. 5; Pl. III, fig. 9). The muscles were always well developed, but on the whole they were always small and irregularly positioned in the orbit. It is remarkable, however, that even in the anophthalmic eyes very little distortion on disarrangement of the extraocular muscles occurred. A fat body was always present around the scleral cyst; but it appeared precociously and irregularly at five days, not at 13 days as in the normal eyes.

The scleral cyst was well defined, but in 13 of the 34 eyes it was burst, or split, so that the contents of the cavity were partially extruded. (Pl. I, fig. 2) Splitting and extrusion of the tissue always occurred in the eyes in which an irregular, broken lens with a fragmented lens epithelium was present. The younger eyes of the series were not always buried in the Harderian gland because the gland does not mature at an early age. Concomitant with the maturing of the gland a degeneration of eye tissue takes place. Eyes that appeared somewhat normal (as a bulge in
the closed eyelid) at 1 day old would gradually be overgrown by glandular tissue, and by the time that the eyelid opened no visible parts of the eyeball were found.

The 34 eyes that were present in the subcategory had many common characteristics. The eyes were always well vascularized and a major artery, the adult ophthalmic, came to the rear of the eyeball and entered the eye in the normal manner. (Pl. III, fig. 9) The sclera had no regular form, but was roughly spheroid with the long axis running in the same direction as the axis of a normal eye. In all of the GMT eyes the sclera had a thickened area, a "cornea," where the conjunctiva folded to meet the eye and which was very nearly always opposite the ophthalmic artery. The conjunctival fold was a narrow, pocket-like fold of skin ectoderm which extended from the conjunctival cavity to the scleral cyst. The fold extended between the lobes of the Harderian gland and was always closed so that the light never reached the eye. Induction by the skin ectoderm of the conjunctival fold probably formed the cornea. (Willier et al., '55, p. 407) Figure 1 of Plate I is a representative GMT eye.

A vacuolated lens, or fragmented lens remains, were found in each of the eyes. In the earlier and in some of the later ages the lens was intact, but it was always vacuolated or internally fractured. The degenerate lenses
usually had broken lens epithelium. (Pl. I, fig. 3 and 4) Some of the lenses in the 1 and 2 day old eyes were almost as large as the lenses of the normal eyes. If, however, the lens showed signs of degeneration near the time of birth it was assumed in this study that the eye never could become a SN eye.

The lens tissue of all the GMA eyes contained leuco-cystes. The leucocytes were usually confined to the epi-trichial cells which were disintegrating. Leucocytes were rarely found in other places except in some rare instances where they were found in the Harderian gland or where the eyelid was opening. The youngest eyes contained the largest number of leucocytes in their lenses, and the number was roughly inversely proportional to the age of the eye and the degree of degeneration of the lens. In most cases well defined lens epithelium was present, even when the lens was in an advanced state of degeneration. The lens was, in most cases, surrounded by a well defined, none cellular, hyaline capsule. At times the detached lens epithelium formed spheres, or lentoids, which could be confused with gland acini. In many cases the lentoids were surrounded by a lens capsule too. (Pl. II, fig. 6)

The lentoids were formed from the lens epithelium which was a normal, well stratified, cuboidal cell type in some eyes. In other GM eyes it was either a low cuboidal,
a columnar, or an irregular, unstratified epithelium. In all instances of highly degenerate lenses some of the epithelium formed into small spheres in which part of the cells differentiated into epitrichial cells; thus, new but smaller lenses or lentoids, were formed. Mitotic figures were found in the lens epithelium of a 7 day old eye, and in the lentoid of a 16 day old eye. In some eyes leucocytes were found both in the main lens, and in the lentoids.

Connective tissue was always present within the scleral cyst. The connective tissue was always well vascularized and a definite chorioid coat was never present. In one case, in a 5 day old eye, tissue appeared to be migrating over the wall of the optic cup into the cavity between the lens and the arrested retina. (Pl. II, fig. 7 and 8) The connective tissue was well vascularized and it was coming from the region of the chorioid. Where the lenses were fractured or vacuolated, connective tissue was sometimes found in the breaks or vacuoles. In every GMT eye the connective tissue was present between the lens and the optic vesicle or intermixed with the broken lens tissue and lentoids, and the tissue was extruded when the scleral wall was broken.

The retina of the eyes, except in the youngest age groups, was always in a state of degeneration or arrested development. In one 50 day old eye, however, there was a section of the retina that had continued to grow and a small
spot contained mature retinal nuclei. One 5 day old eye had a similar mature spot. In the 5 day old eye the pigment layer was a normal low cuboidal type adjacent to the mature spot. For both of the eyes the remaining part of the retinae was in an irregular an arrested state of development. The 50 day old eye had many leucocytes in addition to the mature spot. No optic nerve was present in spite of these continued retinal developments, nor was there any evidence of there ever having been one.

In all of the GMT eyes retinal tissue was present. The retinal tissue in the older eyes was always in an arrested or degenerate state. Never more than one highly irregular cell layer constituted what could be called the pigment layer, and there was never more than several irregular layers in the sensory part of the retina. In many cases between the "pigment" layer and the "sensory" layer a grey to white, translucent, solid substance was present. This substance was undoubtedly the vitreous humor which is secreted by the basal cells of the retina. (Dromm, ’21) The vitreous humor was most probably between the sensory and the pigment layers because connective tissue and lens tissue filled the scleral cavity and the humor could not be secreted to its normal place. Some of the connective tissue was sometimes found growing into the retinal cavity.

A definite change was found in the structure of the
cells of the pigment layer in one of the 3 day old microphthalmic eyes. Where the partially mature retina was in contact with the chorioid the pigment layer was normal low cuboidal cells; where the retina was folded and the pigment layer did not touch the chorioid the pigment layer was of the tall columnar type. The condition was unique.

In all of the 34 cases of microphthalmia a normal development of the conjunctiva, the Harderian glands and all of the other accessory structures was found. Several of the eyes will now be described in detail.

5634-21 (1 day old) This eye was from a unilateral GM animal, and it had a lens that was spherical, but clearly degenerate. The lens epithelium was apparently absent, but a lens capsule was present. Both layers of the retina were one layer thick except in a very small area where ganglionic cells were differenting. The pigment layer was composed of a regular line of low cuboidal cells which were next to the sclera and the retinal layer was an irregular line of cells which lined and adhered to the pigment layer. There was no chorioid layer, but vascularized connective tissue was present in the scleral cavity, and the extraocular muscles were attached to the sclera.

5794-21 (7 days old) This eye was from an unilateral GM rat, and a shattered, irregular lens was present. A single celled pigment layer and a several celled retinal layer were present in the form of a large optic cup. This cup contained lens remains and a well vascularized connective tissue which was supplied by the ophthalmic artery which penetrated an irregular, but intact, sclera. Although some of the 5 day old GMT eyes had fat bodies, no sign of one was found in this 7 day old eye.

5692-51 (10 days old) This eye was from a bilateral GM rat, and it had a highly irregular, broken lens with a well defined lens epithelium. Lentoids, with capsules, were present. The nervous tissue was immature and filled with vitreous humor. Vascularized connective
tissue was present. Both lens cells and connective tissue were extruded from the scleral cavity through a hole in the scleral wall. A fat body was present.

5683-61 (16 days old) Pl. I, fig. 2) This eye was from a bilateral SM eye. The intact part of the lens was vacuolated, but many lens fragments were also present. Connective tissue and nervous tissue with vitreous humor was present. A cornea was present in the sclera. Lens material and connective tissue were extruded from the sclera through a hole in the scleral wall.

5720-52 (35 days old) (Pl. III, fig. 5) This eye was from a bilateral GM rat. Although the lens was broken and had formed many lentoids, one especially large lentoid was present. (Pl. III, fig. 6) No chorioid layer was present, but vascularized connective tissue was abundant. The nervous tissue which was present contained some vitreous humor. A 100 day old eye, 5738-11, was very similar to this 35 day old eye.

The anophthalmic eyes. Four GM eyes were placed in the subcategory, GMA, because of an appreciable lack of eye tissue. In all of the orbits a small fat body was present at the end of the conjunctival infolding where the eye tissue should have been. The location was well vascularized, and a large blood vessel, undoubtedly the adult ophthalamic, was present. Two of the GMA eyes were represented by fat bodies alone.

In a third eye there was some eye tissue, and in a fourth eye a minute amount of eye tissue was present in a small fat pocket surrounded by muscle. Scleral tissue, and possibly retinal tissue could be identified in the fourth eye. In some of the eyes leucocytes were in the tissue where the eye had been located. The eyes were undoubtedly
highly degenerate and highly underdeveloped; that is, they were secondary anophthalmic eyes. All of the eyes were bilateral GM eyes, and individual descriptions follow.

5692-31 (10 days old) and 5740-32 (20 days old) Only small fat bodies were present where the eye tissue should have been. Leucocytes were present in the fat bodies, and the eye locations were well vascularized.

5658-21 (16 days old) In this orbit a small amount of eye tissue in which connective tissue and perhaps retina could be distinguished. A small blood vessel came through the sclera. The eye tissue was embedded in the muscles and a fat body was adjacent to the ocular tissue. The fat body contained leucocytes. The other GMA eye, 5720-12 (35 days old), was very similar to the 16 day old eye, but it contained even less tissue. In all other respects the anophthalmic eyes were comparable to normal.

Eye Anomalies

Two anomalies did not fit into any strict category. The first of them was an anomaly of the eyelid. In a 13 day old bilateral GM rat, the eyelid was split so it looked like an inverted "T." Hair growth was normal all around the "T." The eyelid of the other eye was normal, and the eye beneath the split eyelid was a typical GM eye. No explanation can be given for this anomaly. However, the hair pattern suggests an early developmental defect.

In a second eye, a 5 day old SNA eye, a series of sections revealed a spot at which the iris penetrated the sclera. (Pl. III, fig. 12) Both the nervous layers and the mesodermal layers grew through the sclera in two places. Whether the holes were present and the iris grew through them
or whether there was some active principle within the iris and it formed the hole in the sclera, is unknown. The two eye anomalies were normal in every other respect.

DISCUSSION

I

Before the over-all findings of the research are discussed some particular observations must be reviewed because they contribute to the general picture of rat microphthalmia. The observations include the problem of the vascularized intrascleral connective tissue, retinal irregularities (folds and rosettes), the development of lentoids, Wolffian regeneration, intraocular pressures, the nature of the sclera itself and the general relationship of the factors to each other and to the accessory structures.

In all of the eyes an internal connective tissue was present in the scleral cavity which was always well vascularized by branches from the adult ophthalmic artery. The connective tissue stained brown with Heidenhain's stain as did the chorioid layer of the normal eye. The reticular connective tissue stained a bright blue. With the hematoxylin and eosin there was very little difference between the color of the internal connective tissue, the chorioid of the normal eye and the reticular connective tissue. In all of the SN eyes, except the 100 day anomalies which have been
discussed, a normal chorioid layer was present. In all of the GMT eyes no apparent chorioid layer was found in the scleral cavity. Instead, connective tissue was between the optic vesicle (retina) and the lens.

In the eyes with partially intact lenses and in the eyes which had shattered or vacuolated lenses, the connective tissue was intermingled with lens tissue. In a 5 day old eye in which some connective tissue that could be called chorioid was present, there was what appeared to be a migration of connective tissue over the malformed optic cup tissue from the chorioid area to the space between the lens and the hollow of the optic cup itself. (Pl. II, fig. 7 and 8)

A possible explanation of the growth of the vascularized, chorioid-like connective tissue is that when the connective tissue from the chorioid normally grows from the choroid to form the mesodermal stroma of the iris a possible continuation of growth results because a developed iris is not present; that is, the chorioid tissue receives no "signal" from the underdeveloped or absent iris to stop growing. Eventually the tissue grows and fills the optic cavity between the retina and the lens. As a result an undefined mass of vascularized connective tissue is present in the ocular cavity and there was never a well defined chorioid. Some of the connective tissue can be accounted for by an
ingrowth of tissue while the eye was still at the optic cup stage before the sclera was present.

The retinal tissue that was found in the microphthalmic eyes was either degenerate, arrested in development, or contained retinal irregularities. The SN category contained only three eyes in which the retinae could be considered completely normal. Of all the single eye defects which were found, retinal defects were present in the largest number of eyes. This finding, not only reflects the degree of sensitivity of the retina to the embryonic defect, but also demonstrates that the lens, if it is able to withstand the disruptive influences during the critical stage of development, has more influence over the development of the eye size proper than the retina. The two 100 day anomalies described earlier help reinforce this statement.

Gruenwald ('46, p. 518) states that rosettes are "found in X rayed embryos in a hereditary type of fowl, and rarely in other eyes." The type that occurs in fowl was discovered in the microphthalmic chicks that Jeffery ('41) first described, and which were later described by Gruenwald himself, ('44). In many of the GMT eyes what was believed to be vitreous humor was present in the retinal tissue; it was not unexpected because it is known that the vitreous humor is secreted by the nervous tissue which was always present.
Various types of lentoids have been recorded in the literature. The lentoids have been produced as the by-products of experiments. Lewis ('07) describes some lentoids that he produced in amphibians when he was experimenting on the problem of lens induction by the optic vesicle. According to Werber ('16), King produced lentoids in the body ectoderm after destroying the optic vesicle with a hot needle. Werber also produced lentoids, or lens-like structures, in the head ectoderm by rearing tadpoles in water which contained a small amount of acetone. Werber explains his experimental results by a theory of "blastolysis" in the optic cup substance. The lentoids which were produced in the above experiments were from the skin ectoderm, and can be called primary lentoids.

Schotte and Hummel ('39) actually did experiments to discover if lenses could be produced from implants of lens epithelium or from tissue foreign to the optic cup area; limb blastema, for example. The lentoids that were produced from foreign material did not have lens capsules; the lentoids produced from lens epithelium had capsules. In some experiments where lens epithelium was introduced into the lensless eye lentoids and whole lenses developed; they can be called secondary lentoids and secondary lenses. Lentoids that are products of foreign tissue can be called pseudo-
lentoids. Mann ('28) states that the lens capsule develops from the lens epithelium.

Wolffian lens regeneration, or regeneration of the lens from the iris or the retina, has been recorded in the literature for many years. (Willier et al., '55) Stone and Sapir ('40) report that Wolffian regeneration occurs in urodels and in a fish, but not in anurans. Retinal lentoids, when lens formation is incomplete, is the name applied to the lentoid which is formed by Wolffian regeneration. Reyer ('48, '50) confirms Wolffian lens regeneration in the urodel, but he concludes that "if lens removal is incomplete, the lens fragments remaining in the eye can develop into normal appearing lenses." ('48 p. 256) The secondary lentoids, from lens remains, are different from the retinal lentoids produced in Wolffian regeneration.

Lentoids were found in the presence of degenerating lens tissue in the GM eyes. For the most part they had lens capsules that were well defined, even when the epitrichial cells of the main lens were in an advanced stage of disorganization and disintegration. In some cases the lentoid formed epitrichial cells contained leucocytes. Since the lentoids were found in degenerating lens they can be called secondary degeneration lentoids.

Lentoid formation by Wolffian regeneration in the rat eye is not quite out of the question because a possible
instance of what might be called Wolffian lens regeneration was found. The instance occurred in one of the eyes which was described under the 100 day anomalies. There appeared to be a small piece of lens epithelial cells in the region of the iris. Gruenwald ("44a) mentions some examples of what might be considered Wolffian regeneration in chicks. However, in the example from the 100 day old eyes lens regeneration is improbable because a large lens had been present.

From the examples of the GM rat's eyes studied here, it seems safe to assume that major lentoid formation is from the lens epithelial cells. No evidence was found to demonstrate that the lens material in the disorganized GM lenses is more than disintegrating lens material and secondary degeneration lentoids. No extensive growth of tissue to form a well organized lens to replace the degenerate lenses was ever suspected, so no major role can be assumed to be played by the secondary degeneration lentoids or by Wolffian lens regeneration.

Another interesting phenomenon that is associated with degenerating GM lenses is the condition of the sclera of the eyes. In 13 of the 34 GM eyes an extrusion of the scleral contents from the scleral cavity was present. The lens epithelium was broken into pieces and lentoids were
formed. Lentoids plus connective tissue constituted most of the intraocular contents, especially in some older GM eyes. In many examples the hyaline capsule around the lentoids appeared burst. The vascularized connective tissue which was described previously plus the lentoids were extruded from the relatively intact sclera. In several examples the material was being pushed into the extension of the scleral sheath that surrounds the adult ophthalmic artery. In several cases lentoids seemed to be pushed through the sclera by force. (Pl. III, fig. 10)

To account for some of the intraocular pressure that caused the extrusion of tissue from the scleral cavity, lentoid formation can be cited. The lens was disintegrating; the lens epithelium was not. Since lentoid formation consists of epithelial cells differentiating into larger epitrichial cells, a volume increase will occur (if the formation of epitrichial cells was proceeding faster than lens degeneration, of course.) The intraocular pressure would cause the scleral cavity to give at its weakest point, and the tissue contents would be pushed to other areas.

Extrusion of scleral contents occurs in most of the eyes which had lenses in an advance state of disintegration, and not as much in the eyes in which the lens and the lens epithelium were relatively intact but the lens itself was vacuolated. The importance of the phenomenon to the general
problem of microphthalmia was slight, and the only importance that it could have to the animal is that it might hasten the absorption of the arrested and degenerating eye tissue.

In all of the SN eyes the sclera was a thin, compact structure that was similar to the normal sclera. However, in the GM eyes the sclera was small and not always completely intact. The sclera in the SN eyes was usually a walled sac, it was many times referred to as a scleral cyst. A cornea which was induced by the conjunctival fold of the ectoderm was always present. The sclera of the eye was always very thick in proportion to the size of the cyst as a whole.

According to Weiss and Amprino ('40) the more mechanical stretch (up to a point) that is exerted upon the cartilaginous sclera of a chick, the thinner that the sclera will become. In the GM eyes the sclera was always proportionately thicker than it was in the normal and the SN eyes. Although the sclera of the rat's eye is not cartilaginous a principle similar to the principle of Weiss and Amprino is probably responsible for the phenomenon. Intraocular pressure from lentoid formation is undoubtedly very slight when compared to the pressure of that in a normal eye.

Extraocular muscles were attached to the scleral wall, and the muscles were always large and innervated. The extra-
ocular smooth muscles never reached the scleral wall, but were always present deep in the orbit. All of the other accessory structures which are present in a rat's eye are present in the microphthalmic eye. The Harderian gland, the eyelids and the conjunctiva are all essentially normal, but they are irregularly positioned because of the lack of a large eyeball. The accessory structures were much better developed than the eye itself, because their development is independent of the eye, hence they were not affected by the microphthalmic genes. The same general conclusion in regard to accessory structures of microphthalmic eyes was reached by Gruenwald ('47) and Chase and Chase ('41). A discussion of some of the general principles underlying the problem of the development of the microphthalmic eyes will now be undertaken.

II

The problem of the adult rat eye circulation has been studied by Michaelson ('54) and Janes and Bounds ('55). In his study of the 1 day old eye Michaelson states that "embryonic vitreous vessels" are present and are in a state of disintegration which is nearly complete by the end of the first week of life. The vessels are probably the remains of the embryonic ophthalmic circulation which is described by Leitch ('34) as recorded by Greene ('55). While the
disintegration is underway the adult ophthalmic is developing and Michaelson (*54, p. 64) states that "by the eighth day the main elements of the general adult vascular morphology were found to be present."

Evidence for an even earlier circulation than the one described by Leitch also exists (Browman, unpublished). Three stages in circulatory development can thus be found in the prenatal blood system of the rat. Approximately 12 days after fertilization a primary annular type circulation arises from the anterior carotid of the developing arterial circulatory system and supplies the ocular area. The system is gone by the time that the circulation described by Leitch takes over, approximately 15 days after fertilization. The secondary system degenerates and is replaced by the adult circulation arising from the ophthalmic artery which branches from the palatine portion of the pterygoquadrate. The secondary system is completely supplanted shortly after birth.

Janes and Bounds (*55) give a good picture of the adult circulation which was present in the rat's eyes they studied. Although no special action was taken to stain the blood vessels of the eyes used in the present study, all the basic features of the normal adult circulation were present and the ocular tissue was always well vascularized. A good question to ask at this point is why was there no recovery of the eye tissue after the apparently normal secondary and
adult circulation took over the job of supplying the blood to the growing eye? A well developed blood system was present in the SN and the GM eyes, and a blood system was present in all of the anophthalmic eye locations.

The optic vesicle of the eye is one of the first parts of the eye to develop, and early in the optic vesicle stage the vesicle acts as the inducer of the lens. During, and shortly after, the time that the lens is induced and the lens vesicle is formed, major differentiation takes place in the optic vesicle, and the sensory and pigment layers are developed. The sensory layer very quickly sends nerve fibers to the brain along the optic stalk, and the optic nerve is formed. All of the above development takes place from 12 to 15 days post fertilization.

Earlier, during the time the optic vesicle is growing, the cells of which the vesicle is composed gradually lose the capacity to obtain nutrients from themselves and each other. The loss of capacity to obtain the nutrients does not influence the cells because a concomitant development of a blood system takes place. Twitty ('40, p. 118) states that "these two sets of changes together provide an effective means of insuring constant size adjustment." In a normal eye a state of equilibrium is in effect all during development; in a microphthalmic eye the state of equilibrium is upset. Apparently from the beginning a mutual relationship exists
between the optic cup and the lens. The mutual relationship is very important in both normal and abnormal development as will be demonstrated.

The early circulation is mainly responsible for the development of the early iris and the anterior part of the eye, but what is more important is that it is the early circulation which is responsible for the blood supply to the developing lens. The embryonic tunica vasculosa of the lens is present shortly after the lens has been induced by the optic vesicle, but before there is a reduced requirements of nutrients which is characteristic of the postnatal and the adult lens.

The development of the lens takes place during the time of the change from the primary circulation to the secondary circulation. Much of the work which has been done on the eye is concerned with the development of the lens, especially lens induction by the optic vesicle. Lenses, or the remains of lenses, were found in all but three anophthalmic eyes; and in all of them it was presumed to have been present. Mann ("37) states that aphikia, or true absence of the lens, is very rare. No lens regeneration took place. There is no specific work that has been done on the blood supply to the developing lens.

The optic nerve develops after the lens is induced and the lens vesicle is separated from the overlying ecto-
derm. The chorioid fissure through which the optic nerve must grow to reach the brain develops early. Gruenwald ("45) states that in two conditions in microphthalmic eyes no optic nerve develops. The two conditions are the absence of the retina and the absence of the chorioid fissure. Gruenwald states that the absence of the fissure can occur "even in otherwise perfect eyes." Since the optic nerve cannot grow to the brain if the fissure is closed a SNA, or "otherwise perfect" eye could easily be formed. A defect in the very early embryonic circulation to the optic vesicle could influence the absence or closure of the fissure.

Eyes from which the nerve grows haphazardly from the optic cavity are known to occur in this strain of microphthalmic rats. In some eyes optic nerve fibers have been found to leave the intraocular cavity by growing through the pigment layer and the sclera. For the eyes of the present study the optic nerve was either present or absent; it never grew haphazardly, and never grew through the ocular wall except in the normal manner. The optic nerve will normally grow through the fissure in the majority of cases, so that the condition of the fissure can perhaps determine the presence or absence of the optic nerve.

Beckwith ("27) has observed a relationship between the absence or presence of a lens and the opening or closing
of the chorioid fissure. She states that in the presence of a lens the chorioid fissure will close, and in the absence of a lens it will remain open. Beckwith does not indicate any relationship between the chorioid fissure and the optic nerve, however.

It has already been noted that the lens, or lens material, was found in all of the eyes except three anophthalmic eyes. If the presence of the lens was enough to stimulate the closure of the fissure, then according to Beckwith the fissure would have closed in spite of whether the optic nerve was present or not. If a recovery of the affected, or arrested, retinal tissue occurred after the fissure closed, then a SNA eye would develop. Gruenwald ('44a) describes several normal eyes without optic nerves from chicks. Gruenwald ('45) associated the absence of the optic nerve with the absence of a chorioid fissure.

Microphthalmia occurs as a normal phenomenon in some burrowing and nocturnal animals such as moles and bats. Gubbay ('56) makes a study of the comparative development of the eyes in a large eyed shrew and a reduced eye mole of the order insectivora. The author states that soon after the optic cup is formed there is a reduction or lack of inductive influence and the cup fails to differentiate the ocular structures. The author also notes that lack of ability to differentiate "is most marked in the lens, but
can be traced also in the other parts including the retina." (p. 194)

The findings of Ballard ('39) and Stone and Chase ('40) point to the fact that a reciprocal size relationship exists between the size of the eye and the size of the lens. If the causative agent of microphthalmia is a defect of the blood supply as is inferred by the experiments of Stone and Chase ('41) and suggested by Browman and Ramsey ('43); and in addition, the fact that in the presence of a lens there is a closure of the chorioid fissure, then all the classifications of microphthalmia that were found in the present study can be explained.

In all of the eyes except the anophthalmic eyes, all the major eye tissues were present. The eye tissues, except for the lens, were in an arrested state of development. Degeneration with leucocytes was taking place in the lens epitrichial cells while lentoid regeneration was occurring in the epithelial cells. It is known that all major eye tissues are present during 12½ and 14 days after fertilization. According to Mann ('37) most of the defects of human eyes can be traced to a comparable stage of development because it is one of the most active, complex and rapid stages in the development of the eye.

Early in the development of the rat's eye an early embryonic circulation develops, and it is not completely
supplanted by the adult ophthalmic circulation until after the first week of life. If the causative agent of microphthalmia is a defect in the primary circulation then the postnatal condition of the eye would be influenced by the degree of defect which occurred in the primary circulation (or, perhaps, a difference in the time that the secondary circulation began to assume importance).

In the surrounding optic vesicle tissue the amount of nourishment that is required by the tissue is proportionate to the tissue's growth and undoubtedly increases rapidly with the generalized increase in size of the eye. After an initial depletion of blood supply the recovery of the eyes would depend upon the degree of damage incurred by the various tissues and by their ability to recover. The initial degree of damage done is especially demonstrated by the nervous tissue, which was probably the most greatly affected tissue in the microphthalmic eyes. All of the GM eyes had an arrested or absent retina, and most of the SN eyes with comparable to normal lenses had many irregularities in the sensitive retina.

The 100 day anomalies, eyes with comparable to normal lenses and immature, underdeveloped retinae, are examples which demonstrate that a well developed lens was more important for normal eye development and normal size attainment than the retinae. The lens tissue of the 100 day old eyes
developed normally even though the circulation was defective. Only in the lens tissue is the early circulation absolutely more important than the adult circulation. In the postnatal eyes the lens has a very low metabolism and no major blood supply to the lens tissue is found by the time that the eyes open after birth.

It is here postulated that the causative factor of microphthalmia affects the lens at a critical stage in lens development so that the lens is usually unable to recover. The optic tissue grows only in proportion to the amount of early lens tissue development which is directly influenced by the degree of defect in the embryonic circulation. The lens will either recover completely (SN), or begin an almost certain irreversible degeneration process (GM). Mann ('37) states that smallness of the lens without other abnormalities is known, but rare. Small, normal lenses have been found in the eyes from this strain of microphthalmic rats, but they are rare. The adult circulation has very little influence on the lens once the normal embryonic circulation disappears in both normal and microphthalmic eyes, hence, the lens and the whole eye remain in an arrested stage of development if the lens is adversely affected by the defective early circulation.

If the lens is able to pass the critical stage of development, a SN eye would result. Whether the eye has an
optic nerve or not perhaps depends upon the state of the chorioid fissure. If the fissure were closed there would be no optic nerve; if it were open an optic nerve would develop. If the chorioid fissure closed before the retinal tissue was vascularized by the secondary circulation, or at least while it still had the potential to develop normally, then an eye with a well developed retina but no optic nerve, a SNA eye, would develop. The eye would be normal except for an optic nerve and perhaps a few retinal defects; provided, of course, that the lens was able to develop concomitantly with the eye tissue. The early or late closure of the chorioid fissure was then responsible for the absence or presence of the optic nerve in the SNA and the SNP eyes.

If there were a complete, early defect in the primary circulation, when only a small amount of lens tissue was present, then an anophthalmic eye condition, GMA, would be present in the postnatal rat. If there were a defect in the circulation after the lens were partially formed (or possibly a delay in the development of the secondary circulation) then there would be a degeneration of the lens and the adult circulation would maintain only the eye tissue that had been formed until that time. This fact would account for the near uniformity in size of the GMT eyes.

The conclusion that the lens is one of the more important of the tissues in the developing eye is contrary
to Ramsey ('40). Ramsey states that the retina contains an "organizer principle," and that "severe cases of microphthalmia result when this 'organizer' is lacking in the retina," (p. 37) and that "in mild degrees of microphthalmia partial loss of the organizer in the retina was seen." (p. 38) Among the eyes examined in the present study no eyes had well developed retinae accompanied by broken or irregular lenses. It must not be inferred by this conclusion that the lens alone is responsible for the type of eye development. The lens is only a leading element among the optic tissues, and the mutual size relationship which has been mentioned before is always maintained between the optic cup or the optic tissues and the lens.

Mann ('37), p. 43) states that "practically all germinal defects are bilateral, though the degree of the defect is often slightly different in the two eyes." She also states, as was mentioned before, that it is to an early developmental stage that most of the developmental eye defects can be traced. With a slight difference in degree of defect in the early circulation of the eye, a gross difference such as SN and GM eye in one animal could be possible if a principle such as "critical time of lens development" were taken into account. A very slight difference in the amount, or time of development, of a lens circulation defect has a profound effect on the condition
of the postnatal eye. Similarly a small difference in the closing time of the chorioid fissure in an eye would produce, either an eye with an optic nerve, or an eye without an optic nerve, so the statements by Mann are supported in theory, not contradicted, by the findings of the present research.

In a developmental study of this type it is difficult to state what an arrested eye would have looked like at a later age if it had been allowed to grow to that age. However, when a postnatal series of rat's eyes are used the problem becomes easier because the normal rat's eye should already have formed all of the major tissues before birth, and it is only necessary to look at the microphthalmic eye and evaluate the developmental state of the tissues present and speculate upon their potentiality to become normal. If signs of degeneration were present in the lens of the younger eyes of the series, it was assumed that the eye had reached a point near its maximum growth and it could be accurately and permanently classified as a GM eye. A GMT eye of 100 days of age was only slightly larger than a 1 day old GMT eye, and only a range of variation existed in the whole GMT series.

The only major adult structure or tissue that was completely absent in all except eight of the eyes was the optic nerve which is known to be derived from the sensory
layer of the retina, and the retina was represented in all of the eyes except the three anophthalmic eyes. The microphthalmic eye character is not considered to be 100% homozygous in the microphthalmic strain of rats under study, so that some of the SN eyes were undoubtedly completely normal eyes.

All of the eyes, or remnants of eyes were centered on the normal optic axis. Ostensibly because of the lack of the eye, abnormal growth of the Harderian gland was the only modifying factor of the eye position. The muscles were essentially in a normal position and a normal size in spite of the reduction in the size of the eyeball. Vascularization by way of the adult ophthalmic was present even in the anophthalmic eyes. The principal conclusion from the above findings is that the causative agent that initiated the microphthalmic condition in the eye was active after the primordium of all the major eye tissues were present, but before, or during, optic nerve development.

A statistical analysis of the number of eyes in the different categories used in classifying the eyes would be of little significance due to inadequate, nonrandom sampling. It is believed that all of the major categories of eyes present in the microphthalmic strain of animals was represented. The two major categories of microphthalmia that were used were based solely on the external morphology
of the eye. The two major categories were further subdivided; the first, SN, according to whether an optic nerve was present; and the second, GM, somewhat artificially according to the amount of eye tissue that was present. Ramsey ('40) did not find the same categories, probably because the eyes used in his study were too young and not well differentiated. Ramsey used two classifications for the eyes that he studied—mild and severe microphthalmia. The two cases would fall in the GM category of the present study.

Eyes that do not fit into any of the categories which are described here are found in the microphthalmic strain of rats. Some eyes have been found which have aberrant optic nerves. Some eyes have been found which have intermediate size, normal lenses. Many more anomalies could be described that would not fit into the present categories that were used in this research. The categories are not made less valid by the discovery of an eye that will not fit. The categories are only meant to be a useful guide to the understanding of the general principles of eye development, and the categories are not designed to contain all the possible types of microphthalmic eyes which are found in rats.

The time of occurrence of the microphthalmia under study has been stated to be early in the development of the
rat's eye. Between 12 and 13 days post fertilization the lens vesicle separates from the skin ectoderm, and between 14 and 15 days the ganglionic cells of the sensory layer develop the processes which grow to the brain as the optic nerve. It is during the 12 to 15 day period that the causative agent of microphthalmia is suspected to be present.

It is during an early embryonic stage that the ocular blood system develops. The very early embryonic development of the blood system to the rat's eye is not well understood, and only fragmentary evidence is available as a guide to an understanding of the early circulation. Dr. Browman is presently conducting a study of the early circulatory pattern of both normal and microphthalmic rat eyes, because he believes that the effect of the gene which is responsible for the small eyes is to alter or eliminate part of the early circulatory system.

It has been postulated above that the development of the lens plays a major role in the size of the resulting eye, and that the degree of defect in the early circulation is responsible for the size of the lens. Further work on the early embryonic circulation in the normal and the microphthalmic rat's eye must be undertaken to determine the developmental defect which causes microphthalmia. Only then can the results of the present research be confirmed.
SUMMARY AND CONCLUSION

A study of the postnatal developmental anatomy in a selected series of rat's eyes from a microphthalmic strain of rats was the principal subject of the research. A review of the literature of similar heritable or histologically studied cases of microphthalmia was made along with a brief review of some of the general papers on both normal and abnormal ocular development.

For an adequate background in the normal development of the rat's eye a study of a series of normal eyes (from 11 days post fertilization to 100 days postnatal) was made. An equally broad and comparable series of microphthalmic eyes was used, but for the prenatal ages the information was obtained from an earlier study. From the histological study of the postnatal eyes two major categories, each with two subcategories, were made. The categories were given descriptive names which were seemingly normal (divided into two subcategories, seemingly normal with an optic nerve and seemingly normal without an optic nerve) and grossly microphthalmic (divided into two categories, grossly microphthalmic with tissue and grossly microphthalmic without tissue, or in effect, anophthalmic.)

A general discussion of the findings in each of the
categories was made and a discussion of the various states of development in the principal tissues and structures was undertaken. The retina, lens and lentoids, chorioid and the sclera were the principal subjects of the discussion. The lens and lentoids were discussed at length. Following the discussion of the structures an over-all discussion of some of the general principles of normal and microphthalmic eye development was made, and the applicability of the general principles to the microphthalmia studied here was demonstrated.

From the works of earlier authors it was suggested that a cause of microphthalmia in general, and the microphthalmia studied here in particular, was a defect in the embryonic ocular circulatory system. It was postulated from the present study that the manner in which the microphthalmia was expressed in the postnatal eye was probably directly dependent upon the state of the lens development. If the lens were adversely affected during an early critical phase of development when it was dependent upon the embryonic blood supply then it did not recover and a grossly microphthalmic eye resulted. If it did recover a seemingly normal eye resulted. No intermediate type of eye was found. It was suggested also that the presence of the optic nerve was perhaps dependent upon the status of the chorioid fissure.
As a general conclusion of the present study the statement was made that the condition of the eye in the microphthalmic strain of rats is dependent upon the degree of defect in the partially understood early circulation of the embryonic rat's eye, and only after a study of the embryonic rat's eye circulation is completed can conclusions of the present research be made.
LITERATURE CITED


Leitch, M. S. 1934 Embryology of the principal arterial vessels in the rat. Unpublished manuscript (obtained from Greene, '55)


Fig. 3 and 4. 1. Lens epithelial cells 2. Lens epithelium 3. Lens fragments 4. Break in epithelium 5. Retinal tissue
Fig. 1. An over-all view of a microphthalmic eye showing, sclera, "cornea," retina, broken lens and adult ophthalmic artery. (5683-41; 13 days old; GM)

Fig. 2. An over-all view of a microphthalmic eye showing the extruded scleral contents. (5683-61; 13 days old; GM)

Fig. 3. The lens epithelium of an eye that is beginning to break apart. This eye and the eye in figure 1 are from the same rat. (5683-42; 13 days old; GM)

Fig. 4. A detail of the break in figure 1.
Explanation of Plate II


Fig. 6. 1. Connective tissue 2. Lens epithelium 3. Lens epithelium differentiating into epitrichial cells. 4. Lens capsule

Fig. 7. 1. Harderian gland 2. Striated muscle 3. Chorioid layer 4. Retina 5. Vacuolated lens

Fig. 8. 1. Sclera 2. Large blood vessels 3. Connective tissue 4. Small blood vessels
Fig. 5. An eye with a large and broken lens and an especially large lentoid. (5720-52; 35 days old; GM)

Fig. 6. A detail of the lentoid in figure 5.

Fig. 7. A view of the connective tissue coming from the chorioid layer over the optic cup into the ocular space between the lens and the retina. (5654-41; 2 days old; GM)

Fig. 8. A detail view of the connective tissue mentioned in figure 7.
Explanation of Plate III

Fig. 9. 1. Harderian gland 2. Retina 3. Connective tissue 4. Sclera 5. Extraocular muscle 6. Ophthalmic artery

Fig. 10. 1. Outside of scleral cavity 2. Sclera 3. Scleral cavity 4. Lentoid capsule 5. Lentoid

Fig. 11. 1. Striated muscle 2. Orbitalis muscle of Muller

Fig. 12. 1. Iris 2. Sclera 3. Connective tissue 4. Section of iris penetrating the sclera 5. Vitreous humor
Fig. 9. A cross section of the adult ophthalmic artery entering the ocular tissue. (5794-21; 7 days old; GM)

Fig. 10. A lentoid being extruded from the scleral cavity. (5685-32; 100 days old; GM)

Fig. 11. The orbitalis muscle of Muller, the smooth muscle of the eye, found deep in the orbit. (5683-41; 13 days old; GM)

Fig. 12. The iris penetrating the scleral wall. (5738-11; 5 days old; SNA)