Experimentally Induced Coloboma in the Golden Hamster: A Preliminary Report

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Introduction. The appearance of a coloboma of the iris must have been known long before Walther (15) introduced the term in 1821 since the condition is easily visible. The word coloboma is derived from the Greek and means the part which is taken away in mutilation or injury or the part that is lacking, that is, a defect. In coloboma iridis, therefore, a section of the iris is lacking, often bilaterally. After the invention of the ophthalmoscope by von Helmholtz in 1851, it could be seen that the defect is not restricted to the iris but that lens, retina, choroid, and optic nerve can also be involved. However, little was known about the etiology. A fault in the closure of the "choroid fissure" was first mentioned as the cause of the congenital defect by von Ammon (1) in 1831. At that time the pigment epithelium, the most obviously involved layer, was believed to be part of the choroid, and the cleft was, therefore, called choroid fissure. This is a misnomer since the defect lies primarily in the neural retina and the pigment epithelium, both derived from neuroectoderm. The choroid, a derivative of mesoderm, has not formed at the time of the fissure closure and becomes involved only secondarily. The most commonly employed term now is embryonic fissure.

Various investigators have proposed different theories regarding the cause of the retarded closure or non-closure of the embryonic fissure: lack of degeneration of mesoderm in the fissure (8), inflammation of the optic vesicle (3), pressure from an unusual amount of cerebrospinal fluid (4), abnormally large lens (2), and maternal toxins or malnutrition (10). The actual formation of a coloboma was first observed and described by von Hippel (6) who was able to successfully breed a colobomatous male rabbit and to obtain affected offspring. He found that 18% of the embryos had the same malformation. From his extensive and detailed studies he concluded that the congenital abnormality was hereditary and that all previously postulated theories should be abandoned. Still, opinions differed as to which tissue was primarily involved in coloboma formation. Some researchers (8, 6) believed that the mesodermal tissue within the fissure kept the margins from fusing. Others (12, 13, 7) considered an increase in the mitotic activity of the inner layer of the neuroectodermal optic cup and the subsequent eversion of this layer to be the cause of a non-fusion of the fissure margins. This latter theory is today the most widely accepted one, since the mesodermal tissue seems to disappear from the fissure area before the margins approach each other, prior to actual fusion.

One way to study a congenital defect, besides the breeding of an affected animal, is to induce the abnormality in laboratory animals, a method widely used in teratology. As described in a review article by Tuchmann-Duplessis and Mercier-Parot (14), colobomata have been produced in laboratory animals by means of various teratogenic agents (Table 1).

The golden hamster has also been used in experiments employing teratogens (9, 11); colobomata, however, have not been reported.

The present study represents the second part of a more comprehensive investigation of the morphogenesis of the normal and abnormal embryonic

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TABLE 1.

COLOBOMA INDUCED IN LABORATORY ANIMALS (SELECTED FROM TUCHMANN-DUPLESSIS & MERCIER-PAROT, 1961)

Teratogenic Agent	Animal	Author
X-rays	Rat	Wilson *et al.*, 1953
	Rat	Hicks, 1954
Trypan Blue	Rat	Gilbert and Gillman, 1954
	Mouse	Hamburgh, 1954
Hypoglycemic Sulfonamides	Rat	Tuchmann-Duplessis and Mercier-Parot, 1956-1959
Actinomycin D	Rat	Tuchmann-Duplessis and
	Rabbit	Mercier-Parot, 1956-1959
Folic Acid Deficiency	Rat	Giroud *et al.*, 1954
Vitamin A Deficiency	Rat	Warkany and Schraffenberger, 1946
Vitamin A Excess	Rat	Giroud and Martinet, 1955
	Rabbit	Giroud and Martinet, 1959

fissure. The first part, an electron microscopic study of the normal closure, has already been completed (5). The second part consists of the attempt to induce colobomata by means of hypo- and hypervitaminósis A. If both attempts are successful, the resulting colobomata will then be compared with one another as well as with the sequence of events in the normal closure. It is hoped that this investigation will permit some insight into the cellular phenomena that lead to a congenital coloboma.

Materials and Methods. In the first attempt of this study 25 female golden hamsters, ranging in age from 2 to 6 months, were bred. The middle of a 30-minute mating period was considered time zero. On the 8th day of pregnancy they were given 1 ml with 25,000 IU of vitamin A (US Vitamin and Pharmaceutical Comp.) by stomach tube. Six females, serving as controls, received 1 ml of the carrier solution (sorethytan ester). On the 14th day of pregnancy the animals were sacrificed with sodium pentobarbital. Twenty litters with a total of 204 embryos were obtained from the experimental animals and 6 litters from the control group. The embryos from 15 females were placed in Kahle's fixative for two days and stored in 70% ethanol. The eyes were dissected from the embryos, dehydrated in a graded series of alcohol, aniline and toluene, and embedded in paraffin. Serial sections, made in the equatorial plane, were stained with hematoxylin and eosin. The eyes of the remaining embryos were prepared for electron microscopy. These were fixed in glutaraldehyde (3%) for 1 hour, postfixed in osmium tetroxide (2%) for 1½ hours, dehydrated in acetone, and embedded in Durcupan ACM.

In the second attempt of this study nine female hamsters were used. Exactly seven days after mating they were given 40,000 IU of vitamin A. On the 14th day of pregnancy the females were sacrificed and the embryo eyes processed and embedded in Durcupan ACM as described above. Equatorial sections 1-2 μ thick were stained with toluidine blue for light microscopic examination.

Results. The first attempt to induce a nonclosure of the embryonic fissure by means of hypervitaminosis A was unsuccessful. In addition to some malformations of the mandible and maxilla and a few cases of exencephaly, 124 embryos showed bilateral or unilateral exophthalmos, however, sectioning revealed that all eyes were normal in size and structure (fig. 1). The protrusion was caused by an abnormally shallow orbit, that is, only tissue derived from mesoderm was affected. Since organs derived from neuroectoderm are usually susceptible to the influence of teratogenic agents at a slightly earlier period of development, the time of treatment with vitamin A was changed to seven days after mating. In three females no living embryos but only resorption sites were found. The remaining six females produced 52 embryos, 18 of which had abnormal eyes upon gross examination.

The recent study of the developing fissure in the normal eye showed that it is formed during the 10th day of gestation. The space between its margins is filled with vascular (hyaloid artery) and mesenchymal tissue. The fissure closes during the 12th day, after which no trace of it is left. At that age the eye is approximately 500-600 μ in diameter.

At 14 days (fig. 2), the size of the eye is about 1 mm. The pigment epithelium is thin and heavily pigmented, the retinal layer is quite thick and shows beginning differentiation into inner and outer neuroblastic layer.

Exophthalmic eye with open fissure. Gross examination showed already that the pigmented layer did not form a full circle, that is, a portion in the inferior area was missing or unpigmented.

Equatorial sections (fig. 3) show an eye approximately 750 μ in diameter, with an open fissure containing only traces of mesenchymal cells and small

blood vessels. Although the eye is protruding and "open," that is, without lids and, therefore, appears to be larger, it is actually smaller than the normal eye. This fact demonstrates the influence of an open fissure on the growth of the eye. The exophthalmic eye without coloboma, as obtained in the first attempt with vitamin A, grows normally; the one with the induced open fissure does not. Also obvious is the fact that the neuroretina has separated from the pigment epithelium. This is most likely not an artefact due to shrinkage during fixation as is frequently seen in paraffin sections. In glutaraldehyde-fixed eyes the two layers normally remain attached (fig. 2). At the fissure the inner layer is everted. It seems to have grown faster and has become folded upon itself, thus forming part of the outer layer. The pigmented epithelium is much thicker than it normally is and in several places oblique streaks can be seen which resemble nerve fibers. An identification might be possible by electron microscopy.

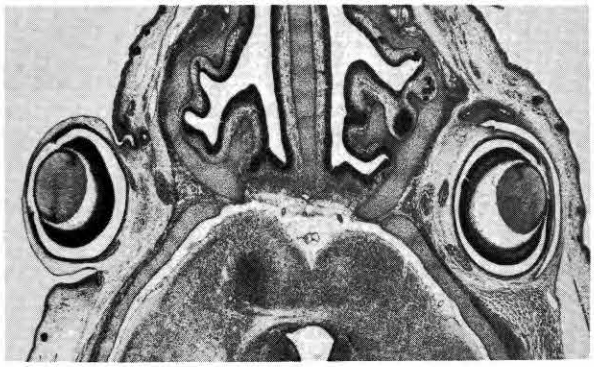

Fig. 1—Horizontal section through the head of a 14-day hamster embryo. The right eye is normal; the exophthalmic left eye is not covered by lids but is otherwise normal. Paraffin section. Hematoxylin and eosin (15 ×).

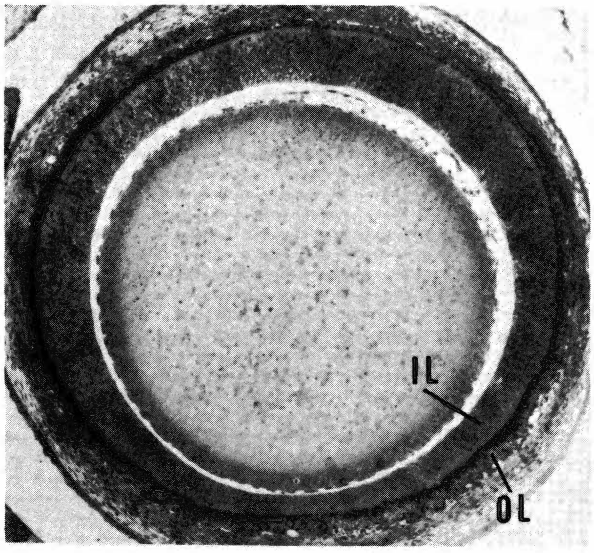

Fig. 2—Equatorial section through a normal eye of a 14-day hamster embryo. IL—inner layer (neural retina); OL—outer layer (pigment epithelium) (75 ×).

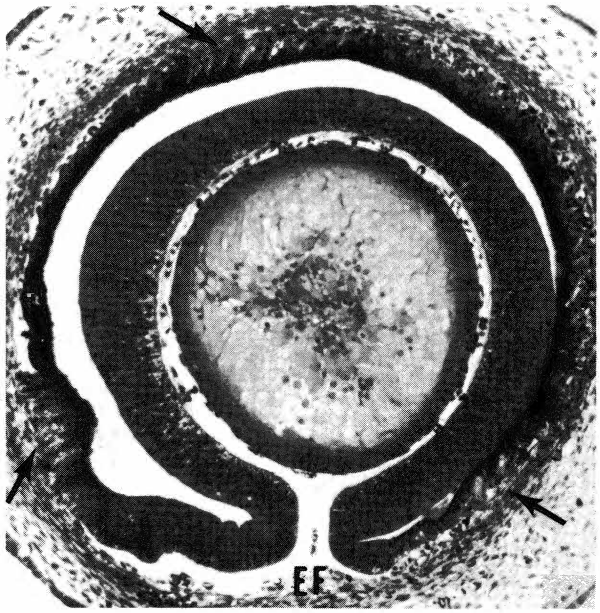

Fig. 3—Eye of a 14-day hamster embryo following treatment with excess vitamin A after seven days of gestation. The embryonic fissure (EF) has not closed, the neural retina is everted at the fissure and forms part of the outer layer. The pigment epithelium is thicker than normal and shows obliquely running streaks (arrows), possibly aberrant nerve fibers. Equatorial epoxy section. Toluidine blue (100 ×).

The lens is small and shows fibers irregular in size and structure. The choroid is being formed but is less condensed and surrounded by much mesenchymal tissue.

Microphthalmic eye with open fissure. In several embryos one eye or both eyes were not visible on gross examination, that is, no pigmented tissue could be seen through the closed lids. Histological examination of one case showed some epithelial tissue with pigment granules. However, the tissue appeared completely unorganized and no definite structures could be recognized. In another case a microphthalmic eye with an open fissure was found. The lids were replaced by a dimple which upon sectioning appeared to change into a deep pit or duct-like structure, that is, the skin never fused as do the lids of the normal eye at this stage of de-

velopment. Sectioning revealed an optic cup much smaller than normal (fig. 4), approximately 250 μ in diameter as compared to about 1,000 μ of a normal eye. The two layers of the optic cup—presumptive neural retina and pigment epithelium—are of similar thickness, that is, the inner layer is much thinner than in normal eyes of this age. The cells of the outer layer contain many pigment granules. Some pigment granules are also present in the retinal layer. The fissure is open, about as wide as on the 10th day when the fissure is being formed by invagination of the optic vesicle. The tissue within and around the cup shows little organization, that is, there is no indication of a formation of lens and choroid. From this first examination the impression is gained that the optic cup developed until it reached a stage similar to that of a normal eye on the 9th or 10th day and that at this stage the normal process became greatly disturbed.

Comments. From these preliminary observations final conclusions cannot be drawn at this time. The results show, however, that a high dose of vitamin A acts as teratogen capable of inducing a typical coloboma in the golden hamster. It will be of interest to compare the induced coloboma with a hereditary one which is being investigated at present (C. Jackson, personal communication, 1972).

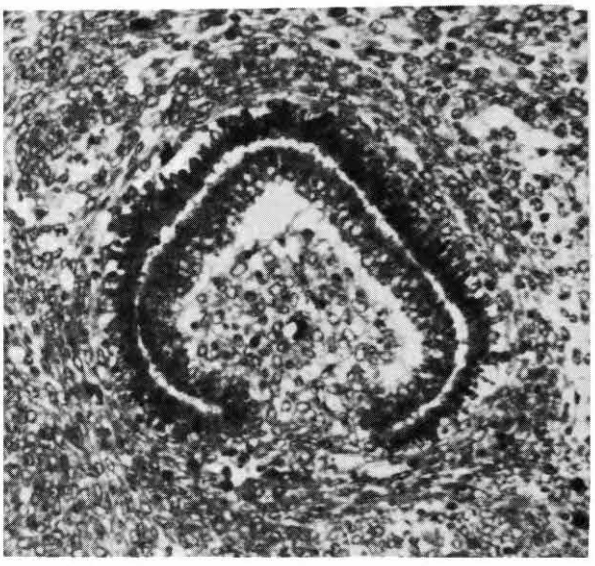

Fig. 4—Equatorial section through a microphthalmic eye of a 14-day hamster embryo, following treatment with excess vitamin A after seven days of gestation. Inner and outer layer are abnormal (see text). Epoxy section. Toluidine blue (200 ×).

This study is being continued and extended by beginning with the younger stages of development, comparable to those examined in the normal eye, and by studying the formation of a coloboma on the ultrastructural level.

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