A Gene causing Ocular Retardation in the Mouse

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WITH TWO PLATES

INTRODUCTION

IN 1955 three mice with very small eyes were observed in a stock segregating for the gene *Patch*. These animals all came from the same mating and on investigation proved to be homozygous for a new gene causing a reduction in the blood-supply of the eyes at a critical embryonic stage. The original animals where also *Ph*/+, but this gene was soon eliminated from the new stock and had nothing to do with the size of the eyes.

A segregating litter of these mice was given to Dr. B. V. Konyukhov of Moscow in 1959 for immunological studies on the development of the lens. Since then Dr. Konyukhov (1961) has carried out genetical (Konyukhov & Glukharev, 1961) and embryological investigations which have partly duplicated the present work. Unfortunately, Dr. Konyukhov has used the unofficial laboratory name of 'blind' (symbol *bl*) for the mutant. This name has already been used for a different eye mutant and the symbol *bl* has been used for the spotting mutant 'blaze'. As the discoverer of the gene I propose to call it 'ocular retardation', symbol *or*.

Genetics

The gene for *ocular retardation* behaves as a normal Mendelian recessive (Table 1). There seems to be a slight deficiency of abnormal animals from the

TABLE 1

Segregation of ocular retardation according to the size of the eyes

Mating	*Normal*	*Abnormal*	*Total*	χ^2_1
or/*or*×+/+	207	0	207	..
+/*or*×+/*or*	538	155	693	2·56
or/*or*×+/*or*	357	291	648	6·72
or/*or*×*or*/*or*	0	219	219	..

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backcross matings, but this is only just significant. Apart from a single doubtful animal there have been no normal overlaps and *or/or* animals can be classified easily at birth from the size of the eyes seen through the semi-transparent eyelids. The gene has been kept on an agouti or black background in order to assist the classification at birth. When the eyes are no longer visible through the skin, classification has to be left until weaning or later when the abnormal animals have only a small slit between the eyelids. Most *or/or* animals are fully fertile and ♀♀ rear their litters. No differences between the eyes of +/*or* and +/+ animals have been observed either in sections of embryos or in the adult.

Material and Methods

Segregating litters of embryos were collected from 12 days until 3 days after birth. The animals could be classified easily from the size of the eyes. Nine-, ten-, and eleven-day embryos were collected from *or/or* × *or/or* matings and matched with normal embryos from CBA/C57BL F_1 mice, as at these stages embryos cannot be classified by the external appearance of the eyes. All the animals were fixed in Bouin's solution and sections stained with Ehrlich's haematoxylin and eosin. Celloidin sections were cut of the heads of newborn, 3-day-old, and adult *or/or* and normal mice. Projection drawings of the eyes were made at ×300 or ×150 of 10-, 11- and 12-day embryos and the mean volumes of the eye-cup and lens vesicle calculated from planimetric measurements. Later, the distribution of blood-vessels, including the smallest capillaries, was added to these drawings. The skeletons of 20 *or/or* and 20 normal litter-mate controls were prepared by the papain maceration method and classified for minor skeletal variants.

Development

9 days

In 9-day-old mouse embryos the optic vesicles are conspicuous outgrowths of the brain reaching to the ectoderm covering the side of the head. The outer surface of the vesicles is starting to sink inwards and the overlying ectoderm to invaginate at the lens placode. No differences have been discovered between the normal and *or/or* optic vesicles or lens placodes at this age.

10 days

By 10 days the optic vesicles are cup-shaped with the choroid fissure forming and the intra-retinal space becoming obliterated; the optic stalk is becoming slimmer (Plate 1, figs. A, B). There is already a difference in the thickness of the inner presumptive retinal layer of cells which is nearly 3 times as thick as the outer layer. The lens placode has sunk well below the surface and is rounding off to form a vesicle which, however, is still open to the exterior. The lens epithelium and the internal layer of the retina are not separated by any cells. There are numerous blood-capillaries near the back of the optic cup and round

the rim at the junction of the lens epithelium and the overlying ectoderm. No differences between normal and *or/or* eyes have been discovered at this age and measurements of representative sections failed to reveal any differences in size which were statistically significant (Table 2).

TABLE 2

Mean measurements from central sections of 20 normal and 20 or/or *eyes at 10 days (in mm.)*

Measurement	*Normal*	*or/or*
1. Width of lens vesicle . . .	0·1055	0·1041
2. Depth of lens invagination . .	0·0525	0·0647
3. Thickness of lens placode . .	0·0327	0·0321
4. Diameter of intra-retinal space .	0·1582	0·1564

11 days

By 11 days the optic cup is complete in normal embryos and the choroid fissure is starting to be obliterated except where it joins the optic stalk. The intra-retinal space is also nearly obliterated except near the rim and along the choroid fissure. The inner retinal layer is now about 5 times as thick as the outer layer where pigment is starting to form. The lens vesicle is complete, but is still attached to the overlying ectoderm. In most embryos the inner lens cells are starting to elongate and grow into the central space making the latter crescent-shaped. The internal retinal layer of cells and the lens epithelium are still in contact in places, notably near the rim along the dorsal side, but ventrally blood-vessels and mesenchymatous cells are invading the space between the lens and retina over the outer rim of the eye-cup. There are numerous blood-capillaries both behind the back of the eye and inside it between the lens and the retina. The optic stalk is grooved (in cross-section) near the eye-cup, and blood-vessels are present in this groove. They probably pass into the eye-cup, but the choroid fissure is very narrow at this age and there is little space for them.

TABLE 3

Mean volumes in cubic millimetres of normal and or/or *eye-cups and lens vesicles*

	Eye-cup		*Lens vesicle*		*Ratio lens/eye-cup*	
Age (days)	*Normal*	or/or	*Normal*	or/or	*Normal*	*or/or*
10	0·0048	0·0048	0·00062	0·00070	0·13	0·14
11	0·013	0·010	0·0023	0·0017	0·18	0·17
12	0·041	0·016	0·0102	0·0039	0·25	0·24

At this age the *or/or* eyes are still very similar to the normal eyes and there are no obvious differences in the growth of any of the structures mentioned above. In order to find out if there were differences in size, the volumes of eye-cup and lens were calculated for 10-, 11-, and 12-day embryos from sections

(Table 3). These show that at 11 days the *or/or* eyes are just starting to lag behind the normals in size.

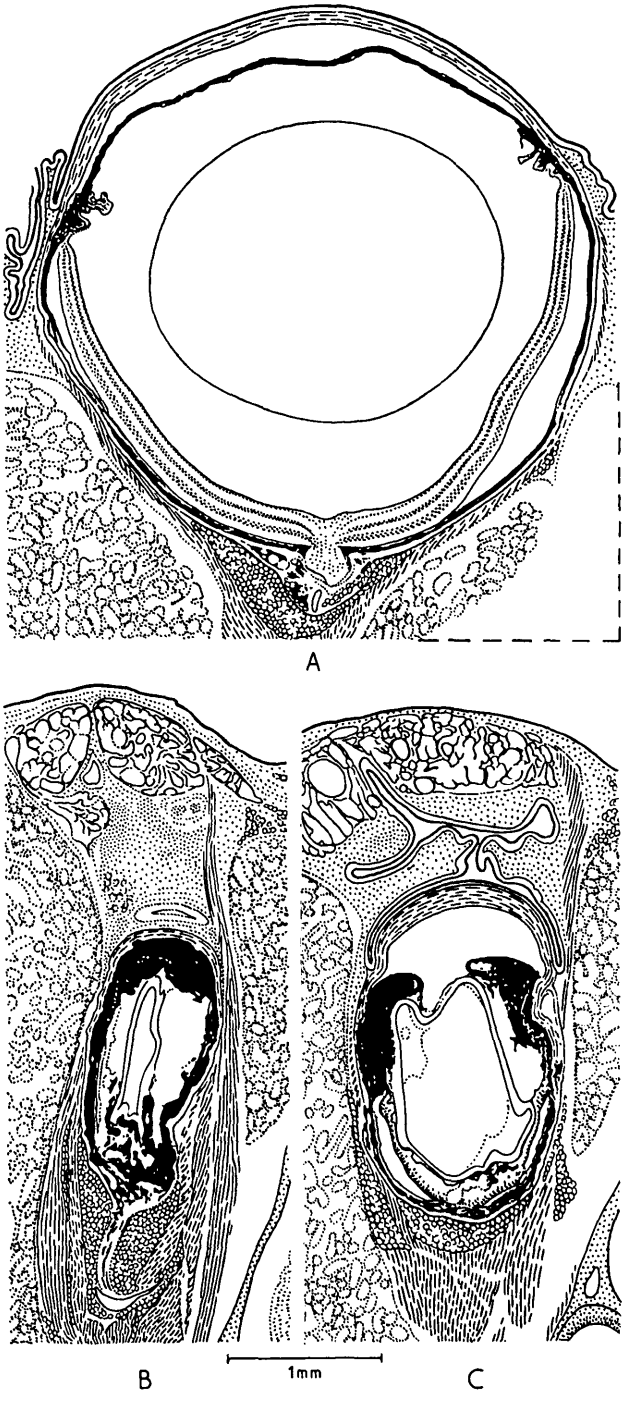
12 days

In normal embryos the eyes have grown considerably and the retinal layer is now about 8 times as thick as the outer layer (Plate 1, figs. C, D). Nerve fibres are differentiating and are concentrated at the choroid fissure and already growing out along the optic nerve. The choroid fissure has practically closed except at the junction with the optic stalk where the central artery and vein are present (Plate 1, fig. C). In the retina this line of fusion is marked by pycnotic cells which are normally present when any organ changes its shape during development (Glücksmann, 1951). The lens has increased in size and is now a solid structure with fibres stretching up to the lens epithelium. It is separated from the retina at the back by blood-capillaries, but is still in contact with the retina just under the rim of the eye-cup and is also in contact with the presumptive cornea.

The *or/or* eyes at 12 days can be distinguished from normal eyes not only in sections, but also by their smaller size in fixed embryos. Measurements of the eye-cup and lens at this age confirm this impression (Table 3), and the study of sections reveals that the eyes are retarded in their development. The most obvious abnormalities are the relatively thin retinal layer of the eye-cup and the lack of nerve fibres and blood-vessels in the choroid fissure where it joins the optic stalk. In some embryos nerve fibres start to develop and grow out from the inner surface of the retina, but they do not penetrate past the outer retinal layer. This latter layer is very similar to that of normal eyes and it alone is continuous with the optic stalk. The inner layer is only about 4 times as thick as the outer layer, and the intra-retinal space is present round part of the rim and opposite to the junction of the outer retinal layer of cells with the optic stalk (Plate 1, fig. D). In most animals the lens still has a crescent-shaped cavity in which there are often large dying cells, but the inner lens cells are starting to form fibres. As in the normal eyes the lens is in contact with the eye-cup along the dorsal rim and is also still in contact with the presumptive cornea. At the back and over the ventral rim, blood-vessels and mesenchymatous cells form a cushion between the lens and the eye-cup, and these are the only blood-vessels entering the eye in *or/or* embryos. The choroid fissure has practically closed completely and the retinal layers have started to fuse together throughout their length, although the join can still be traced in sections by the increased number of dying cells. In the *or/or* eyes the normal pathway for the central artery and vein through the retina to the inner layers is occluded in this process.

Later embryos

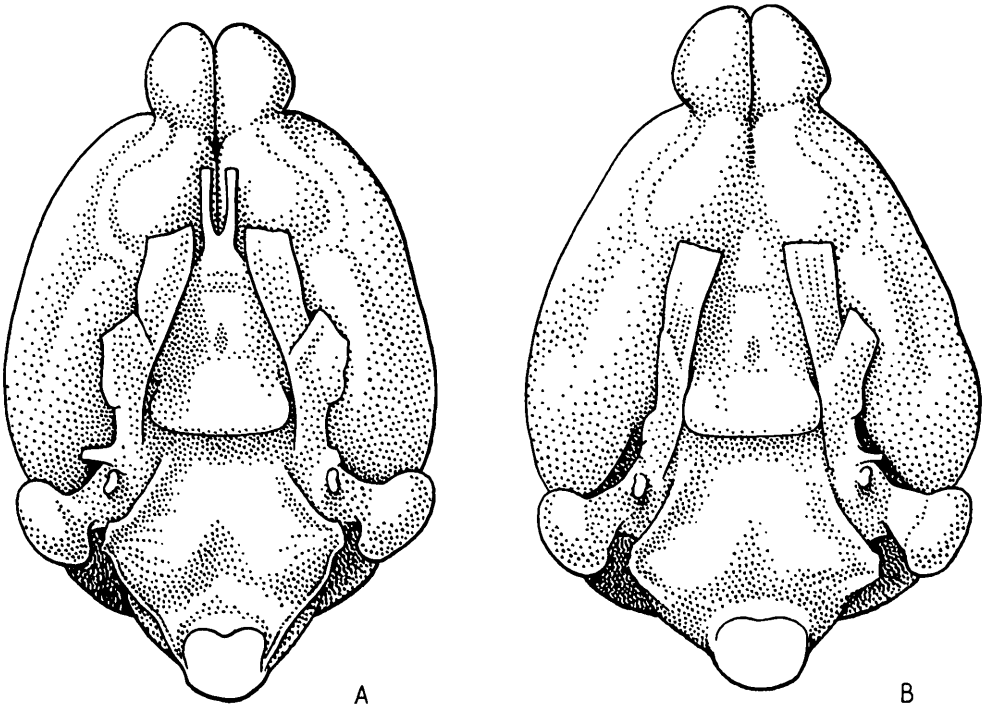
After 12 days there is a rapid increase in the size and differentiation of normal eyes. The presumptive retina becomes thicker and the various cellular layers are



TEXT-FIG. 1. Drawings of sections of an adult normal (A) and an *or*/*or* (B and C) eye.

determined. There are more nerve fibres growing out along the optic stalk to the brain and the gap left by the embryonic choroid fissure is enlarged to accommodate the larger blood-vessels which normally provide the blood-supply to the inner retinal layers and the back of the lens. The rim of the eye-cup and the edge and front of the lens are supplied by capillaries from the annular vessels, but these normally do not penetrate deeply into the eye.

In *or/or* mice eye development lags further and further behind that of normal animals as the choroid fissure is gradually completely obliterated. The only blood-vessels which enter the *or/or* eyes are those from the annular vessels over the rim of the eye-cup. The lens remains small and either retains the original lens cavity or secondary cavities develop under the lens epithelium. The retina remains thin and the few nerve fibres that differentiate soon degenerate (Plate 2, figs. E, F). The optic stalk also gradually regresses as no nerve fibres grow down it.



TEXT-FIG. 2. Ventral view of normal (A) and *or/or* (B) brains showing the lack of an optic chiasma.

Birth and later

In new-born mice, eyes which are normally pigmented can be seen easily through the semitransparent eyelids, and normal and *or/or* animals can be distinguished by the great reduction in the size of the eyes of the latter. In sections the lens is normally large and fairly uniform in structure, but in *or/or* mice it has many large vesicles (Plate 2, figs. G, H) and although there are some

fibres these usually disappear and in the adult the lens is soft and irregular in shape (Text-fig. 1). The retina remains thin and undifferentiated, but is full of pigment in the adult. There is practically no optic stalk at birth and in the adult it has disappeared completely and there is no optic chiasma (Text-fig. 2). The adult orbit which appears to be of normal size is filled by the hypertrophied Harderian and lachrymal glands. The eyelids are thickened and often have ingrowing eyelashes, but they are never completely fused. No skeletal variants connected with the gene have been discovered.

Discussion

The normal development of the eyes depends not only on competent tissues being in the right place at the right time, but also on the presence of an adequate source of nutrition for later development. Chase & Chase (1941) in their work on *anophthalmia* found that if the primary optic vesicles were too small and did not reach the overlying head ectoderm, no lens was formed and this in turn had a profound influence on the development of the rest of the eye. Nevertheless, in spite of very variable manifestation from anophthalmia to microphthalmia, where the eyes were almost normal (if smaller in size), they maintained that there was always a morphologically correct blood-supply. The differences were due to defects in the primary optic vesicles in the presence of an adequate blood-supply.

In *microphthalmia* (*mi*), where the optic vesicle fails to form a proper cup and the choroid fissure remains permanently open, there is also a normal blood-supply (Müller, 1950, 1951). The eyes gradually regress and optic nerve fibres do not differentiate in the retina and grow along the optic stalk. This gene has other effects, though, besides those on the size of the eyes and in *mi*/+ mice the amount of pigment in both the eyes and in the hair is reduced, while in *mi*/*mi* animals the skeleton is also affected.

In the rat, Browman (1961) suggested that failure of the early blood-supply was correlated with varying degrees of microphthalmia. He did not observe any histological defects in the optic primordia of 10- and 11-day-old rat embryos. He noticed the first defects in 12-day-old embryos and found increasing numbers of eye defects at 13 days, suggesting that there was a critical period at about the 11th day and reaching a threshold during the 12th day. There was a consistent absence of a normal blood-supply to the eye region in the microphthalmic strain of rats; the most common defect being the absence of a central artery and often of an ophthalmic artery. There was a range from normal eyes to complete anophthalmia.

In many respects the gene for *ocular retardation* shows features which are common to these observations in the rat. The gene is far less variable in its manifestation, but the time at which the first defects are found is very similar to that in the rat. The distribution of the major blood-vessels from the internal carotid artery and to the anterior cardinal vein appears to be normal up to the

age of 11 days, but after that time the central artery and vein are missing from their definitive positions along the choroid fissure. The latter closes completely and the only blood-supply for the inner retinal layers is from branches of the annular vessels which enter the eye-cup over the rim, between it and the lens. The retina fails to differentiate and nerve fibres fail to grow out along the optic stalk to the brain; they start to develop, but usually do not penetrate through the outer pigmented layer. This latter layer is the only one which remains in contact with the optic stalk, but as nerve fibres fail to develop the optic stalk degenerates and is missing in the adult. Unlike some of the rat embryos abortive attempts by these nerves to grow out in abnormal places (Browman, 1961) do not appear to take place.

The reduction in the blood-supply to the inner layers of the eye due to the lack of the central artery and vein cannot be compensated for by the branches from the annular vessels which enter the eye-cup over the rim. However, they allow the eyes to go on developing at a much slower rate than normal and some differentiation takes place. The eyes do not degenerate completely, but are severely retarded after a failure in their blood-supply at a critical age.

These findings are in agreement with those of Konyukhov (1961) and Konyukhov & Glukharev (1961) where the embryonic development is dealt with only briefly, although a diagrammatic representation of the probable course of events is given in Konyukhov (1961).

Summary

1. The development of the eyes in mice homozygous for the gene *ocular retardation* (*or*) is described here.

2. Up to the age of 11 days the eyes of *or*/*or* embryos appear to be normal in their size and general proportions, but after this time they are reduced in size. This is due to the failure of the central artery and vein to establish a pathway along the choroid fissure, which closes completely.

3. In the adult the lens is never crystalline and is always distorted in shape, while the retina contains no sensory cells, no optic nerves develop, and there is no optic chiasma. An orbit of normal size is filled with hypertrophied Harderian and lachrymal glands.

Résumé

Sur un gène provoquant un retard du développement de l'œil chez la souris

1. On décrit le développement des yeux chez des souris homozygotes pour le gène *ocular retardation* (*or*).

2. Jusqu'à l'âge de 11 jours, les yeux des embryons *or*/*or* ont une taille et des proportions générales normales, mais ensuite leur taille se réduit. Ceci est dû au fait que l'artère et la veine centrale ne peuvent se placer le long de la fissure choroïdienne, qui se referme complètement.

3. Chez l'adulte, le cristallin n'est jamais transparent et est toujours distordu; la rétine ne renferme pas de cellules sensorielles, il ne se forme pas de nerf optique, et il n'y a pas de chiasma optique. L'orbite, de taille normale, est occupée par les glandes de Harder et lacrymales hypertrophiées.

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EXPLANATION OF PLATES

PLATE 1

FIG. A. Normal right eye from 10-day embryo.
FIG. B. Right eye from *or/or* 10-day embryo.
FIG. C. Normal left eye from 12-day embryo.
FIG. D. Left eye from *or/or* embryo, litter mate of C.

PLATE 2

FIG. E. Normal right eye from 14-day embryo.
FIG. F. Right eye from *or/or* embryo, litter mate of E.
FIG. G. Normal right eye from new-born mouse.
FIG. H. Left eye from *or/or* new-born mouse, litter mate of G.

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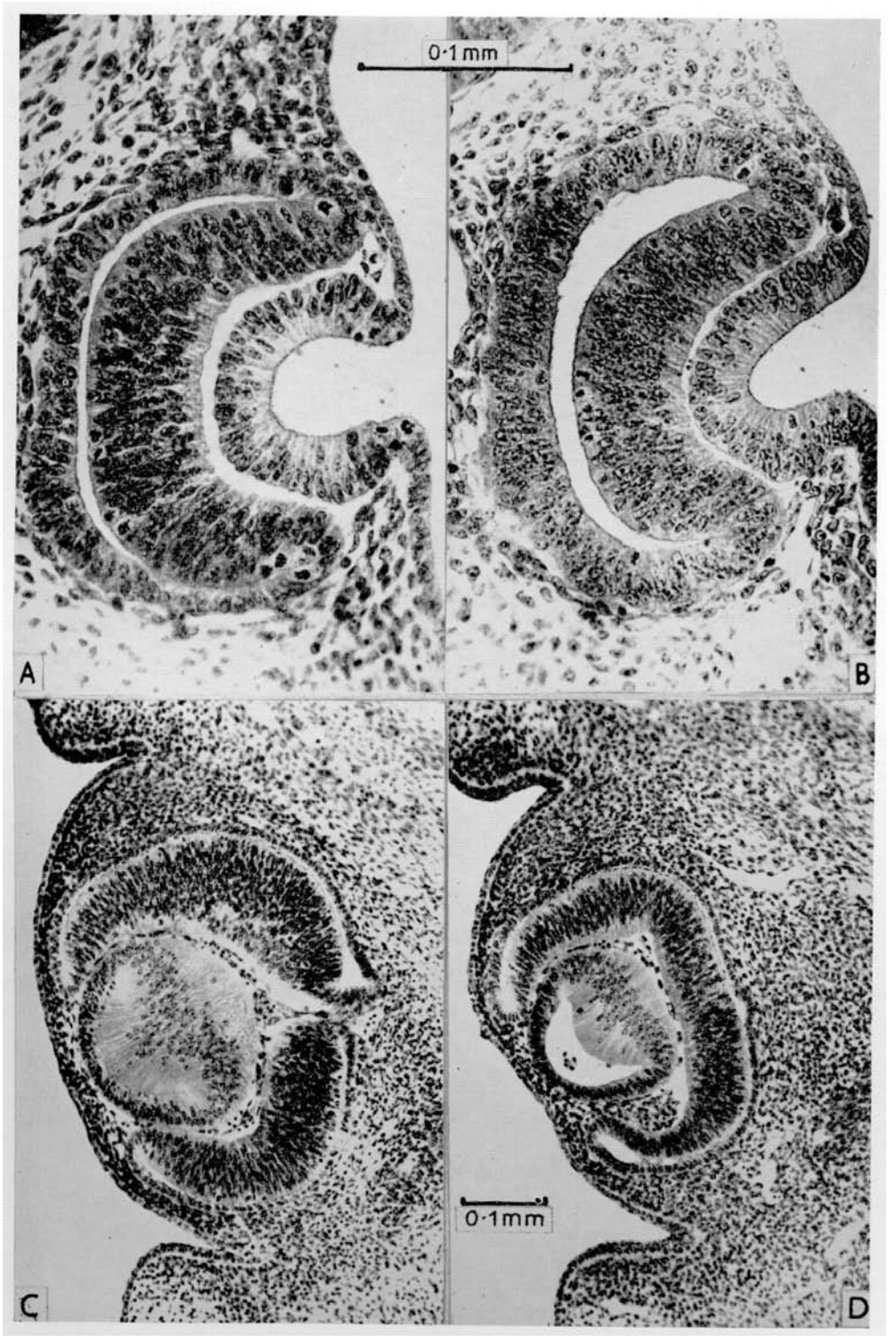

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Plate 1

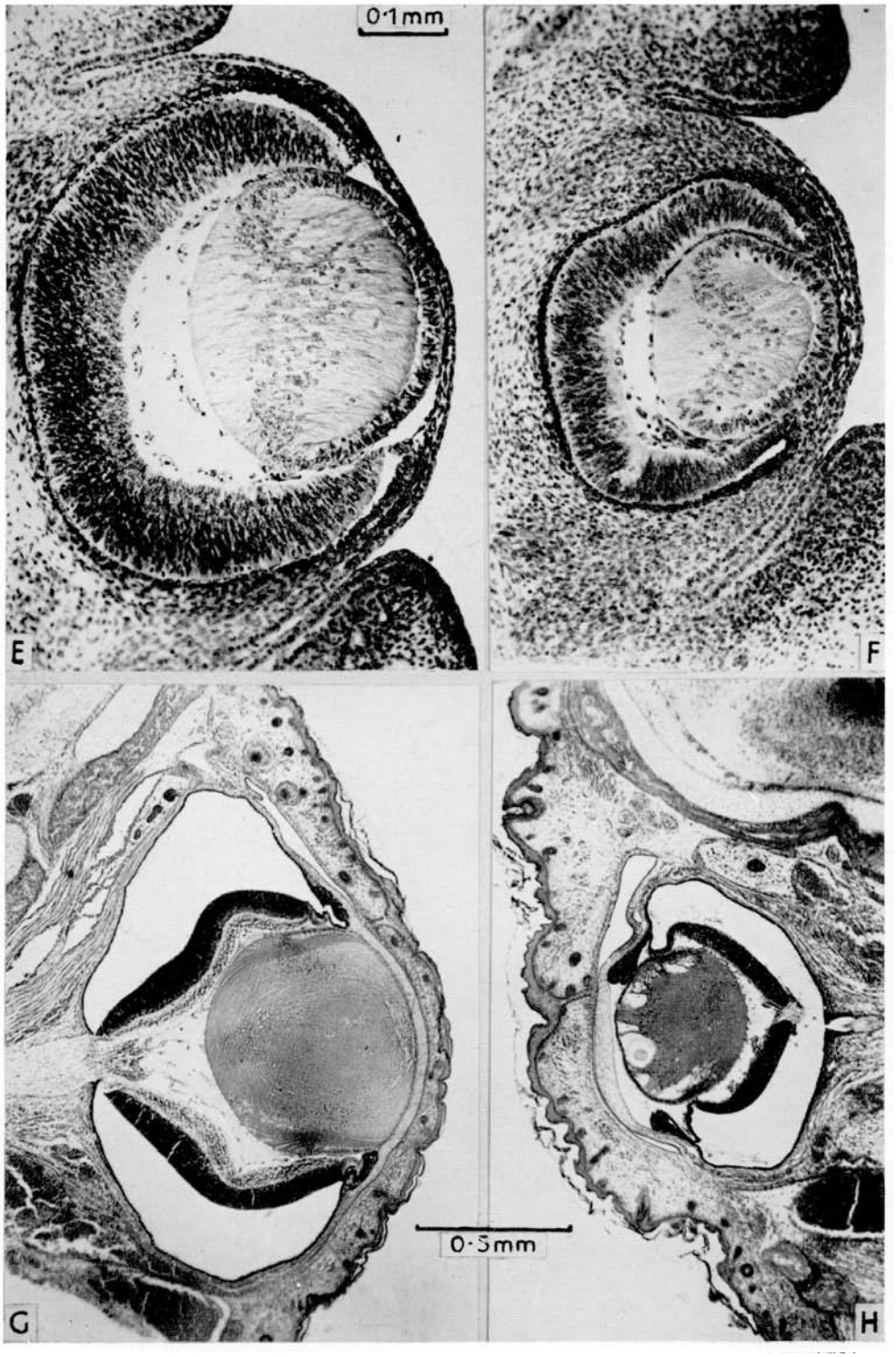

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Plate 2