THE GENETIC AND MORPHOLOGICAL CHARACTERISTICS OF MICROPHTHALMIC MICE (BLIND MUTANTS)

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Laboratory mice have developed many mutant lines having hereditary eye defects. They include mutants with anophthalmia, microphthalmia, retinal degeneration, absence of photoreceptors, cataract, aplasia of the optic nerve, and others [8]. A very detailed genetic analysis and embryological study of mutant mouse lines have been made of mice with microphthalmia [7, 9, 11], and with anophthalmia [2-6]. It has been shown that these two conditions are inherited as recessive characters. In those with microphthalmia (symbol mi) in the homozygote, the condition is (mi/mi), and there is a microphthalmia with a coloboma of the retina, complete absence of pigmentation, abnormal skeletal development, and in particular incomplete development of the teeth with the result that they normally die at an early age, shortly after birth. The mutant line of mice with anophthalmia shows a 98% population of animals with anophthalmia, and 2% with microphthalmia [5], and mice of this line do not differ in fertility from normal laboratory mice [2].

In 1956, in Grüneberg's Laboratory in London, Truslov found a new spontaneous mutation of microphthalmic mice, which he called "blind" (symbol bl). In 1959, in the same laboratory, we found a mouse of this mutant line of an agouti* color. We here give a morphological and genetical description of this line.

A Morphology of the "Blind" Line

The blind mutants in the homozygous condition (bl/bl) are microphthalmic, and in the heterozygous condition (+/bl) had normal eyes. When homozygous (bl/bl) mice of agouti color were crossed with albino mice having normal eyes (+/+) we obtained microphthalmic albino offspring. It would be expected that the retina of the latter would be more abnormal, because it is known that in albinos, retinal differentiation is somewhat delayed [10].

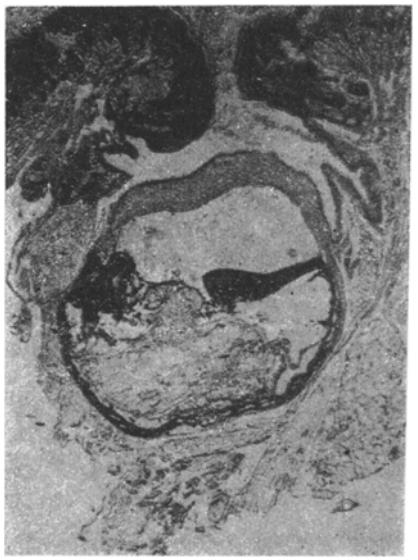
In the homozygous microphthalmic animals there was a great reduction in the overall size of the eyes. In some animals, the lids were stuck together, but most frequently there was a small slit between them, through which the reduced eye could be seen.

In many cases, the lids were open, and a more or less formed eye could be seen. Very frequently in addition to the microphthalmia, the cornea and lens were somewhat clouded. Measurements made on a large number of animals of the diameter of the eye (along the optic axis) showed that the length was 1.1-2.6 mm. Also, the measurements of the eyes of mice of the inbred line C57BL, which we used as controls, varied from 2.8 to 3.3 mm. Most frequently (in about 50% of the cases) in animals with microphthalmia, the eye diameter was about 1.7 mm, whereas the mean diameter of a normal eye is 3.1 mm. Considerable differences in eye size between normal and microphthalmic animals are clearly evident at birth. It should be noted that the variation in the size of the abnormal eyes has been observed also in new-born mice.

The structure of the microphthalmic eyes is definitely abnormal, and to an extent which varies according to the animals. In many mice, such malformed eyes had a more or less well-differentiated cornea and lens. In most animals, the retina was completely undifferentiated into layers, and contained no photoreceptors (no rods); the tunica propria of the cornea was greatly thickened, the lens was grossly malformed, and there was no ciliary body

*We are grateful to Professors Grüneberg and Truslov for supplying us with a stock of mutant lines of blind and microphthalmic mice.

or iris. The pigment epithelium was greatly thickened, and frequently the peripheral parts of the thin undifferentiated retina were infiltrated with pigment. In many mice, the lens was completely degenerate, and some portions of the retina were undifferentiated (see figure).



Section through the microphthalmic eye of an adult mouse; the lids have not fused, the eye is greatly reduced.

In some cases we observed almost complete degeneration of the eye as a whole, in an animal only one month old. Microphthalmia is associated with developmental failure and degeneration of the optic nerve.

It should be noted that joined eyelids are not an indication of complete degeneration in microphthalmia. In one animal which had fused lids, we observed a comparatively large eye with a more or less differentiated retina, lens, cornea and other structures.

Microphthalmic animals are fully viable; their fertility is even somewhat greater than that of mice of the inbred line C57BL. As a rule, the microphthalmic animals were about 10% smaller in size and weight than offspring from the same litter with normal eyes. However, for the first month of post-embryonic development, all appreciable differences in size and weight disappeared. A study of the skeleton and viscera of microphthalmic animals showed no notable differences from the normal inbred line C57BL. Measurements of the bones of the skull of the axial skeleton, and the limbs of mutants and imbred mice disclosed no differences. In particular, the relative measurements of the orbit in the microphthalmic and inbred mice were approximately the same. It should be noted that in three of the 510 microphthalmic animals the axial skeleton was bent. From these observations, we cannot infer that in the mutant line 'blind' there was any malformation of the skeleton as there is in the mutant line 'microphthalmia' [1, 7].

Genetic Analysis of the Mutant Line "Blind"

When crossing microphthalmic animals with each other, in all cases the offspring were abnormal (Table 1).

TABLE 1. Segregation of bl in the Offspring from Various Crosses

Cross ♀♀ ♂♂	No. of pairs	No. of litters	Offspring		
			total	with normal eyes	with microphthalmia
bl/bl×bl/bl	19	37	287	—	287
bl/bl×+/+	24	29	222	222	—
+/+×bl/bl	20	22	173	173	—
+/bl×+/bl	41	54	430	316	114
bl/bl×+/bl	35	39	295	154	141
+/bl×bl/bl	51	63	469	253	216

Nevertheless, in the F_1 generation from a reciprocal cross of mice with microphthalmia (bl/bl) and of inbred mice with normal eyes (+/+) there were no microphthalmic animals. From the cross F_1(+/bl × +/bl) there was a segregation of the characters of the F_2-offspring: 114 mice had microphthalmia, and 316 were phenotypically normal animals. The ratio of the two groups was 1 : 2.8, i.e., it approached the theoretically expected result for a monohybrid cross. Reciprocal crossing of homozygotes (bl/bl) with the heterozygote (+/bl) gave approximately the same number of animals with microphthalmia (141 and 216) as with normal eyes (154 and 253).

Thus, the results given in Table 1 show that microphthalmia in the "blind line" is inherited as a recessive character. Confirmation was obtained by crossing hybrid pigmented mice (agouti color) with microphthalmic animals and with normal-eyed albinos (Table 2). In the F_1 generation of such a cross there were 363 normal-eyed agouti animals. In the next cross of the F_1 generation, in F_2 there were 185 mice, comprising four phenotypes: 105 pigmented animals with normal eyes, 35 pigmented with microphthalmia, 33 albinos with normal eyes, and 12 microphthalmic albinos. The relative numbers in these groups approached the expected values of 9 : 3 : 3 : 1. The results

TABLE 2. Results of Crossing Pigmented Microphthalmic Hybrids (bl/bl C/C) with Normal-Eyed Albinos (+/+ c/c)

Cross ♂♂ ♀♀	No. of pairs	Generation	No. of litters	Total	Offspring: Phenotype[1]			
					Pigmented		Albinos	
					with normal eyes	with microphthalmia	with normal eyes	with microphthalmia
bl/blC/C×+/+ c/c	32	F_1	47	363	363		—	—
+/blC/c×+/bl C/c	61	F_2	21	185	105	35	33	12

Genotype																							
+/+ C/c 25		+/+C/C 12		+/blC/c 49		+/blC/C 19		bl/bl C/c 25		bl/blC/C 10		+/+ c/c 13		+ /bl c/c 20		bl/bl c/c 12							
♀♀ 13	♂♂ 12	♀♀ 5	♂♂ 7	♀♀ 28	♂♂ 21	♀♀ 10	♂♂ 9	♀♀ 15	♂♂ 10	♀♀ 5	♂♂ 5	♀♀ 6	♂♂ 7	♀♀ 11	♂♂ 9	♀♀ 6	♂♂ 6						

[1] $X^2=0.115$; N=3; P=0.99.

of the cross of F_2 showed that the ratio of the groups of the animals with different genotypes also approached tne theoretical value expected for a double hybrid (Table 2). These results indicate the independent inheritance of microphthalmia and albinism in mutant mice of the "blind" line. From Table 2 it can be seen that microphthalmia is not sex-linked.

Thus, the results of the analysis showed that microphthalmia in the mutant line "blind" is inherited as a recessive character, and is not sex-linked.

The cross of the mutant "blind" and microphthalmic showed that the double heterozygotes (+/bl, +/mi) have normal eyes. This result confirms the fact that bl and mi are not alleles. The results of the next cross of the double heterozygotes between each other showed that the factors bl and mi segregate independently, and evidently form part of different groups. Further, the other crosses showed no linkage between bl and A (agouti). Consequently, the results enable us to distinguish blind I (albinism), V (agouti), and XI (microphthalmia) linkage groups.

Thus, a morphological study of mice of the mutant line "blind" showed that the inherited anomaly is only that of microphthalmia, and is not associated with visible abnormalities of other organs. It should be noted that the inheritance of the microphthalmia is of the human type as far as the degree of damage to the eye is concerned. Therefore, microphthalmia in mice may be a good model for the study of the mechanisms of the origin, development, and transmission of the condition in man. Such a model makes it possible to carry out a detailed experimental analysis of the pathogenesis of the inherited anomaly in man. Thus, through our researches it has been shown that in mice, microphthalmia of the line "blind" is inherited as a recessive character, and is not sex-linked. Microphthalmia is not associated with any visible anomalies of other organs, or with any reduction of viability or fertility.

SUMMARY

Microphthalmia in mice of the mutant stock "blind" is inherited as an autosomic recessive character with complete penetrance. It was shown that bl does not belong to the I, V, or XI linkage groups; bl and mi are not alleles. The eye structure of the microphthalmic animals shows a marked deviation from the normal, the abnormality exhibiting a wide diversity. Microphthalmia was not accompanied by any visible anomalies of other organs, or by decreased viability or fertility.

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