A new allele of microphthalmia induced in the mouse: microphthalmia – defective iris (mi^dl)

BY JOHN D. WEST†*, GRAHAM FISHER†, JOHN F. LOUTIT†, MICHAEL J. MARSHALL‡, NORMAN W. NISBET‡ AND V. HUGH PERRY§

† MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, England
‡ Charles Salt Research Centre, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire SY10 7AG, England
§ Department of Experimental Psychology, South Parks Road, Oxford, OX1 3UD, England

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SUMMARY

A new allele of microphthalmia (mi) in the mouse was discovered among the progeny of a male that had been treated with the potent mutagen ethylnitrosourea. Homozygotes have white coats, mildly defective bone resorption and small eyes (about 60% of the normal size) with very little pigmentation. The iris and retina are abnormal, there is no vitreous body and iris pigmentation is restricted to a rim around the pupil. No haematopoietic defect was detected. Genetic studies showed that the mutation is linked to lurcher (Lc) on chromosome 6 and crosses with Mi^wh/+ and mi/+ mice indicate that the mutation is allelic with these two alleles of the microphthalmia (mi) locus. We designate the new allele microphthalmia-defective iris (mi^dl). Some mi^dl/+ heterozygotes (including the original mutant animal) showed a bright ‘red-reflex’ when light was shone directly into the eye and this may have been caused by reduced choroidal pigmentation. Otherwise mi^dl/+ mice appeared normal. The mi^dl/mi compound heterozygotes had white coats, small eyes, and small teeth; bone resorption was more severely defective than in mi^dl/mi homozygotes. The osteopetrosis was corrected by treatment of mi^dl/mi mice with parental mi/+ bone marrow which suggests that the defect is intrinsic to mi^dl/mi marrow cells. The coats of mi^dl/Mi^wh compound heterozygotes were white; the irises were more symmetrical and iris pigmentation was less severely reduced than in mi^dl/mi homozygotes but pupil dilation appeared to be restricted. Partial complementation occurred in the mi^dl/Mi^wh compound heterozygotes with respect to eye size, which was usually near normal.

1. INTRODUCTION

The microphthalmia locus (mi) on chromosome 6 of the mouse is probably a complex locus of at least two closely linked and functionally related genes (e.g.

* Present address: Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, Scotland.
Eight alleles are known (mi, mibw, mirw, misp, misws, Mitb, Mitor and Mitwh) and these have various pleiotropic effects on eye size and anatomy, pigmentation and the capacity for secondary bone resorption. Green (1981, pp. 158–161) has summarized the pleiotropic effects of the various alleles and Packer (1967) has suggested that they are caused by a basic defect that affects cell division.

A new allele of microphthalmia was discovered at Harwell (West & Fisher, 1985a) among the progeny of a male that had been treated with the potent mutagen ethylnitrosourea (West & Fisher, 1985b). Homozygotes have white coats and small eyes with abnormal irises. We have designated the new allele microphthalmia-defective iris (mitdi). In this paper we present genetic evidence that the new mutation is an allele of mi and we describe its effect on pigmentation, the eye, haematopoiesis and bone resorption.

2. MATERIALS AND METHODS

(i) Mice

The mitdi mutation and the Mitwh allele were maintained at Harwell on outbred genetic backgrounds. The mi allele was maintained at Oswestry on an inbred strain (G) developed from mice from Professor H. Grüneberg. Other Harwell stocks that were used include an outbred stock carrying the genes lurcher (Lc) and sightless (Sig), and the outbred PT stock (homozygous for non-agouti (a), brown (b), chinchilla (cch), dilute (d), pink-eyed dilution (p), short-ear (se) and piebald (s)). C3H/HeH, C57BL/Ola and DBA/20la inbred mice and (C3H/HeH × 101/H) F1 hybrids were also used in various crosses at Harwell and CBA/Ca was used at Oswestry.

(ii) Examination of eyes

Pupils were dilated with a drop of 1% (w/v) atropine sulphate and eyes were examined in situ with a Zeiss 30SL/M slit lamp, as described by West & Fisher (1985b, c). This was used for observations of the iris pigmentation, iris abnormalities and the red-reflex. White light directed into the pupil when the slit illuminator was directly in line with the corneal microscope of the slit lamp caused the pupil to glow red in mice with reduced eye pigmentation. This response is referred to as a red reflex.

Mice were killed by cervical dislocation and the eyes removed for histology, weighing or examination under a dissecting microscope. Eyes and other tissues prepared for histology at Harwell were fixed in neutralized formol saline (4% formaldehyde), embedded in paraffin wax, sectioned at 7 μm and stained with haematoxylin and eosin. Those prepared for histology in Oxford were fixed in formol alcohol (85 ml 45% ethanol plus 10 ml 40% formaldehyde plus 5 ml glacial acetic acid), embedded in paraffin wax, serially sectioned at 10 μm and stained with cresyl violet.

Mice were weighed, at 6 weeks of age, to an accuracy of 0.01 g on an electronic, top-loading balance (Sartorius 1205 MP) and eyes were weighed to an accuracy of 0.1 mg on a mechanical balance (Sartorius 2842). Some eyes were fixed in Bouin’s fixative for later examination.

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(iii) Bone resorption studies

Animals homozygous for the \textit{mi}\textsuperscript{dl} allele were crossed with G-strain \textit{mi}/+ mice to produce the compound heterozygote \textit{mi}\textsuperscript{dl}/\textit{mi}. Bone marrow was washed out of the femurs and tibias of G-strain \textit{mi}/+ or the H-2 compatible allogeneic CBA/Ca mice with medium 199 (Flow Laboratories). 4.5–4.6 \times 10^6 nucleated bone marrow cells were injected into the tail vein of \textit{mi}\textsuperscript{dl}/\textit{mi} recipients. Diagnostic radiography was used at 3 and 8 weeks post transplant to evaluate bone resorption. Mice were anaesthetized with ether and films were exposed for 1 s with minimum filtration at 40 kV from a mobile X-ray apparatus.

(iv) Statistical tests

Statistical tests were done on a Hewlett-Packard programmable calculator, programmed by Mr D. G. Papworth of the MRC Radiobiology Unit to compute \(\chi^2\) with Yates’ correction, the correlation coefficient (\(r\)), Student’s \(t\)-test and Smith and Satterthwaite’s variation of Welch and Aspin’s \(t\) test for samples with different variances (Satterthwaite, 1946). Values for the Welch and Aspin \(t\) test are shown as \(t'\) and this test was used in preference to Student’s \(t\) test when sample variances were significantly different at the 1\% level.

3. ORIGIN OF THE \textit{mi}\textsuperscript{dl} MUTATION

The \textit{mi}\textsuperscript{dl} mutation was found among the progeny of a cross between an untreated female of the PT strain and a C3H/HeH male that had been treated with 250 mg/kg of the potent mutagen ethylnitrosourea. The proband was a male born 5 months after paternal treatment with ethylnitrosourea. This male was examined with a slit lamp at 3 weeks of age, as part of a screen for dominant cataract mutations (West & Fisher, 1985b), and showed an unusually bright red-reflex (pupillary reflex) when the slit illuminator was directly in line with the corneal microscope of the slit lamp. He was outcrossed to a (C3H/HeH \(\varphi\) \times 101/H \(\delta\)) F\textsubscript{1} hybrid female and then the progeny with bright red-reflexes, were intercrossed or backcrossed to their father.

A bright red-reflex was found in 5/27 of the progeny of the outcross between the proband and the (C3H/HeH \(\varphi\) \times 101/H \(\delta\)) F\textsubscript{1} female. A novel phenotype (white coat and small eyes with abnormal irises) occurred in 8/12 offspring of the backcross to the proband and in 4/22 of the intercross progeny. This phenotype was inherited by 10/10 progeny when two small-eyed, white mice were mated. (These preliminary data are not included in the Tables.)

4. GROSS PHENOTYPE AND INHERITANCE OF \textit{mi}\textsuperscript{dl}

The inheritance pattern in the initial crosses (discussed above) and the subsequent crosses (1–8 in Table 1) show that \textit{mi}\textsuperscript{dl} is inherited as a simple Mendelian recessive gene. Although the frequency of \textit{mi}\textsuperscript{dl}/\textit{mi}\textsuperscript{dl} homozygotes is sometimes slightly lower than expected, this deficiency is only statistically significant in cross 5. The breeding performance tended to be poor when \textit{mi}\textsuperscript{dl}/\textit{mi}\textsuperscript{dl} homozygotes were
intercrossed but this impression is based on few matings and other crosses showed that both male and female homozygotes were fertile.

The gross phenotype of the \( mi^{dl}/mi^{dl} \) homozygote is shown in Plate 1a. The coat is completely white and the eye is small. Examination of the irises of such mice with a slit lamp revealed that the irises were unpigmented apart from a rim of pigment around the pupil (Plate 2a). The irises were usually misshapen, often incomplete or absent ventrally and failed to dilate in response to the application of atropine. (Eye size and iris shape seemed less severely abnormal among \( mi^{dl}/mi^{dl} \) homozygotes that were produced subsequently, by several generations of crosses with C57BL/Ola inbred mice, but no systematic comparison was made.) Cataracts could be seen in some \( mi^{dl}/mi^{dl} \) homozygotes but because the proband had been outcrossed to a (C3H/HeH \times 101/H) \( F_1 \) female it is not clear whether the cataract is caused by the \( lop-2 \) mutation, present in the 101/H strain (West & Fisher, 1985c), or by the \( mi^{dl} \) gene itself. (Cataracts were also seen in some \( il^{il}/il^{il} \) homozygotes and have been observed, by Tost (1958), in \( mi/mi \) homozygotes.)

Initially it was not clear whether the white coat and eye abnormalities were the result of the same gene or whether, for example, a spontaneous mutation, that caused the eye abnormalities, had arisen in the untreated PT-strain mother, close to the genes \( p \) and \( c^{ch} \) on chromosome 7. (In this case the ‘white’ coat would have been the product of the \( p c^{ch}/p c^{ch} \) genotype.) This second possibility was excluded by allelism tests involving crosses between PT mice (homozygous for \( c^{ch}, p, a, b, d, s \) and \( se \)) and either the small-eyed, white mice or their \( F_1 \) progeny. No white mice were produced.

Other allelism tests (crosses 9 and 10 in Table 1) indicated that the new mutation was either allelic with \( M^{wh} \) (white) or interacted with that gene, since the putative double heterozygotes were white and had mild eye abnormalities (Plates 1b and 2b). (The eyes were more normal in size, the iris pigment was less severely reduced

### Table 1. Inheritance of \( mi^{dl} \)

| Cross (♀ × ♂) | white:coloured | % white | Expected % white | Significance |
|---------------|----------------|---------|-----------------|-------------|
| 1. \(+/+ \times mi^{dl}/mi^{dl}\) | 0:119 | 0 | 0 | — |
| 2. \(+/+ \times mi^{dl}/+\) | 0:38 | 0 | 0 | — |
| 3. \(mi^{dl}/mi^{dl} \times +/+\) | 0:56 | 0 | 0 | — |
| 4. \(mi^{dl}/+ \times +/+\) | 0:31 | 0 | 0 | — |
| 5. \(mi^{dl}/+ \times mi^{dl}/mi^{dl}\) | 61:80 | 40:7 | 50 | \(\chi^2 = 4.86, P = 0.027\) |
| 6. \(mi^{dl}/mi^{dl} \times mi^{dl}/+\) | 15:19 | 44:1 | 50 | \(\chi^2 = 0.26, P = 0.61\) |
| 7. \(mi^{dl}/+ \times mi^{dl}/+\) | 38:144 | 20:9 | 25 | \(\chi^2 = 1.44, P = 0.23\) |
| 8. \(mi^{dl}/mi^{dl} \times mi^{dl}/mi^{dl}\) | 18:0 | 100 | 100 | — |

Allelism tests with \( M^{wh} \) and \( mi \)

| Cross | white:coloured | % white | Expected % white | Significance |
|-------|----------------|---------|-----------------|-------------|
| 9. \(M^{wh}/+ \times mi^{dl}/mi^{dl}\) | 22:27 | 44:9 | 50 | \(\chi^2 = 0.33, P = 0.57\) |
| 10. \(mi^{dl}/mi^{dl} \times M^{wh}/+\) | 25:25 | 50:0 | 50 | — |
| 11. \(mi^{dl}/mi^{dl} \times mi^{dl}/M^{wh}\) | 44:0 | 100 | 100 | — |
| 12. \(mi^{dl}/M^{wh} \times mi^{dl}/mi^{dl}\) | 47:0 | 100 | 100 | — |
| 13. \(mi^{dl}/+ \times mi^{dl}/+\) | 53:164 | 24:4 | 25 | \(\chi^2 = 0.01, P = 0.92\) |
A new microphthalmia allele in the mouse

although the rest of the eye appeared unpigmented; the irises were more symmetrical but failed to dilate fully in response to atropine.) The absence of pigmented mice among the progeny when mi\textsuperscript{di} /Mi\textsuperscript{wh} animals were backcrossed to mi\textsuperscript{di} /mi\textsuperscript{di} (crosses 11 and 12 in Table 1) further suggested that mi\textsuperscript{di} was allelic with Mi\textsuperscript{wh} and was therefore a new mutation at the microphthalmia complex locus on chromosome 6.

Table 2. Genetic Linkage of mi\textsuperscript{di} with Lc and Sig

| Progeny | Cross 1* | Cross 2* | Total |
|---------|---------|---------|-------|
| Parental chromosomes† | | | |
| 1. \(+ + \text{mi}^{\text{di}}\) | 13 | 15 | 28 |
| 2. \(\text{Sig} \text{Lc}+\) | 15 | 18 | 33 |
| Recombinant chromosomes† | | | |
| 3. \(+ \text{Lc} +\) | 5 | 4 | 9 |
| 4. \(\text{Sig} + \text{mi}^{\text{di}}\) | 10 | 8 | 18 |
| 5. \(+ + +\) | 9 | 6 | 15 |
| 6. \(\text{Sig Lc mi}^{\text{di}}\) | 3 | 1 | 4 |
| 7. \(+ \text{Lc mi}^{\text{di}}\) | 0 | 0 | 0 |
| 8. \(\text{Sig} + +\) | 0 | 0 | 0 |
| Total | 55 | 52 | 107 |

Recombination (%)

\[
\text{Sig-Lc} = \frac{15/55 = 27.3\pm 6.0\% }{12/52 = 23.1\pm 5.8\% } = \frac{27/107 = 25.2\pm 4.2\% }{12/55 = 21.8\pm 5.6\% } = \frac{7/52 = 13.5\pm 4.7\% }{19/107 = 17.8\pm 3.7\% }
\]

\[* \text{Cross 1 = } \frac{\text{Sig} \text{Lc} +}{\text{Sig} \text{Lc} +} \times \frac{+ + \text{mi}^{\text{di}}}{+ + \text{mi}^{\text{di}}} = \delta.\]

\[\text{Cross 2 = } \frac{\text{Sig} \text{Lc} +}{\text{Sig} \text{Lc} +} \times \frac{+ + \text{mi}^{\text{di}}}{+ + \text{mi}^{\text{di}}} = \delta.\]

† The homologous chromosomes (+ + mi\textsuperscript{di}) are not shown.

Similarly, cross 13 (Table 1), between \(\text{mi}/+\) and \(\text{mi}^{\text{di}}/+\) mice suggested allelism of \(\text{mi}^{\text{di}}\) and \(\text{mi}\). The putative \(\text{mi}^{\text{di}}/\text{mi}\) compound heterozygotes were white with rudimentary eyes, seemed to have normal teeth and, although fully viable, were apparently of reduced fertility. (Homozygous \(\text{mi}/\text{mi}\) mice are white with rudimentary eyes, teeth that fail to erupt and generally die around weaning unless specially fed.) The fertility of \(\text{mi}^{\text{di}}/\text{mi}\) males and females seemed to be intermediate between the very low fertility of \(\text{mi}/\text{mi}\) and the moderate fertility of \(\text{mi}^{\text{di}}/\text{mi}^{\text{di}}\) homozygotes.

Allelism of \(\text{mi}^{\text{di}}\) and the microphthalmia (\(\text{mi}\)) locus was also supported by the three point linkage test, with the chromosome 6 marker genes, sightless (\(\text{Sig}\)) and lurcher (\(\text{Lc}\)), shown in Table 2. The results show that the three genes are linked in the gene order \(\text{Sig-Lc-mi}^{\text{di}}\) which is in agreement with the established linkage relationship for \(\text{Sig}, \text{Lc}\) and \(\text{mi}\). The recombination frequencies are reasonably close to the expected values. Roderick & Davisson (1981) cite recombination frequencies for \(\text{Lc}\) and \(\text{mi}\) of 11.0 ± 1.1% in females and 10.9 ± 1.1% in males. Equivalent frequencies for \(\text{Sig}\) and \(\text{mi}\) (calculated by adding the \(\text{Sig-hpy}\) and \(\text{hpy-mi}\)
frequencies) are given as 42.1% for females and 37.1% for males. The assignment of \( m^d_1 \) to chromosome 6, the gene order of \( \text{Sig-Lc-} m^d_1 \) and the recombination frequencies between these loci confirm that \( m^d_1 \) is an allele of the \( m \) locus.

5. PHENOTYPE OF \( m^d_1/+ \) HETEROZYGOTES

Although the \( m^d_1/+ \) mice are fully pigmented and grossly indistinguishable from ++/+ individuals (Plate 1c), the \( m^d_1 \) mutation was discovered in a ++/\( m^d_1 \) individual that exhibited an unusually bright red-reflex. Examination of other

Table 3. Genotypes and presence of bright red-reflex among coloured
\((m^d_1/+ \times m^d_1/+ ) \ F_1\) offspring

| Mouse number and sex | Presence of bright red-reflex | Genotype established by progeny testing |
|----------------------|-------------------------------|----------------------------------------|
| Mouse number and sex | Presence of bright red-reflex | Genotype established by progeny testing |
| Number | Presence | Genotype | Establish | by progeny testing |
| 1 ♀ | + | \( m^d_1/+ \) | a | + | b | +/+ | +/+ | d | +/+ | +/+ | se/+ |
| 2 ♂ | + | \( m^d_1/+ \) | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ |
| 3 ♀ | — | \( m^d_1/+ \) | a | + | b | +/+ | +/+ | d | +/+ | +/+ | +/+ |
| 4 ♂ | — | \( m^d_1/+ \) | +/+ | a | + | b | +/+ | +/+ | d | +/+ | +/+ | se/+ |

\( m^d_1/+ \) heterozygotes with a slit lamp showed that the intensity of the red-reflex was very variable and often no greater than in ++/+ mice. Since a particularly bright red-reflex was seen in mice homozygous for either brown (b) or piebald (s), we investigated the possibility that the bright red-reflex, seen in some \( m^d_1/+ \) mice, was dependent on heterozygosity at one or more of the six coat colour genes (a, b, c<sub>b</sub>, p, d and s) that are present in the PT stock. Crosses between PT females and either \( m^d_1/mi_1 \) or ++/+(C3H/HeH strain) males showed that heterozygosity for the PT genes alone is insufficient to produce a bright red-reflex. (A bright red-reflex was seen in 0/28 (PT ♀ x × ++/+ ♂) \( F_1 \) and 39/44 (PT ♀ x \( m^d_1/mi_1 \)) \( F_1 \) offspring.) Some offspring with bright red-reflexes were also produced from crosses between \( m^d_1/mi_1 \) homozygotes and either a/a b/b d/d (DBA/2Ola strain) or ++/+ (C3H/HeH strain) mice. However, it was difficult to evaluate whether any coat colour genes were necessary to produce a bright red-reflex in \( m^d_1/+ \) heterozygotes because the \( m^d_1/mi_1 \) parents also carried various coat colour genes of PT origin.

Genetic analysis of four pigmented offspring from a \( m^d_1/+ \times \times m^d_1/+ \) cross, however, suggests that heterozygosity for these coat colour genes is not required for production of a bright red-reflex. Two of the four pigmented offspring had a bright red-reflex. All four mice were crossed first to \( m^d_1/mi_1 \) homozygotes, to establish whether they were \( m^d_1/+ \) or +/+ , and then crossed to PT strain mice, to determine which coat colour genes they carried. The results are shown in Table 3. Significantly, the second mouse shown was \( m^d_1/+ \), produced a bright red-reflex but failed to transmit any of the seven PT genes to any of his 39 (PT ♀ x \( m^d_1/+ \)) \( F_1 \) offspring.

Superficial examination of a few excised eyes with a dissecting microscope revealed areas where the choroidal pigment was reduced or absent in \( m^d_1/+ \) individuals. Although pigmentation generally seemed more complete in +/+ eyes,
some variation was present and a systematic comparison was not done. However, it is well established that $M^{wh}/+ \text{ and } mi/+ \text{ heterozygotes have unpigmented patches in the choroid}$ (Grüneberg, 1948; Deol, 1973) and it seems likely that $\text{mi}^{dl}/+ \text{ heterozygotes have similar patches. When large enough, such patches of reduced choroidal pigmentation would probably produce a bright red-reflex when a light is shone directly into the eye. It remains to be determined whether this effect is influenced by the genetic background.}$ Grüneberg (1948) pointed out that the choroidal pigmentation is increased in non-agouti $a/a$ mice, so it might be predicted that $\text{mi}^{dl}/a/a$ mice would be less likely to show a bright red-reflex than $\text{mi}^{dl}/+/+ \text{ or } \text{mi}^{dl}/+ a/+ \text{ individuals.}$

Fig. 1. Plots of left and right eye weights with line showing expectation when left and right eyes are equal in weight. The left graph shows that eyes from $\text{mi}^{dl}/\text{mi}^{dl}$ homozygotes ($\bullet$) are lighter and more variable in weight than those from $+/\text{mi}^{dl}$ heterozygotes ($O$). The right graph shows that eyes from $\text{mi}^{dl}/M^{wh}$ compound heterozygotes ($\bullet$) are more similar in weight to those from $\text{mi}^{dl}/+$ heterozygotes ($O$). However some $\text{mi}^{dl}/M^{wh}$ eyes are lighter and one mouse has a very small left eye.

6. EYE SIZE

Eyes are reduced to about 60% of the normal size in $\text{mi}^{dl}/\text{mi}^{dl}$ homozygotes but the reduction is variable and often asymmetric in degree. The eyes of $\text{mi}^{dl}/M^{wh}$ compound heterozygotes span a large range of sizes but the majority are almost normal in size. This is illustrated by the graphs of eye weights (Fig. 1) and detailed statistics of eye weights are shown in Tables 4–7.

Tables 4 and 5 show eye and body weights for various genotypes at 6 weeks of age. In most cases the coefficient of variation is not significantly reduced by calculating eye weight as a function of body weight so the statistical analyses (Tables 6 and 7) are done on uncorrected eye weights. Comparison of $\text{mi}^{dl}/\text{mi}^{dl}$ and $\text{mi}^{dl}/+$ genotypes showed that the eyes of $\text{mi}^{dl}/\text{mi}^{dl}$ were significantly lighter in weight but the body weights of the two genotypes were not significantly different (Table 6). Both eye weights and body weights of $\text{mi}^{dl}/+$ mice were actually slightly
Table 4. Effect of mi<sup>1</sup> on eye weight at 6 weeks

| Genotype | Females | Males |
|----------|---------|-------|
|          | Body weight (g) | Left eye (mg) | Right eye (mg) | Left eye/body (mg/g) | Right eye/body (mg/g) |
|          | Weight ± S.E. | Coefficient of variation, % | Weight ± S.E. | Coefficient of variation, % | Weight ± S.E. | Coefficient of variation, % |
| +/+/+   | 18.87 ± 0.46 (7.7) | +/+/+ | 18.95 ± 0.20 (3.3) | 19.22 ± 0.22 (3.7) | 101 ± 0.02 (5.3) |
| -/+/+   | 19.24 ± 0.57 (9.4) | -/+/+ | 20.51 ± 0.32 (3.4) | 20.03 ± 0.28 (4.4) | 107 ± 0.02 (9.5) |
| 10       | 19.11 ± 0.72 (12.0) | 12.24 ± 0.75 (19.9) | 11.87 ± 0.72 (19.1) | 0.66 ± 0.06 (22.9) |
| +/mi<sup>1</sup> | 10       | 19.30 ± 0.52 (7.1) | 20.09 ± 0.31 (15.0) | 19.73 ± 0.29 (2.9) | 0.87 ± 0.01 (5.1) |
|          | 10       | 24.52 ± 0.42 (5.5) | 21.88 ± 0.28 (4.1) | 21.54 ± 0.23 (3.3) | 0.68 ± 0.02 (5.2) |
|          | 10       | 24.38 ± 0.47 (8.7) | 21.65 ± 0.26 (14.1) | 21.21 ± 0.25 (15.3) | 0.50 ± 0.03 (18.1) |

*Coefficient of variation, % = standard deviation/mean x 100%.
Table 5. *Eye weights of mi<sup>dl</sup>/Mi<sup>wh</sup> compounds at 6 weeks*

| Genotype | No. | Body weight (g) | Left eye (mg) | Right eye (mg) | Left eye/body (mg/g) | Right eye/body (mg/g) |
|----------|-----|----------------|---------------|---------------|----------------------|----------------------|
| M<sup>dl</sup>/M<sup>wh</sup> | 9 | 21.52 ± 0.37 (5.1) | 21.23 ± 0.32 (4.6) | 21.03 ± 0.28 (4.0) | 0.99 ± 0.02 (6.1) | 0.96 ± 0.02 (5.6) |
| M<sup>dl</sup>/M<sup>wh</sup> | 10 | 22.07 ± 0.04 (5.5) | 21.65 ± 0.31 (4.6) | 20.92 ± 0.42 (6.4) | 0.98 ± 0.01 (4.0) | 0.95 ± 0.01 (4.7) |
| mi<sup>dl</sup>/+ | 10 | 20.00 ± 0.68 (10.8) | 19.42 ± 0.63 (10.2) | 19.30 ± 0.40 (6.5) | 0.97 ± 0.02 (7.5) | 0.97 ± 0.02 (7.3) |
| mi<sup>dl</sup>/+* | 9 | 20.43 ± 0.59 (8.7) | 20.01 ± 0.24 (3.5) | 19.60 ± 0.29 (4.4) | 0.98 ± 0.02 (6.9) | 0.96 ± 0.02 (7.5) |
| mi<sup>dl</sup>/Mi<sup>wh</sup> | 10 | 19.44 ± 0.70 (11.4) | 18.29 ± 0.87 (15.0) | 17.47 ± 0.83 (14.9) | 0.96 ± 0.07 (21.6) | 0.91 ± 0.05 (18.5) |

* The second group of mi<sup>dl</sup>/+ females is the same as the first group with one abnormal female excluded. The female was small with small eyes and had a severe cataract in the left eye. (Body weight, 16.16 g; left eye, 14.1 mg; right eye, 16.6 mg.)
higher than for the (C3H/HeH × 101/H) F₁ mice used as +/+ controls (Table 4). Although Miₜ⁹/miₜ¹ eyes tended to weigh less than miₜ¹/+ eyes (Table 5) this difference was only statistically significant for one of the eight comparisons shown in Table 6. This probably reflects the sporadic occurrence of small eyes among miₜ¹/Miₜ⁹ mice which is also illustrated in Fig. 1. Correlations between left and right eye weights and between body and eye weights were usually positive, and

Table 6. Statistical significance of effect of miₜ on eye and body weights

| Comparison | Females | Males |
|------------|---------|-------|
|            | Mean    | t or t' | Mean    | t or t' |
| +/miₜ² × miₜ¹/miₜ² - | 19.24 | t₁₈ = 0.140 | 24.52 | t₁₈ = 0.241 |
| +/miₜ¹ | 19.11 |     | 24.33 |     |
| miₜ²/miₜ¹ |     |     |     |     |
| +/miₜ² × miₜ¹/miₜ² + | 20.51 | t₁₂ = 10.541** | 21.86 | t₁₂ = 13.539** |
| +/miₜ² | 12.24 |     | 12.05 |     |
| miₜ¹/miₜ² | 20.03 | t₁₂ = 10.649** | 21.54 | t₁₂ = 14.770** |
| miₜ²/miₜ¹ | 11.87 |     | 12.21 |     |
| +/Miₜ⁹ × miₜ¹/miₜ¹ - | 21.23 | t₁₇ = 0.923 | 21.16 | t₁₅ = 2.336* |
| +/miₜ¹ | 21.65 |     | 19.70 |     |
| miₜ¹/miₜ¹ | 21.03 | t₁₇ = 0.218 | 20.97 | t₇₃ = 1.716 |
| Miₜ⁹/miₜ¹ | 20.92 |     | 18.84 |     |
| miₜ¹/miₜ¹ × Miₜ⁹/+ - | 19.42† | t₁₈ = 1.057 | 20.96 | t₁₁ = 1.565 |
| miₜ¹/+ | 18.29 |     | 19.43 |     |
| Miₜ⁹/miₜ¹ | 19.30† | t₁₈ = 2.000 | 20.74 | t₁₈ = 0.580 |
| miₜ¹/miₜ¹ | 17.47 |     | 20.40 |     |

* P < 0.05; ** P < 0.001.
† Includes one abnormally small female. See footnote to Table 5.

sometimes significantly so, except for mi₁/mi₁ homozygotes, where correlations were generally much lower and none was statistically significant (Table 7). This reflects the greater variation among miₜ/miₜ eyes which is also shown in Fig. 1.

7. EYE HISTOLOGY

The iris abnormalities already discussed (Section 4 above) are illustrated in Plate 2. The miₜ/miₜ eyes have misshapen irises with restricted pupil dilation and only a rim of pigmentation around the pupil. The miₜ/miₜ irises are more symmetrical, have more pigment but are still less pigmented than normal, and have restricted pupil dilation.

Representative histological sections are illustrated in Plate 3. In all respects the


Table 7. Correlation coefficients (r) for body and eye weights

| Genotype                        | Body and left eye |       |       | Body and right eye |       |       | Left and right eyes |       |       |
|---------------------------------|-------------------|-------|-------|-------------------|-------|-------|---------------------|-------|-------|
|                                 | ♀♀                 | ♂♂    |       | ♀♀               | ♂♂    |       | ♀♀               | ♂♂    |       |
| +/+♀ × +/+♂                     | r₆ = +0·811**      | r₆ = +0·779** |       | r₆ = +0·761*      | r₆ = +0·158 |       | r₆ = +0·884*** | r₆ = +0·391 |       |
| +/+                               |                   |       |       | r₆ = +0·512       | r₆ = +0·431 |       | r₆ = +0·705*      | r₆ = +0·656* |       |
| +/mi²⅛ ♂ × mi²⅛/mi²⅛ ♂          | r₆ = +0·096        | r₆ = -0·318 |       | r₆ = +0·775**     | r₆ = +0·700* |       | r₆ = +0·730*      | r₆ = +0·804** |       |
| -/mi²⅛                           | r₆ = +0·281        | r₆ = +0·135 |       | r₆ = +0·196       | r₆ = -0·156 |       | r₆ = -0·151       | r₆ = -0·228 |       |
| mi²⅛/mi²⅛                         | r₆ = +0·701        | r₆ = +0·722 |       | r₆ = +0·376       | r₆ = +0·954*** |       | r₆ = +0·906*** | r₆ = +0·867** |       |
| +/mi²⅛ wh ♂ × mi²⅛/mi²⅛ ♂        | r₆ = +0·261        | r₆ = +0·315 |       | r₆ = +0·705*      | r₆ = +0·954*** |       | r₆ = +0·906*** | r₆ = +0·967** |       |
| -/mi²⅛ wh                        | r₆ = +0·701        | r₆ = +0·722 |       | r₆ = +0·376       | r₆ = +0·954*** |       | r₆ = +0·906*** | r₆ = +0·967** |       |
| mi²⅛/mi²⅛ wh × mi²⅛ wh/♂          | r₆ = +0·693*       | r₆ = +0·770** |       | r₆ = +0·705*      | r₆ = +0·954*** |       | r₆ = +0·906*** | r₆ = +0·967** |       |
| mi²⅛/♂                            | r₆ = -0·072        | r₆ = +0·215 |       | r₆ = +0·656*      | r₆ = +0·660* |       | r₆ = +0·845**      | r₆ = +0·903*** |       |
| mi²⅛/Mi²wh                        |                   |       |       | r₆ = +0·118       | r₆ = +0·677* |       | r₆ = +0·167       | r₆ = +0·450 |       |

* P < 0·05; ** P < 0·01; *** P < 0·001.
† The group of mi²⅛/♀ females includes one abnormally small female. See footnote to Table 5.
eyes of \( m^{dl}/+ \) are similar to those of \(+/+\) individuals from the C57BL/Ola strain. The formation of the optic disk and the vitreous body is normal (Plates 3a, b). The thickness of the retinal layers (Plates 3d, e) and the pigmentation and width of the retinal epithelium and the choroid (Plates 3d, e) and pigmentation of the iris (Plates 3h, i) are all indistinguishable from the normal. (Although some regions of choroidal pigmentation seemed somewhat thinner than normal in certain \( m^{dl}/+ \) eyes (see Section 5 above) this was not always apparent and was not true of the eye shown in Plate 3e.)

The eyes of \( mi^{dl}/mi^{dl} \) mice are abnormal in many respects other than their small size. A low power micrograph of a region through the optic disk reveals that the vitreous body is virtually absent and the lens abuts the surface of the retina (Plate 3c). It is also apparent in this figure that the formation of the optic disk is grossly abnormal and the retinal lamination highly distorted. In regions removed from the optic disk the retinal layers vary greatly in the extent to which they are disordered. Plate 3f shows a relatively normal field but it is clear that the thickness of the layers is abnormal; the ganglion cell layer varies from a single layer to several layers thick, the inner nuclear layer is abnormally thick and the outer nuclear layer abnormally thin. It is also notable that the inner segments of the photoreceptors are poorly developed and the outer segments are absent. The separation of the neural retina from the underlying retinal epithelium is a common feature of these eyes and cannot be simply attributed to a histological artefact since this was not found in \(+/+\) or \( mi^{dl}/+ \) eyes. In many parts of the retina of \( mi^{dl}/mi^{dl} \) eyes rosetting of the outer nuclear layer was observed (Plate 3g) and in some parts the outer nuclear layer was entirely absent.

The retinal epithelium of the \( mi^{dl}/mi^{dl} \) eye not only lacks pigment but shows additional abnormalities. In \(+/+\) and \( mi^{dl}/+ \) eyes the epithelial cells lie as a monolayer of columnar epithelium, with little variation in their size and spacing. The retinal epithelium of \( mi^{dl}/mi^{dl} \) homozygotes varies in thickness and in some parts the epithelial cells are piled up in an apparently random fashion. The pigmentation of the iris was also dramatically reduced (Plate 3j) as noted previously.

Limited observations of histological sections of a single \( mi^{dl}/Mi^{wh} \) eye (not shown) revealed that pigmentation was restricted to the iris and that the neural retina was abnormal. The neural retina was similar to that in \( mi^{dl}/mi^{dl} \) homozygotes except there were no rosettes. In places the number of photoreceptors was quite large but the inner and outer segments had failed to develop. In other parts the photoreceptors were absent (as in mice homozygous for retinal degeneration, \( rd \)) and the inner nuclear layer was abnormal.

8. SKELETAL AND HAEMATOPOIETIC TISSUES

(i) X-ray examination and bone resorption studies

Homozygous \( mi^{dl}/mi^{dl} \) mice had a minor defect in bone resorption such that osteopetrosis was seen only where bone growth was most active. This contrasts with the classical osteopetrosis of \( mi/mi \) homozygous mice which is manifest at all sites of endosteal growth. None of the half dozen \( mi^{dl}/mi^{dl} \) mice examined
radiologically showed any abnormality of the skeletal pattern; marrow cavities were clear cut. Minor histological abnormalities are discussed below (Section iv).

Osteopetrosis in \( mi^{dl}/mi \) compound heterozygotes, however, was easily detected radiographically at 3 weeks of age and appeared as an opaque band behind the growth plate. Obliteration of the marrow cavities was not as extensive as it was in the \( mi/mi \) homozygote. The \( mi^{dl}/mi \) mouse has a normal life span but remains osteopetrotic.

Treatment of seven \( mi^{dl}/mi \) compound heterozygotes with parental, G-strain \( mi/+ \) bone marrow resulted in radiological clearing of osteopetrotic bone by 8 weeks though not at 3 weeks (Plate 4). Seven \( mi^{dl}/mi \) recipients of immunologically ‘foreign’ CBA/Ca bone marrow and 8 untreated \( mi^{dl}/mi \) controls showed no evidence of resorption at 3 or 8 weeks. These observations suggest that the defect in bone resorption is intrinsic to \( mi^{dl}/mi \) bone marrow cells (presumably progenitors of osteoclasts) and can be corrected by the survival of normal, immunologically compatible bone marrow cells.

(ii) Blood count

None of the 6 adult \( mi^{dl}/mi^{dl} \) mice showed markedly abnormal counts. Means (and range) for red corpuscles were \( 9.9 \times 10^{9} \text{ ml}^{-1} \) (8.7–10.9), for haematocrit 48.7% (45–53) and mean corpuscular volume 49 (44–54).

(iii) Radiosensitivity

Homozygous \( mi/mi \) mice in our hands have similarly normal values for peripheral blood; nevertheless they are unduly radiosensitive to X- and \( \gamma \)-irradiation – \( LD_{95} 6 \text{ Gy for } ^{60}\text{Co } \gamma\text{-rays (Walker, 1975) in mice based on C57BL/6J and similarly in Harwell stock derived from Grüneberg 6 Gy 250 kV X-rays (Nisbet, Menage & Loutit, 1979). This increased radiosensitivity suggests a defect of the haematopoietic stem cell. Homozygous } mi^{dl}/mi^{dl} \text{ mice on the mixed background do not exhibit the phenomenon – 1 out of 4 mice died after both 7 and 7.5 Gy and 2 out of 7 after 8 Gy, much the same as } mi^{dl}/+ \text{ and normal hybrid stock.}

(iv) Histology

Only one of 6 adult \( mi^{dl}/mi^{dl} \) mice examined showed skeletal abnormality. At the metaphyses of upper tibia and lower femur was a band of atypical osteopetrotic bone about 1 mm broad. None of the other centres of ossification examined in sternum, vertebrae and tarsus–metatarsus showed this, nor did the other 5 mice. Two homozygotes killed at each of the days 5, 12, 19 after birth were not distinguishable from normal counterparts. Bone marrow was of normal appearance and cell-density.

Thymus and lymph nodes were of normal appearance. In the spleen, however, Malpighian bodies were lush, some with germinal centres (rarely seen in Harwell stock) and notably the red pulp showed broad bands of myeloid tissue (also unusual in Harwell stock).
9. DISCUSSION

Genetic studies have shown that \( mi^{dl} \) is a recessive allele at the microphthalmia (mi) locus on chromosome 6. Like other alleles at this locus it affects pigmentation, eye size, eye structure, and bone resorption. In \( mi^{dl}/mi^{dl} \) homozygotes pigment is absent from the coat and eye, apart from a ring of iris pigment around the pupil. Eye size is variable but it is typically reduced to about 60\% of its normal size. The iris is structurally abnormal and pupil dilation is impaired. The vitreous body is absent, the retinal pigment epithelium is of uneven thickness and there are gross abnormalities of the neural retina. The defect in bone resorption is relatively minor in \( mi^{dl}/mi^{dl} \) homozygotes but more severe in \( mi^{dl}/mi \) compound heterozygotes. The minor defect in \( mi^{dl}/mi^{dl} \) mice was not detected radiologically and only seen once histologically. Thus, although \( mi^{dl}/mi^{dl} \) homozygotes have osteopetrotic potential they are often unaffected.

Heterozygous, \( mi^{dl}/+ \) mice appeared normal, except that some showed a bright red-reflex (pupillary reflex). This was probably associated with reduced pigmentation in the choroid and may have been potentiated by involvement of various genes affecting coat colour (see Grünberg, 1948) but this is not clear.

Although \( mi^{dl}/Mi^{wh} \) compound heterozygotes had white coats, they had almost normal size eyes with more iris pigmentation, and less severe eye abnormalities than \( mi^{dl}/mi^{dl} \) homozygotes. Since eyes of \( Mi^{wh}/Mi^{wh} \) homozygotes are small (e.g. Grobman & Charles, 1947; Packer, 1967; Konyukhov & Osipov, 1968), the near-normal eye size of \( mi^{dl}/Mi^{wh} \) compound heterozygotes implies that inter-allelic complementation occurs between \( mi^{dl} \) and \( Mi^{wh} \). Iris pigmentation was less severely reduced in \( mi^{dl}/Mi^{wh} \) compound heterozygotes than in either \( mi^{dl}/mi^{dl} \) or \( Mi^{wh}/Mi^{wh} \) homozygotes. (Observations on \( Mi^{wh}/Mi^{wh} \) homozygotes were limited to a few mice but Grünberg (1953) also describes \( Mi^{wh}/Mi^{wh} \) iris pigmentation as a slender pigment ring.) This implies that partial complementation also occurs in \( mi^{dl}/Mi^{wh} \) compound heterozygotes with respect to iris pigmentation. In contrast, no obvious complementation was noticed in \( mi^{dl}/mi \) compound heterozygotes.

Partial complementation in \( Mi^{wh}/mi \) compound heterozygotes has been reported by Konyukhov & Osipov (1968) with respect to body size, eye weight, volume of the spinal ganglia, size of the adrenals and thickness of the dermis. Grünberg also reported the partial complementation of eye size in \( Mi^{wh}/mi \) compound heterozygotes (Grünberg, 1952) and implied that \( Mi^{wh}/mi \) compounds generally had more iris pigmentation than \( Mi^{wh}/Mi^{wh} \) or \( mi/mi \) homozygotes (Grünberg, 1952, 1953). Deol (1973) confirmed that \( Mi^{wh}/mi \) compound heterozygotes had more iris pigmentation than \( Mi^{wh}/Mi^{wh} \) homozygotes and showed that in both genotypes the iris pigmentation was restricted to the inner layer of the iris, which is an extension of the retinal pigment epithelium.

Partial complementation has also been reported for other compound heterozygotes. This was reported for coat colour in \( Mi^{b}/Mi^{wh} \) compound heterozygotes (Larsen, 1966; Konyukhov & Osipov, 1968) but not, for example, in \( Mi^{b}/Mi^{or} \) or \( Mi^{or}/mi \) compounds (Larsen, 1966; Stelzner, 1966). Hollander (1968) has also reported partial complementation for coat pigmentation, eye pigmentation and eye size in \( Mi^{wh}/mi^{ws} \) compound heterozygotes.
A new microphthalmia allele in the mouse

These observations have been used to suggest that the microphthalmia locus is a complex locus of closely linked and functionally related genes (e.g. Hollander, 1968). Present evidence shows that $M$ $\text{wrh}$ belongs to a different complementation group from $mi$, $mi^\text{ws}$ and $Mk^b$. Our own observations show that $mi^2$ belongs to the second group. Further genetic studies of the relations of the various alleles at this complex locus are needed.

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EXPLANATION OF PLATES

PLATE 1
Head of (a) mi<il/mi<il homozygote, (b) mi<il/Mi<wh compound heterozygote and (c) mi<il/+ heterozygote. Note the small eye and white coat of mi<il/mi<il and the normal size eye and white coat of the mi<il/Mi<wh genotype. The mi<il/+ heterozygote has a normal size eye and normal (non-agouti) coat pigmentation.

PLATE 2
Eyes from (a) mi<il/mi<il, (b) mi<il/Mi<wh and (c) mi<il/+ mice. The mi<il/mi<il eye (a) has a misshapen iris and the pigment is restricted to the rim around the pupil. The mi<il/Mi<wh eye (b) has a more normally shaped iris and intermediate levels of iris pigmentation; the rest of the eye is unpigmented. The mi<il/+ eye (c) is normal.

PLATE 3
Plates 3a–c show sections through the optic nerve head (onh) of (a) +/+ (C57BL/Ola strain), (b) mi<il/+ and (c) mi<il/mi<il eyes. In (a) and (b) the vitreous body (v) separates the lens from the retinal surface but is virtually absent in (c). Note also that in (a) and (b) the retinal layers are normal but in (c) are highly distorted. Scale bar = 100 μm. Plates 3d–g show higher power fields of sections from (d) +/+ , (e) mi<il/+ , (f and g) mi<il/mi<il. Note that (e) is very similar to (d) but (f and g) show a number of abnormalities. See text for further details. gel, ganglion cell layer; inl, inner nuclear layer; onl, outer nuclear layer; ph, photoreceptors. Small arrow indicates the retinal epithelium, the large arrow the choroid. Scale bar = 25 μm. Plates 3h–j show the iris pigmentation of (h) +/+ , (i) mi<il/+ and (j) mi<il/mi<il eyes. Note the paucity of pigmentation in (j). Scale bar = 25 μm.

PLATE 4
Diagnostic X-ray radiography of an adult mi<il/mi mouse (a) before and (b) 8 weeks after treatment with mi/+ bone marrow. The first radiograph (a) shows dense endosteal shadows in the growth metaphyses of femora, tibiae, ilia, humeri and radii, accentuation of the rib shadows and poles of the vertebrae. These features are no longer present in the second radiograph (b), taken 8 weeks after bone marrow transplantation, but the polar densities in the caudal vertebrae persist (owing to slow bone turnover there) and the bulbous deformities at the major metaphyses remain. (Remoulding of the shape is limited in mature animals.)
