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Absence of mitochondrial malic enzyme in mice carrying two complementing lethal albino alleles

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SUMMARY

Mitochondrial malic enzyme (MOD-2) was found to be missing in partially complementing genotypes between lethal deletion alleles at the albino locus in Chromosome 7 of the mouse. Since such partial complementers survive to adulthood, the absence of normal mitochondrial malic enzyme is compatible with life; however, the sterility of both females and males may be correlated with this enzyme deficiency. Of the six radiation-induced lethal albino mutations, five include deletions for the *Mod-2* locus mapping 1 centimorgan distally from *c*. Our data indicate that the maximum genetic distance occupied by any of these deletions is 6 centimorgans.

1. INTRODUCTION

A syndrome of biochemical, morphogenetic and ultrastructural abnormalities has been studied and described in mice homozygous for several X-ray-induced mutations at the albino (*c*) locus. The investigations originated with an analysis of the nature of lethal effects of these mutations identified and isolated in radiation experiments at Oak Ridge and Harwell (Erickson, Gluecksohn-Waelsch & Cori, 1968). Death of homozygotes for the mutations c^{14Cos}, c^{3H}, c^{65K} and c^{112K} occurs shortly after birth and is associated with deficiencies of up to five liver enzymes, three serum proteins and ultrastructural abnormalities of subcellular organelles of liver and kidney cells (Gluecksohn-Waelsch & Cori, 1970; Thorndike *et al.* 1973; Trigg & Gluecksohn-Waelsch, 1973; Garland *et al.* 1976). Additional mutations, c^{25H} and c^{6H}, were found to cause pre-implantation (Lewis, 1978) and post-implantation (Lewis, Gluecksohn-Waelsch & Turchin, 1976) death of homozygous embryos, respectively.

These mutations have been shown to be physical deletions of chromosomal material including the albino locus by both genetic (Erickson, Eicher & Gluecksohn-Waelsch, 1974) and cytological analysis (Miller *et al.* 1974; Jagiello *et al.* 1976). The present studies were undertaken to complete the detailed mapping for all six deletions relative to the three closely linked Chromosome 7 loci *tp* (taupe), *sh-1* (shaker-1) and *Mod-2* (mitochondrial malic enzyme) and to investigate the expression of the MOD-2 (mitochondrial malic enzyme) deficiency in partially complementing viable genotypes produced by intercrosses of the various deletions.

2. MATERIALS AND METHODS

(i) *Complementation tests*

The six X-ray-induced lethal albino deletions used in this study are c^{3H}, c^{6H}, c^{25H}, c^{65K}, c^{112K} and c^{14Cos}.

Testing of the mutations c^{3H}, c^{112K} and c^{65K} for presence or absence of the *Mod-2* locus was carried out as follows. A mouse heterozygous for c^{ch} (chinchilla, allelic to *c*) and one of the deletions, e.g. c^{3H}, was mated to a mouse of the SM/J strain, homozygous for *Mod-2*^{a} (Table 1, cross 1). All strains in which the various albino deletions are carried have been shown to express *Mod-2*^{b} only. The F_1 resulting from this cross must be heterozygous either for c^{ch} and +, as well as *Mod-2*^{b} and *Mod-2*^{a}, or for c^{3H} and + and *Mod-2*^{a} and *Mod2*^{b}, if the latter is intact in the c^{3H} deletion (Tables 1, 2). Distinction between F_1's carrying c^{ch} and those with c^{3H} was made by mating them to mice from the BALB/cJ strain homozygous for *c* (Tables 1, 2). A total of eight non-albino offspring was considered evidence ($P < 0{\cdot}01$) that the parental mouse had inherited c^{ch} rather than c^{3H} (Tables 1, 2). Distinction between presence and absence of the *Mod-2* locus in the deletions depended on the possible electrophoretic demonstration of MOD-2B in the heterozygote (Tables 1, 2*b*), which would show the MOD-2A band only if *Mod-2* is deleted.

The two deletions c^{6H} and c^{25H} known to include the *Mod-2* locus (Erickson, Eicher & Gluecksohn-Waelsch, 1974) were tested for presence or absence of the two closely linked loci *tp* and *sh-1* as follows. Mice heterozygous for one of the two lethal albino deletions and c^{ch} were mated to homozygous *tp* mice. The coat colour of F_1 offspring was inspected for the expression of *tp*. Similar matings were made between heterozygotes (c^{ch}/c^{6H} or c^{ch}/c^{25H}) and mice of the FS/Ei inbred strain homozygous for the four Chromosome 7 mutant alleles *p*, c^{ch}, *sh-1* and *fr* (see Fig. 1 for full gene names and their position on the linkage map). F_1 mice with diluted pigmentation, thus known to carry an albino mutant allele, were inspected for evidence of *sh-1* expression by observation of behaviour, auditory response and swimming ability.

Table 1.

Cross 1 $\quad \dfrac{c^{ch}\ Mod\text{-}2^b}{c^{3H}\ Mod\text{-}2^b(?)} \times \dfrac{+\ Mod\text{-}2^a}{+\ Mod\text{-}2^a}$

Cross 2* $\quad F_1\ \dfrac{c^{ch}\ Mod\text{-}2^b}{+\ Mod\text{-}2^a}$ *or*† $\dfrac{c^{3H}\ Mod\text{-}2^b(?)}{+\ Mod\text{-}2^a} \times \dfrac{c}{c}$ (BALB)

* Produces coloured offspring only (c^{ch}/c and $+/c$).
† Produces 1 coloured ($+/c$) to 1 albino (c^{3H}/c) offspring.

(ii) *Electrophoresis*

After confirmation of genotype (i.e. $c^{ch}/+$ or $c^{3H}/+$, cf. Tables 1, 2) the mice were killed by cervical dislocation, and their MOD-2 phenotypes (MOD-2AB versus MOD-2A) determined on cellulose acetate gels as described by Eicher &

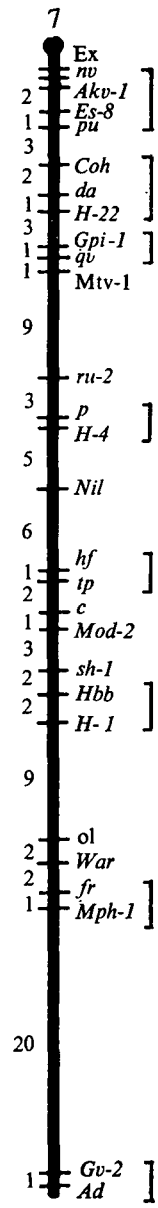

Fig. 1. Linkage map of mouse Chromosome 7, as modified after Green (1975). [The distance from the centromere to the *Gpi-1*, glucose phosphate isomerase-1 locus is shown as approximately 11 map units (Eicher, 1977).] The gene symbols of interest relative to this report are *p* (pink-eyed dilution), *tp* (taupe), *c* (albino), *Mod-2* (mitochondrial malic enzyme), and *sh-1* (shaker-1). The complete names of other gene loci can be found in a recent issue of *Mouse News Letter*.

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Coleman (1977), but using a pH 8·5 Tris-glycine buffer (3·0 g Trizma base, Sigma, 14·4 g glycine per litre solution). Controls included known *Mod-2*^{b}/*Mod2*^{b}, *Mod-2*^{a}/*Mod-2*^{b} and *Mod-2*^{a}/*Mod-2*^{a} individuals. Supernatants were prepared from fresh or frozen whole hearts, kidneys and brains. No differences were noted in gel patterns prepared from fresh versus frozen whole organs.

Presence or absence of MOD-2 in c^{3H}/c^{3H} or c^{14Cos}/c^{14Cos} newborn individuals as well as adults of the partially complementing genotypes c^{6H}/c^{3H}, c^{6H}/c^{112K} and c^{6H}/c^{65K} was determined by electrophoresis of supernatants prepared from a single adult heart or two to three pooled newborn hearts.

3. RESULTS

As mentioned above, all of the six parental *c*-deletion stocks expressed only the *Mod-2*^{b} allele. Table 2 and Fig. 2 show that the three newly tested albino deletions (c^{3H}, c^{65K} and c^{112K}) include the *Mod-2* locus: we never observed the heterozygous phenotype MOD-2AB expected in F_1 individuals carrying the *Mod-2*^{a} allele obtained from their SM/J parent and an albino deletion from the parent strain carrying *Mod-2*^{b} (cf. Tables 1, 2). Instead, the MOD-2 phenotype of F_1's was identical to that of known *Mod-2*^{a} homozygotes (SM/J), indicating absence of the *Mod-2*^{b} allele. In the c^{14Cos} mutation the *Mod-2* locus was confirmed to be intact (Fig. 2) (Erickson, Eicher & Gluecksohn-Waelsch, 1974).

Table 2. MOD-2 *phenotypes of mice carrying lethal albino alleles*

No. mice analysed	Mice	Genotype	MOD-2 phenotype
14	SM/J	+/+	A
3	c^{6H} line	c^{ch}/c^{6H}	B
5*	F_1 (with SM/J)	$+/c^{6H}$	A
3*		$+/c^{ch}$	AB
5	c^{3H} line	c^{ch}/c^{3H}	B
3	F_1 (with SM/J)	$+/c^{3H}$	A
3		$+/c^{ch}$	AB
1	c^{65K} line	c^{ch}/c^{65K}	B
4	F_1 (with SM/J)	$+/c^{65K}$	A
2		c^{ch}/c^{65K}	AB
7	c^{112K} line	c^{ch}/c^{112K}	B
4	F_1 (with SM/J)	$+/c^{112K}$	A
3		$+/c^{ch}$	AB
14	Complementers	c^{6H}/c^{3H}	Void
3		c^{6H}/c^{65K}	Void
5		c^{6H}/c^{112K}	Void
7	Newborn	c^{ch}/c^{3H}	B
15		c^{3H}/c^{3H}	Void
9		c^{14Cos}/c^{14Cos}	B
7		c^{ch}/c^{14Cos}	B
9		c^{ch}/c^{ch}	B

* Data from Erickson, Eicher & Gluecksohn-Waelsch (1974).

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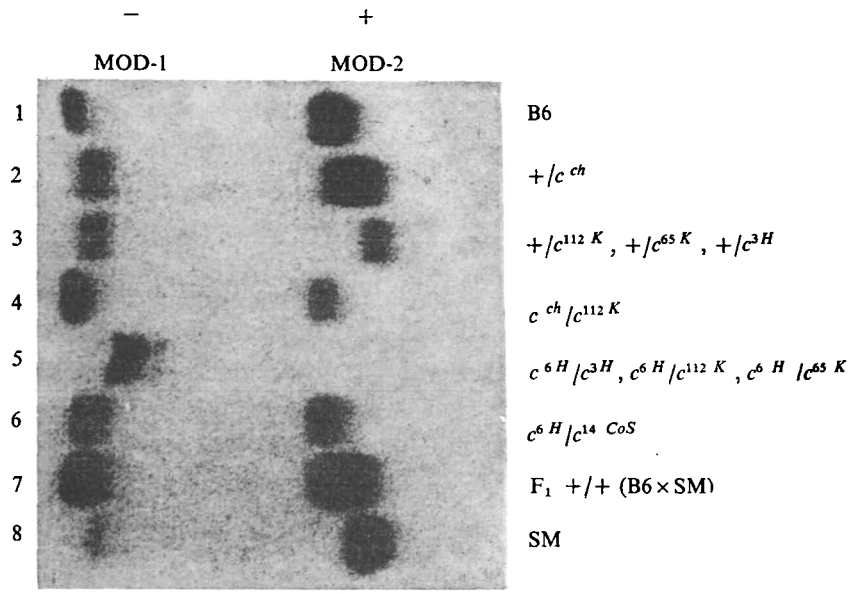

Fig. 2. Representative electrophoretic gel stained for malic enzyme. The supernatant (MOD-1) and mitochondrial (MOD-2) forms migrate to the cathode and anode, respectively. Samples that could have occupied slots 1–8 are listed to the right. The abbreviations B6 and SM represent the inbred strains C57BL/6J and SM/J, respectively. The fast migrating form of MOD-1, MOD-1B, is shown in slots 1 and 4, the slow form, MOD-1A, in slots 5 and 8, and the heterozygous form, MOD-1AB, in slots 2, 3, 6 and 7. Note the additional bands trailing between the normal position for MOD-1A and the origin in slot 5. The fast-migrating form of MOD-2, MOD-2A is in slots 3 and 8, slow form, MOD-2B, in slots 1, 4 and 6, and heterozygous form, MOD-2AB, in slots 2 and 7. Note presence of MOD-2A phenotype in slot 3 due to deletion of *Mod-2* locus in Chr 7 carrying c^{112K}, c^{65K}, or c^{3H} allele and absence of MOD-2 in slot 5 when c^{6H} allele is combined with the c^{112K}, c^{65K} or c^{3H} allele.

Mice of any of the three partially complementing genotypes (c^{6H}/c^{3H}, c^{6H}/c^{65K} and c^{6H}/c^{112K}) failed to show malic enzyme activity on electrophoretic gels in the region of MOD-2 but did show activity in the region of MOD-1 (supernatant malic enzyme) (Table 2, Fig. 2). c^{6H}/c^{3H} is homozygous for $Mod\text{-}1^a$, while c^{6H}/c^{112K} and c^{6H}/c^{65K} are heterozygous for the alleles $Mod\text{-}1^a$ and $Mod\text{-}1^b$ which are located on Chromosome 9. A series of multiple bands between the Mod-1 region and the origin were observed in the majority of c^{6H}/c^{3H} individuals, but never in gels from control mice nor in those from other complementing types, e.g. c^{6H}/c^{112K} or c^{6H}/c^{65K}. These additional bands were heaviest near the MOD-1 band and trailed off in intensity in the direction of the origin. The presence of multiple bands appeared to be age-dependent as mice younger than 6 weeks tended to have fewer (2–3) and lighter bands than older individuals (3–4 distinct bands). Multiple bands were demonstrable in heart but not in kidney or in brain tissue of c^{6H}/c^{3H} complementers. They were absent in heart when malic acid was omitted from the staining mixture, or when the gels were stained for malic dehydrogenase or isocitrate dehydrogenase.

Both c^{6H} and c^{25H} complemented the *tp* and *sh-1* mutations, indicating that the loss of chromosomal material in each does not extend as far as either of these closely linked loci. These results together with those previously published indicate that none of these six X-ray induced mutations extend from *c* towards the centromere as far as *tp* or away from the centromere as far as *sh-1* (see Fig. 1). Thus, none of these deletions is greater than 6 centimorgans in length.

4. DISCUSSION

Deletion mapping for six of the X-ray-induced lethal albino mutations has now been extended. Our results together with previous ones (Gluecksohn-Waelsch & Cori, 1970) show that none of these Chromosome-7 deletions extends beyond albino (*c*) toward the centromere to include taupe (*tp*). Five of the six include the nearest known locus distal to *c*, mitochondrial malic enzyme (*Mod-2*) (Fig. 1), but none includes the next distally mapped locus shaker-1 (*sh-1*).

Of particular interest is our finding that all three of the viable complementing genotypes, c^{6H}/c^{112K}, c^{6H}/c^{65K} and c^{6H}/c^{3H}, lack normal MOD-2 activity in heart, kidney and brain tissue as determined on cellulose acetate gels. This absence of normal mitochondrial malic enzyme is a new inborn error of metabolism, unique in its genetic causation, i.e. in a complementing viable double heterozygote. Thus, the enzyme deficiency is not lethal, as previously suggested by Erickson *et al.* (1974). However, the sterility of mice of these three genotypes may be related to MOD-2 deficiency.

The presence of multiple bands in the region of MOD-1 on electrophoretic gels prepared from hearts of the majority of c^{6H}/c^{3H} mice remains unexplained at this time. Their trailing off from the MOD-1 region may indicate that they are a form of supernatant malic enzyme. However, it cannot be ruled out that they reflect the presence of residual proteins of the mitochondrial enzyme type. The bands are reminiscent of the secondary isozymes discussed by Harris (1975). In our material this phenomenon appears to be related to an ageing process specific for the heart because a greater number of more strongly stained bands were observed in hearts from older than from younger c^{6H}/c^{3H} mice. It is noteworthy that the additional bands are characteristic of c^{6H}/c^{3H} and never appeared in any of the other complementing genotypes nor in controls.

The availability of mice without normal mitochondrial malic enzyme may be useful for defining the possible physiological and biochemical effects resulting from its absence. In addition, viable mice that lack all or most of the chromosomal DNA coding for both the *Mod-2* and *c* gene products could be useful for the isolation of one or both of these genes.

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