Complementation Studies of Lethal Alleles in the Mouse Causing Deficiencies of Glucose-6-Phosphatase, Tyrosine Aminotransferase, and Serine Dehydratase

(microsomal membrane abnormalities/enzyme differentiation/allelic complementation/chromosomal deletions)

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ABSTRACT The pleiotropic effects of six radiation-induced lethal alleles at the albino locus in the mouse include deficiencies of various enzymes and of serum proteins. Closely correlated with the biochemical deficiencies are severe ultrastructural abnormalities of endoplasmic reticulum membranes and the Golgi apparatus in liver and kidney cells. Complementation studies led to the distinction of four different complementation groups among the six lethal alleles. The results are compatible with the interpretation that the six lethal alleles represent overlapping deletions of various sizes at and around the albino locus in the mouse, in a region concerned with the control of biochemical and structural differentiation.

A series of six radiation-induced lethal alleles at the albino locus of the mouse show pleiotropic effects which include enzyme deficiencies in liver and kidney and ultrastructural abnormalities of the endoplasmic reticulum in these organs (1-4). Although many similarities exist between the effects of the various alleles, they differ from each other in the stage of lethal effects in homozygotes and in the severity of enzyme deficiencies. Differences are also found in the extent and severity of the ultrastructural abnormalities caused by the different alleles, although the lesions are qualitatively similar (4).

The multiplicity of the mutant effects and their induction through radiation made it seem likely that the alleles represent deletions of different sizes. Complementation studies were undertaken in order to gain further insight into the fine genetic structure of the chromosomal region involved.

MATERIALS AND METHODS

Of the six alleles used in this study, three were obtained from Oak Ridge (c^{14CoS} , c^{112K} , c^{65K}) and three from Harwell, England (c^{3H} , c^{6H} , c^{25H}). Identification of the mutations was based on interaction with the known recessive albino allele in linkage group I, Chromosome 7, of the mouse: animals carrying the original albino allele and one of the new radiation induced mutations at the same locus have the albino phenotype. Homozygotes for any of these new alleles are albino also, but lethal, four of them dying perinatally, and two (c^{6H} and c^{25H}) during early embryogenesis. Animals carrying two non-identical, radiation-induced albino alleles also have the albino phenotype.

The mutations must be maintained in the heterozygous state. Their presence in one dose in combination with the chinchilla allele at the same locus (c^{ch}/c) is expressed by a clearly recognizable dilution of the chinchilla pigmentation in comparison with the pigmentation of the homozygote (c^{ch}/c^{ch}). This dosage effect eliminates the necessity for progeny testing of heterozygotes.

Enzyme tests were carried out on livers of newborn animals which were frozen immediately after dissection and analyzed not longer than 3 weeks after freezing. Occasional checks on unfrozen livers were carried out.

Glucose-6-phosphatase (D-Glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) was determined in a 1:10 liver homogenate in 0.25 M sucrose as previously described (3). Tyrosine aminotransferase (L-Tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) was determined in the supernatant of the same homogenate after centrifugation at $31,000 \times g$ (30 min), using the method of Lin *et al.* (5) as previously described. The determination of serine dehydratase [L-serine hydro-lyase(deaminating), EC 4.2.1.13] in a centrifuged liver homogenate was based on the method of Suda and Nakagawa (6), except that incubation was at pH 9 which was found to be optimal. The pyruvate formed in the reaction was converted to the hydrazone and the optical density was read at 450 nm in a Zeiss spectrophotometer. Enzyme activity remained linear up to 15 min of incubation and was proportional to enzyme concentration between 5 and 20 mg of tissue. Added pyruvate was not destroyed during incubation. A blank consisted of an incubation mixture without added serine.

NADH-cytochrome *c* reductase [NADH:(acceptor) oxidoreductase, EC 1.6.99.3] was determined by the method of Greengard and Dewey (7) on an isolated microsomal fraction as previously described (3).

For serum protein determinations, blood was collected by decapitation and allowed to clot at room temperature for 1 hr. The clot was removed by centrifugation and serum of albino and colored littermates from each litter was pooled. An aliquot of the serum was taken for protein determination. Because of the occasional appearance of hemolysis, a modified biuret method was used to measure actual serum protein in a hemolytic sample (8).

RESULTS

Viability, Growth, and Fertility. Table 1 summarizes the results of interline crosses. In the majority of the crosses the

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TABLE 1. Results of interline crosses

Matings	Offspring		
	Colored	Albino	Total
1. $c^{8H}/c^{ch} \times c^{14CoS}/c^{ch}$	151	55	206
2. $c^{8H}/c^{ch} \times c^{65K}/c^{ch}$	133	43	176
3. $c^{8H}/c^{ch} \times c^{112K}/c^{ch}$	375	110	485
4. $c^{8H}/c^{ch} \times c^{3H}/c^{ch}$	557	155	712
5. $c^{85H}/c^{ch} \times c^{14CoS}/c^{ch}$	273	80	353
6. $c^{85H}/c^{ch} \times c^{65K}/c^{ch}$	97	20	117
7. $c^{85H}/c^{ch} \times c^{112K}/c^{ch}$	332	75	407
8. $c^{85H}/c^{ch} \times c^{3H}/c^{ch}$	380	84	464
9. $c^{85H}/c^{ch} \times c^{8H}/c^{ch}$	70	0	70

observed ratios of colored to albino offspring do not differ significantly from the expected 3:1 ratios. However, crosses of $c^{85H} \times c^{65K}$, c^{112K} and c^{3H} (Matings 6, 7, and 8) resulted in a significant deficiency of albino types, presumably as the result of prenatal death of a certain proportion of such fetuses. The complete absence of albino offspring in crosses of $c^{85H}/c^{ch} \times c^{8H}/c^{ch}$ (Mating 9) indicates prenatal death of the c^{85H}/c^{8H} genotype.

Viability is affected differently in various combinations of alleles (Table 2). In the case of c^{14CoS} , only one of the five possible types combining two different alleles is fully viable,

TABLE 2. Viability of crosses with different alleles

Alleles	c^{14CoS}	c^{3H}	c^{112K}	c^{65K}	c^{8H}	c^{85H}
c^{14CoS}	0	0	0	0	Fully viable	Not viable, but exceptionally may live up to 2 days
c^{3H}	0	0	0	0	Viable with reduced life span, runt	0
c^{112K}	0	0	0	0	Viable with reduced life span, runt	0
c^{65K}	0	0	0	0	Viable with reduced life span, runt	0

namely c^{8H}/c^{14CoS} . All others die perinatally. Animals carrying the c^{3H} , c^{112K} , and c^{65K} alleles in combination with the c^{8H} allele are viable, but there is increased mortality in the pre-weaning and weaning stages and a significantly shortened life span. In addition, the animals appear runt. The c^{8H}/c^{65K} combination was the least viable, the animals rarely reaching maturity.

Growth curves for the c^{8H}/c^{14CoS} mutants showed no difference from normal littermate controls up to the time of weaning, but thereafter their growth was retarded, and they weighed 11 g at 30 days as compared to 14 g for the controls. The growth curves of the c^{8H}/c^{112K} and c^{8H}/c^{3H} mutants begin to diverge from the controls at about 7–10 days of age, and the weight of the mutants at 30 days of age is often only one half of that of the controls. Some representative growth curves are shown in Fig. 1.

A summary of tests for fertility appears in Table 3. c^{14CoS} and c^{8H} complement fully, except for pigmentation, and double heterozygotes (c^{8H}/c^{14CoS}) are albino and fertile. They breed in a balanced lethal system with enforced heterozygosis since only the heterozygotes survive beyond birth. The other combinations shown in Table 3 are not fertile in either sex; some

FIG. 1. Growth curves of complementing albinos and their litter mate controls.

TABLE 3. Tests for fertility

Genotype	Result
c^{8H}/c^{14CoS}	Fertile in both sexes.
c^{8H}/c^{3H}	Reproductive activity but infertile in both sexes. 1 female on dissection had 10 disintegrating embryos at about 8 days of gestation.
c^{8H}/c^{112K}	Reproductive activity but infertile in both sexes. 4 females had disintegrating embryos at about 8 days of gestation.
c^{8H}/c^{65K}	No reproductive activity. Infertile.

Males or females of the genotype shown were caged for several months with either fertile normal females or normal males. The females were checked daily for vaginal plugs.

TABLE 4. Serine dehydratase activity in the liver of the mutant c^{14CoS}

Age	Enzyme activity ($\mu\text{mol}/\text{min per g}$)			
	Albino	Genotype	Colored	Genotype
1-6 hr	0.32 ± 0.06 (11)	c^{14CoS}/c^{14CoS}	2.16 ± 0.48 (12)	*
Several days	—		5.75 ± 0.79 (5)	c^{ch}/c^{14Co}
			7.68 ± 0.68 (4)	c^{ch}/c^{ch}
3 Months	—		57.0 ± 5.0 (3)	c^{ch}/c^{14CoS}
			51.5 ± 5.8 (3)	c^{ch}/c^{ch}

* Heterozygous and homozygous normal mice cannot be distinguished at this age on the basis of eye and fur color.

of the double heterozygous females become pregnant, when mated to normal males, but their embryos die at an early gestation period. The mechanism responsible for sterility is not known in either males or females. Preliminary observations of the males show morphologically abnormal seminal vesicles and also abnormalities of sperm morphology.

Biochemical observations

It was of interest to find out whether the defects in viability, growth, and fertility of the complementing genotypes were reflected in the activities of the enzymes known to be affected by the alleles. The three enzymes studied were glucose-6-phosphatase, tyrosine aminotransferase, and serine dehydratase. Since a deficiency of the last-named enzyme has not been described previously, some pertinent data are given below in a separate section.

Serine Dehydratase. A development curve of the enzyme in normal albino mice has been described (9). The enzyme activity is low in newborn and rises rather slowly (compared to the other two enzymes mentioned above) in the post-natal period. An adult value some 20 times larger than the newborn value is not reached until about 2 months after birth.

Table 4 shows that the enzyme activity in the livers of

newborn albinos of the mutant c^{14CoS} is only one seventh that of the colored littermate controls. The albinos rarely survive in good condition longer than 6-8 hr. At a later age, when heterozygous and homozygous normal mice can be distinguished, the developmental curve appears to be the same in the two types of animals. As with the other enzyme deficiencies in these mutants, the serine dehydratase activity does not show a gene dosage effect.

Enzyme Activity and Serum Protein of Complementing Types.

A preliminary series of experiments has been published previously (3). It showed that the albinos of the c^{6H}/c^{112K} genotype had normal levels of glucose-6-phosphatase activity at birth and at 1.5 months of age. Similarly, the c^{6H}/c^{3H} albinos showed normal enzyme levels for glucose-6-phosphatase and tyrosine aminotransferase at 2.5-5 months of age. The more extensive data in Table 5 which also include the c^{6H}/c^{65K} genotype confirm this and they show in addition that serine dehydratase is also normal in the albinos in each of the three crosses. Thus, these double heterozygotes, which according to Tables 2 and 3 are viable but runted and infertile, show complementation of enzyme levels. Not shown in Table 5 are determinations of the three enzyme levels in adult albinos of the c^{6H}/c^{14CoS} genotype, which according to Tables 2 and 3, is fully viable and fertile. As expected, the enzyme levels were entirely normal. Electronmicroscopic studies of these complementation types have shown a completely normal structure of the endoplasmic reticulum.

A quite different result is obtained in crosses with the c^{25H} allele (Table 6). Here, albinos which die a few hours after birth all show low activity for the three enzymes when compared with littermate controls. Thus, the c^{25H} allele in contrast to the c^{6H} allele does not complement the other alleles for enzyme activity.

In previous experiments, the serum proteins were found to be 22% lower in lethal albinos than in controls, a statistically significant difference (3). In the present series, the serum protein levels of complementing types paralleled the enzyme activity levels. Thus, in Table 5 the serum proteins were the same in albinos and controls, whereas in Table 6 the serum proteins in each of the crosses were significantly ($P < 0.01$) lower in albinos than in controls. It will be of interest to determine whether a particular serum protein is missing in the lethal albinos.

TABLE 5. Enzyme levels and serum proteins in interline crosses of the c^{6H} allele

	c^{6H}		c^{112K}		c^{65K}	
	Albino	Colored	Albino	Colored	Albino	Colored
Glucose-6-phosphatase	17.0 ± 0.45 (5)	17.3 ± 0.74 (5)	6.97 ± 1.16 (8)	8.8 ± 1.6 (5)	3.0 (3)	2.26 (3)
Tyrosine amino-transferase	1.01 ± 0.22 (5)	0.94 ± 0.15 (5)	1.10 ± 0.15 (6)	1.22 ± 0.20 (6)	1.62 (3)	1.74 (3)
Serine dehydratase	1.96 ± 0.35 (6)	1.97 ± 0.45 (6)	2.77 ± 0.37 (8)	3.4 ± 0.30 (6)	2.30 ± 0.30 (4)	2.12 ± 0.15 (4)
Serum protein	30.2 ± 1.26 (6)	30.3 ± 1.32 (6)	31.0 ± 0.44 (5)	31.2 ± 0.59 (5)	29.2 ± 1.25 (6)	29.0 ± 1.16 (6)

The animals were newborn to a few hours old, except for glucose-6-phosphatase of the c^{3H} series which was determined on 1- to 2-day-old mice. In each series, the albinos and colored were litter mates. Enzymatic activity is in $\mu\text{mol}/\text{min per g}$ of liver. The average $\pm$ SEM is recorded with the number of animals in parentheses. In the case of serum protein, the number of pooled serum samples used is indicated.

FIG. 2. Deletions around the albino locus, chromosome 7 (linkage group I) of the mouse. *sh-1* = shaker 1. *mod-2* = mitochondrial malic enzyme. *c* = albino. *tp* = taupe. *EMV* = genomic region concerned with control of certain enzymes, microsomal membrane structure and viability. *F*, *G*, and *D* = genomic regions concerned, respectively, with fertility, growth, and early embryonic differentiation.

NADH-Cytochrome *c* Reductase. In a preceding paper (3), this enzyme appeared to be higher in lethal albinos than in controls, but, as stated in a note added in proof, the difference turned out not to be statistically significant (3). In the present series, c^{25H}/c^{3H} albinos gave an average value (in $\mu\text{mol/g}$ of microsomal protein per min) of 193 ± 28 (7 animals) versus 137 ± 21 (5 animals) for control littermates ($P > 0.05$). A smaller series for c^{25H}/c^{14CoS} and c^{25H}/c^{112K} gave similar results, that is, the differences from controls were not statistically significant.

DISCUSSION

The present complementation studies extend previous observations about the nonidentity of the radiation induced lethal alleles at the albino locus in the mouse. Thus, the order of severity of enzyme deficiencies and membrane ultrastructural abnormalities in homozygous newborn is $c^{14CoS} < c^{112K}$, $c^{65K} < c^{3H}$. These alleles do not show any striking complementation with each other, except for combinations including the c^{14CoS} allele which survive somewhat longer than the respective homozygotes (1). Of particular interest are the c^{6H} and c^{25H} alleles. Both of these homozygotes die as early embryos soon after implantation, but c^{6H} forms a balanced lethal system with c^{14CoS} and double heterozygotes (c^{6H}/c^{14CoS}) live and breed, though with a decreased lifespan. Their enzyme

and serum protein values are within the normal range at birth and continue to be so through adult life. With the other alleles, (c^{3H} , c^{65K} , and c^{112K}), c^{6H} achieves only partial complementation: double heterozygotes live longer than the homozygotes but they are runted and infertile and die prematurely, even though their enzyme and serum protein levels are within the normal range. Whereas c^{25H} resembles c^{6H} in the early prenatal mortality of homozygotes, it differs from it in its failure to complement fully with c^{14CoS} and partially with the other alleles. All combinations with c^{25H} die perinatally, with severe enzyme and serum protein deficiencies. Because of the embryonic death of c^{6H} and c^{25H} homozygotes, the effects of these alleles on enzyme activities cannot be ascertained directly, but only indirectly by their interaction with other alleles as determined in complementation studies.

The available data serve to distinguish four complementation groups among the six alleles: 1. c^{6H} , 2. c^{25H} , 3. c^{14CoS} , and 4. c^{3H} , c^{65K} , c^{112K} . They also support the assumption that the six alleles represent overlapping deletions of different sizes. As a first approximation, the diagram in Fig. 2 representing these deletions indicates their probable extent in the individual alleles. In addition to the albino locus, the deletions include the genome concerned with the control of certain enzymes and serum proteins, microsomal ultrastructure, viability, growth, fertility, and morphogenesis. Enzyme activities, membrane structure, and viability are always associated in this system and have not been found to be affected separately in any of the allelic combinations. It cannot be determined if these effects are causally related to each other or if they trace back separately to a common, genetically caused defect. They are grouped together here without regard to the possible fine structure and sequence of the relevant genome. Fertility, growth, and morphogenesis appear to be affected independently of the former group. Thus, the pleiotropic effects of the albino alleles are separable to some extent by these complementation studies.

The failure to detect a visible deletion in Chromosome 7, linkage group I, in mouse embryos heterozygous for one of the albino alleles, c^{3H} , (11) can be ascribed to the fact that this deletion would be below the level of resolution with present cytological techniques.

A recent test of the marker *mod-2*, one crossover unit removed from the *c*-locus (10), has shown this locus not to be

TABLE 6. Enzyme levels and serum proteins in interline crosses of the c^{3H} allele

	c^{14CoS}		c^{3H}		c^{112K}		c^{65K}	
	Albino	Colored	Albino	Colored	Albino	Colored	Albino	Colored
Glucose-6-phosphatase	1.16 ± 0.11 (11)	4.07 ± 0.96 (9)	0.24 ± 0.02 (11)	2.46 ± 0.38 (9)	0.54 ± 0.06 (5)	3.16 ± 0.47 (4)	0.44 ± 0.06 (6)	3.62 ± 0.69 (4)
Tyrosine amino-transferase	0.18 ± 0.04 (10)	0.66 ± 0.11 (8)	0.06 ± 0.03 (6)	0.93 ± 0.09 (7)	0.23 ± 0.08 (5)	0.83 ± 0.1 (5)	0.12 ± 0.04 (4)	0.73 ± 0.42 (4)
Serine dehydratase	0.54 ± 0.18 (6)	2.65 ± 0.69 (6)	0.24 ± 0.04 (6)	1.68 ± 0.56 (6)	0.59 ± 0.08 (6)	1.86 ± 0.58 (6)	0.39 ± 0.13 (4)	2.20 ± 0.43 (4)
Serum protein	24.2 ± 1.04 (4)	31.4 ± 1.13 (4)	24.9 ± 1.0 (8)	31.1 ± 0.71 (8)	23.4 ± 0.91 (3)	30.6 ± 1.28 (3)	22.0 ± 1.18 (3)	30.3 ± 0.88 (3)

The animals were newborn to a few hours old. The rest of the legend as in Table 5.

deleted in the c^{14CoS} allele. This allele represents no doubt the least extensive deletion including only the genome for the three enzymes, serum protein, viability, and microsomal membrane structure. The counterpart of c^{14CoS} is the c^{6H} deletion with a normal genome for the complex of enzymes-membrane structure-viability but with deletions in the genome concerned with morphogenesis and growth as well as fertility. The c^{6H} allele includes a deletion of the *mod-2* locus (10) and complements fully with c^{14CoS} . Its failure to do the same with c^{3H} , c^{65K} , and c^{112K} indicates that the latter three alleles are deleted in a portion of the genome concerned with growth and fertility, in addition to the deletion of the enzyme-membrane structure-viability complex. Finally, c^{25H} appears to be the most extensive deletion, lacking the genome for the enzyme-membrane structure-viability complex and that controlling growth and fertility as shown by its failure to complement c^{14CoS} , c^{3H} , c^{65K} , and c^{112K} ; it also appears to be deleted in the region concerned with embryonic differentiation since it does not complement c^{6H} . The marker *mod-2* has been shown to be deleted in the c^{25H} allele as well (10).

In the interpretation of the deletion mapping as presented in Fig. 2, it must be emphasized that it is not possible to estimate the exact extent of the genomic regions which are defined only by the phenotypes controlled by the albino alleles. The regions D G F and E M V might be composed of one or many genetic loci. Obviously, complementation mapping of the type described here with genetic markers widely spaced does not have the resolving power to permit estimation of the number of loci concerned, or their map distances. It does,

however, serve to promote the further genetic dissection of a chromosomal region implicated in the control of biochemical and morphogenetic differentiation.

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