ANALYSIS OF THE ALBINO-LOCUS REGION OF THE MOUSE: IV. CHARACTERIZATION OF 34 DEFICIENCIES[a]

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ABSTRACT

Thirty-four independent nonviable *c*-locus mutations (types c^{al}, albino lethal and c^{as}, albino subvital), derived from radiation experiments, were tested for involvement of nearby markers *tp*, *Mod-2*, *sh-1*, and *Hbb*: 10, 22, and 2 involved, respectively, none of these markers, *Mod-2* alone, and *Mod-2* plus *sh-1*. When classified on this basis, as well as according to developmental stage at which homozygotes die, and by limited complementation results, the 34 independent mutations fell into 12 groups. From results of a full-scale complementation grid of all 435 possible crosses among 30 of the mutations, we were able to postulate an alignment of eight functional units by which the 12 groups fit a linear pattern. Abnormal phenotypes utilized in the complementation study were deaths at various stages of prenatal or postnatal development, body weight, and reduction or absence of various enzymes. Some of these phenotypes can be separated by complementation (*e.g.*, there is no evidence that mitochondrial malic enzyme influences survival at any age); others cannot thus be separated (e.g., glucose-6-phosphatase deficiency and neonatal death).—We conclude that all of the nonviable albino mutations are deficiencies overlapping at *c*, and ranging in size from <2cM to 6-11 cM. The characterization of this array of deficiencies should provide useful tools for gene-dosage studies, recombinant-DNA fine-structure analyses, etc. Since many of the combinations of lethals produce viable albino animals that resemble the standard *c*/*c* type, we conclude (a) that the *c* locus contains no sites essential for survival, and (b) that viable *non*albino *c*-locus mutations (c^{xv}) are the result of mutations within the *c* cistron. Viable albinos (c^{av}, the majority of radiation-induced *c*-locus mutations) may be intracistronic mutations or very small deficiencies.

MUTATIONS recovered in mutagenesis experiments using the specific-locus method (W. L. RUSSELL 1951) provide a potential wealth of material for the study of gene structure and function in the mouse. For each of seven loci, any genetic event is detectable that affects the wild-type allele in such a way as to produce a visible phenotype in heterozygotes that received a recessive marker from the other parent. Several types of genetic events meet this qualification; for any of the marked loci, the array of mutants is potentially useful for exploring the locus itself, as well as the region surrounding it.

Following our studies of *d* (dilute), *se* (short-ear), and *d se* mutations in

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Chromosome *9* (RUSSELL 1971), we have concentrated on the *c* locus in Chromosome *7*. This locus is of interest because it codes for a well-characterized enzyme, tyrosinase (COLEMAN 1962), because nearby markers have been mapped (RODERICK and DAVISSON 1981), and because there are available various independent chromosomal aberrations involving the *c* region, such as a tandem duplication (RUSSELL *et al.* 1975) and several *X*-autosome translocations (RUSSELL and CACHEIRO 1978), which present possibilities for gene-dosage and other studies.

Radiation experiments yielded, in over 3,600,000 offspring observed, altogether 119 presumed mutations involving the *c* locus, of which 107 were studied for phenotype and viability of heterozygotes and homozygotes (RUSSELL, RUSSELL and KELLY 1979). A subset of these mutations, all homozygous viable and all probably of spontaneous origin, was recovered in 16 mosaic individuals (RUSSELL 1979). Special emphasis has been placed on the study of over 30 lethal *c*-locus mutations, several of which were early shown to complement for lethality; the complementation pattern and other findings provided evidence that these lethal mutations were small deficiencies (RUSSELL and DEHAMER 1972, 1973). Two of these lethals, along with three others derived from specific-locus experiments at Harwell, have been used in extensive studies by GLUECKSOHN-WAELSCH and her group (1979, review).

Some of the *c*-mutant homozygotes die neonatally and others prenatally (RUSSELL and DEHAMER 1973), the latter deaths occurring either before implantation, or shortly after (or at) implantation (RUSSELL, DEHAMER and BORMAN 1974; RUSSELL and RAYMER 1979). One mutant of each of the last two types has been studied for developmental morphology (LEWIS, TURCHIN and GLUECKSOHN-WAELSCH 1976; LEWIS 1978). The neonatally lethal mutants are deficient for glucose-6-phosphatase (G6Pase) activity (ERICKSON, GLUECKSOHN-WAELSCH and CORI 1968; RUSSELL and DEHAMER 1973; DEHAMER 1975), and this phenotype could not be separated from neonatal death by complementation (RUSSELL, DEHAMER and BORMAN 1974). Heterozygotes for 22 of the *c* lethals were found to have reduced activity for mitochondrial malic enzyme (MOD-2) and were thus concluded to be deficient for the closely-linked marker *Mod-2*; and crosses between mutants indicated that a total absence of MOD-2 was compatible with survival, at least to weaning age (RUSSELL, DEHAMER and BORMAN 1974; DEHAMER 1975). Two Harwell mutants were also deficient for *Mod-2* (ERICKSON, EICHER, and GLUECKSOHN-WAELSCH 1974). A quantitative study of enzyme activity has shown exact dosage effects for *Mod-2* (BERNSTINE, RUSSELL and CAIN 1978); and a *cis*-acting regulatory gene, *Mdr-1*, has been discovered between *c* and *Hbb* (BERNSTINE, KOH, and LOVELACE 1979; BERNSTINE 1979; BERNSTINE and KOH 1980). Two of the more than 30 Oak Ridge *c*-lethals are deficient for *shaker-1*, *sh-1*, which is 6 cM from *c* (RUSSELL and CACHEIRO 1977), and these two, as well as two other *c* lethals are cytologically detectable (RUSSELL and CACHEIRO 1977; MILLER *et al.* 1974).

In the present investigation, 34 independently induced nonviable *c*-locus

mutations derived from Oak Ridge radiation experiments were tested by deficiency mapping and entered into an extensive complementation grid of 465 combinations. From the phenotypes—death at various stages during intrauterine and postnatal life, reduced weight, reduction or absence of various enzymes, and uncovering of nearby markers—it was possible to postulate eight functional units in the *c* region. The mutations fell into 12 groups that show no deviations from a linear map and are concluded to represent overlapping deficiencies.

MATERIALS AND METHODS

All of the albino-locus mutations referred to in this paper were derived from radiation experiments using the specific-locus method. In this procedure, genetically uniform homozygous wild-type mice [(101/Rl × C3H/Rl)F_1 or (C3H/Rl × 101/Rl)F_1] are mated to the noninbred T stock, which is homozygous recessive at seven marker loci, including the *c* locus. The general symbol c^* will be used generically to designate any mutation involving that locus. Details concerning detection of the mutations, allelism testing, and establishment and maintenance of stocks are given in the first paper of this series (RUSSELL, RUSSELL and KELLY 1979). That paper also established the following system of superscripts: (a) the first superscript indicates whether, with regard to pigment phenotype, the mutation mimics the standard albino allele, *c* ("c^a"), or is intermediate ("c^x"); (b) the second indicates whether homozygotes are viable ("v"), subvital ("s"), or lethal prenatally or neonatally ("l"). Following these first two superscripts, additional superscripts can be added to provide identification of individual mutants.

All of the mutations used for the major portion of the present study were of the c^{al} type (7 neonatally lethal and 26 prenatally lethal), except for one which was c^{as}. All were whole-body mutants. The prenatally lethal c^{al}'s have been further analyzed as to time of death of the homozygotes: in 13 of the stocks, death occurred before implantation and, in 13 others, at, or shortly after, implantation (RUSSELL and RAYMER 1979). In Table 1, the mutants are listed by the mutagenic treatment that produced them and by viability of the homozygote. Two of the mutant stocks, 65K and 112K, descended from sib offspring of an irradiated animal and are assumed to represent a cluster derived from but a single mutation in a spermatogonial stem cell. The 35 stocks listed in Table 1 therefore represent 34 independent mutations.

In addition to the mutants shown in Table 1, two viable (c^{av}) stocks were used in some of the crosses. These were 62DSD and 38R145L, derived from irradiation of spermatogonia with 90 R/min X rays (100 + 500 R, 24 hr interval) and neutrons (145 rads at 0.16 rad/min), respectively.

Two major groups of experiments were carried out: deficiency mapping and complementation mapping. The former involved crosses of each of the mutants listed in Table 1 with the following nearby markers on Chromosome 7: *tp* (taupe), *sh-1* (shaker 1), and *Mod-2* (mitochondrial malic enzyme); and of two of the mutants with *Hbb* (hemoglobin, β chain). Stocks used for this purpose were TP/Rl (*a/a*; *tp/+*, inbred), CASH/Rl (*a/a*; *b/b*; *Ca/+*, *p +/p sh-1*; *s/s*), SM/JRl (*Mod-2*^{b}/*Mod-2*^{b}, inbred), and SEC/Rl (*a/a*; *b/b*; c^{ch} Hbb^s/c^{ch} Hbb^s; *++/d se*, inbred).

The complementation mapping involved all possible crosses between all but four of the mutants listed in Table 1, as well as several crosses using the two c^{av} stocks (see above). Between one and 22 matings were made for each cross between mutants, over 1300 matings in all, or, an average of about three matings per combination. During the first year-and-a-half of the experiment, all cages that had pregnant females were checked twice daily for new litters, and some of the newborns were sacrificed for biochemical studies (DEHAMER 1975). Subsequently, cages were checked less frequently, but at least twice a week, and none of the

TABLE 1

Origin and death time of nonviable c-locus mutants

Germ-cell stage irradiated	Mutagenic treatment Type of radiation	Dose rate and exposure	Mutants, by viability of homozygote			
			Subvital	Neonatally lethal	Lethal at or soon after implantation	Preimplantation lethal
Spermatogonia	γ ray	48 R/min, single		14 Cos		
Spermatogonia	X ray	>70 R/min, single		65 K ¶	2YPS$_J$	146G
				112 K	3YPS$_D$	4PB
				16 HATh		
Spermatogonia	X ray	9 R/min, single				39SAS
Spermatogonia	X ray	>70 R/min, multiple*		LA	AL	26DVT
					19DTR	
					23DVT	
					11DSD	
Spermatogonia	neutrons	various†, single		15R60L	24R145L	3R60L
				10R75M	7R250H	
Postspermatogonial stages	X ray	90 R/min, single			68G	
					202G	
Postspermatogonial stages	X ray	90 R/min, multiple‡			1DThW$_b$	
Postspermatogonial stages	neutrons	0.16 rad/min, single				2R145L
						3R145L

TABLE 1—Continued

Germ-cell stage irradiated	Mutagenic treatment: Type of radiation	Dose rate and exposure	Mutants, by viability of homozygote: Subvital	Neonatally lethal	Lethal at or soon after implantation	Preimplantation lethal
Oocytes	X ray	90 R/min, single	20FATw‖	$1FaFy_h$		
Oocytes	X ray	90 R/min, multiple§				$1FDFoHr_e$
Oocytes	neutrons	0.16 rad/min, single				10FR60L
Oocytes	neutrons	100 rad/min, single			4FR60Hd	$1FR60H_b$
					5FR60Hg	$9FR60H_b$
						$12FR60H_b$
						$14FR60H_b$

* LA, AL: 4 × 500R (28-day intervals).
19DTR: 2 × 500R (2-hr interval).
23DVT, 26DVT: 5 × 200R (24-hr intervals).
11DSD: 100 + 500R (24-hr interval).
† 15R60L, 24R145L, 3R60L: 0.16 rad/min.
10R75M: 1.0 rad/min.
7R250H: 100 rad/min.
‡ 6 × 50R (7-day intervals).
§ 8 × 50R (75-min intervals).
‖ Of several available 20FATw lines (RUSSELL *et al.* 1979), the one used in the present study has homozygotes that generally die between birth and weaning.
¶ Cluster, see Table 2, footnote †.

animals were sacrificed. Under either regime, all surviving offspring remained with their mother until about 4 weeks old, at which time all were weighed, and albinos as well as roughly equal numbers of c^{ch}/c and c^{ch}/c^{ch} littermates were stored until they died of natural causes or until one year of age, whichever came sooner.

Because weaning weight varies with sex, number of littermates, and other factors, there is considerable inter-litter variation, and comparisons of overall averages for the segregating genotypes can be misleading. We therefore averaged the weights of all like-sexed animals of a given genotype within each litter and paired the albino male average with the littermate c^{ch}/c male average, and similarly for females. Where there were no like-sexed c^{ch}/c littermates (14.9% of the cases), we used the c^{ch}/c^{ch} like-sexed littermate average for comparison; and where there were neither c^{ch}/c nor c^{ch}/c^{ch} like-sexed littermates, the albino weight was not used at all.

To clarify certain features of the results, it became necessary to examine the uterine contents of females involved in many crosses (altogether 232 females from 44 types of cross). Mated primiparous females were checked each morning for vaginal plugs and were sacrificed between days 14 and 17 following the finding of a plug (plug = day zero). Observations were made of the numbers of corpora lutea (indicative of ovulations), live fetuses (classified as to eye color), dead embryos (with estimates of age at death), and moles. Evidence discussed elsewhere (RUSSELL and RAYMER 1979) indicates that moles represent death just before, during, or very shortly after implantation. For brevity, this possible array of events will be referred to as "implantation" deaths. The difference between the numbers of corpora lutea and total implants (live and dead) represents either unfertilized eggs or death of conceptuses early during the preimplantation period. If matings that produce c^{al}/c^{al} segregants show an excess in this deficit, the excess is assumed to result from the latter cause, and is referred to as "preimplantation" death.

Further details of procedure are described together with the results.

RESULTS

A. Deficiency Mapping

Four loci in the vicinity of the *c* locus were used for deficiency mapping.

TABLE 2

Deficiency mapping with markers near the c *locus*

c-Locus mutant		Locus tested				
Group symbol	Stock	*tp* segregation	*Mod-2* Activity ratio§	*Mod-2* gel band‖	*sh-1* segregation	*Hbb* gel band††
A1	14CoS	0/36‡	1.0	++¶	0/26**	
A2	1FAFyh	0/30	0.9	++	0/52	
A3	16HaTh	0/56	1.0	++	0/32	
A4	15R60L	0/25	1.1	++	0/30	
A5	10R75M	0/36	1.0	++	0/30	
A6	LA*	0/73	1.0		0/35	
Ai1	$1DThW_b$	0/68	0.9	++	0/54	
Ai2	23DVT	0/53	1.1	++¶	0/21	
Ai3	$3YPS_D$*	0/25	1.0	++	0/42	
C1	20FATw	0/58	1.1	++¶	0/25	
Bi1	11DSD	0/54	0.5	+	0/41	
Bi2	$4FR60H_d$	0/49	0.4	+	0/52	

These loci, and their relation to *c* are as follows (from proximal to distal): *tp*-(3)-*c*-(2)-*Mod-2*-(4)-*sh-1*-(2)-*Hbb* (RODERICK and DAVISSON 1981). The tests were done for 35 albino stocks (34 independent mutants). Results are shown in Table 2.

1. Taupe (*tp*): From crosses of $c^{ch}/c^{*} \times tp/tp$, altogether 1688 offspring

TABLE 2—Continued

c-Locus mutant		Locus tested				
Group symbol	Stock	*tp* segregation	*Mod-2* Activity ratio§	*Mod-2* gel band‖	*sh-1* segregation	*Hbb* gel band††
Bi3	5FR60Hg	0/26	0.5	+	0/23	
Bi4	2YPS$_J$*	0/48		+	0/38	
Bp1	1FDFoHr$_c$	0/42	0.5	+¶	0/62	
Bp2	1FR60H$_b$	0/93	0.5	+	0/39	
Bp3	9FR60H$_b$	0/45	0.5	+	0/42	
Bp4	14FR60H$_b$	0/58	0.5	+	0/53	
Bp5	4PB	0/42	0.5	+	0/33	
Bp6	3R60L	0/32	0.6	+	0/53	
Bp7	2R145L	0/33	0.5	+	0/22	
Bp8	3R145L	0/43	0.6	+	0/82	
Di1	202G	0/52	0.4	+	0/72	
Di2	24R145L	0/63	0.5	+	0/30	
Di3	7R250H	0/49	0.5	+	0/34	
Di4	AL*	0/45	0.5		0/36	
Dp1	10FR60L	0/69	0.5	+	0/26	
Dj1	19DTR	0/58	0.4	+	0/40	
Dj2	68G	0/29	0.4	+	0/42	
Dq1	146G	0/79	0.4	+	0/74	
Dq2	39SAS	0/60	0.4	+¶	0/73	
E1	65K†	0/25	0.5	+	0/22	
E1′	112K†	0/39	0.4	+¶	0/10	
Fp1	26DVT	0/51	0.5	+	5/18	++
Fq1	12FR60H$_b$	0/44	0.5	+	10/30	++

* Not tested in the full complementation grid. Group assignment based on criteria shown in Figure 1 (RESULTS, Section A5).

† 65K and 112K are derived from the same male in matings sampling irradiated spermatogonial stem cells. They are presumed to have resulted from a single mutational event.

‡ Numerator: offspring with taupe phenotype. Denominator: total number of offspring observed in matings of c^{ch}/c^{*} with tp/tp.

§ Based on data of BERNSTINE *et al.* 1978, except ratio for LA and AL (BERNSTINE, personal communication).

‖ ++, MOD-2 band of c^{ch}/c^{*} as heavy as corresponding band of c^{ch}/c^{ch} littermate. +, MOD-2 band of c^{ch}/c^{*} clearly lighter than corresponding band of littermate. Data of DEHAMER 1975.

¶ Confirmatory data available from comparison between $+^{c}$ *Mod-2*$^{b}/c^{ch}$ *Mod-2*^{a} and $+^{c}$ *Mod-2*$^{b}/c^{*}$. Mutants, maintained on *Mod-2*^{a} background, were crossed to SM/JRl.

** Numerator: offspring with shaker and/or deaf phenotype. Denominator: total number offspring observed in matings of c^{ch}/c^{*} with *sh-1*/*sh-1*.

†† ++, relative staining of Hbb^{d} and Hbb^{s} bands same in $c^{ch}Hbb^{s}/c^{*}Hbb^{d}$ and $c^{ch}Hbb^{s}/c^{ch}Hbb^{d}$ segregants.

were classified for coat color. None of the matings produced nonwild-type progeny, indicating that if any of the mutants are deficiencies, these do not include the *tp* locus.

2. *Mitochondrial malic enzyme* (*Mod-2*): Segregants of the types c^{ch}/c^{*} and c^{ch}/c^{ch} from crosses within each of the stocks were compared with respect to specific activities of MOD-2 in heart mitochondrial fractions (BERNSTINE, RUSSELL and CAIN 1978). A c^{ch}/c^{*}:c^{ch}/c^{ch} ratio of about 0.5 indicates that the c^{al} mutant is probably a deficiency that includes the *Mod-2* locus, whereas a ratio of about 1.0 suggests that the *Mod-2* locus is not involved. In most of the stocks, an earlier comparison had been made between c^{ch}/c^{*} and c^{ch}/c^{ch} littermates with respect to the staining intensity of the MOD-2 band on starch gels (DEHAMER 1975). In addition, starch-gel comparisons had been made between $+^{c}$ $Mod\text{-}2^{b}/c^{ch}$ $Mod\text{-}2^{a}$ and $+^{c}$ $Mod\text{-}2^{b}/c^{*}$ littermates in six c^{*}-stock outcrosses (DEHAMER 1975). As indicated in Table 2, the enzyme activity measurements confirmed all of the earlier starch-gel information.

3. *Shaker-1* (*sh-1*): Crosses were of the type $c^{ch}/c^{*} \times sh\text{-}1/sh\text{-}1$. Since the shaker phenotype is not always clearly expressed by weaning age, offspring from these matings were kept until they were at least 6 weeks old, at which time all that were not shaking were tested for hearing ability (response to a sharp sound). Altogether 1394 offspring were classified in this manner. Two of the c^{al} stocks produced shaker offspring from the mating to *sh-1*/*sh-1*, indicating that these mutants are probably deficiencies that include the *sh-1* locus.

4. *Hemoglobin β chain* (*Hbb*): The two *c*-lethal mutants that appear to be deficiencies involving *sh-1* were further tested with respect to *Hbb*. Within each stock, $c^{ch}Hbb^{s}/c^{ch}Hbb^{d}$ and $c^{ch}Hbb^{s}/c^{*}$ segregants were compared with respect to staining intensity of the Hbb^{d} bands (relative to the Hbb^{s} band) in polyacrylamide gels in the presence of cystamine. No differences could be detected (BERNSTINE, unpublished).

5. *Conclusions from deficiency mapping*: On the basis of deficiency mapping alone, the mutants fall into three groups: (a) not deficient for any nearby marker (10 mutants); (b) deficient for *Mod-2*, but not *tp*, *sh-1*, or *Hbb* (23 stocks, representing 22 independent mutations); and (c) deficient for *Mod-2* and *sh-1*, but not *tp* or *Hbb* (two mutants).

From the results of the deficiency mapping (three categories) and the information shown in Table 1 on the developmental stage at death of homozygotes (four categories), it is possible to construct a matrix with 12 fields. The mutants studied occupy 7 of these 12 fields. Three of the 7 fields are subdivisible (Figure 1) on the basis of complementation tests with groups A and Ai (see below). When such subdivisions are imposed on the matrix, the mutants fall into 12 categories, designated as groups A, Ai, Bi, Bp, C, Di, Dp, Dj, Dq, E, Fp, Fq. Within a group, each mutant has been designated by a number following the group symbol, *e.g.*, A1, A2, . . . etc., and these designations are shown, together with the original stock names, in Table 2. The complete symbols for mutants A1, A2, etc., are $c^{al\text{-}A1R1}$, $c^{al\text{-}A2R1}$, etc. In the text of the paper, we

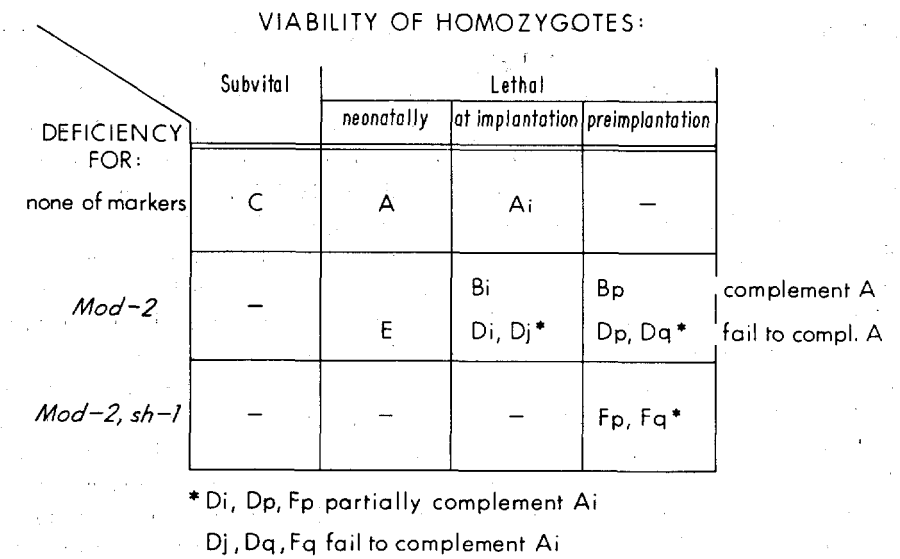

FIGURE 1.—Classification of nonviable albino mutations by involvement of nearby markers, and by developmental stage of death of homozygotes.

occasionally refer to mutants in groups A, Ai, Bi, . . . etc. as c^{A}, c^{Ai}, c^{Bi} mutants generically. In our earlier publications (RUSSELL *et al.* 1979; RUSSELL and RAYMER 1979), and occasionally in this paper, Bi and Bp mutants are jointly referred to as B; Di, Dp, Dj, and Dq mutants as D; Fp and Fq mutants as F. Ai mutants were earlier referred to as A′.

The four mutants that were not tested in the subsequent complementation grid may be tentatively assigned to groups on the basis of the matrix shown in Figure 1. Thus, mutants LA and $3YPS_D$—which are not deficient for any of the markers (Table 2) and are lethal at birth and at implantation, respectively (Table 1)—can be assigned to groups A and Ai, respectively. Mutants $2YPS_J$ and AL, which are deficient for *Mod-2* but not *sh-1*, were each crossed with one A-group and one Ai-group mutant. For $2YPS_J$, both combinations were fully viable, and this mutant is thus tentatively assigned to group Bi. With AL, both combinations yielded albinos that died perinatally, indicating that AL is probably a Di-group mutant.

B. Complementation mapping: postnatal observations

For 31 of the 35 stocks listed in Table 1 (all except $2YPS_J$, $3YPS_D$, LA, and AL), every one of the 465 possible crosses between stocks yielded information. Data were combined for the two cluster mutations (65K and 112K), resulting in 435 combinations of independent *c*-locus mutations. In addition, to provide a control for survival parameters, one or both of two viable albino mutants (62DSD and 38R145L) were crossed with 22 of the *c* lethals, for a total of 34 c^{av}/c^{al} combinations, yielding altogether 309 newborns.

In the crosses between the various stocks of nonviable *c*-locus mutants,

a pattern of four general types of results emerged. These may be summarized as follows (letting c^{*1} and c^{*2} designate any two different nonviable albino mutants). (a) Survival of the c^{*1}/c^{*2} albinos was as good as that of the c^{av}/c^{al} control, with most albinos surviving for at least 120 days; and weaning weights of c^{*1}/c^{*2} relative to littermates were no lower than in the case of controls. This pattern is referred to as "good survival," or Pattern I. (b) c^{*1}/c^{*2} albinos usually died during the second through fifth weeks, although some died neonatally, and occasional ones lived beyond the fifth week; the weaning weight of c^{*1}/c^{*2} was greatly reduced. This pattern is referred to as "poor survival," or Pattern II. (c) c^{*1}/c^{*2} albinos were stillborn, or died on the day of birth or soon thereafter ("neonatal death," or Pattern III). (d) No c^{*1}/c^{*2} albinos were observed among a large number of offspring classified "prenatal death," or Pattern IV. Results for certain individual mutants that were assigned to a group according to the criteria of Figure 1 deviated from the general pattern for the group. In some cases, the deviation was apparent rather than real (as explained in more detail in the following sections). In no case could the sporadically deviant results be fitted into a consistent pattern that would lead to reassignment of the mutant to a different complementation group, or establishment of an additional group. However, as explained in the DISCUSSION, subdivision of some of the complementation groups by criteria other than those here summarized may become possible in the future.

1. Combinations giving good survival (Pattern I): The matrix of 84 combinations that yielded "good" survival is shown in Table 3. Altogether 5566 offspring were observed at birth, and there were 1021 opportunities for albinos to

TABLE 3

Combinations giving good survival (Pattern I)

	A(5)* × Bi,Bp(11)	Ai(2) × Bi,Bp(11)
No. combinations conforming//nonconforming	53//2†	20//2‡
Offspring observed at birth	3581 [25–164]§	1480 [36–119]
Albino survival opportunities	735 [2–32]§	231 [2–22]
	A(5) × C(1)	Ai(2) × C(1)
No. combinations conforming//nonconforming	5//0	2//0
Offspring observed at birth	385 [33–134]	120 [30–90]
Albino survival opportunities	86 [4–42]	20 [7–13]

* Figure in parentheses following complementation-group symbol indicates number of independent mutations completely tested in complementation grid.

† Cross Bp8 × A3 yielded only 2.5% albinos among 80 newborns; both died before day 7. Cross Bp7 × A5 yielded 25.0% albinos; however, of 7 available for follow-up, none survived past day 56.

‡ Low proportions of albinos at birth:
Bp8 × Ai1: 8/94; all 5 available for follow-up died neonatally.
Bp8 × Ai2: 6/111; of 2 available for follow-up, one survived over 1 year.

§ Figures in brackets indicate range for individual crosses.

be scored for long-term survival; the ranges for individual crosses are given in the table. (Note that not all albinos born could be studied for long-term survival since many were killed at birth for enzyme studies; therefore the numbers given are "opportunities.")

The distribution of combinations with respect to proportion of albinos observed within 3 days after birth (Figure 2) approached a normal curve, slightly skewed to the left. The overall mean proportion of albinos at birth was 23.8%, as compared to 20.8% in control crosses yielding c^{av}/c^{al}. For 80 of the 84 combinations, fewer than 30% of albino offspring died neonatally (*i.e.*, days 0, 1, or 2); in fact, for 59 combinations, there were no neonatal albino deaths at all. The overall proportion of albinos surviving to day 120 or beyond was at least as good as among c^{av}/c^{al}, namely 66.6% (of 1021), as compared to 56.6% (of 53) in the control. Figure 3 shows the distribution of individual combinations.

Weaning weight computations, derived by paired comparisons (see MATERIALS AND METHODS), are shown in the top portion of Table 4 for four groups of Pattern-I combinations. Overall, albinos weighed about 84% as much as their

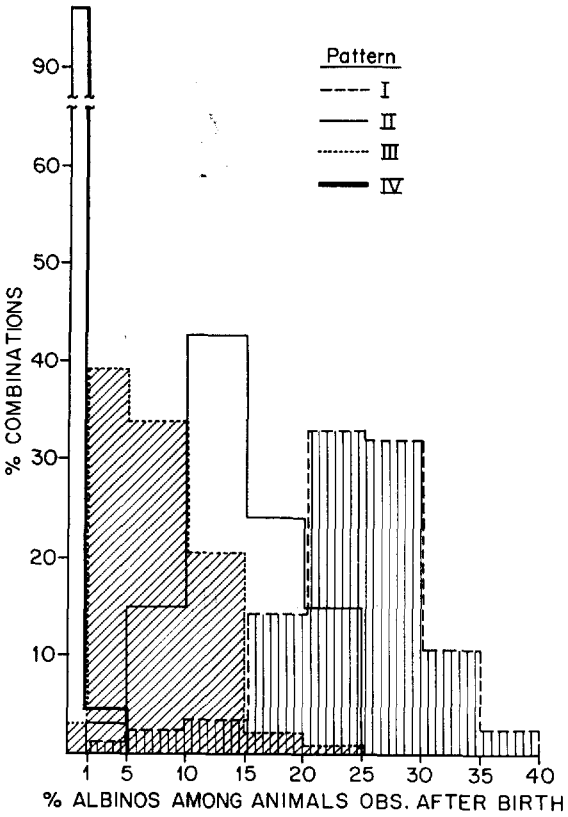

FIGURE 2.—Distribution of Pattern-I, -II, -III, and -IV combinations with respect to the frequency of albinos among animals observed on the day of birth or within 3 days thereafter.

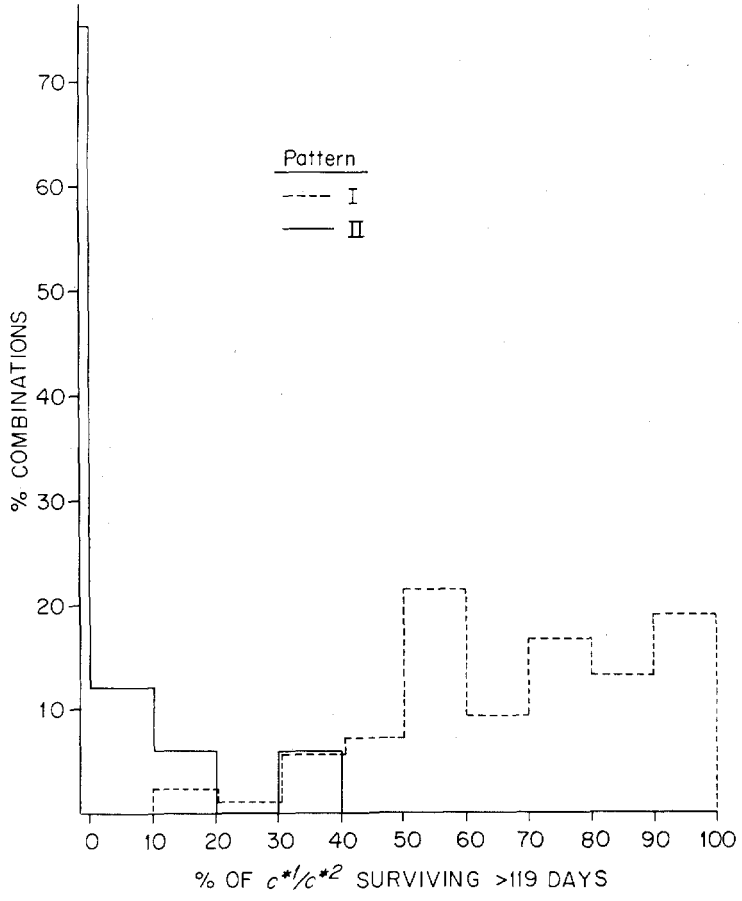

FIGURE 3.—Distribution of 84 Pattern-I and 33 Pattern-II combinations with respect to proportion of c^{*1}/c^{*2} animals surviving more than 119 days after birth.

like-sexed nonalbino littermates, and this ratio was very similar for all the separate combinations and for both sexes within them. It did not differ significantly from the control ratio, derived from paired comparisons of c^{av}/c^{al} with their like-sexed nonalbino littermates.

Four combinations that did not clearly conform to the general "good survival" pattern outlined are indicated in Table 3, with details in the footnotes. Three of these combinations had a low proportion of albinos at birth, and thus only few long-term-survival opportunities. In each of these three, one parent was Bp8; however, four other Bp8/A combinations behaved according to Pattern I, and the mutant cannot be assigned to one of the other designated complementation groups.

2. Combinations giving poor survival (Pattern II): Table 5 lists the 33 combinations which yield albinos that generally die before day 120. Altogether

TABLE 4

Relative weaning weights of albino offspring resulting from combinations of nonviable mutants

Pattern	Cross	No. animals weighed	♂♂ No. pairs*	♂♂ albino/nonalbino %	♀♀ No. pairs*	♀♀ albino/nonalbino %
I	A × Bi,Bp	1038	108	82.3	120	84.6
	A × C	162	17	86.8	19	87.7
	Ai × Bi,Bp	277	20	83.9	30	84.3
	Ai × C	6	1	70.9	1	89.9
	Total	1483	146	83.0	170	84.9
II	Bi,Bp × E	417	37	50.9	43	50.3
	C × Bi,Bp	41	1	47.9	6	47.1
	C × Di,Dj	67	4	34.4	8	40.0
	C × E	15	2	40.6	—	—
	Total	540	44	48.7	57	48.5
Control	$c^{av} \times c^{al}$	63	6	85.9	8	82.6

* A pair consists of (a) the average weight of albino males (or females) in a litter, and (b) the average weight of like-sexed c^{ch}/c^* littermates (or of like-sexed c^{ch}/c^{ch} littermates where no c^{ch}/c^* was available).

3458 offspring were observed at birth, and 374 albinos were observed for long-term survival.

The distinction between the "poor" and "good" survival syndrome is a relative one. Although in 25 of the 33 "poor" survival combinations none of the albinos lived beyond day 119 (Figure 3), some of the combinations did yield a few long-term survivors. In all of the 33 combinations, however, more than 40% of the albinos died between days 7 and 119. It should be noted (Figure 2) that a smaller frequency of albinos was observed at birth than in the case of Pattern-I combinations, where the mean was close to the expected 25% (Section B.1). In the Pattern-II combinations, the mean was 14.1% and the range from 2.2 to 22.0%, suggesting that some albinos were stillborn and eaten. It thus appears as if "poor survival," rather than clearly affecting any specific age, may decrease overall viability throughout.

Table 5 lists five combinations that fall near the edges of the range. None is a clear exception: two combinations (one each c^C/c^E and c^{Bi}/c^E) are better than average, overlapping with the tail end of the Pattern-I distribution with respect to survival past day 119; and three combinations failed to have albinos survive the neonatal period, but the opportunities were few (3 + 2 + 2).

Pattern-II albinos are clearly of reduced body size. As shown in the lower portion of Table 4, paired comparisons indicate that such albinos have weaning weights less than half as great as those of like-sexed nonalbino littermates. Most of the results come from Bi × E and Bp × E crosses, but the limited data from other Pattern-II combinations are similar.

3. Combinations producing neonatal death (Pattern III): The matrix of 97

TABLE 5

Combinations giving poor survival (Pattern II)

	C(1)*×Bi,Bp(11)	C(1)×Di,Dp(4)	C(1)×Dj,Dq(4)	C(1)×E(1)	C(1)×Fp(1)	C(1)×Fq(1)
No. of combinations conforming//not conforming	10//1†	4//0	4//0	0//1‡	1//0	0//1†
Offspring observed at birth	638[31–116]§	293[47–95]	314[45–123]	83	115	92
Albino survival opportunities	69[2–11]§	34[6–11]	37[4–23]	17	9	1
	E(1)×Bi,Bp(11)					
No. of combinations conforming//not conforming	9//1†+1‡					
Offspring observed at birth	1923[52–774]					
Albino survival opportunities	207[2–67]					

* See Table 3, footnote (*).

† Survival poorer than pattern. In crosses C1 × Bp1, C1 × Fq1, and E1 × Bp8, respectively, 8.3% of 36, 2.2% of 92, and 10.6% of 85 newborns were c^{*1}/c^{*2}. All albinos not killed at birth—3, 2, and 2, respectively—died neonatally.

‡ Survival better than pattern. In crosses E1 × Bi1 and E1 × C1, 37.5 and 35.3% albinos (of 16 and 17 opportunities, respectively) survived past day 120.

§ See Table 3, footnote (§).

TABLE 6

Combinations causing neonatal death (Pattern III)

	A(5)*×A(5)	A(5)×Ai(2)	A(5)×Di,Dp(4)	A(5)×Dj,Dq(4)	A(5)×E(1)	A(5)×Fp(1)	A(5)×Fq(1)
No. of combinations conforming//not conforming	10//0	9//1‡	20//0	20//0	4//1‡	5//0	5//0
Offspring observed at birth	601[34–118]†	749[27–116]	1562[42–128]	1335[37–109]	328[51–104]	404[61–118]	320[47–80]
			Ai(2)×Di,Dp(4)		Ai(2)×E(1)	Ai(2)×Fp(1)	
No. of combinations conforming//not conforming			8//0		2//0	2//0	
Offspring at birth			566[45–103]		240[111–129]	172[75–97]	
			E(1)×Di,Dp(4)	E(1)×Dj,Dq(4)		E(1)×Fp(1)	E(1)×Fq(1)
No. of combinations conforming//not conforming			4//0	4//0		1//0	0//1‡
Offspring observed at birth			332[58–104]	323[53–99]		85	35

* See Table 3, footnote (*).
† See Table 3, footnote (§).
‡ No albino offspring observed at birth. However, uterine-dissection experiment (Table 8 and RESULTS Section C1) indicates that albino combination survives to birth.

combinations of mutants which produce albinos that die neonatally is shown in Table 6. Altogether 7052 offspring were observed at birth, with the range for individual crosses given in the table. The proportion of albinos at birth is even smaller than in the Pattern-II combinations (Figure 2), presumably because some stillborn albinos are eaten by their mothers prior to observation. Thus, the mean was 6.6 and the range 0 to 23.2%.

Table 6 shows three apparent exceptions, *i.e.*, combinations for which no albinos were observed at birth in 35-51 offspring. However, as shown in a uterine-dissection experiment (Section C.), these crosses, too, conform to the pattern of neonatal death.

4. Combinations producing prenatal death (Pattern IV): In 221 of the 435 possible combinations of independent *c* mutants tested, the albino offspring die prenatally (Table 7). A total of 13,209 offspring was observed at birth (or soon thereafter), with the range for individual combinations listed in the table.

Among the 221 combinations, nine yielded exceptional results, namely, single albino newborns among (generally) large numbers of nonalbinos (see footnote to Table 7). These crosses were further investigated in uterine-dissection experiments (Section C.).

C. Complementation mapping: prenatal observations

Uterine-dissection experiments were done for three purposes: (1) to obtain further information for combinations that appeared to be exceptions to the neonatal-death pattern by failing to yield albino newborns; (2) to obtain information for combinations that appeared to be exceptions to the prenatal-death pattern by yielding rare albino newborns; and (3) to provide more detailed definition of the functional units in the complementation map.

(1) Presumably because of cannibalism, the average proportion of albinos observed within a few days after birth is only 6.6% for Pattern-III combinations, instead of the expected 25% (Section B3, and Figure 2). Thus, it was theoretically possible that crosses that appeared to be exceptions to a neonatal-death syndrome by failing to yield albino newborns merely represented the tail end of the distribution. For seven crosses (Table 8), the proportion of living albino fetuses was, in fact, found to fluctuate around 25% (mean, 24.2%), despite the fact that the average neonatal proportion for these seven had been only 1.5%, with three of the crosses having altogether failed to yield postnatally observable albinos (Table 6). None of these crosses are, therefore, exceptions to the neonatal-death pattern. Another apparently exceptional group of crosses involved Ai with some of the mutations in groups that had earlier (RUSSELL and RAYMER 1979) been classified merely as D and F. In these crosses, unlike in certain other Ai × D or Ai × F crosses, no living albino fetuses were found (Table 9A), and this resulted in our defining additional complementation groups Dj, Dq, and Fq (Figure 1 and Section A5). The death of c^{Dj}/c^{Ai}, c^{Dq}/c^{Ai}, and c^{Fq}/c^{Ai} animals occurs at or shortly after implantation of the embryo.

(2) Apparent exceptions to the prenatal-death pattern were found in the case of nine crosses (of 221) each of which yielded one albino newborn in an

TABLE 7

Combinations causing prenatal death (Pattern IV)

	Ai(2)* × Ai(2)			Ai(2) × Dj,Dq(4)		Ai(2) × Fq(1)
No. of combinations conforming//not conforming	1//0			8//0		2//0
Offspring observed at birth	131			600[41–101]		197[93–104]
		Bi,Bp(11) × Bi,Bp(11)	Bi,Bp(11) × Di,Dp(4)	Bi,Bp(11) × Dj,Dq(4)	Bi,Bp(11) × Fp(1)	Bi,Bp(11) × Fq(1)
No. of combinations conforming//not conforming		54//1†	41//3§	41//3‖	11//0	11//0
Offspring observed at birth		3418[22–119]‡	2803[39–88]	2864[43–105]	588[39–89]	550[27–71]
			Di,Dp(4) × Di,Dp(4)	Di,Dp(4) × Dj,Dq(4)	Di,Dp(4) × Fp(1)	Di,Dp(4) × Fq(1)
No. of combinations conforming//not conforming			6//0	15//1¶	4//0	4//0
Offspring observed at birth			229[17–57]	831[29–76]	213[37–67]	174[27–58]
				Dj,Dq(4) × Dj,Dq(4)	Dj,Dq(4) × Fp(1)	Dj,Dq(4) × Fq(1)
No. of combinations conforming//not conforming				6//0	4//0	3//1**
Offspring observed at birth				258[28–61]	162[29–50]	169[31–59]
						Fp(1) × Fq(1)
No. of combinations conforming//not conforming						1//0
Offspring observed at birth						22

* See Table 3, footnote (*).
† Bp5 × Bi1: one albino among 87 newborns; it died neonatally.
‡ See Table 3, footnote (§).
§ Bp3 × Di1, Bi1 × Di1, Bi1 × Di2, each had one albino among 88, 40, and 82 newborns, respectively; these died day 6, neonatally, and neonatally.
‖ Bp6 × Dj2, Bp7 × Dq1, Bp8 × Dq2, each had one albino among 105, 68, and 51 newborns, respectively; all died neonatally.
¶ Di1 × Dq2 had one albino among 50 newborns; it died neonatally.
** Dq2 × Fq1 had one albino among 43 newborns; it died neonatally.

TABLE 8

Results of in utero *examinations in Pattern-III crosses in which no albino newborns had been observed*

Cross	Pregnant ♀♀ (No.)	Living fetuses, total (No.)	Living fetuses lacking pigment* (%)	Embryos dead after day 9 p.c. (No.)
A2 × A4	6	36	19.4	0
A2 × Ai2	4	29	24.1	0
A2 × Di1	4	22	22.7	0
A2 × Dj2	4	24	29.2	0
A2 × E1	5	25	24.0	0
A2 × E1′	4	40	27.5	0
E1′ × Fq1†	3	6	16.7	1
Total	30	182	24.2	1

* Presumed to be c^{*1}/c^{*2}.

† In the Pattern-III cross of E1′ × Fp1, which had not failed to yield albino newborns (Table 6), the uteri of six pregnant females contained 34 living fetuses, of which 29.4% lacked eye pigment; no embryos died after day 9 p.c.

average number of 68.2 classified offspring. Two alternative situations could account for this phenomenon. (a) There is partial complementation, so that c^{*1}/c^{*2} animals in these (and perhaps other) crosses die late in fetal life, rather than early in gestation, as do the c^{*1}/c^{*1} and c^{*2}/c^{*2} types; the rare albinos then would represent those few that are not cannibalized. (b) Alternatively, there is no complementation, and c^{*1}/c^{*2} animals die early in gestation; but some mechanism exists to allow occasional animals to become exceptions to this early mortality. The results of the uterine dissection experiment are shown in Table 9B. In none of the nine crosses were any albino fetuses observed among a total of 204 living fetuses. Nor was there any evidence of albinos dying after day 9 postconception. Excess death (in comparison with controls) was found to occur either preimplantation ("pre" in the last column of the table), or at or shortly after implantation ("imp"). The c^{*1}/c^{*2} combinations therefore die well before the midpoint of gestation, and there is no partial complementation. The occasional albinos observed at birth appear to be escapees from this early death.

(3) In order to develop the complementation map with respect to functions that permit survival to certain stages in embryonic development, all mutants of the original B group (Bi and Bp) and original D group (Di, Dp, Dj, Dq) were examined in the hemizygous state. This was accomplished by combining them with $c^{al\text{-}Fp/R1}$, which is known to be deficient for the pertinent region, since it lacks *sh-1* as well as *Mod-2* and *c*. The results of the uterine dissections for these 19 crosses are shown in Table 9C.

For these crosses, as well as for those listed in Tables 9A and 9B, to determine at what stage of development an excess loss was occurring, the results for each combination were compared with the average values for the $c^*/c^{ch} \times c^{ch}/c^{ch}$ crosses of the two parental mutants (RUSSELL and RAYMER 1979, Table

TABLE 9

Results of in utero *examinations in crosses between lethal albino mutants*

Cross*	No. c.l.†	Living fetuses		Dead after day 9 p.c.	Moles		Preimpl. loss§		Time of death‖	
		Pigment	No pigment		Combin.	Contr. av.‡	Combin.	Contr. av.‡	Combin.	Parental homoz.¶
A										
Ai1 × Dj1	42	47.6	0	0	38.1	(7.7)	14.3	(15.7)	imp	imp, imp
Ai1 × Dj2	28	42.9	0	3.6	32.1	(9.0)	21.4	(15.3)	imp	imp, imp
Ai1 × Dq1	45	51.1	0	0	33.3	(8.4)	15.6	(8.4)	imp	imp, pre
Ai1 × Dq2	31	38.7	0	0	45.2	(7.5)	16.1	(17.3)	imp	imp, pre
Ai1 × Fq1	41	46.3	0	0	43.9	(12.9)	9.8	(16.4)	imp	imp, pre
Ai2 × Dj2	30	33.3	0	0	23.3	(11.5)	43.3	(15.7)	imp+pre	imp, imp
Ai2 × Dq1	41	65.8	0	2.4	22.0	(10.9)	9.8	(8.8)	imp	imp, pre
Ai2 × Dq2	38	42.1	0	0	39.5	(10.0)	18.4	(17.7)	imp	imp, pre
Ai2 × Fq1	42	47.6	0	2.4	35.7	(15.4)	14.3	(16.8)	imp	imp, pre
B										
Bi1 × Bp5	40	52.5	0	0	32.5	(21.3)	15.0	(16.8)	imp	imp, pre
Bi1 × Di1	39	56.4	0	0	28.2	(12.7)	15.4	(15.5)	imp	imp, imp
Bi1 × Di2	39	66.7	0	0	20.5	(12.2)	12.8	(19.4)	imp	imp, imp
Bp3 × Di1	57	66.7	0	0	19.3	(10.7)	14.0	(13.4)	imp	pre, imp
Bp6 × Dj2	43	65.1	0	2.3	25.6	(12.9)	7.0	(18.1)	imp	pre, imp
Bp7 × Dq1	33	54.5	0	0	0	(11.1)	45.5	(10.4)	pre	pre, pre
Bp8 × Dq2	42	50.0	0	4.8	11.9	(18.4)	33.3	(14.9)	pre	pre, pre
Di1 × Dq2	53	50.9	0	0	32.1	(8.8)	17.0	(20.7)	imp	imp, pre
Dq2 × Fq1	13	23.1	0	7.7	7.7	(16.2)	61.2	(25.2)	pre	pre, pre

TABLE 9—Continued

Cross*	No. c.l.†	Living fetuses: Pigment	Living fetuses: No pigment	Dead after day 9 p.c.	Moles: Combin.	Moles: Contr. av.‡	Preimpl. loss§: Combin.	Preimpl. loss§: Contr. av.‡	Time of death‖: Combin.	Time of death‖: Parental homoz.¶
C										
Fp1 × Bi1	115	57.4	0.9	0.9**	24.3	(18.1)	16.5	(15.7)	imp	pre, imp
Fp1 × Bi2	44	61.4	0	0	29.5	(13.8)	9.1	(16.4)	imp	pre, imp
Fp1 × Bi3	50	48.0	0	0	30.0	(18.9)	22.0	(12.6)	imp?	pre, imp
Fp1 × Bp1	37	51.4	0	0	21.6	(20.0)	27.0	(13.9)	pre	pre, pre
Fp1 × Bp2	39	55.3	0	0	7.7	(12.3)	38.5	(16.2)	pre	pre, pre
Fp1 × Bp3	36	55.6	0	0	2.8	(16.2)	41.7	(13.6)	pre	pre, pre
Fp1 × Bp4	45	60.0	0	0	15.6	(17.0)	24.4	(12.6)	pre	pre, pre
Fp1 × Bp5	40	42.5	0	0	22.5	(20.9)	35.0	(16.8)	pre	pre, pre
Fp1 × Bp6	83	60.2	0	0	16.9	(14.9)	22.9	(14.8)	pre	pre, pre
Fp1 × Bp7	45	46.7	0	0	11.1	(13.7)	42.2	(14.1)	pre	pre, pre
Fp1 × Bp8	42	61.9	0	0	7.1	(21.8)	31.0	(9.6)	pre	pre, pre
Fp1 × Di1	59	64.4	0	1.7	22.0	(12.3)	11.9	(15.5)	imp	pre, imp
Fp1 × Di2	57	43.9	0	1.8	36.8	(11.8)	17.5	(19.3)	imp	pre, imp
Fp1 × Di3	77	66.2	0	0	23.4	(18.5)	10.4	(17.9)	imp?	pre, imp
Fp1 × Dj1	67	65.7	0	1.5	17.9	(14.5)	14.9	(19.3)	imp?	pre, imp
Fp1 × Dj2	31	54.8	0	3.2	32.3	(15.7)	9.7	(18.9)	imp	pre, imp
Fp1 × Dp1	34	44.1	0	0	11.8	(14.8)	44.1	(16.9)	pre	pre, pre
Fp1 × Dq1	40	57.5	0	0	10.0	(15.1)	32.5	(12.0)	pre	pre, pre
Fp1 × Dq2	57	56.1	0	0	5.3	(14.3)	38.6	(20.9)	pre	pre, pre

* Crosses are grouped as follows: *Table 9A*, Ai combinations that are prenatally lethal. *Table 9B*, Pattern-IV combinations that had yielded occasional albino newborns. *Table 9C*, combinations of the long deficiency, Fp1, with Bi, Bp, Di, Dp, Dj, and Dq-group mutants.

† c.l. = corpora lutea, taken as indicative of number of ovulations. All other numbers (living fetuses, dead embryos, moles, preimplantation loss) are expressed as percentage of corpora lutea.

‡ Computed as the average value for the $c^{+}/c^{ch} \times c^{ch}/c^{ch}$ crosses of the two parental mutants (RUSSELL and RAYMER 1979).

§ Number of corpora lutea minus total implants (living, dead, and moles).

‖ pre = before implantation; imp = at or shortly after implantation.

¶ From RUSSELL and RAYMER 1979.

** One embryo, which died about day 13, had no eye pigment.

1 control matings). The death stage of the combination, c^{*1}/c^{*2} was then compared with the death stages of the parental homozygotes, c^{*1}/c^{*1} and c^{*2}/c^{*2} (RUSSELL and RAYMER 1979; Table 1, last column). These comparisons indicate that the "imp" type of death behaves as if dominant to the "pre" type of death, *i.e.*, "imp" × "imp" and "imp" × "pre" crosses produce "imp" combinations, while "pre" × "pre" crosses produce "pre" combinations. Of the 37 crosses analyzed in this manner, 33 clearly conformed to this pattern, three probably conformed, and one gave inconclusive results.

DISCUSSION

The abnormal phenotypes utilized in the complementation study were deaths at various stages of development (preimplantation, at or shortly after implantation, neonatal, juvenile), weaning weights, the reduction or absence of various enzymes (mitochondrial malic enzyme, glucose-6-phosphatase, and tyrosinase as surmised by pigment condition), and the uncovering of recessive markers. The mutant groupings that were originally based on involvement of certain nearby markers and death time of homozygotes (see matrix, Figure 1) held up in the full-scale complementation tests. It is possible to postulate an alignment of functional units by which all analyzed *c*-locus mutations fit a linear pattern (Figure 4). This was not so for *d*- and *se*-region mutations, a few of which could not be fitted into a linear map (RUSSELL 1971).

Mapping against previously known markers (*Mod-2*, *sh-1*, and *tp*) indicates that 24 of the 34 independent nonviable *c*-locus mutations studied must be deficiencies. That many of the mutants were deficient for *Mod-2*, and two of them for *sh-1* as well as *Mod-2*, was already known from earlier studies (RUSSELL, DEHAMER and BORMAN 1974; RUSSELL and CACHEIRO 1977; BERNSTINE, RUSSELL and CAIN 1978). In accordance with mouse genetic nomenclature rules, these deficiencies may be designated Df(7)*c Mod-2* or Df(7)*c Mod-2 sh-1*, each followed by the symbol for the individual mutant (Table 2, column 1).

Groups A, Ai, and C, containing altogether ten of the nonviable mutants, are not deficient for any previously mapped marker. There are, however, several reasons for concluding that these mutants too are deficiencies [Df(7)*c*]. The functional unit for G6Pase and neonatal survival can be complemented separately from that for pigment. If that functional unit represented a site within the *c* locus, it would have to be supposed from the large number of A-Bi, A-Bp, A-C, Ai-Bi, Ai-Bp, Ai-C complementations that many other sites in that locus could be lost without disturbance of the G6Pase and neonatal-survival functions; whereas the loss of any of a large number of other sites within the locus would lead to loss of tyrosinase function. This is unlikely. A similar argument leads to the conclusion that the juvenile-survival function is probably controlled by a site outside, rather than within, the *c* locus.

The functions for G6Pase and neonatal survival have not been separated. Of 97 combinations of independent mutations that produce neonatal lethality

(Table 6), 70 were tested by DEHAMER (1975); all were severely deficient for G6Pase. Of 117 combinations giving good or poor postnatal survival (Tables 3 and 5), 87 were tested by DEHAMER, and all of these were found to have G6-Pase activity within the range of normal littermates. On a more limited scale, this nonseparation is also seen in the results of GLUECKSOHN-WAELSCH (1979) who described altogether ten combinations of five independent *c*-locus mutations (including two used in the present study). Three of the six neonatally lethal combinations were tested for G6Pase and found to be low; all three of the combinations that gave good or poor survival (by our terminology) were normal with respect to G6Pase. For the six tested combinations, the abnormality, or normality, respectively, of two other liver-specific enzymes (tyrosine aminotransferase and serine dehydratase), of serum protein, and of the structure of subcellular membrane organelles paralleled the status of G6Pase (GLUECKSOHN-WAELSCH *et al.* 1974).

While possible causes of the neonatal death have thus been identified, the other types of death used as phenotypes in our complementation mapping result from functional disturbances that are yet to be elucidated. The two separable sites controlling implantation survival (Figure 4) presumably govern distinct developmental processes. Homozygotes for a mutation that is deficient for one of these sites exhibit abnormalities of the ectoplacental cone and parietal endoderm detectable days 6½–7 of gestation (LEWIS, TURCHIN, and GLUECKSON-WAELSCH 1976).

No specific cause is known for "juvenile death" and associated runtiness. We have shown for 33 combinations of independent mutants (Section B-2 and Figures 2 and 3) that there is no sharp age demarcation for this mortality, but that viability, in general, is decreased between birth and about 4 months of age, by which time virtually all Pattern-II death has occurred. Body size is also markedly reduced. The two shortened-lifespan combinations studied by GLUECKSOHN-WAELSCH *et al.* (1974), probably fit this same pattern; both were also infertile. (Fertility was not tested in our 33 combinations.)

The juvenile-survival function is separable from the MOD-2 function by virtue of the C-group mutant $c^{as\text{-}c1Rl}$ which is *non*deficient for *Mod-2*. Combinations of $c^{as\text{-}c1Rl}$ with 22 independent *Mod-2*-deficient *c*-lethal mutations (*i.e.*, combinations possessing one dose of MOD-2, such as is known from other evidence to be compatible with full survival) produce a Pattern-II phenotype that is indistinguishable from that of MOD-2-lacking E-Bi or E-Bp combinations (Table 5). Had it not been for this finding, one could have concluded that poor survival and runtiness might be the result of a zero-dose of MOD-2. An earlier suggestion (ERICKSON, EICHER, and GLUECKSOHN-WAELSCH 1974) that mitochondrial malic enzyme might be essential in embryonic development was disproved by the finding that the neonatally-lethal cluster mutations 65K-112K (Group E) are deficient for *Mod-2*, and that E-B combinations can survive at least to weaning age (RUSSELL, DEHAMER and BORMAN 1974; BERNSTINE, RUSSELL and CAIN 1978). The C-group-combination results now remove the

need for assuming that mitochondrial malic enzyme influences survival at any age.

Thus, of the many phenotypes that have been found associated with the *c*-locus mutants, some can and others cannot be separated by complementation. Altogether, eight separable functional units have been identified, which control the following phenotypes: (1) implantation (or early postimplantation) survival; (2) neonatal survival, G6Pase, certain other enzymes, serum proteins, membrane organelles; (3) tyrosinase [*c* locus]; (4) juvenile survival, normal size, and fertility; (5) mitochondrial malic enzyme [*Mod-2* locus]; (6) implantation (or early postimplantation) survival, normal ectoplacental cone, parietal endoderm; (7) preimplantation survival; (8) labyrinth of ear [*sh-1* locus]. The most proximal of the functional units is distal to *tp*, and the most distal is at the *sh-1* locus. Thus, two, one, and two units have been identified between *tp* and *c*, between *c* and *Mod-2*, and between *Mod-2* and *sh-1*, respectively.

Our mutants can be placed into 13 groups (including viables) which span between one and eight of these functional units. By virtue of the method for detecting these mutants (RUSSELL, RUSSELL and KELLY 1979), all groups span the unit for tyrosinase (pigment). Five of the 13 groups contain but a single independent mutant; and one group of lethals, Bp, has as many as eight mutants. In examining results reported by GLUECKSOHN-WAELSCH (1979), it appears that Harwell mutants c^{5H}, c^{6H} and c^{25H} fit into our groups E, Bi, and Dp (or Dq), respectively. (The other two independent mutants used by her, c^{14Cv8} and $c^{65,112K}$, came from Oak Ridge and are A and E group, as shown in the present paper.)

All nonviable groups of *c* mutants are considered deficiencies which, for the most part, act as recessives with respect to the stated function they eliminate. Thus, in reading Figure 4, two lines (deficiencies) must overlap at a given function before that function appears to be affected by the combination of mutations. The exceptions are (a) the MOD-2 function, which is presumably halved by deficiency in only one of the chromosomes (although the direct evidence for this conclusion comes from $c^*/+$, rather than c^{*1}/c^{*2}, genotypes), and (b) possibly the tyrosinase function (unpublished results of SCHIFFMAN, quoted by GLUECKSOHN-WAELSCH 1979), although, with respect to visible pigment, the deficiencies here too act recessively. In the case of the G6Pase deficiency and associated defects, GLUECKSOHN-WAELSCH (1979) has taken the absence of a dosage effect as evidence for the involvement of regulatory gene(s). It would be premature to draw similar conclusions concerning the other functions that appear unaffected by a single deficiency.

Undoubtedly, many additional functional units are identifiable for the 6- to 11-cM-long region of Chromosome 7 that is covered by the various deficiencies. (A recombinational length of 1cM can contain a large number of "typical" genes coding for polypeptides.) Additional functional units could be defined (a) by future inclusion of new mutants in the complementation grid,

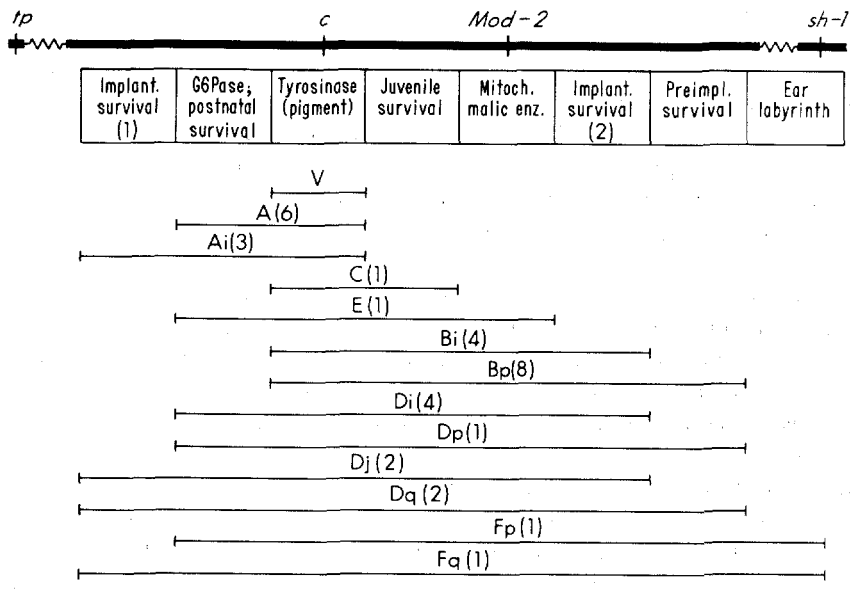

FIGURE 4.—Complementation map of *c*-locus mutants. With postulated functional units (shown in boxes below the genetic map), all mutants grouped by the criteria of Figure 1 fit a linear pattern. The number shown in parentheses following each complementation-group designation indicates the number of independent Oak Ridge mutations in the group. In addition, Harwell mutants c^{3H}, c^{6H}, and c^{25H}, can probably be added to groups E, Bi, and Dp (or Dq), respectively (see text). "V" indicates viable albino mutants of which there were 52 among 90 nonmosaic *c*-locus mutations found in the progeny of irradiated mice.

or (b) by studies of more detailed functions in the existing grid. For example, by means of embryological studies, it was possible to separate prenatal survival into "implantation survival 1", "implantation survival 2", and "preimplantation survival" (Figure 4); and the original B, D, and F groups (RUSSELL and DEHAMER 1973) could be divided into Bi, Bp, Di, Dj, Dp, Dq, Fp, and Fq groups. Studies of biochemical, morphological, and developmental characters would probably also produce finer subdivisions. The finding that E-group homozygotes can have kidney and thymus abnormalities (ERICKSON, GLUECKSOHN-WAELSCH and CORI 1968) could lead to definition of a new functional unit depending on which, if any, of E-Ai, E-C, or E-Bi combinations showed these phenotypes.

It should be noted, however, that there is a limit to the definition of further functional units distal to "preimplantation survival" when *c*-locus deficiencies are used in complementation studies: any combinations involving such sites would also be homozygous deficient for more proximal regions and thus die before implantation, greatly limiting the phenotypes that could be observed. There may thus be hidden heterogeneity within the Bp and Dp categories, possibly accounting for the fact that there are more mutants in one of these groups (Bp) than in any other.

It may be particularly interesting to look for further functional units between the *c*-locus and the neonatal and juvenile survival units. With the level of observation used in the present investigation, there were no detectable phenotypes in about 80 distinct fully-complementing (Pattern-I) combinations. If more detailed studies also fail to reveal such phenotypes, one may conclude that the sites controlling the neonatal and juvenile survival functions (Figure 4) are either very close to the *c* locus, or are separated from it by noncoding DNA.

Since overlapping deficiencies can produce viable albino animals that resemble the standard *c/c* type, it may be concluded that the *c* locus contains no sites essential for survival. In analogy with the argument that has been made for the *rosy* locus in Drosophila (CHOVNIK, GELBART and MCCARRON 1977), the *c* locus is presumably not part of a complex involving adjacent units. While the present investigation has concerned itself only with nonviable mutations, the majority of radiation-induced mutations involving the *c* locus are in fact homozygous viable, namely, 52 (=57.8%) of the 90 independent nonmosaic *c*-locus mutations found in the progeny of an irradiated parent (RUSSELL, RUSSELL and KELLY 1979). Such viable mutations could be (a) deficiencies extending beyond the *c* locus on either side or both, but not far enough to involve the already identified functional units, or (b) deficiencies involving only the *c* locus or portions of it, or (c) site mutations with the *c* cistron. Since overlapping deficiencies (*i.e.*, total absence of the locus) produce a viable albino phenotype, the easiest explanation for viable *non*albino (*i.e.*, "intermediate," or c^{x}) alleles is that they are the result of mutations within the *c* cistron. Whether a different site, or sites, can mutate to the null state, or whether such nulls are deficiencies of a major part of, or the whole locus, could be determined by recombination experiments of a probably impractical magnitude.

In that connection, it may be noted that the rare exceptional albino survivors to birth of combinations of deficiencies that normally kill prior to the midpoint of intra-uterine life (see Pattern IV, Table 7) are unlikely to be the result of simple recombinational events. Only two of the nine exceptions could be explained in such a manner, one each from crosses of $c^{ch}/c^{al\text{-}Bp} \times c^{ch}/c^{al\text{-}Di}$ and $c^{ch}/c^{al\text{-}Di} \times c^{ch}/c^{al\text{-}Dq}$. In the first case, a crossover could have occurred between the normal (c^{ch}) chromosome and the $c^{al\text{-}Bp}$ deficiency in the region between *c* and the functional unit for "implantation survival 2" (Figure 4), yielding in combination with $c^{al\text{-}Di}$ an albino poor survivor. The second case could be explained by a crossover in the same region of either parent, yielding a neonatally-lethal albino. However, none of the other seven cases fits into similar simple recombinational explanations. There is also no evidence for simple "leakiness" of the mutations in that the exceptionals do not merely represent the tail end of a spread in death times but are clear escapees from a very much earlier death.

The characterization, here accomplished, of an array of deficiencies involving several loci with established functions (and others with as yet unknown functions), and the existence of other rearrangements for that region (RUSSELL

et al. 1975; RUSSELL and CACHEIRO 1978) provide possible tools for future research. The system can, for example, be manipulated to furnish dosage series and *cis-trans* comparisons, and should also prove useful in recombinant-DNA studies designed to provide a fine-structure analysis of portions of the mouse genome. Such studies have been initiated RUSSELL and BERNSTINE 1981).

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