# ANALYSIS OF THE ALBINO-LOCUS REGION OF THE MOUSE. I. ORIGIN AND VIABILITY*

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ABSTRACT

Numerous specific-locus experiments designed to test the mutagenic effect of external radiation have yielded, in over 3,600,000 animals observed, altogether 119 presumed mutations involving the *c* locus. Of these, 55 were viable and albino ($c^{av}$), 13 were viable and of various intermediate pigment types ($c^{xv}$), four were subvital ($c^{as}$ and $c^{xs}$), seven were neonatally lethal albinos ($c^{al}$), 28 prenatally lethal albinos ($c^{al}$); 12 died untested. All of the prenatally lethal and at least one of the neonatally lethal *c*-locus mutations ($c^{al}$ classes) are probably deficiencies that we have analyzed extensively in other experiments. Since absence of the locus mimics albino in phenotype, the intermediates ($c^{xv}$ and $c^{xs}$ groups) probably resulted from intragenic changes. The class of viable albino mutants ($c^{av}$) might include, in addition to intragenic changes, some extremely small deficiencies.—The effects on viability of *c*-locus lethals ($c^{al}$'s) in heterozygous condition are not drastic enough to be perceived in stocks of mixed genetic background except in the case of the two longest known deficiencies and a few others.—Analysis of the relation between radiation treatment and type of *c*-locus mutants obtained shows that the relative frequency of viable mutations, for each germ-cell type, is greater for low-LET than for neutron irradiation; however, the difference for any individual cell type is not significant. The majority (66.7%) of mutations derived from X- or $\gamma$-ray irradiated spermatogonia are viable, and the proportion of "intermediates" among these viables is similar to that among presumed spontaneous *c*-locus mutations. No significant dose-rate effect on the proportion of lethals could be demonstrated within the set of mutants induced by low-LET irradiation of spermatogonia. Although sets from other germ-cell stages are too small for statistical tests, the results for oocytes are similar, as far as they go. Furthermore, most of the *c*-locus mutations induced in spermatogonia, even by high-dose-rate X-ray or $\gamma$ irradiation, are of a type most likely to result from single-track events (62% $c^{xv}$, $c^{xs}$, and $c^{av}$; plus 16% presumed deficiencies not involving the closest marker). These results support the view that most of the reduction in mutation frequency at low dose rates is not due to a change in relative proportion of two-track and one-track ionizing events.

SPECIFIC-LOCUS mutation-rate experiments that have been in progress at our laboratory for many years (W. L. RUSSELL 1951) have yielded large numbers of mutations involving one (or, occasionally, two) of seven loci located

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in five linkage groups. In addition to supplying quantitative information on mutation rates associated with various radiation or chemical treatments, the mutants provide valuable material for qualitative studies on the nature of induced and spontaneous mutations in the mouse. Such studies have two objectives: (1) to shed light on the genetic organization of the mouse through detailed exploration of small chromosomal regions; and (2) to illuminate any possible relationships between the variables of the mutagenic treatment and the type of mutations induced.

Two of the seven loci at which recessive mutations can be detected in our specific-locus experiments—dilute, *d*, and short-ear, *se*—are very closely linked (0.1% recombination, GREEN 1966), and 102 mutations that involved either one or both of them have been analyzed by complementation and deficiency mapping (L. B. RUSSELL 1971). Two other specific loci, albino, *c*, and pink-eye *p*, are more loosely linked (16% recombination in females, 12% in males, GREEN 1966). The albino locus and mutations recovered at it present a number of features that make analysis of mutants particularly interesting, as well as convenient: (1) most new *c*-locus mutations are not repeats of the $c^{ch}$ allele that is used as marker in the cross (see below), so that they can be separated with certainty from the marker; (2) loci closely linked to *c* on both sides are known, which facilitates deficiency mapping; and (3) the *c*-locus and neighboring regions are involved in several known rearrangements, such as reciprocal *X-7* translocations (L. B. RUSSELL and BANGHAM 1960; L. B. RUSSELL and MONTGOMERY 1970), an *X* insertion (CATTANACH 1961), and a tandem duplication (L. B. RUSSELL *et al.* 1975).

The present investigation concerns *c*-locus mutants recovered in the course of a large number of specific-locus experiments carried out over a period of several years to test the mutagenic effect of various types of externally administered radiations. Mutations induced by internal radiation emitters or by chemicals are not included. The mutations have been analyzed (1) according to their origin; (2) with respect to their viability in heterozygous and homozygous condition; (3) by means of deficiency and complementation mapping; (4) for their biochemical effects (DEHAMER 1975; BERNSTINE, RUSSELL and CAIN 1978); and (5) for their cytological properties. Brief reports of several of these studies have been published as abstracts (L. B. RUSSELL *et al.* 1972; L. B. RUSSELL and DEHAMER 1973; L. B. RUSSELL, DEHAMER and BORMAN 1974; L. B. RUSSELL and CACHEIRO 1977). Two of our *c*-locus mutations (in three stocks) and three others have been studied by GLUECKSOHN-WAELSCH and her associates (ERICKSON, GLUECKSOHN-WAELSCH and CORI 1968; ERICKSON, EICHER and GLUECKSOHN-WAELSCH 1974; TRIGG and GLUECKSOHN-WAELSCH 1973; GLUECKSOHN-WAELSCH *et al.*, 1974). The present paper presents information on the external phenotype and viability of 119 independent *c*-locus mutants produced in our laboratory, and relates these findings to the radiation history of the mutants. The second paper of the series presents certain special features of one subset of these 119 mutants, namely 16 mosaics, or fractionals (L. B. RUSSELL 1979). The third paper (L. B. RUSSELL and RAYMER 1979) describes embryological findings

on the time of death of the prenatally lethal *c*-locus mutants. Subsequent publications will establish the existence of several complementation groups and describe their properties.

## MATERIALS AND METHODS

All but three of the 119 independent *c*-locus mutations mentioned in this paper were derived from experiments in which genetically uniform homozygous wild-type mice [(101/Rl × C3H/Rl)$F_1$ or (C3H/Rl × 101/Rl)$F_1$] were mated to the noninbred T stock, which is homozygous recessive at seven marker loci, including the *c* locus (*a/a*; *b/b*; $c^{ch}p/c^{ch}p$; *d se/d se*; *s/s*—*a* = nonagouti; *b* = brown; $c^{ch}$ = chinchilla; *p* = pink-eyed dilution; *d* = dilute; *se* = short-ear; *s* = piebald spotting). Mutations at any of these loci are detected by external examination in first-generation offspring from such a cross. The remaining three *c*-locus mutants came from crosses in which the $c^{ch}$ marker was introduced by a stock other than T.

If the wild-type allele at the *c*-locus (to be referred to as $+^c$, or *C*) has mutated, pigmentation of the hairs, and usually of the eyes as well, is reduced. Since the mutation is recovered in combination with the intermediate allele, $c^{ch}$, mutant animals heterozygous for new amorphs resemble the "gray" phenotype of $c^{ch}/c$ (hair base, café-au-lait; subterminal band, cream; eye-pigment ring in newborns, narrower than in wild type). Mutant animals carrying new intermediate alleles can have darker hair and/or eyes than $c^{ch}/c$ (Figure 1). The phenotype of homozygotes is described under RESULTS.

Some of the standard genotypes that will be referred to in the course of the description of breeding tests have the following phenotypes: *C/–*, black eyes, black body hairs (with yellow subterminal bands and yellowish belly if the mouse also carries certain agouti alleles), yellow pinna hairs; $c^{ch}/c^{ch}$, black eyes, sepia body hairs (with cream subterminal bands and cream belly in case of certain agouti backgrounds), cream pinna hairs; $c^{ch}/c$, ruby eyes, café-au-lait body hairs (with ivory subterminal bands and ivory belly in case of certain agouti backgrounds); *c/c*, pink eyes, white hairs everywhere. The genetic map of the portion of chromosome 7 that contains the *c*-locus and other nearby loci to be mentioned in this paper is shown in Figure 2.

The *c*-locus mutants were found in 54 experimental series in which the wild-type parent in the treated groups received external irradiation of various types (X ray, gamma ray, or neutron) in various dose distributions (acute, protracted, or fractionated by a variety of regimes) during

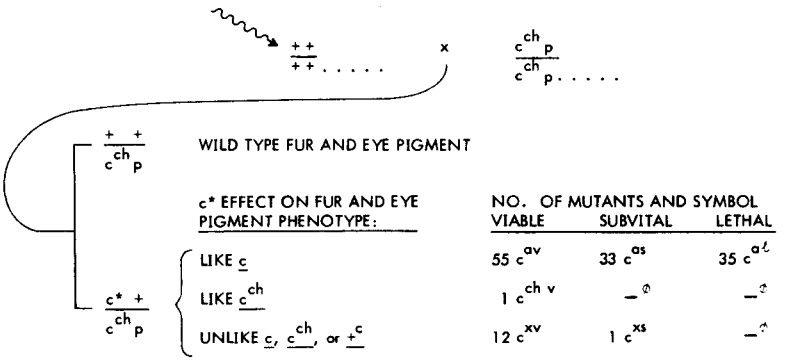


FIGURE 1.—Detection and classification of *c*-locus mutants. The wavy arrow indicates the irradiated parent; the dots following the chromosome-7 markers indicate that other marked loci are not shown in this diagram.

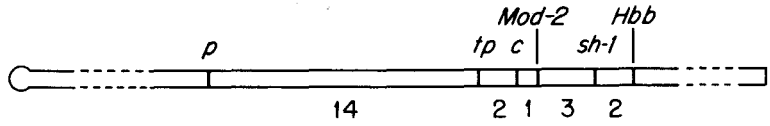


FIGURE 2.—Genetic map for a portion of chromosome 7. Recombination frequencies shown below chromosome. *p*, pink-eyed dilution; *tp*, taupe; *c*, albino; *Mod-2*, mitochondrial malic enzyme; *sh-1*, shaker-1; *Hbb*, hemoglobin β-chain.

certain germ-cell stages (spermatogonia, spermatocytes, spermatids, spermatozoa, maturing or mature oocytes). Altogether 2,292,547 $F_1$ animals were screened in these experiments. An additional 65 experimental series in which 1,330,267 $F_1$ individuals were screened failed to yield *c*-locus mutations. Mutation-rate data for many, but not all, of these various experiments have been published (W. L. RUSSELL 1963, 1965a,b, 1967, 1968, 1972, 1977).

If the phenotype of a presumed mutant indicated that the animal might be $c^*+/c^{ch}p$ (where $c^*$ is any mutation involving the *c*-locus), testing proceeded as follows. Except for the case where $c^*$ might be a repeat mutation to $c^{ch}$ (see below), the proband was mated to $c^{ch}/c^{ch}$, and as many progeny as practicable were collected to establish allelism, as indicated by the recovery of only $c^*/c^{ch}$ and $c^{ch}/c^{ch}$ offspring. The former segregants were again mated to $c^{ch}/c^{ch}$ to establish a stock. After three generations of continued outcrossing to $c^{ch}/c^{ch}$ (when the chance of having retained *p* is small), $c^*/c^{ch}$ animals were mated *inter se* to determine viability and phenotype of $c^*/c^*$ homozygotes, with observations beginning at the time of birth. The presumed $c^*/c^*$ obtained were test-mated with *c/c*. (Note: this tests the possibility that the presumed $c^*/c^*$ animals could, in fact, be $c^*p/c^{ch}p$ which is white, or nearly white, and pink-eyed, as are most of the viable $c^*/c^*$'s). Where $c^*/c^*$ was viable and fertile, the stock was maintained in homozygous condition; where it was lethal, subvital, or poorly fertile, the stock was maintained by continued outcrossing of $c^*/c^{ch}$ to $c^{ch}/c^{ch}$. Data derived from $c^*/c^{ch} \times c^{ch}/c^{ch}$ and $c^*/c^{ch} \times c^*/c^{ch}$ crosses during the various stock-keeping and testing procedures supply information concerning the relative viability of *c*-locus mutations in the heterozygous and homozygous states.

If it appeared, either on the basis of phenotype or as a result of the initial cross described above, that the mutant might be "$c^{ch}$"$+/c^{ch}p$ (where "$c^{ch}$" is a repeat mutation to $c^{ch}$), the proband was mated to $+^c p/+^c p$. Six wild-type sons (*ca.* 86% of which are expected to be "$c^{ch}$"$+/+ p$, and *ca.* 14% $c^{ch}+/+ p$, if there is no selection against "$c^{ch}$") were then again outcrossed to $+^c p/+^c p$, and subsequently mated to those of their own wild-type daughters ($F_2$ of the proband) that had been tested to carry "$c^{ch}$" (or $c^{ch}$). Such backcross matings are thus expected to be "$c^{ch}$"$+/+ p$ *inter se* (*ca.* 86% of the lines), or $c^{ch}+/+ p$ *inter se* (*ca.* 14% of the lines). If each one of the six lines derived from a given mutant yielded chinchilla in such a cross, the mutation was assumed to be a viable repeat of $c^{ch}$, since the probability that all six derive from $c^{ch}+/+ p$ recombinant sons of the mutant is only $(0.14)^6$ or 0.000008.

It is occasionally necessary in this paper to refer to mutant groupings that have been established on the basis of complementation and deficiency mapping. Briefly, properties of these groups may be summarized as follows on the basis of deficiency for adjacent loci (L. B. RUSSELL and DEHAMER 1973; RUSSELL, DEHAMER and BORMAN 1974; DEHAMER 1975; L. B. RUSSELL and CACHEIRO 1977; BERNSTINE, RUSSELL and CAIN 1978) and time of death of homozygotes (this paper; and L. B. RUSSELL and RAYMER 1979): not deficient for *Mod-2*, groups C, A, A′, with homozygotes dying, respectively, early postnatally, neonatally, and prenatally; deficient for *Mod-2*, groups E, B. D, with homozygotes dying neonatally in the case of E, and prenatally in the case of B and D, which are further distinguishable because B, unlike D, complements A for mortality; deficient for *Mod-2* and *sh-1*, group F.

## RESULTS

*Types of mutations and their origin*: Of 119 independent presumed *c*-locus mutations scored, 107 were fully tested for allelism and homozygous viability.

Of the remaining 12, one was tested for allelism only, four died before they were old enough to reproduce, and seven were not mated. The 107 tested mutations may be subdivided (see Figure 1) on the basis of (1) viability in the homozygous condition (viable, subvital, and neonatally or prenatally lethal—designated with *v*, *s*, and *l*, respectively, as part of their superscript), and (2) whether, with respect to pigment phenotype in various combinations, they mimicked the "albino" allele (mutants designated with "*a*" as part of their superscript) or were intermediate between *c* and *C* (designated with "*ch*" or "*x*" as part of their superscript). The intermediate type was not found among *c*-locus lethals.

The number of mutants in the various viability-pigment classes is shown in Figure 1. The lethal class ($c^{al}$) was further subdivided into seven neonatal lethals (homozygotes either stillborn, or liveborn but not surviving beyond the second day after birth), and 35 prenatal lethals (no evidence for homozygotes ever being born). More extensive evidence on this classification is presented by RUSSELL and RAYMER (1979). Table 1 shows the origin of all *c*-locus mutants according to their radiation history. Three of the 119 mutations, all derived from irradiated spermatogonia, were recovered as clusters of two, two, and three, respectively.

The $c^{av}$ class appears to be relatively homogeneous. Because of the gradual loss of heterosis as the age of a stock increases, and because the different stocks have widely varying genetic backgrounds, quantitative comparisons between $c^{av}$ stocks is not very meaningful. The $c^{x}$ classes are composed of mutants having various phenotypes. Only one of these mutants is a repeat of $c^{ch}$. Another resembles $c^{ch}$, but, in combination with *c*, often produces darker than $c^{ch}/c$ hairs at the tip of the snout and base of the tail. Two (including the $c^{xs}$ mutant) are intermediate between $c^{ch}$ and wild type. One resembles *c* except for the occurrence of occasional dark spots in both $c^{x}/c^{x}$ and $c^{x}/c$ animals. The remainder are of various degrees of intermediacy between *c* and $c^{ch}$ with respect to hair and/or eye pigment, some having Himalayan features, others occasional dark patches.

Only four of the 107 tested *c*-locus mutations have been classified as "subvital," on the basis of rather heterogeneous criteria. Many animals homozygous for the mutations 20FATw (induced by X rays in oocytes) or 19R75H (induced by neutrons in spermatogonia) die between birth and weaning, although a small percentage may survive—sterile—for as much as a year. In the case of 20FATw, while the presently maintained line may thus be classified as subvital, another line, now extinct, did not yield any homozygotes in weekly observations of breeding pens. Homozygotes in that line may thus be assumed to have been prenatally lethal, or neonatally lethal and quickly cannibalized. In two other (now extinct) lines of 20FATw, homozygotes almost invariably survived past weaning, indicating that the time of death of this mutant stock is strongly influenced by genetic background. A third subvital mutant, 28DTW, characterized by color intermediate between $c^{ch}$ and +, produces homozygotes in lower than expected frequencies, and these homozygotes yield small litters. The fourth subvital, 135G, also yields homozygotes in reduced numbers, and these generally die at an early age or are sterile. Of these four subvitals, only one, 20FATw, has so far been studied in more detailed tests.

TABLE 1

*c-locus mutants recovered in irradiation experiments*

| Germ-cell stage | Treatment of parent: Type of irradiation | Dose rate (R/min) | Classification of mutants* $c^{av}$ | $c^{xv}$ | $c^{as}$ | $c^{xs}$ | $c^{al}$ (neonatal) | $c^{al}$ (prenatal) | Incomplete test |
|---|---|---|---|---|---|---|---|---|---|
| —— | control | —— | $1 + 4^{m}$†‡ | $1 + 2^{m}$ | $0 + 1^{m}$ | . | . | . | $1^{m}$ ($c^{a}$) |
| sp'gonia | X or γ | >45 | 10 | 3‡ + $1^{m}$ | . | . | 3§ | 4 | 7§ |
| sp'gonia | X | 9 | 2 | . | . | . | . | 1 | |
| sp'gonia | γ | <1 | $5 + 1^{m}$ | 3‖ | . | . | . | 1 | 1 |
| sp'gonia | X | fractionated | $1 + 1^{m}$ | 2 | . | 1 | 1‡ | 2‡ | . |
| sp'gonia | X | fractionated, 24-hr interval | 8¶ | . | . | . | . | 4 | . |
| sp'gonia | neutrons | various | $9 + 3^{m}$ | $0 + 1^{m}$ | 1 | . | 2 | 3 | . |
| postgonia | X | various | 2 | . | . | . | . | 2 | . |
| postgonia | X | fractionated | . | . | . | . | . | 1 | . |
| postgonia | neutrons | various | . | . | . | . | . | 2 | 1 |
| oocytes | X or γ | various | $4 + 1^{m}$ | . | 1 | . | 1 | 1 | 1 |
| oocytes | neutrons | various | 2 | . | . | . | . | 7 | 1 |

* See Figure 1 and text. The first superscript designates phenotype: $a$ = albino, $x$ = intermediate allele. The second superscript designates homozygous viability: $v$ = viable, $s$ = subvital, $l$ = lethal.

† Superscript $m$ denotes mosaic, or fractional, mutant.

‡ One of these not derived from cross of (101 × C3H)$F_1$, or (C3H × 101)$F_1$ × T. See MATERIALS AND METHODS for derivation.

§ One of these occurred as a cluster of two.

‖ One of these was $c^{ch\,v}$. All others in this column are intermediate alleles other than $c^{ch\,v}$.

¶ One of these occurred as a cluster of three.

*Relation between radiation treatment and viability of induced* c-*locus mutations*: We have shown elsewhere (L. B. RUSSELL 1979) that the *c*-locus fractionals are not radiation induced. Because of this, the fractional mutants that were found in various radiation groups (Table 1) have been removed from these groups and included with the controls prior to calculating distributions into viability-phenotype classes (Table 2). The numbers of mutations in some of the subgroups, especially irradiated postspermatogonial stages, are small.

It may be noted that, within each of the germ-cell stage groupings, the relative frequency of homozygous viable mutations was greater for X or $\gamma$ irradiation than for neutron irradiation (though only slightly so in the case of spermatogonia). Of the three major germ-cell stage groups, spermatogonia, overall, yielded the highest frequency of viable mutations. Intermediates ($c^{xv}$ and $c^{xs}$) were found only among spontaneous mutations and those presumed to have been induced in spermatogonia.

*Viability information from breeding data*: Crosses made in the course of stock-keeping and testing procedures (see MATERIALS AND METHODS) yielded information on the viability of mutants. Progeny data from such matings have been tabulated in Table 3 for all seven neonatally lethal mutants, 26 of the 28 presumed prenatally lethal ones, and one subvital. These 34 mutants include three (designated $c^{al}$-?) for which complementation and deficiency mapping has not yet been done. Two viable ($c^{av}$) mutants are also tabulated for comparison.

The $c^*/c^{ch} \times c^*/c^{ch}$ matings provide the initial indication for groupings according to viability of homozygotes; and this has subsequently been verified and refined by intrauterine observations (L. B. RUSSELL and RAYMER 1979). In 26 of the 33 lethal ($c^{al}$) mutants listed in Table 3, and in the lethal line of $c^{as}$ mutant 20FATw (see above), all offspring classified at birth had eye pigment; and only $c^*/c^{ch}$ and $c^{ch}/c^{ch}$ progeny were found at weaning. In each of eight other lethal ($c^{al}$) lines (representing seven independent mutations), pink-eyed, presumed $c^*/c^*$, offspring were observed at birth. These were either already

TABLE 2

*Distribution of major classes of* c-*locus mutations*

| Germ-cell stage irradiated | Type of radiation | No. of mutations | Type of mutation | | | $\frac{(c^{xv}+c^{xs})}{(c^{av}+c^{as}+c^{xv}+c^{xs})}$ |
|---|---|---|---|---|---|---|
| | | | Viable | Subvital | Lethal* | |
| Spontaneous† | —— | 18‡ | 94.1 | 5.9 | 0 | 0.28 |
| Spermatogonia | X or $\gamma$ | 51 | 66.7 | 2.0 | 31.4 | 0.26 |
| | neutrons | 15 | 60.0 | 6.7 | 33.3 | 0 |
| Postgonial | X or $\gamma$ | 5 | 40.0 | 0 | 60.0 | 0 |
| | neutrons | 3 | 33.3 | 0 | 66.7 | 0 |
| Oocytes | X or $\gamma$ | 7 | 57.1 | 14.3 | 28.6 | 0 |
| | neutrons | 9 | 22.2 | 0 | 77.8 | 0 |

* Includes prenatal and neonatal lethals.
† Control group, plus mosaic mutants from all groups (see text).
‡ One of these (type $c^a$) not tested for homozygous viability.

TABLE 3

*Survival of homozygotes and heterozygotes in c-lethal lines*

| Mutant type and complementation group | No. of mutants† | No. offspring in cross of: $c^*/c^{ch} \times c^*/c^{ch}$ (Range)‡ | No. offspring in cross of: $c^*/c^{ch} \times c^{ch}/c^{ch}$ (Range)‡ | No. of lines with $c^*/c^*$ born | No. of lines with $c^*/c^*$ alive at 3 days | No. of lines with $c^*/c^{ch}$ viability signif. reduced§ |
|---|---|---|---|---|---|---|
| $c^{al}$-A | 5 | 454–1927 | 519–1138 | 5 | 0 | 0 |
| $c^{al}$-A′ | 3 | 434–940 | 536–768 | 0 | – | 0 |
| $c^{al}$-B | 11 | 85–974 | 515–1070 | 0 | – | 2 |
| $c^{as}$-C | 1 (2 lines)‖ | 538–590 | 251–524 | 1 | 1¶ | 0 |
| $c^{al}$-D | 8 | 128–1263 | 522–1231 | 0 | – | 0 |
| $c^{al}$-E | 1 (2 lines)** | 2623–4254 | 529–532 | 2 | 0 | 0 |
| $c^{al}$-F | 2 | 44–612 | 486–1211 | 0 | – | 2 |
| $c^{al}$-? | 3 | 146–451 | 147–505 | 1 | 0 | 1 |
| $c^{av}$ | 2 | 316–358 | 489–606 | 2 | 2 | 0 |

† One line of each, unless otherwise indicated.
‡ Range for the various lines within a group.
§ $P < 0.01$ in a one-tailed test (in either cross or both) in comparison with expectation for equal viability of $c^*/c^{ch}$ and $c^{ch}/c^{ch}$ segregants. Classification at weaning.
‖ Lethal line and subvital line (see text).
¶ Of 150 presumed $c^*/c^*$ born, 35 survived to weaning but died shortly thereafter.
** Cluster of two: mutants derived from same irradiated father.

dead, or died within two days, and their frequency was considerably less than the expected 25% as a result of cannibalism. In the subvital line of stock 20FATw, the $c^*/c^*$ offspring usually died at about one week, but occasionally survived past weaning.

Preliminary information concerning heterozygous viability of *c*-locus mutants can be obtained from both the $c^*/c^{ch}$ *inter se* matings and the $c^*/c^{ch}$ outcrosses to $c^{ch}/c^{ch}$ (stock-keeping). The frequency of heterozygotes among non-$c^*/c^*$ offspring should be 66.7% and 50.0%, respectively, if $c^*/c^{ch}$ and $c^{ch}/c^{ch}$ segregants are equally viable. Of 76 frequencies computed, only five were significantly lower than expectation ($P < 0.01$). In nine of the stocks, both ratios were noticeably (though not necessarily significantly) decreased. Such effects were confined to the F group (both mutants), B group (seven of 11 mutants) and D group (one mutant). It thus appears that the majority of *c*-locus lethals do not affect survival in heterozygous condition drastically enough to be detectable in mixed genetic backgrounds. More subtle effects would undoubtedly be demonstrable by the use of co-isogenic stocks in experiments specifically designed for this purpose.

## DISCUSSION

As was the case at the *d* and *se* loci (L. B. RUSSELL 1971; L. B. RUSSELL and W. L. RUSSELL 1960), mutants involving the *c*-locus can be divided into several classes by viability and phenotype, and then further subdivided on the basis of deficiency and complementation mapping.

At the albino locus, as at the dilute and short-ear loci, viable mutants include a class that resemble the known viable "bottom" allele—*c*, *d*, and *se*, respectively—as well as a heterogeneous class that was phenotypically intermediate between the bottom allele and wild type. By contrast, lethal mutants at all three of these loci do not include an "intermediate" class. Many of the lethals have been shown to be very small deficiencies, on the basis of the absence either of a known neighboring locus (L. B. RUSSELL, DEHAMER and BORMAN 1974; ERICKSON, EICHER and GLUECKSOHN-WAELSCH 1974; BERNSTINE, RUSSELL and CAIN 1978) or of a functional unit established by complementation analysis (L. B. RUSSELL 1971). Absence of the *c*-locus, presumed to be the structural locus for tyrosinase (WOLFE and COLEMAN 1966), thus mimics the standard *c* allele with respect to coat-color phenotype in various combinations, indicating that *c* is, in fact, a null mutation. These findings contradict an earlier suggestion (DUNN 1934) that *c* could be a hypomorph, but are in keeping with measurements of enzyme activity (COLEMAN 1962). (It is conceivable that the standard *c* allele could be a spontaneous deficiency, but there are no *a priori* reasons for assuming this.)

Since it may, therefore, be concluded that the "intermediates" ($c^{av}$ and $c^{as}$ classes, by our designation) are not associated with absence of the albino locus, it is highly probable that they result from intragenic changes at this locus. The same conclusion cannot automatically be drawn about the $c^{av}$ class. Complementation tests make it likely that the viable albino animals obtained in intercrosses of certain lethals are the result of combinations of small deficiencies that overlap at the *c*-locus but do not overlap at presumed adjacent functional lethals (L. B. RUSSELL, DEHAMER and BORMAN 1974; RUSSELL and CACHEIRO 1977). One may therefore conclude that homozygous absence of the albino locus alone is viable and albino. While some of the $c^{av}$ mutants could theoretically result from small deficiencies, rather than re-mutations to the *c* allele, such deficiencies would have to be small enough to exclude functional units already mapped near the albino locus. It is unlikely that the question can be completely resolved by present techniques, except perhaps by laborious attempts to induce reverse mutations. [Spontaneous reverse mutations of the *c* allele have not been observed in over 3,000,000 animals (SCHLAGER and DICKIE 1971).] In the meantime, one may assume that $c^{av}$ mutants are either intragenic mutations at the *c*-locus, or deficiencies involving barely more than the *c*-locus alone.

Little can be concluded, at present, about the small, heterogeneous group of subvitals except that one, which was of intermediate color, 28DTW, presumably does not involve deficiency of the albino locus. At least one of the group, 20FATw, acts like a leaky mutant.

At the albino locus, as at the *d* locus, lethal mutants fall into subgroups. One major group dies at birth, or shortly after, and it is possible that there may be slight viability and morphological differences within this neonatally lethal group. Thus, ERICKSON, GLUECKSOHN-WAELSCH and CORI (1968) found $c^{65K}$ and $c^{112K}$ to be lower in survival to birth and higher in frequency of thymus abnormalities than $c^{14CoS}$ and $c^{3H}$. However, 65K and 112 K have subsequently been found by

us to represent the cluster mutation of a new complementation group, E, which is deficient for *Mod-2* as well as *c* (L. B. RUSSELL, DEHAMER and BORMAN 1974). This result indicates (1) that MOD-2 is not required for intrauterine survival, and (2) that a deficiency of at least 1 cM in length can be homozygous viable until birth (L. B. RUSSELL and RAYMER 1979).

The effects on viability of *c*-locus lethals in heterozygous condition (with $c^{ch}$) are, in the majority of the mutants, not drastic enough to be perceived in stocks of mixed genetic background. The proper measurement of more subtle heterozygous effects, which could have greater selective importance than homozygous lethality, will require the development of co-isogenic stocks. Only some of the mutants, primarily in complementation groups F and B, produce heterozygous viability depressions large enough to be clearly detectable by the present crude indicators. It is of interest that of nine stocks in which the frequency of heterozygotes was lower than expected (though not necessarily significantly so) in both of the crosses made, eight are preimplantation lethals in homozygous condition (RUSSELL and RAYMER 1979). The F-group mutants are deficiencies involving, in addition to *c* and *Mod-2*, also the *sh-1* locus, but not *tp* or *Hbb* (see Figure 2), and are thus at least 4 but less than 8 cM long (RUSSELL and CACHEIRO 1977). The fact that many mutants belonging to the B, D, or E complementation groups, which are deficiencies at least 1 but less than 6 cM in length, produced no consistent measurable effects in heterozygous condition may be contrasted with the findings for the *d-se* region, where marked heterozygous viability depression was found for a *se*-deficiency mutant that is less than 2 cM long (L. B. RUSSELL 1972), as well as for several *d se* deficiencies that are in the 0.16 to 6.7 cM range (L. B. RUSSELL, STEELE and THOMPSON 1966; L. B. RUSSELL 1971). This illustrates that, as might be expected, the length of deficiencies that can be tolerated without major adverse dominant effects, is not a constant parameter throughout the mouse genome, but depends on the content of specific chromosome regions.

It is of interest that not only were all but one of the control *c*-locus mutations viable, but the fractionals that are distributed among the irradiated groups and are presumed to be of spontaneous origin also (L. B. RUSSELL 1979) were all within viable categories. That is, among 18 probably spontaneous *c*-locus mutations, none was lethal and only one was subvital. This finding appears to contrast with results obtained at the *d*-locus (L. B. RUSSELL 1971), where only three of 15 tested spontaneous mutations were viable. Most of the remainder, however, were of the $d^{op}$ type, which could represent an intragenic mutation.

In comparing mutational spectra between groups it should be noted that the bulk of the spontaneous mutations, at least at the *c* locus, probably have a stage of origin that differs from that of the bulk of the induced mutations. Specifically, the fractional mutants must have arisen postmeiotically or in the zygote (L. B. RUSSELL 1979); while mutations recovered after radiation of spermatogonia or oocytes were presumably induced before the meiotic divisions. Any qualitative differences could thus be due to this as well as other reasons.

The relative proportions of intermediate-type ($c^{xv}$ and $c^{xs}$) and albino ($c^{av}$ and $c^{as}$) among presumed spontaneous mutations (28% intermediate type) were very similar to those among 35 nonlethal, whole-body mutants derived from X- or $\gamma$-irradiated spermatogonia (26% intermediates). On the other hand, none of the ten nonlethal whole-body mutants derived from neutron-irradiated spermatogonia was of the $c^{xv}$ or $c^{xs}$ type. It appears that low-LET irradiation of spermatogonia increases the absolute frequency of viable *c*-locus mutations without changing the spectrum from that of spontaneous ones.

In addition to increasing the frequency of viable *c* mutations, irradiation appears to produce types that have not, to date, occurred spontaneously, namely, the neonatal and postnatal lethals. The proportion of lethals following low-LET irradiation of oocytes is not dissimilar to that from spermatogonia. The proportion of lethals apears to be increased after irradiation of postspermatogonial stages in general, but the number of mutations derived from these stages is too small for analysis. Neutron irradiation, for each germ-cell type, yields a higher proportion of lethals than does low-LET irradiation; but the difference for any individual cell type is not significant. Subdivision of the spermatogonial data into single and fractionated exposures yields nonsignificant differences, both for the proportion of viables ($P > 0.2$) and for the frequency of intermediates among the viables ($P > 0.4$).

Within the set of mutants induced by low-LET irradiation of spermatogonia, a number of different groupings have been tried in order to test for the possible existence of a dose-protraction effect on the distribution of viable and lethal mutants. None of these comparisons has revealed a statistically significant difference. For example, a test of the significance of the difference in the proportion of lethals between results from experiments using a dose rate of 48 R per min, or higher, and those using a dose rate of 0.8 R per min, or lower, yields a *P* value of 0.20. (In this comparison, the high dose-rate exposures excluded an experiment in which the dose was given in small fractions.) These analyses indicate that the qualitative distribution of spermatogonially derived mutants does not change in a major way with dose rate. This supports the view of W. L. RUSSELL (1964, 1965a, 1968a, 1969) that the major portion of the reduction in frequency of specific-locus mutations at low dose rates is not, as ABRAHAMSON and WOLFF (1976) have suggested, due to a change in the proportion of two-track and one-track ionization events.

Furthermore, the hypothesis of ABRAHAMSON and WOLFF requires that most of the mutations induced by high-dose-rate irradiation be two-track in origin. An examination of the nature of the mutations does not support this assumption. In the X- or $\gamma$-ray high dose-rate irradiated spermatogonial groups, more than 60% of the mutations were of the $c^{xv}$, $c^{xs}$ and $c^{av}$ types. The $c^{xv}$ and $c^{xs}$ mutations are probably intragenic changes, as noted; and the $c^{av}$ mutations are either intragenic changes or deficiencies involving barely more than the *c*-locus alone. All of the above nonlethal mutations are presumptive single-track events. Moreover, for those mutations that are lethals and, therefore, presumed to be deficiencies, more than one-third have been shown not to involve the closest asso-

ciated marker gene. It seems likely that at least some of these, and perhaps some of the genetically detected small deficiencies as well, may be single-track events. In any case, the high proportion of viables cited above indicates that most of the *c*-locus mutations induced in spermatogonia, even by high-dose-rate irradiation, resulted from single-track events. The *c*-locus mutations derived from oocytes are much less numerous, but it is noteworthy that the two induced by high-dose-rate exposure to single large doses are both viable.

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