ACUTE RADIATION RESPONSE OF MICE FROM A CROSS BETWEEN RADIOSENSITIVE AND RADIORESISTANT STRAINS[1]

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THE existence of genetic differences in the radiosensitivity of inbred mouse strains has been well established under controlled experimental conditions (KOHN and KALLMAN 1956; GRAHN and HAMILTON 1957). These studies have indicated that strain BALB/c is the most radiosensitive of those investigated and that strain C57BL is relatively resistant. As a consequence of this known difference, strains BALB/c and C57BL were selected for a study designed to give information on the mode of inheritance of radiosensitivity.

In toxicity testing, observed sensitivity is assumed to be a continuously distributed variable (MATHER 1946). When rectified, this distribution is described by the dosage-mortality slope—the latter being inversely proportional to the standard deviation of the distribution. The mean or median of the distribution (LD_{50}) and a measure of dispersion (the dosage-mortality slope) will be used in this study to describe the acute lethal response. The slope has previously been employed in the estimation of phenotypic variances for inbred strains (GRAHN and HAMILTON 1957) and should provide, for segregating generations, a means of estimating the genetic variance.

MATERIALS AND METHODS

Both reciprocal F_1 generations were produced from parent strains BALB/c and C57BL/6. These are designated as the CB F_1, from the BALB/c dam, and the BC F_1, from the C57BL/6 dam. Second generation hybrids were produced from each reciprocal F_1 by mating *inter se* and are designated as the CB and BC F_2. A small group of F_3 mice was also produced. In this instance, the matings were made within the five coat color types that segregated in the F_2. One additional group of animals was produced from an outcross of the F_1 dam with the strain A/Jax sire ($F_1 \times A$). Though these mice were not originally intended to be a part of the present study, they provide some useful information.

The mice were exposed to single doses of whole-body 200 kvp X-irradiation when 60–110 days of age. A summary of the experimental design is given in Table 1. A series of independent tests to estimate the LD_{50} values was carried out on the several generations; 17 for the BALB/c, 16 for the C57BL/6, 20 for the F_1, 15 for the F_2 and 5 for the F_3. Each test generally consisted of three dose groups with an average of 14–15 mice per dose. The average within-test regression of arcsine-mortality on dosage in roentgens (dosage-mortality slope) was estimated

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TABLE 1

Summary of experimental design: First number, number of independent tests employing given dose; second number, total number of mice; third number, percent mortality from pooled test results

Dose	BALB/c	C57BL/6	F_1	F_2	F_3
420	4–59–7	.....	.....	.....	.....
435	4–60–15	.....	.....	.....	.....
450	4–60–25	.....	.....	.....	.....
(465)	.....	.....	.....	.....	.....
480	4–53–38	.....	.....	5–75–4	.....
495	4–58–41	.....	.....	.....	5–72–13
510	4–50–52	.....	.....	6–85–2	.....
525	4–57–61	.....	.....	.....	.....
540	4–51–59	.....	.....	6–89–16	.....
555	4–54–89	.....	8–113–1	.....	.....
570	4–57–84	6–89–10	.....	7–95–27	.....
585	4–54–87	6–87–23	9–131–17	.....	.....
600	4–59–95	6–89–20	.....	.....	5–71–46
615	.....	6–82–32	9–131–20	.....	.....
630	.....	6–81–56	.....	7–103–55	.....
645	.....	6–84–57	9–128–41	.....	.....
660	.....	6–88–74	.....	.....	.....
675	.....	6–87–89	9–130–56	.....	.....
690	.....	.....	.....	6–83–71	.....
705	.....	.....	7–106–69	.....	5–72–82
720	.....	.....	.....	6–86–83	.....
735	.....	.....	6–88–84	5–75–92	.....
750	.....	.....	5–69–96	.....	.....
Total mice	672	687	896	691	215

by a weighted covariance analysis for each group. This slope is used to derive the LD_{50} value for each test.

The earlier investigation of the parent inbreds (GRAHN and HAMILTON 1957) had indicated that age was an important variable in the acute lethal response while the litter seriation and litter size associated with the exposed mice were of little consequence. On the basis of those results, only the relationship of age to mortality was derived for the F_1 and F_2 generations. The F_3 data were not sufficient to warrant an estimate.

The regression of mortality on age can be used to predict expected mortality at any age within the limits of the data (60 ~ 110 days). If we assume the dosage-mortality slope value to be constant in the above age range, then $LD_{50/30}$ values can be estimated from the predicted mortalities. Age changes in sensitivity are given below (see Figure 1) in terms of the LD_{50} value because of its clarity of expression.

The linear regression of mortality on age was used to adjust test responses to a constant age at exposure of 85 days. The individual test LD_{50} values were then estimated from the age-adjusted test means. The standard error of the LD_{50} was

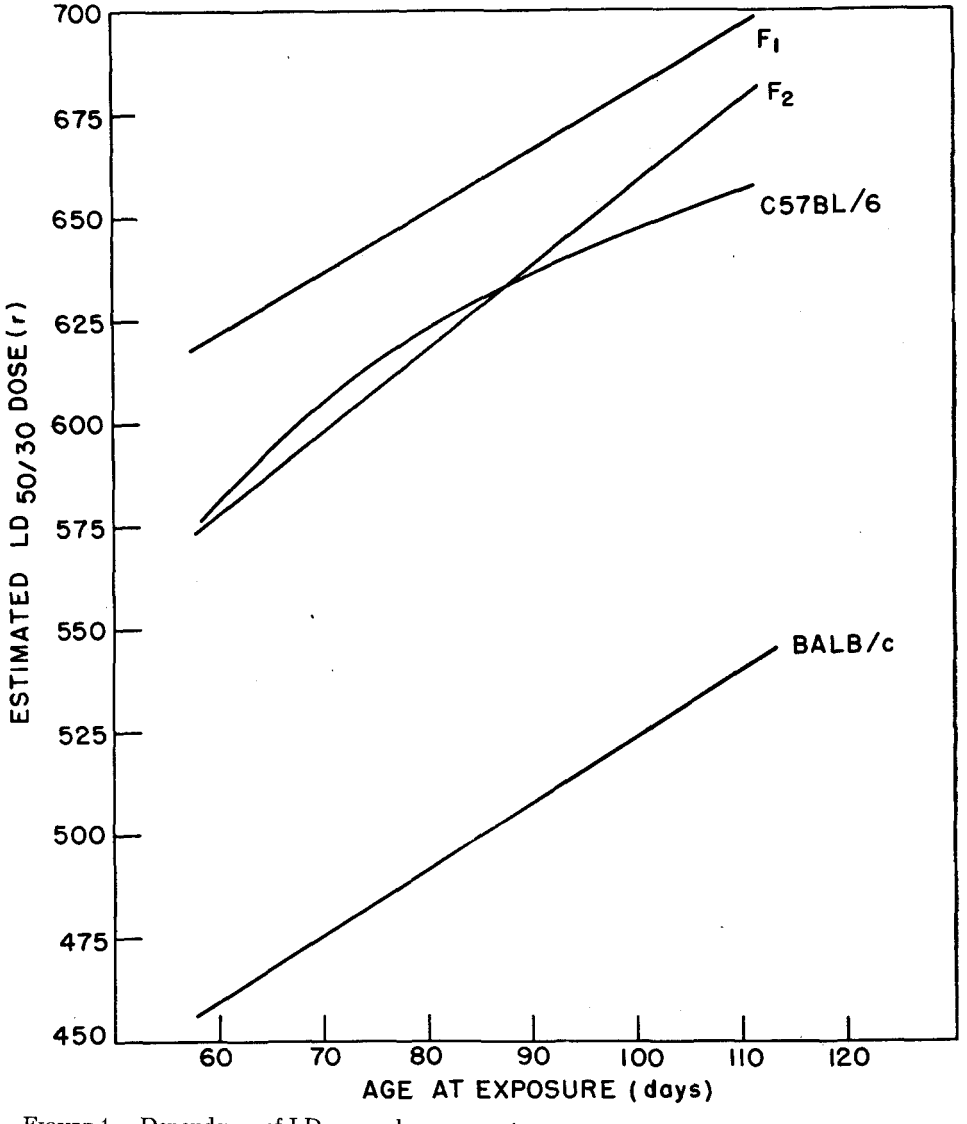

FIGURE 1.—Dependence of $LD_{50/30}$ value on age at exposure.

derived from the distribution of the test estimates. A more complete description of the statistical procedures and methods of distributing the mice among the doses in each test was given in the previous report (GRAHN and HAMILTON 1957).

In order to estimate the LD_{50} values for certain subclasses, i.e., sex, reciprocal crosses, and coat color type, the data for the subclass in question were pooled within dose across all individual tests. Estimates of the dosage-mortality slope, the LD_{50} value and their standard errors were derived from these pooled data by a weighted least squares linear regression analysis. The method of pooling was

required since the within-test within-dose subclass sample sizes were too small (generally fewer than eight mice per test dose) to permit the form of analysis described above for the principal estimates.

With reference to the mortality data given in Table 1, it may be noted that these are also pooled data figures. Because the data are nearly equally distributed on either side of the 50 percent mortality point, LD_{50} doses derived from these data will vary by only a few roentgens from the mean test values given in this study. However, slope values obtained from pooled data are consistently lower than the within-test estimates, since pooled data estimates are not independent of variation among tests. Nevertheless, the comparative magnitude of the slopes in the different generations and their linearity can be readily checked.

First generation hybrids

The $LD_{50/30}$ dose at 85 days of age is estimated to be 659.5±6.6r and the within-test dosage-mortality slope is + 0.404±.028. (These values are presented in Table 4 with the parental values for comparative purposes.) A breakdown of the F_1 mice into sex and reciprocal subclasses is given in Table 2. A significant sex

TABLE 2

$LD_{50/30}$ values by sex and reciprocal for BALB/c : C57BL/6 F_1 hybrids

Reciprocal cross	Female	Male	Combined sexes
CB (BALB/c ♀ × C57BL/6 ♂) F_1	676.4r	648.3r	661.6r
BC (C57BL/6 ♀ × BALB/c ♂) F_1	675.8r	654.1r	662.4r
Combined reciprocals	676.7±7.0r	651.5±5.3r	

difference ($P < .025$) is apparent; the female has an LD_{50} 25.2±8.8r higher than the male. No significant sex differences were detected in the parent strains (GRAHN and HAMILTON 1957).

There is no difference between reciprocals, within or across sex, which indicates that no maternal influence or sex linkage can be detected in the response.

The LD_{50} dose increases linearly with age between 60 and 110 days; rising from 622r to 696r in that interval (Figure 1).

Second generation hybrids

The F_2 LD_{50} value is 627.8±6.6r. The within-test dosage-mortality slope is + 0.274±.026. The sharp drop in the slope value (see Table 4) is an indication of increased variability, most of which can be assumed due to the genetic variation expected in a segregating generation. There are no sex or reciprocal differences in the F_2 mice. Age changes in the LD_{50} in this generation also appear linear with age and are given in Figure 1.

The F_1 mice are heterozygous for agouti, black and albino (*AaBbCc*). The F_2 generation segregates five coat color types: black agouti, brown agouti, black, brown and albino in an expected ratio of 27:9:9:3:16, respectively. Nonagouti,

black and full color are derived from the C57BL parent, and agouti, brown and albino come from the BALB/c parent.

To examine the possibility of a non random segregation or "association" of sensitivity factors with the color genes, the F_2 mice were subdivided into the color classes and comparisons were made on an alternative allele basis with reference to the parent inbred that contributed the gene. The results are given in Table 3. Only minor differences are apparent at the agouti and black loci, but a

TABLE 3

$LD_{50/30}$ values for coat color comparisons in BALB/c : C57BL/6 F_2 number of mice of given color type shown in parentheses

BALB/c contribution	C57BL/6 contribution	Difference
Agouti (384)	Nonagouti (134)	
640.1r	635.5r	4.6r
Brown (157)	Black (361)	
635.6r	642.0r	6.4r
Albino (173)	Nonalbino (518)	
602.3±8.6r	639.3±7.9r	37.0r*

* Difference signicant at one percent level.

TABLE 4

Comparative data of parental and hybrid generations

Strain	Observed $LD_{50/30}$ ± SE	Observed slope ± SE	Reciprocal slope
BALB/c	500.1±6.9r	.368±.032	2.72r
C57BL/6	630.3±4.1r	.516±.041	1.94r
F_1	659.5±6.6r	.404±.028	2.48r
F_2	627.8±6.6r	.274±.026	3.65r
F_3	613.3±28.0r	.264±.020	3.79r

significant difference is evident at the albino locus, with albino associated with sensitivity.

The relative sensitivity of the albino is confirmed in part by the results of the irradiation of the F_1 × A outcross progeny. Since strain A is homozygous recessive for the color genes involved (*aabbcc*), the progeny of this cross segregated 1:1 for albino and nonalbino. The LD_{50} for the albinos is 615r (116 mice tested) and for the nonalbinos it is 650r (119 tested). Because of the small samples involved, the difference is within the limits of one standard error. However, the magnitude and direction of the difference is similar to that seen in the F_2 segregants.

Third generation hybrids

The F_3 mice were produced principally to ascertain whether the F_2 mice were fully expressing their expected genetic variability as measured by the dosage-mortality slope. It was considered possible that the uniform vigor of the F_1 dam

could have suppressed the full expression of variation in the F_2. Additionally, the matings were made within the five color classes in an effort to obtain additional information on the albino effect on sensitivity.

The LD_{50} is 613.3±28.0r and the dosage-mortality slope is + 0.264±.020. The latter is nearly identical to the F_2 slope and indicates that the expression of genetic variability in the F_2 is not affected by maternal factors.

The F_3 mice included 56 albinos and 159 nonalbinos. These were distributed equally across the three test doses and gave mortalities of 46.4 percent and 47.2 percent for albino and nonalbino, respectively. Among the several color families, black agouti moved to a position of relative resistance (36 percent mortality) and brown agouti to a position of relative sensitivity (53 percent mortality). Thus, the F_3 data appear to bear little resemblance to those of the F_2. However, an adequate genetic analysis of this generation would require a very broad sampling of F_2 genotypes for matings within all color segregants. As this type of toxicity testing is relatively uneconomical for mice, the production of the F_3 was discontinued.

Comparative analysis of parent and hybrid generations

In Table 4, the observed LD_{50} values and dosage-mortality slopes (see also Figure 2) are presented for both parent strains and the three hybrid generations.

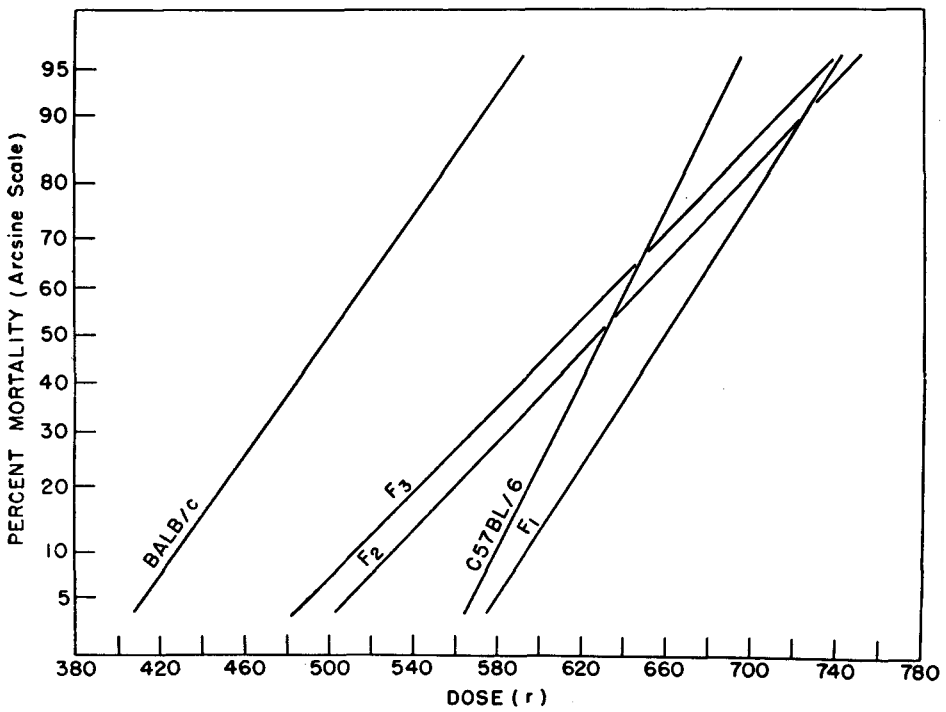

FIGURE 2.—Relationship of dosage-mortality slopes for parent and hybrid generations.

A comparison of LD_{50} values shows that the F_1, with an LD_{50} nearly 30r above the high parent, expresses a significant degree of hybrid vigor. In the F_2, the value is almost equal to the high parent, while in the F_3, the LD_{50} is slightly, but not significantly, below the F_2. These estimates suggest that, genetically, radioresistance is dominant to sensitivity, or, in other terms, is the function of a nonadditive genetic system.

The dosage-mortality slopes support a different genetic interpretation. In the F_1, the slope is intermediate to the two parents, while the F_2 and F_3 slopes fall below the low parent, and manifest a sharp increase in the variability of response. Since the squared inverse slope is directly proportional to the variance of the population, the slope data are typical of a characteristic controlled by an additive genetic system, wherein the F_1 variance is equal to the parental variance and the F_2 expresses the added variance due to genetic segregation.

Estimation of heritability of radioresistance

Heritability can be estimated from these data by the relation: $h^2 = (\sigma^2_{F_2} - \sigma^2_{F_1})/\sigma^2_{F_2}$. This assumes the F_1 variance to be an unbiased estimate of the environmental variance (σ^2_E), and the F_2 variance to include only additive genetic variance (σ^2_G) in addition to σ^2_E. The arithmetic mean of the parental variances also estimates σ^2_E.

The variances, in this instance, are derived from the dosage-mortality slopes. The arcsine slope can be converted to probits by taking the product of the arcsine slope and the regression of probit values on arcsine values (+ .0481) as described by GRAHN and HAMILTON (1957). The reciprocal of the squared probit slope provides a direct estimate of the variance of the distribution. The variances are: BALB/c, 3192; C57BL/6, 1624; F_1, 2652; F_2, 5761.

Using $\sigma^2_{F_1} = \sigma^2_E$, h^2 is 0.54. If σ^2_E is derived from the mean parental variance, $h^2 = 0.58$. These figures clearly indicate that a great amount of genetic variability in radiation resistance does exist in a heterogeneous population.

DISCUSSION

Resistance to the acute lethal effects of whole-body X-irradiation is a heritable characteristic that shows both additive and nonadditive genetic qualities. In terms of the LD_{50} dose, genetically determined resistance is nonadditive. This property was also present in the X-ray data of KOHN and KALLMAN (1956) for strains BALB/c, A/He and their F_1 hybrid. Dominance of resistance is, in fact, a general finding in the first generation offspring of crosses between resistant and susceptible strains within a number of plant and animal species when subject to test by a range of infectious or toxic agents (see review by GOWEN 1948). It should be emphasized, however, that in the F_2 of the present study, the degree of dominance varies with dose (see Figure 2). For example, an LD_{10} is 50r below the high parent, while an LD_{90} is 40r above it. A very incomplete picture is derived by using a single level of either response or dose, and genetic interpretations will vary accordingly.

It is unfortunate that the dose-response slope has not received more attention, since it can convey information beyond that noted above. In an F_2, a linear slope that is shallower than the F_1 or parent values provides a measure of genetic variability and also suggests that the character being investigated is under additive genetic control. This is seen in the present data and in the response of Drosophila to DDT (KING 1955). A nonlinear, or stepped, slope, such as observed by LICHTWARDT (1956) for the response of houseflies to DDT, indicates the presence of one or two major dominant genes.

In addition to the slope data, a second parameter, recovery rate, adheres to the additive scheme. For strains BALB/c, A/He and their F_1, KOHN and KALLMAN (1957) gave the following recovery rates as measured by the paired dose technique: BALB/c, 21 percent/day; A/He, 47 percent/day; F_1, 35 percent/day (percent recovery from a first dose of 350r).

To the extent of the limited available data, the additive component of resistance appears to find more general expression, while the nonadditive component seems limited to expression on the dose scale. This may indicate the latter component to be primarily a heterotic manifestation that acts to raise the dose-response threshold. A fully adequate interpretation of the nonadditive component cannot be made without data on the response of a wide variety of interstrain crosses.

The high heritability estimate of .54–.58 indicates that a program of selection for radioresistance or sensitivity could be successfully carried out, although the nonadditive contribution might obscure the results in early generations. Though no selection experiment has been attempted, SCHOTT (1932) reported successful selection for resistance to mouse typhoid, which has been shown to have a high genetic correlation with radiation resistance (GRAHN 1954; STADLER and GOWEN 1957). SCHOTT raised resistance from 18 percent to 75 percent in only six generations. This could only have been accomplished if heritability was high.

The significant association between the albino locus and radiosensitivity that was observed in the F_2 requires further evaluation. Its possible presence in the testcross mice ($F_1 \times A$) tends to implicate the albino gene itself, while the apparent loss of this association in the F_3 indirectly infers that a linkage could be involved. Because of the small sample sizes employed, and in the absence of a direct test for linkage, no positive genetic interpretation can be brought forth.

In view of the existing data, a program of recurrent backcrossing to the sensitive parent, BALB/c, has been instituted. This procedure should provide additional information on both the additive and nonadditive genetic components, and, as well, should help elucidate the relationship between albinism and sensitivity.

SUMMARY

1. The acute lethal response to whole body X-irradiation of F_1, F_2 and F_3 generations from parent strains BALB/c and C57BL/6 is shown to manifest both nonadditive and additive genetic effects. The variance of sensitivity, measured by the dosage-mortality slope, is additive. The $LD_{50/30}$ dose is nonadditive. It is

suggested that the nonadditive component may be primarily a heterotic expression.

2. No maternal influence or sex linkage is detected in the acute response.

3. Radiosensitivity is associated with the albino gene, although the data do not permit discrimination between pleiotropism or linkage as the basis of this association.

4. In the F_2, the genetic component of variance (heritability) accounts for about 55 percent of the variation in radiation sensitivity.

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