GENETIC VARIATION IN THE ACUTE LETHAL RESPONSE OF FOUR INBRED MOUSE STRAINS TO WHOLE BODY X-IRRADIATION[1]

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THE existence of genetic variation in radiation sensitivity within a species has been acknowledged since HENSHAW (1944) reported a difference in the acute lethal response of C3H and LAF_1 mice to X-radiation. Since that time, strain or genetic differences in the radiation response of small laboratory mammals have been indicated for the sterilization dose (LORENZ *et al.* 1947), susceptibility to leukemia induction (KIRSCHBAUM and MIXER 1947), changes in blood chemistry (KOHN 1951), hematopoietic response after spleen protection (KAPLAN and PAULL 1952), changes in body and organ weight (GRAHN 1954a,b) and for the reduction of life expectancy (HENSHAW *et al.* 1947; EVANS 1948; SACHER 1950; GOWEN 1956).

Although the single dose LD_{50} value is often used in radiobiological studies as an experimental end point, information concerning genetic variation in this value is inadequate. Strain differences in the LD_{50} following X-irradiation are known to exist in mice (LORENZ *et al.* 1952) and rats (KOHN 1951). RUGH (1953), reviewing several independent studies, indicated that the LD_{50} value in mice may vary from 400r to 650r of X-rays, depending upon the strain employed. This range of LD_{50} values is confounded, however, with differences in age at exposure, radiation quality, and unaccountable environmental factors that certainly must vary among laboratories. No attempt has been made to quantify, in a single experimental effort, strain differences in acute lethality. Consequently, certain features such as age changes in sensitivity, dosage-mortality slopes and sex differences are known only in general terms.

MATERIALS AND METHODS

In order to describe quantitatively strain or genetic differences in radiation response, three separate inbred mouse strains and two sublines of a fourth strain were exposed to single whole-body doses of X-irradiation. In addition to data on strain differences of LD_{50} values, dosage-mortality slopes and sex effects, some information on age effects was obtained by deliberately employing a limited amount of variation in age at time of exposure. To facilitate interlaboratory comparison, strains were chosen which have been used frequently in experimental radiobiology, cancer genetics and general mouse genetics.

Inbred strains BALB/c, $C3H_f$/He, C57BL/6 and sublines A/Jax and A/He of the A strain were used in this study. All mice were bred and maintained by full-sib matings in this laboratory under conditions of constant temperature and humidity.

The data for each strain are composed of a series of 15 to 20 independent tests

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TABLE 1

Summary of experimental design for each strain

Strain	Total number of mice	Number of doses	Dose range	Number of tests	Ave. number of mice/test
BALB/c	672	12	420–600r	17	40
A (Combined)	1008	13	480–660r	30	34
A/Jax	522	11	480–645r	15	35
A/He	486	12	495–660r	15	32
$C3H_f$/He	845	10	525–660r	19	44
C57BL/6	687	8	570–675r	16	43

or replications. With the exception of several preliminary single point tests, the replication or test group was subdivided into dose groups of an average size of 14 mice with both sexes being represented in about equal numbers. From two to five dose groups, depending on the number of animals available, were exposed in each test. All of the mice of a test dose group were irradiated in a single exposure run of approximately 30 minutes duration.

After the dose range had been ascertained over which a fractionally lethal response would occur (from approximately 5 to 95 percent mortality), the data were progressively built up, test by test, during a period of nearly two years. At least three independent measures of mortality were made at each dose employed for a given strain although in the more prolific strains it was possible to obtain five or six assays per dose. Within each strain, however, approximately equal numbers were exposed to all doses.

The doses were separated by a 15r interval, starting with the lowest dose of 420r. Table 1 presents a summary of the experimental features for all strains.

Since litter-mates, within-sex, were caged together, the doses delivered in each test were systematically distributed across mice of the same litter. This procedure effectively balanced the contribution of age and all factors pertinent to a litter (i.e. parity and litter size) across all doses within a test, and circumvented the heterogeneity that can arise (KAPLAN and BROWN 1952; SMITH *et al.* 1954a) when litter differences are confounded with dose.

However, as a result of chance, each replication or test had a mean age, parity and litter size that differed slightly from all other tests. The removal of this variation from the final estimates of the LD_{50} is discussed below.

Because of the distribution process carried out within tests, doses automatically became balanced across cages, the latter usually holding from 8 to 14 mice. The two sexes and all strains were caged separately. A 30-day observation period was used for determining acute lethality. Survivors are being kept for the study of strain differences in the lifetime effects of single doses.

All exposures were done with a half-wave self-rectified X-ray generator operating at 200 kvp and 15 ma. With a HVL of 1.1 mm Cu and a target distance of 27 inches to the mid-point of the animal, dose rates of 21 to 23r/minute in air were achieved.

For statistical analyses, each test-dose percentage mortality value was transformed to the arcsine scale in order to make the mortality response linear to dosage

expressed on the arithmetic scale. All values were weighted by the pertinent number of animals.

In order to estimate the $LD_{50/30}$ values (the dose expected to kill 50 percent of the animals in 30 days) a dosage mortality slope, $b_{m.d}$, or the regression of arcsine mortality on dose was derived. In this study, the slope employed for each strain is the average within test slope obtained by means of covariance analysis techniques described by SNEDECOR (1946). The within-test slope is preferred as it is independent of any variation in age or litter factors.

The LD_{50} value for each test was then estimated by the following formula:

$$LD_{50} = \bar{d} + \frac{45.0 - \bar{m}}{b_{m.d}},$$

where $\bar{d}$ is the test mean dose, $\bar{m}$ is the test mean arcsine mortality, and 45.0 is the arcsine value for 50 percent. Preliminary estimates of the strain LD_{50} values were obtained by deriving the weighted mean of all the individual test LD_{50} values.

To establish the effect of variation in age, parity and litter size upon mortality, the data for each strain were broken down into four subclasses for each of these three variates. Effort was made to retain rough numerical equality among subclasses while attempting to maximize the total range between mid-points of the subclass intervals. The resulting $4 \times 4 \times 4$ tables generally had $50 \sim 55$ cells represented. Within each cell, the weighted means for dose and for response were estimated so that the response could be adjusted to a constant dose by means of the dosage-mortality slope. For each strain, the preliminary estimate of the LD_{50} value was used as the constant dose in order to keep the mortality data in the middle of the percentage scale. On the assumption that the errors from the adjustment process were self-cancelling, a weighted multivariate analysis was executed according to methods described by SNEDECOR (1946). In these data age ranged from $60 \sim 110$ days, parity from $1 \sim 10$ and litter-size from $2 \sim 12$.

In strains BALB/c, A and $C3H_f$, the most linear interrelationships were obtained on the arithmetic scale for all variables. A log transform of mortality was used in the C57BL strain. By means of the derived multiple regression equations, the test mean mortality values were adjusted to a constant age, parity and litter size for all strains. Final estimates of the test LD_{50} values were then obtained from these adjusted mortality values.

In each strain the variance among test LD_{50} values and the standard error of the mean were calculated in a routine manner.

RESULTS

The final estimates of the strain $LD_{50/30}$ values and their standard errors are presented in table 2. Preliminary analysis had indicated that the two sublines of the A strain were nearly identical in response (A/Jax LD_{50}: 548 $\pm$ 7r; A/He LD_{50}: 557 $\pm$ 6r); consequently, the data were combined to provide a single estimate listed for strain A.

The maximum difference in LD_{50} values observed among the four strains is 130r, a difference of about 26 percent in the LD_{50}s of the C57BL and BALB/c strains.

TABLE 2

Weighted mean test $LD_{50/30}$ values, variances among tests and within-test dosage-mortality slopes after removal of variation in age, parity and litter size

Strain	$LD_{50/30} \pm SE$	Variance among tests	$b_{m \cdot d} \pm SE$
BALB/c	500.1 ± 6.9r	809.79	.368 ± .032
A	552.3 ± 4.4r	583.96	.417 ± .037
$C3H_f/He$	593.2 ± 6.2r	735.31	.433 ± .050
C57BL/6	630.3 ± 4.1r	267.46	.516 ± .041

(Since this manuscript was prepared, a fifth mouse strain, C57L/He, has been subject to test. This strain, which has a common origin with strain C57BL, has the highest LD_{50} value among the five strains; 677.8 ± 8.5r. The range of genetic variation in LD_{50} values is thus increased to over 175r. The dosage-mortality slope is .426 ± .041, which represents a recession from the high value seen in C57BL mice. However, these new observations do not seriously alter the conclusion that susceptibility and variability are concomitant measures of genetic differences in radiation sensitivity.) Inspection of the data of table 2 reveals that a close and direct relationship exists between the LD_{50} and dosage-mortality slope. This relationship denotes that strain differences are not constant, but tend to increase as lower levels of mortality are approached. Of greater significance, however, is the indication that the variability of response, measured in terms of dosage, must increase with susceptibility because a low slope defines a broad distribution of sensitivities in the population tested

Figure 1 presents a frequency distribution of the predicted individual test LD_{50} values for the four strains. Since the average test employed from 34 to 44 mice, the precision of test estimates is comparable for all strains.

The multiple regression equations relating mortality to changes in age, parity and litter size are given in table 3. While variation in mortality was significantly reduced by this analysis, inspection of the partial correlations indicates that the success of the analysis was due primarily to the correction for variation in age at exposure. All strains show a negative correlation between age at exposure and mortality.

Figure 2 expresses this age-dependence of acute lethality in terms of estimated LD_{50} values at a constant parity and litter size for all strains. The data show that strain differences in the LD_{50} vary quite extensively with variation in age at test.

The observed curvilinear relation between arithmetic mortality and age in the C57BL strain is fairly consistent with the data of ABRAMS (1951) on the same strain involving a range from 30 to 90 days of age. His observations integrated with those given here strongly suggest that a rapid rate of increase of the LD_{50} occurs as age increases from 30 to 60 days. Both sets of data indicate a progressively declining rate of increase beyond 60 days of age. The other three strains are similar in having a linear dependence of the LD_{50} on age. No data are available to ascertain the nature of the curve prior to or beyond the given age interval. It is possible that all strains have the same basic age dependence curve, but the comparability of response occurs over a different chronological age interval in each strain.

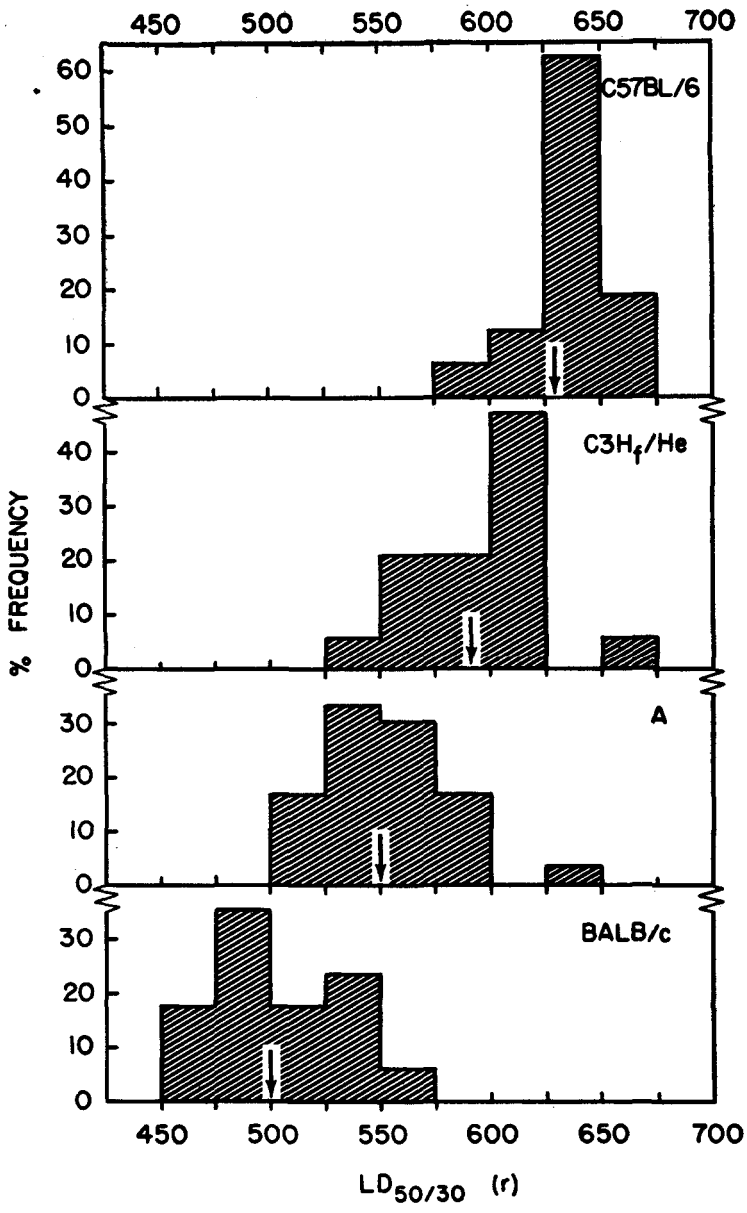

FIGURE 1.—Frequency distribution of individual test $LD_{50/30}$ values by class intervals of 25r for all strains after removal of variation in age, parity and litter size. Arrows indicate position of means.

In parity and litter size there is no consistent effect from strain to strain. This analysis may not be the most efficient in regard to these variables since litter size generally shows a shallow curvilinear relation to parity. If a linear relationship is employed the precision of estimates involving either parity or litter size is reduced.

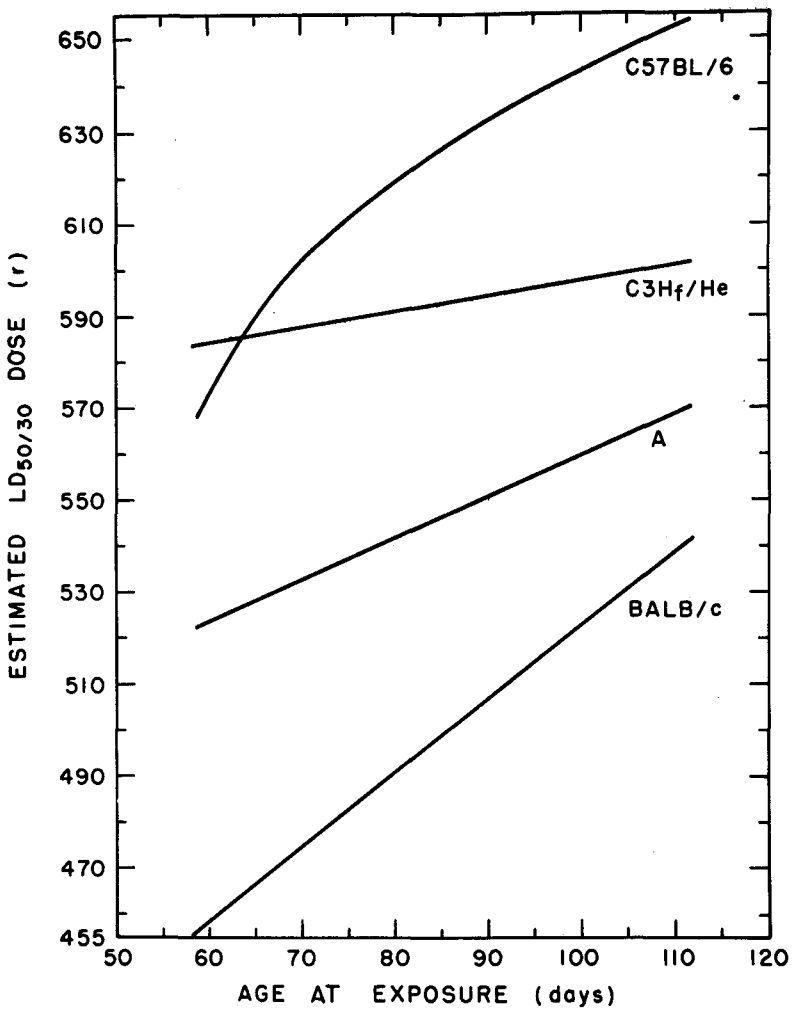

FIGURE 2.—Dependence of $LD_{50/30}$ values on age at exposure for all strains at a constant parity and litter size of 3.5 and 6.3, respectively.

In particular, the significance of all partial correlations is uncertain. They are presented, however, because deviations from linearity with regard to parity and litter size are not excessive. In all instances, age:response relationships are linear on the scale indicated in table 3.

LD_{50} values and dosage-mortality slopes were also estimated for both sexes in each strain. Sex differences in these values were not significant.

DISCUSSION

The underlying assumption in the use of a linearizing transformation of toxicity data is that individual sensitivity is a continuously and normally distributed variable (MATHER 1946). The derived dosage-mortality slope describes this distribution since

TABLE 3

Multiple regression equations and partial correlations of percent mortality on age, parity and litter size

Strain	Regression equations:	Strain means		
		Age x_1	Parity x_2	Litter size x_3
BALB/c	$Y = 115.99 - 0.990x_1 + 2.174x_2 + 1.825x_3$	85.2	3.60	5.85
A	$Y = 118.45 - 0.640x_1 - 1.191x_2 - 0.878x_3$	87.2	3.37	5.66
$C3H_f$/He	$Y = 73.04 - 0.246x_1 - 0.765x_2 + 0.135x_3$	84.1	3.78	6.74
C57BL/6	$\log Y = 2.473 - 0.010x_1 + 0.035x_2 - 0.009x_3$	85.3	3.38	7.18

	Partial correlations:		
	$r_{Y1\cdot23}$	$r_{Y2\cdot13}$	$r_{Y3\cdot12}$
BALB/c	−.486	+.244	+.213
A	−.508	−.144	−.101
$C3H_f$/He	−.335	−.161	+.025
C57BL/6	−.589	+.512	−.156

the inverse slope is proportional to its standard deviation. With the probit transform, the inverse slope directly produces the standard deviation. The arcsine transform used in this study can be re-expressed in probit terms by means of a conversion factor that relates the two transforms to each other. As indicated by FINNEY (1952), a nearly linear relation exists between the probit and arcsine transformations for percentage values between five and 95. The linear regression coefficient of probit values on arcsine values is 0.048. The product of this factor and the arcsine-mortality slope provides a close approximation of the probit-mortality slope which is then used to derive the standard deviation in terms of roentgens.

Standard deviations calculated in the above manner for the four strains are: BALB/c: ±56.5r; A: ±50.0r; $C3H_f$: ±48.1r; C57BL: ±40.3r. These values are descriptive of the variability of expression of each genotype on the dose scale and can be considered as manifestations of the within-strain phenotypic variances.

The results of the investigation lead, therefore, to one principal conclusion: the radiosensitivity of each strain is characterized by two parameters, a mean (or LD_{50}) and a variance (or dosage-mortality slope). Both parameters are equally significant as quantities that describe the fundamental properties of the strain. In these data, susceptibility to the acute lethal effects of X-irradiation is associated with a greater phenotypic variance since the slope and LD_{50} decrease concomitantly.

The magnitude of the phenotypic variance, defined above, is presumed to be an innate characteristic of the genotype and not a function of genetic heterogeneity. All of the strains tested have been inbred by full-sib matings for at least 50 generations. It is therefore reasonable to assume that any residual genetic variation existing in a strain would make only an insignificant contribution to the total variability of response to X-irradiation.

With respect to the radiation response, it is assumed that the primary event, the absorption of energy, leads to identical primary injury in all strains. The initial expression of injury, occurring one to five days after exposure, is undoubtedly very

similar in all strains. Studies by GRAHN (1954) on the genetic variation in body weight response after X-irradiation showed that for the first two days very little strain difference existed. After five days postirradiation strain variation in time and rate of recovery from the initial expression of injury became apparent. It is similarly concluded here that strain differences in the LD_{50} value are a function of different rates of recovery of physiological systems important in the manifestation of acute radiation lethality. In this regard, the hematopoietic system is of primary importance, as indicated by the success of spleen and marrow protection in enhancing survival (JACOBSON 1952). In addition, SMITH *et al.* were able to show with mice that survival rate between four and 19 days after irradiation is closely related to the granulocyte count under routine experimental conditions (1954a) and also when the animals were challenged with a *Pseudomonas aeruginosa* infection (1954b). During this period, hematopoietic recovery is most active.

Although no data are yet available on the postirradiation blood counts for these strains, it is postulated that the time and rate of recovery of the hematopoietic system are major variables in the expression of strain differences in acute lethality. The mode of gene action under this postulate may be the control of the rate of production of blood cells during recovery. Additionally, it is possible that the more resistant genotype may act to create a greater physiological sensitivity to feed-back impulses arising from the depleting blood system.

If susceptible mice remain at a critical physiological level for longer periods of time as a result of slower recovery rates, then two features of the survival time data should emerge in support. One, the survival time of decedents in the 30-day interval should be greater (SACHER 1951). Two, during the modal period of deaths from the hematopoietic syndrome, which has been described by BOND *et al.* (1954), mortality should be sustained at a higher rate to a later time in the 30-day interval. A preliminary analysis of the survival time data shows that strains BALB/c, $C3H_f$ and C57BL are in close adherence to expectation for both criteria. They have mean survival times of 14.6, 13.9 and 13.3 days respectively, while the crude daily rate of mortality remains above two percent per day until about 21, 18 and 17 days. There is a secondary contribution to the mortality between 20 and 30 days, generally running at a rate less than two percent per day. Strain A mice, which have a shorter survival time than all other strains, have an almost negligible incidence of death in this latter period. A more detailed analysis of the time rates of mortality, to be reported subsequently, may provide insight into the apparent inconsistency of the A strain.

Increases in susceptibility that are concurrent with increases in survival time are also noted between species of widely different radiosensitivity (SACHER 1951). BRECHER and CRONKITE (1952) gave evidence that, here too, the rate of recovery of the hematopoietic system is of primary significance in accounting for species differences. Thus, there is good general adherence between and within species to the hypothesis that greater susceptibility to irradiation is a consequence of slower rates of recovery, particularly of the hematopoietic system. The general hypothesis will be given formal treatment by SACHER.

A comparison of the results of this study with those from other laboratories is

difficult because of wide variation in experimental procedure. There is apparently no comparative data on the BALB/c strain, although a related strain, BALB/Gw, is known to be relatively radiosensitive (GRAHN 1954). LORENZ *et al.* (1952) gave LD_{50} values for 3-month old mice of strains A and $C3H_f$ as 560r and 600r, respectively, after exposure to 186 kvp X-rays. The similarity with the results of the present study undoubtedly rests upon the comparability of age and radiation factors.

KAPLAN and BROWN (1952) report an LD_{50} value for 33-day old C57BL mice of 486r after exposure to 120 kvp X-rays. This value may well be consistent with the present data in light of the rapid rate of change of the LD_{50} with age in this strain.

In consideration of the fact that variation in X-ray quality alone can alter the LD_{50} for mice by as much as 30 percent (GRAHN *et al.* 1956), it cannot be overemphasized that for useful interlaboratory comparison radiation quality, in addition to strain, age and sex, must be clearly defined. When knowledge of the dosage-mortality slope is available, this should be given because of its significance in defining the basic variability of response.

SUMMARY

1. Following single whole-body exposure to X-irradiation, the $LD_{50/30}$ value for four inbred strains of mice is shown to vary from 500r for the most susceptible strain to 630r for the most resistant strain.

2. The existence, between strains, of a positive relationship between the dosage-mortality slope and the LD_{50} value indicates that the basic within-strain phenotypic variance is positively correlated with susceptibility.

3. Age is a major variable which affects response with resistance increasing progressively between 60 and 110 days of age. Parity and litter size are inconsistent and minor in effect.

4. No significant sex differences were indicated either in the LD_{50} values or in the dosage-mortality slopes.

5. It is postulated that the time and rate of recovery of the hematopoietic system are the major physiological variables in the expression of genetic differences in the acute radiation response. Certain indirect evidence is presented to support this postulate.

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LITERATURE CITED

ABRAMS, H. L., 1951 Influence of age, body weight and sex on susceptibility of mice to the lethal effects of X-radiation. Proc. Soc. Exp. Biol. Med. **76:** 729–732.

BOND, V. P., M. S. SILVERMAN, and E. P. CRONKITE, 1954 Pathogenesis and pathology of post-irradiation infection. Radiation Res. **1:** 389–400.

BRECHER, G., and E. P. CRONKITE, 1952 Correlation of bone marrow injury with mortality in different species of irradiated animals. Am. J. Clin. Pathol. **28:** 551 (Abst.).

EVANS, T. C., 1948 Effects of small daily doses of fast neutrons on mice. Radiology **50:** 811–834.

FINNEY, D. J., 1952 *Statistical Method in Biological Assay.* Hafner Publishing Co. New York.

GOWEN, J. W., 1956 Effects of whole body exposure to nuclear or X-ray energy on life span and life efficiency. A/Conf/8/P/256. International Conference on the Peaceful Uses of Atomic Energy. Geneva. 1955. To be published in Proceedings of the Conference.

GRAHN, D., 1954a Genetic variation in the response of mice to total body X-irradiation. I. Body weight response of six inbred strains. J. Exp. Zool. **125:** 39–61.

1954b Genetic variation in the response of mice to total body X-irradiation. II. Organ weight response of six inbred strains. J. Exp. Zool. **125:** 63–83.

GRAHN, D., G. A. SACHER, and H. WALTON, JR., 1956 Comparative effectiveness of several X-ray qualities for acute lethality in mice and rabbits. Radiation Res. **4:** 228–242.

HENSHAW, P. S., 1944 Experimental roentgen injury. II. Changes produced with intermediate-range doses and a comparison of the relative susceptibility of different kinds of animals. J. Natl. Cancer Inst. **4:** 485–501.

HENSHAW, P. S., E. F. RILEY, and G. E. STAPLETON, 1947 The biologic effects of pile radiation. Radiology **49:** 349–360.

JACOBSON, L. O., 1952 Evidence for a humoral factor (or factors) concerned in recovery from radiation injury: a review. Cancer Research **12:** 315–325.

KAPLAN, H. S., and M. B. BROWN, 1952 Mortality of mice after total-body irradiation as influenced by alterations in total dose fractionation and periodicity of treatment. J. Natl. Cancer Inst. **12:** 765–775.

KAPLAN, H. S., and J. PAULL, 1952 Genetic modification of response to spleen shielding in irradiated mice. Proc. Soc. Exp. Biol. Med. **79:** 670–672.

KIRSCHBAUM, A., and H. W. MIXER, 1947 Induction of leukemia in eight inbred stocks of mice varying in susceptibility to the spontaneous disease. J. Lab. Clin. Med. **32:** 720–731.

KOHN, H. I., 1951 Changes in composition of blood plasma of the rat during acute radiation syndrome, and their partial mitigation by dibenamine and cortin. Am. J. Physiol. **165:** 27–42.

LORENZ, E., W. E. HESTON, A. B. ESCHENBRENNER, and M. K. DERINGER, 1947 Biological studies in the tolerance range. Radiology **49:** 274–285.

LORENZ, E., CONGDON, and D. UPHOFF, 1952 Modification of acute irradiation injury in mice and guinea pigs by bone marrow injections. Radiology **58:** 863–877.

MATHER, K., 1946 *Statistical Analysis in Biology.* Interscience Publishers, Inc. New York.

RUGH, R., 1953 Radiobiology, irradiation lethality and protection. Military Surgeon **112:** 395–413.

SACHER, G. A., 1950 The survival of mice under duration-of-life exposure to X-rays at various dose rates. Univ. Chicago Metallurgical Lab. Report CH **3900.**

1951 Remarks on the relation between single LD_{50} and the mean survival times of decedents. Quart. Report Biol. and Med. Research Div. Argonne Nat. Lab. ANL **4571:** 109–114.

A mathematical analysis of acute radiation injury and lethality. In preparation.

SMITH, W. W., L. GONSBERG, I. ALDERMAN, and J. CORNFIELD, 1954a Effect of granulocyte count and litter on survival of irradiated mice. Am. J. Physiol. **178:** 474–476.

SMITH, W. W., R. Q. MARSTON, H. J. RUTH, and J. CORNFIELD, 1954b Granulocyte count, resistance to experimental infection and spleen homogenate treatment in mice. Am. J. Physiol. **178:** 288–292.

SNEDECOR, G. W., 1946 *Statistical Methods.* 4th edition. Iowa State College Press. Ames.