Title
Animal Selection in Radiobiological Research

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ANIMAL SELECTION IN RADIOBIOLOGICAL RESEARCH[1]

DISCLAIMER

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Running Title: Animal Selection in Radiobiology......

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Summary. The pattern of body weight changes of mice before irradiation modified their responses to doses of gamma radiation in the midlethal range. A significant increase in mortality compared to control mice was found for mice that had either lost weight prior to irradiation, or had gained more weight than the average weight gain of the population. The results emphasize that selection criteria in animal radiobiological experiments can influence results.

Mammalian sensitivity to radiation is often indicated by the LD_{50}, that is, the amount of radiation needed to kill half of the original population in a specified time, usually 30 days. Mortality is a consequence of radiation damage to bone marrow stem cells (1). For results to be maximally reproducible between experiments and to be comparable to data reported by other investigators, there should be strict criteria used to select animals from a stock population for use in radiation experimentation. For example, it has been shown that there is a definite "cage" effect that strongly influences mortality of irradiated mice, in that the mortality after a given dose of radiation was not independent of the number of mice per cage (2). The reason for the effect was not determined. This effect has also been reported by Hahn and Howland (3), who found an increased 30 day mortality for multiply housed versus single housed rats. The authors felt that social pressures exerted by multiple housing increased radiosensitivity. In this research, the mortality of mice to irradiation as a function of body weight changes from 8 to 10 weeks of age have been examined.

MATERIALS AND METHODS

Animals: Animals used were male Swiss Webster noninbred mice, obtained from the Simonsen Breeding Laboratories, Gilroy, California. Nutrient agar cultures of heart blood of randomly selected mice showed no *Pseudomonas aeruginosa* or *Salmonella* sp. organisms present prior to irradiation. Fecal samples were also negative. Animals were maintained on laboratory mouse food and chlorinated water *ad libitum* for the duration of the experiments.

Animal Selection Procedures: Mice were received from the supplier at 49 ± 3 days of age, and were housed (15 per cage) in large cages. For the first week after receipt, 1 teaspoon of Terramycin powder (Charles Pfizer Co.) was added to the water bottles to act as a broad spectrum antibiotic in an effort to insure a healthy preirradiation mouse colony. At 8 weeks of age, each mouse was housed in a pint Mason jar with cedar chip bedding and 100 ml water bottles (Fig 1). From the eighth through the tenth weeks of age, the mice were weighed three times over a period of 10 days.

For the purpose of testing the effects of body weight changes on radiosensitivity, the following groups were intercompared:

Controls: These mice had gained weight from the eighth to the tenth weeks and their weights were within ± 4 grams of the mean weight of the entire population at the tenth week. These mice comprise our usual experimental group and usually constitute about 70 percent of the original number of mice.

Reject 1 (R1): Even though the mean weight of the population increases over the weighing interval, there are mice that lose weight during this time. These mice comprise the R1 experimental group, although the proviso was made that the weight of these mice at the tenth week also had to be within ± 4 grams of the mean weight of the population.

Reject 2 (R2): This group of mice had body weights that remained constant from the eighth to the tenth week, and also had a weight at irradiation within ± 4 grams of the mean population weight.

Reject 3 (R3): These mice gained weight from the eighth to the tenth weeks, but their weight at irradiation was more than 4 grams below the mean weight of the population.

Reject 4 (R4): These mice had gained weight from the eighth to the tenth weeks, but their weights were more than 4 grams above the mean weight of the population at the time of irradiation.

Irradiation Procedures: Mice were irradiated, 6 at a time, with a cobalt-60 -

gamma-ray source of 1500 Curies strength. Mice were 70 cm from the source, and a 5 mm lucite shield was placed immediately in front of the source to screen out any interference from secondary electron production. The air dose rate at the position of the mouse was measured with a Victoreen R-meter and was found to be about 70 Roentgens per minute. The conversion from air Roentgen dose to the absorbed tissue dose in rads was obtained by multiplying the Roentgen dose by 0.875. Radiation doses were given which produce damage primarily to the hematopoietic system (740-835 rads), and mice with this type of injury die from the consequences of bone marrow failure, normally within 9-17 days after irradiation with an average survival time of approximately 11 days.

Hematological Studies: To see if observed mortality differences between groups could be related to the preirradiation hematopoietic status of the blood forming tissues, blood was obtained from the orbital sinus venous complex in unanesthetized mice and packed cell volumes, white blood cell counts, and differential white blood cell counts were done. Also, mice were killed by cervical dislocation, one humerus was removed and the bone was washed out by use of Hank's balanced salt solution. The cell suspension was aspirated repeatedly to obtain a single cell suspension and was then centrifuged at 1200 g. The pellet was resuspended in a small amount of fetal calf serum, and the suspension was streaked on glass slides with a camelhair brush, air dried, and fixed with absolute methanol. Differential counts of bone marrow cells were performed on 500 cells, stained with Wright's stain. Due to the small number of animals in the reject groups, postirradiation analysis of bone marrow and peripheral blood changes were not done.

RESULTS AND DISCUSSION

In Table 1 are shown the weight data for 8 different radiation experiments over a time period of about 1 year. In Fig 2 are plotted the mean weight values of the 8 experiments for each of the three weighings performed, along with their standard errors. The average weight of the mice increased by 2.8 grams over the ten-day weighing period. A two-tailed t-test of the average weights of the eight different experiments versus the time of weighing showed that the average weights were significantly different from one another at the 0.05 level of probability.

With regard to the effects of preirradiation weight change patterns on subsequent radiation-produced mortality, the relative radiosensitivities of the groups obtained from the original mouse population are shown in Table 2. The slopes and standard deviations of the mortality curves were calculated using the method of Litchfield and Wilcoxon (4) and differences were evaluated using a t-test. Of the four reject groups, there are two (R2 and R3) that do not show a radiosensitivity different than found in the control mice. The respective $LD_{50/30}$ values are 796 rads (controls); 801 rads (R2); and 808 rads (R3). However, there are two groups that do show a significantly greater radiosensitivity as compared to the control group. These are the R1 and R4 groups with $LD_{50/30}$ values of 741 rads and 744 rads respectively. These are mice that have either lost weight (R1), or have gained more weight than the control mice (R4) during the preirradiation weighing period.

Although a difference in radiation sensitivity is found for the R1 and R4 groups of mice as compared to controls, the reason for such a difference in sensitivity is not known. As radiation mortality at these dose levels is related to depression in hematological status, it was hoped that examination of the peripheral blood and the bone marrow preirradiation might show differences that could be related to subsequent mortality. However, as the data in Tables 3 and 4 show, there are no significant differences in the values of several hematological parameters between experimental groups. Therefore, the difference in mortality must tentatively be attributed to other than primary differences in hematological integrity, and these indirect influences are not known.

It would seem that if radiation experimentation with mice involves an estimate of a $LD_{50/30}$ value, then some type of preirradiation culling of animals prior to irradiation appears necessary. For example, in the Swiss Webster mice, the proportions of the total population that fall into the control, R1, R2, R3 and R4 categories are respectively about 70, 10, 5, 10 and 5 percent. Given their respective $LD_{50/30}$ values, this would give a weighted average $LD_{50/30}$ of about 788 rads, a value not significantly different than the $LD_{50/30}$ value for the control population (796 rads). However, it would certainly enlarge the error variance of the estimation of the $LD_{50/30}$ value.

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TABLE I

Pattern of weight change of mice over a 10 day interval from the eighth to the tenth weeks of age

Experiment Number	Weighing No. 1	Weighing No. 2	Weighing No. 3	Experimental Date
1	28.0 ± 0.9[a] (259)[b]	28.8 ± 0.8	30.6 ± 0.8	6/12/68
2	27.8 ± 0.9 (263)	28.8 ± 0.9	31.4 ± 1.0	8/1/68
3	28.0 ± 0.8 (247)	28.9 ± 0.9 (	33.0 ± 1.1	10/1/68
4	30.0 ± 0.5 (410)	30.9 ± 0.6	32.4 ± 0.4	12/1/68
5	29.5 ± 0.4 (735)	30.4 ± 0.5	30.9 ± 0.6	2/1/69
6	29.8 ± 0.8 (466)	30.7 ± 1.0	32.5 ± 0.3	3/1/69
7	28.6 ± 0.7 (659)	29.0 ± 0.2	31.0 ± 0.6	4/1/69
8	28.1 ± 0.5 (893)	29.3 ± 0.4	30.5 ± 0.3	6/1/69
Average Weights	28.7 ± 0.3[c]	29.6 ± 0.3	31.5 ± 0.4	

[a]Value indicates the standard deviation of the mean weight of the population.

[b]Values in parentheses indicate the number of mice used in each experiment.

[c]Indicates the standard error of the average mean weight of the 8 experiments.

TABLE 2

Thirty day postirradiation mortality of mice irradiated with gamma-rays at 10 weeks of age as a function of weight changes in a 2 week period preirradiation

Group	A	B	Dose (rads)	No. Dead/ No. Irradiated	Percent Mortality (95% confidence limits)	Mean Survival time (days)
Controls	+	100 ± 12.7	740	14/48	29 (17-44)	11.7
	+	100 ± 12.7	796	24/48	50 (35-65)	13.5
	+	100 ± 12.7	853	34/48	71 (56-83)	12.8
Estimated $LD_{50/30}$ for Controls is 796 rads (752-840 ; 95% confidence limits)						
R1	-	100 ± 12.7	740	11/22	50 (27-69)	14.2
	-	100 ± 12.7	796	21/30	70 (51-85)	13.5
	-	100 ± 12.7	853	29/32	91 (75-98)	14.0
Estimated $LD_{50/30}$ for the R1 group is 741 rads (700-783)						
R2	0	100 ± 12.7	740	5/18	28 (10-54)	12.2
	0	100 ± 12.7	796	8/18	44 (21-69)	13.1
	0	100 ± 12.7	853	13/18	72 (46-90)	13.9
Estimated $LD_{50/30}$ for the R2 group is 801 rads (760-844)						
R3	+	87.3	740	6/24	25 (9-46)	11.9
	+	87.3	796	15/33	45 (28-64)	12.7
	+	87.3	853	16/24	67 (45-86)	15.0
Estimated $LD_{50/30}$ for the R3 group is 808 rads (763-854)						
R4	+	112.7	740	15/30	50 (31-69)	14.3
	+	112.7	796	15/21	71 (48-89)	13.3
	+	112.7	853	31/36	86 (70-96)	.2.0
Estimated $LD_{50/30}$ for the R4 group is 744 rads (701-788)						

A Indicates direction of weight change from the eighth to the tenth weeks of age; + indicates weight gain; 0 indicates no change in weight; - indicates weight loss as compared to controls.

B Weight (relative to controls) of group at the last weighting before irradiation.

TABLE 3

Preirradiation peripheral blood hematological values of Swiss Webster male mice separated into groups on the basis of weight gain prior to irradiation

Group	No. of mice	White Blood Count 1,000's/mm^3	Neutrophils percent	Lymphocytes percent	Monocytes percent	Eosinophils percent	Packed Cell Volume, percent
Controls	13	3.85 ± 0.4[a]	12.0 ± 1.1	85.0 ± 1.8	0.8 ± 0.4	2.2 ± 0.5	50.6 ± 0.7
Reject 1	5	5.20 ± 0.5	8.8 ± 1.3	86.2 ± 3.7	1.2 ± 0.4	3.8 ± 1.0	49.2 ± 1.4
Reject 2	5	4.65 ± 0.8	14.0 ± 1.5	83.6 ± 4.0	0.8 ± 0.5	1.6 ± 0.7	48.6 ± 0.9
Reject 3	5	3.90 ± 0.7	20.2 ± 1.6	76.6 ± 2.5	0.6 ± 0.4	2.6 ± 0.9	50.2 ± 1.5
Reject 4	5	4.98 ± 0.6	10.0 ± 1.4	88.0 ± 3.0	1.0 ± 0.6	1.0 ± 0.4	48.7 ± 0.7
Reject 5	5	5.05 ± 0.8	13.3 ± 1.2	83.8 ± 3.1	0.9 ± 0.4	2.2 ± 1.2	49.1 ± 1.4

[a] Values represent the mean of the observations and the standard error of the mean.

TABLE 4

Preirradiation differential count of nucleated cells in the bone marrow of male Swiss Webster mice separated into groups on the basis of weight gain prior to irradiation

	Group					
Cell Type	Controls (13)[a]	Reject 1 (5)	Reject 2 (5)	Reject 3 (5)	Reject 4 (5)	Reject 5 (5)
Total Neutrophils	41.1 ± 1.2[b]	41.9 ± 3.0	45.4 ± 2.4	34.8 ± 5.2	38.3 ± 2.8	44.5 ± 2.1
Myeloblasts	1.9 ± 0.4	2.3 ± 2.0	1.0 ± 0.6	3.2 ± 1.7	2.6 ± 1.3	1.3 ± 0.8
Promyelocytes	6.6 ± 0.5	7.6 ± 3.2	6.6 ± 2.1	4.5 ± 1.2	4.4 ± 0.6	3.5 ± 1.2
Myelocytes	7.9 ± 0.6	6.6 ± 2.4	8.9 ± 3.2	5.8 ± 1.7	6.8 ± 2.1	7.1 ± 1.4
Metamyelocytes	6.5 ± 0.6	3.4 ± 1.8	9.1 ± 1.8	3.2 ± 0.7	5.5 ± 1.4	7.7 ± 1.6
Bands	13.5 ± 0.7	15.0 ± 1.4	16.0 ± 1.3	13.3 ± 1.4	12.8 ± 1.8	14.4 ± 1.1
Segmented	4.7 ± 0.3	7.0 ± 0.9	3.8 ± 1.4	4.8 ± 1.1	6.2 ± 1.2	5.5 ± 1.3
Eosinophils	5.3 ± 0.8	6.1 ± 1.5	9.0 ± 1.5	8.5 ± 1.3	6.7 ± 1.1	5.6 ± 1.1
Erythroid Cells	21.2 ± 2.0	22.0 ± 4.0	19.0 ± 1.4	24.5 ± 4.1	26.2 ± 3.5	18.8 ± 1.6
Lymphocytes	31.4 ± 1.4	29.0 ± 1.1	27.0 ± 2.4	31.2 ± 6.8	28.2 ± 1.9	30.1 ± 3.0

[a] Indicates the number of mice used.

[b] Values are mean values and the standard error of the mean.

Fig 1. Photograph of the single housing condition used for mice in radiation experiments to minimize animal fighting and cross-contamination.

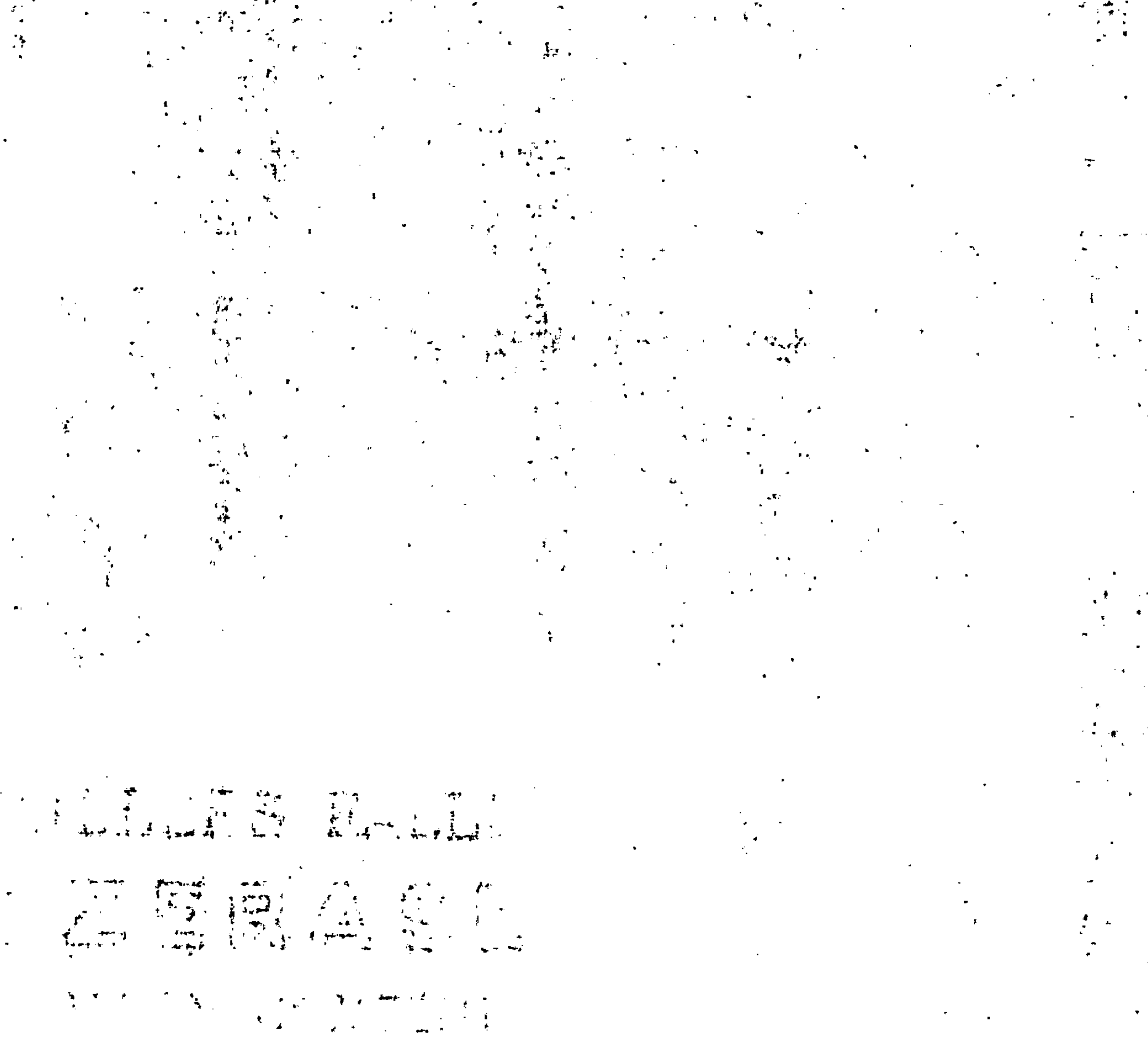
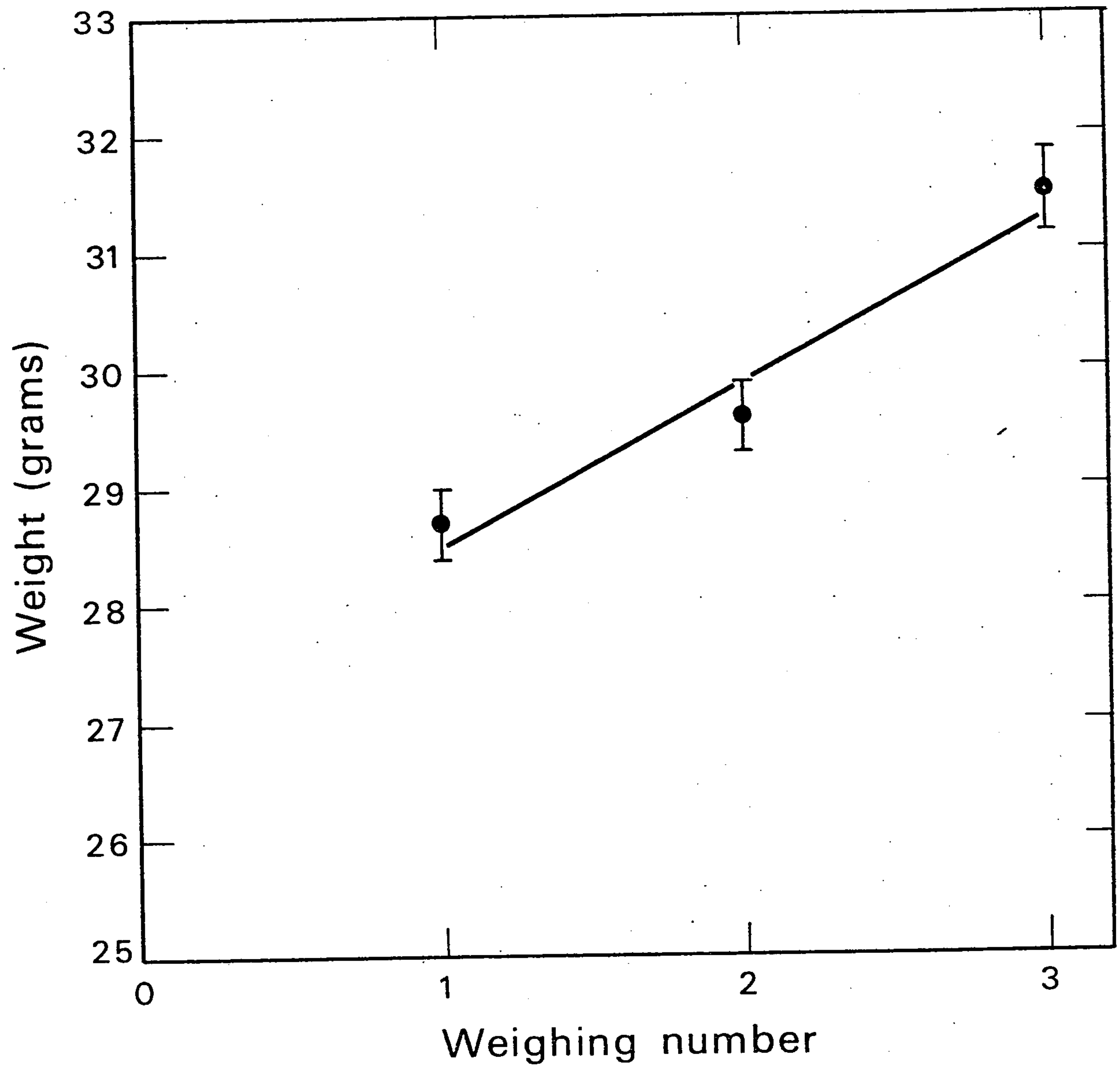


Fig 2. Weight gain of mice over 3 weighings from the eighth to the tenth weeks of age. Each point is the mean and standard error of eight experiments. The linear least-squares regression equation of the line is Y = 27.1 + 1.4 X. A chi-square analysis of the data showed that the deviation of the points from a linear relationship is not significant over this time interval.



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