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The effects of x-irradiation on quantitative genetic traits was investigated by comparing the response of irradiated mice to selection for litter size and 28-day weight with the response to selection for the same two traits in non-irradiated lines. Irradiated lines were maintained at three levels of x-irradiation, 25 r, 50 r and 100 r, administered to both males and females immediately before pairing. No matings were made later than 14 days after irradiation so that only the effects of x-irradiation upon the post-meiotic stages of gametogenesis were studied.

The lines receiving 50 r and 100 r were all lost by the fourth generation of selection. An interaction of genetic and physiological factors was assumed to be responsible for the reduced fertility leading to the termination of these lines.

Selection response for litter size was negative in five of the six irradiated lines. This was attributed to reduced selection

differentials due to lower fertility and to reduced heritability due to physiological masking of the additive genes for litter size. It appears that the accumulation of recessive lethals also may have played a role in reducing heritability in the irradiated lines.

Selection response for 28-day weight was positive in four of the six irradiated lines, although less than in the non-irradiated controls in most cases. Three of the irradiated lines had realized heritability values greater than either of the controls. Some increase in usable genetic variance for 28-day weight was indicated although reduced selection differentials prohibited increased selection response.

Irradiation of females in metestrus or one day after metestrus increased the size of their litters. Females mating later than one week after irradiation produced fewer litters and litters of smaller size than control females. Most of the fertility problems encountered in irradiated animals could be attributed to the females. Histological examination eight months post-irradiation revealed serious radiation damage in ovaries while spermatogenesis appeared normal. Females receiving 50 r and 100 r had a significantly higher incidence of mammary gland tumors at ten months of age than the non-irradiated controls.

Effects of Radiation on Selection Progress in Mice

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TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	3
MATERIALS AND METHODS	13
RESULTS AND DISCUSSION	19
Litter Size	19
Twenty-eight Day Weight	29
Reproductive Fitness	35
Tumor Incidence	48
SUMMARY AND CONCLUSIONS	50
BIBLIOGRAPHY	54

LIST OF FIGURES

Figure		Page
1.	Response of individual lines to five generations of selection for large litters.	21
2.	Response of different treatment groups (replicated lines combined) to five generations of selection for large litters.	22
3.	Increase in mean inbreeding coefficients for individual lines during five generations of selection.	24
4.	Response of individual lines to five generations of upward selection for 28-day weight.	31
5.	Response of different treatment groups (replicated lines combined) to five generations of upward selection for 28-day weight.	32
6.	Average size of litters of lines receiving 0 r, 50 r and 100 r of x-irradiation born on various days after pairing of dam and sire.	36
7.	Number of litters born in different treatment groups (replicas combined) by days after pairing.	39
8.	Number of litters born by days after pairing (non-irradiated lines combined and irradiated lines combined).	40
9.	Photomicrographs of seminiferous tubules from a non-irradiated male (left) and a male receiving 100 r x-irradiation (right) showing restoration of spermatogenesis eight months after irradiation.	46
10.	Photomicrographs of ovaries from mice receiving 0 r (A), 25 r (B), 50 r (C) and 100 r (D) of x-irradiation showing the relative degrees of radiation damage eight months after irradiation.	46

LIST OF TABLES

Table		Page
1.	Selection intensity (expressed in standard deviations) for litter size during five generations of selection.	19
2.	Means and standard deviations for litter size during five generations of selection.	20
3.	Selection response and realized heritability for litter size during five generations of selection.	25
4.	Selection intensity (expressed in standard deviations) for 28-day weight during five generations of selection.	30
5.	Means and standard deviations for 28-day weight during five generations of selection.	30
6.	Selection response and realized heritability for 28-day weight during five generations of selection.	34
7.	Number of litters born per generation in each of five treatment groups.	37
8.	Means and standard errors of pituitary, thyroid, and adrenal weights expressed in mgs. per gm. body weight.	41
9.	Means and standard errors of the diameter of the three largest follicles, diameter of the adrenal medulla, width of the adrenal cortex, and height of the seminal vesicle epithelium in mice from each level of radiation treatment.	43
10.	Means and standard errors of ovary, uterus, testicle and seminal vesicle weights in mice from each level of radiation treatment.	44
11.	Means and standard errors of the number of developing follicles and corpora lutea in ovaries from mice of different levels of radiation treatment.	47

LIST OF TABLES (CONTINUED)

Table		Page
12.	Means and standard errors of the size of the largest follicle and largest corpus luteum in ovaries from mice receiving 0 r and 25 r of x-irradiation.	48
13.	Incidence of mammary gland tumors in ten month old mice treated at four levels of irradiation.	49

EFFECTS OF RADIATION ON SELECTION PROGRESS IN MICE

INTRODUCTION

Selection, or differential-reproduction, is recognized as an important force in the alteration of a heriditary trait that is influenced by a multiplicity of genes. Selection progress, however, is often slow and disappointing, especially when the number of genes controlling the trait of interest is quite large. Selection limits have also been encountered in which progress is made at a diminishing rate as the limit is approached, after which there is a "plateau" at a level of progress which seemingly cannot be improved by further selection. This may be due to one of at least three different situations:

a. When selection favors individuals heterozygous at some loci.

b. When natural selection opposes the direction of artificial selection.

c. Or, when genetic variance is exhausted.

Most attempts to accelerate response to selection or to avoid restricting limits have been based upon the idea of providing more usable genetic variation to the population.

Several methods such as crossing previously selected lines, outcrossing, using new mating systems and inducing new genetic

variation by mutagenic agents have been used to accelerate selection progress or circumvent plateaus. The present study is an investigation of the effects of x-irradiation on response to selection for 28-day weight and litter size in mice and an exploration into some of the problems involved in experiments of this nature. These problems include the induction of sterility, tumors and other physiological conditions which affect the fitness of individuals in the population.

The importance of such a study is not limited to the scientific knowledge obtained concerning the genetic effects of irradiation but also has economic implications to plant and animal breeders. The economic value of improving plants and animals by irradiation and selection will increase as nuclear energy becomes more readily available and less expensive and as our knowledge of its proper use increases.

REVIEW OF LITERATURE

Artificial selection has probably been practiced to some degree since man first domesticated animals and plants. However, it was not until around 1920 that a sound theoretical basis for quantitative genetics was established. The work of Fisher (1918), Wright (1921), Haldane (1924), and others united Mendelian inheritance with quantitative inheritance by postulating the simultaneous segregation of genes at many loci. Working upon this foundation, Zeleny (1922), MacArthur (1949), Falconer (1955), Fraser and Kindred (1960), Wolfe (1960) and many others have performed selection experiments and explained their results in accordance with Mendelian laws. Good summaries of selection studies and explanations for the results of selection are given by Bogart (1959) and Falconer (1960a).

The discovery of the mutagenic effect of ionizing radiations gave birth to many new realms of study in the field of genetics. Such mutagenic effects were postulated very early in the history of genetics. It is quite likely that the wing mutations obtained by Morgan (1911) after exposing Drosophila to radium, were of the induced type. However, he realized the importance of the genetic purity of the material used in this study and was reluctant to accept this isolated example as induced mutation. Repeatable demonstration of mutation induction was not possible until Muller (1927)

published his well known CIB technique for detecting sex-linked recessive lethals and showed, without question, that x-irradiation did increase the occurrence of such lethals.

Attempts to utilize radiation induced variation in accelerating selection progress came within a decade of Muller's work. Serebrovsky (1935) and Rokizky (1936) were probably the first to study the effect of radiation on the genetic variation affecting a quantitative trait. By treating Drosophila with x-rays they were able to increase the variance in the number of sternopleural bristles. They were unable, however, to increase response to selection in the irradiated lines. A high level of inbreeding was attained and Clayton and Robertson (1964) suggest this as an explanation for their largely negative results.

Buzzati-Traverso (1953) and Scossiroli (1954) reported an entirely different outcome from their studies. By alternating irradiation (3,000 r per generation) and selection they almost doubled the number of sternopleural bristles in a population of Drosophila that had presumably reached a plateau for the trait under previous selection. Selection for a low number of bristles however, showed little further response under the same treatment. Scossiroli later repeated the experiment (Scossiroli and Scossiroli, 1959) using isogenic lines and a population based on crossing two isogenic lines. He was able to increase sternopleural bristle number from about

17 to 25 with ten generations of selection and irradiation with 3,000 r of x-rays given to both males and females. He also compared the effect of irradiation in isogenic and hybrid lines and was able to conclude that x-ray-induced increase in recombination rates was not an important factor in producing new genetic variability, at least in this experiment.

Clayton and Robertson (1955) reported only modest gains in selection response and genetic variation for abdominal bristles in highly inbred lines of Drosophila derived from their Kaduna population. They later employed a technique similar to Scossiroli's (Clayton and Robertson, 1964) on two distinct sets of plateaued populations. It differed slightly in that only 1,800 r was administered to adults each cycle and the irradiated flies were mass mated rather than pair mated. Both sets of populations were derived from the Kaduna population as in their 1955 study. Their results were once again modest and they attributed Scossiroli's spectacular results to a phenomenon peculiar to his line of flies rather than a general effect of irradiation. From these studies they estimated the dose required to introduce new variation equal to that in a standard outbred population to be 500,000 r. An additional aspect of this study was the appearance of a major gene, 'scabrous'. This gene is known to increase sternital bristle number by 15-20. Since this same gene had been found in another line derived without irradiation from the

same base stock, its occurrence could not be ascribed to irradiation.

Although the work of Yamada and Kitagawa (1961) was designed to estimate the x-ray doubling dose for genetic variance of quantitative traits, some of their experiments showed selection responses in bristle characters of Drosophila that were attributed to irradiation. They also show an increase in variance and from their figures a value of 60,000 r is estimated to be required to introduce new variation equal to that in a standard outbred population. Though this is much less than the 500,000 r mentioned above, either figure is large and suggests difficulty in obtaining detectable variance in mammalian populations where the size of the dose must be restricted.

In a recent study of bristle number in Drosophila, Jones (1967) reported a greater response in irradiated lines than in unirradiated controls and a much higher phenotypic variance in the irradiated lines. He postulated that this extra response and increased variance were caused by a few genes each with a large effect on bristle number and suggested that mutations at only five or six loci with effects of half a standard deviation could have produced the difference obtained in his experiment. His results were also complicated by the 'scabrous' gene which increased bristle number by a factor of 1.2 in heterozygotes and 1.65 in homozygotes for both males and females in the background of two of his irradiated lines at generation 30. He also presented evidence that some of those genes which produced

increased bristle number were lethal in the homozygous state or closely linked to lethal genes and consequently, selection for bristle number worked in opposition to the fitness of the population.

The most extensive attempts to induce beneficial mutations have been by the plant breeders. Many agricultural and horticultural crop plants have been subjected to mutagenic agents by workers in Sweden. Excellent reviews of this work are given by Gustafsson et al. (1960), Nybom (1960), MacKey (1960), and Smith (1958). Similar work by scientists in East Germany and the United States is reported by Gaul (1960). Although many mutants have been recovered, few could be called useful and most of these have been macro-mutations. Such mutants as early ripening, stiff straw, larger grains, and disease resistance have been induced in some cereal species. However, it is doubtful that any of these could have not been found in natural populations and the effort involved in isolating induced mutations is probably greater than that required to screen large populations for spontaneous mutants. Relatively little success has been attained in selecting plants for quantitative variation produced by radiations.

The work of Gregory (1955, 1956, 1960) on yield in peanuts demonstrates that quantitative variation beyond natural variability can be induced by radiation so that an increase in selection efficacy is realized. This work had particular significance to plant breeders since most of the agronomically important characteristics are controlled by polygenic systems.

Oka et al. (1958) reported an increase in variability for both heading date and plant height in rice under x-irradiation with no significant deviation from the mean of the controls. The treated plots gave higher estimated heritabilities although no selection was practiced. The fact that the mean was unchanged although variability was increased was attributed to the induction of mutations with both plus and minus phenotypic effects.

Only a few months later, Rawlings et al. (1958) published similar results with soybeans. Irradiation of seeds of Adams and Hawkeye soybeans with x-rays and thermal neutrons significantly increased the genetic variability for yield, plant height, maturity, and seed size. The estimates of genetic variance in the irradiated lines averaged five times as large as those in the controls, and the predicted genetic gains from selection indicated that an advance could be made within each population for all characters except plant height. As in the rice experiment, no selection was actually attempted.

Other than Drosophila and plant studies, attempts to improve selection response with radiation have been few. Abplanalp et al. (1964) began a study in 1952 with a population of chickens that appeared to be approaching a plateau in response to selection for egg production. A total of 8,000 r of x-radiation was administered to semen over seven generations at doses of 1,000 to 1,500 r per generation. Six generations of selection followed the irradiation

phase. His results suggest that genetic damage during the irradiation phase was pronounced for hatchability, egg production, and other traits associated with reproduction, while average egg size was relatively unchanged. Selection following the radiation phase restored hatchability and egg production to levels only slightly below controls within two generations, suggesting that dominant deleterious genes were eliminated at that stage. Response to selection for egg number in subsequent generations was no different or perhaps a little less than controls. They concluded that 8,000 r was not sufficient to induce substantial amounts of genetic variation accessible to selection for high egg production. Inbreeding results from brother × sister matings in the last generation of selection indicate that the irradiated populations carry a larger load of recessive deleterious genes than controls. These effects would probably go unnoticed in a non-inbred selected population.

Tribolium castaneum has become a popular organism for selection experiments in recent years. Bartlett et al. (1966) studied the effects of selection and x-radiation on pupal weight in this organism and observed correlated responses in reproductive fitness for 11 generations. Random and selected populations were maintained under treatments of 0, 100, and 1,000 r per generation. High selection contributed to significant responses in pupal weight, but the degree of response was negatively correlated with the level of irradiation.

Even though an increase in heritability and phenotypic variance for pupal weight was observed in some irradiated lines, a decline in reproductive fitness contributed to smaller selection differentials and in turn less selection response. A difference in the degree of resistance to deleterious effects of irradiation was noted in the two strains of material used. This was attributed to the previous selection history of the two strains.

Roberts (1967) attempted to overcome an apparent plateau in response to selection for six-week weight in mice. Although conclusive proof was lacking, he suggested that most loci contributing to variance in body weight had been fixed in the population. This suggestion was based on the fact that selection progress had stopped, there was no significant regression of the weight of the offspring on that of the sire, and reversed selection failed to bring about any decrease in body weight. His attempt to introduce new genetic variance was by irradiating the scrotal region of the males with 600 r of x-irradiation. The treated line was split at the second generation and sib matings were made to exploit any recessive mutations that might have been induced. Both irradiated lines showed an increase in body weight over the supposed plateau value. The experiment was complicated, however, by the fact that the control line also increased above its previous value. Roberts postulated a rare recombinational event to be responsible for this rise in the control

line. If this is accepted, the x-ray treatment may be considered successful in producing new usable genetic variance in genes controlling six-week body weight. The author makes the following suggestions for future investigations:

a. A single dose of 600 r is too small.

b. Success may be sporadic and unpredictable; therefore, several replicates should be used.

c. The control line should also be replicated to safeguard against fortuitous shifts as found in his control line.

d. Twenty or more generations may be necessary to allow clear patterns to emerge from such an experiment.

In addition to these suggestions by Roberts, several other generalities may be suggested by the present literature review:

a. There is wide variation among organisms with regard to radiation sensitivity.

b. Within species there is considerable difference in response to radiation and selection, depending on the genetic background of the stock.

c. Response to radiation and selection depends on the number of genes affecting a trait and the relative contribution of each.

d. Qualitative genes such as 'scabrous' in Drosophila may complicate quantitative studies.

e. Selection progress is often hampered by induced lethals or other genes that are deleterious for fitness.

MATERIALS AND METHODS

All mice used in this experiment were obtained from the Oregon State University Small Animal Laboratory. This colony was established in 1964 from Swiss-Webster (random bred albino) mice obtained from Simonsen Laboratories in Gilroy, California. For the duration of this experiment (July, 1966 to November, 1967), they were housed in the small animal room of the Oregon State University Radiation Center. An automatic timer turned the room lights on daily at 7:00 a. m. and off at 7:00 p. m. and the temperature was thermostatically maintained at 72 degrees F. After weaning at 21 days, each mouse was reared individually in a 6" × 6" compartment of a galvanized cage. Clean water and Purina Lab Chow were provided daily and cages were cleaned and sawdust bedding added every three days.

Five treatment groups, each replicated, were maintained as closed lines with four males and 12 females selected as breeding animals each generation. These breeding animals were individually selected for 28-day weight and litter size. Twenty-eight day weights were adjusted for size of litter at weaning and to a mid-sex value. Litter size was defined as the number of live-born mice. In an attempt to place equal emphasis on each trait, scores for selection were based on the following index:

$$I = \frac{w - \overline{w}}{\sigma_w} + \frac{LS - \overline{LS}}{\sigma_{LS}}$$

I = score.

w = 28-day weight of the individual.

$\overline{w}$ = the mean 28-day weight of the population from which the individual was selected.

σ_w = standard deviation of the population for 28-day weight.

LS = litter size of the individual.

$\overline{LS}$ = the mean litter size of the population from which the individual was selected.

σ_{LS} = standard deviation of the population for litter size.

Lines 1A and 1B were maintained as replicated controls with breeding animals being selected at random. Lines 2A and 2B were maintained for five generations with all breeding animals selected according to their index scores each generation. Lines 3A and 3B were also selected but both males and females were treated with 25 r of x-irradiation immediately before mating. Lines 4A and 4B, also selected lines, received 50 r and 5A and 5B were selected and treated with 100 r each generation. This design gave an unselected control, a selected control, and lines at three treatment levels, all replicated.

Matings were made at random, immediately after treatment.

Each male was caged with three females for a period of two weeks. Thus, in the treated animals, sperm which were in the postmeiotic stages at the time of irradiation were used. The female germ cells used were also treated as mature oocytes since females must be irradiated as fetuses to expose oogonia (Green and Roderick, 1966). After two weeks, males were removed and females placed in individual cages. Females reared their litter to an age of 21 days when the young were weaned and toe clipped for identification. The young were then housed individually and fed the standard Purina Lab Chow until they were 28 days old. They were then weighed and placed in large holding cages (sexes separated) until the youngest mice were 60 days old. Then the selected animals were treated and mated to repeat the cycle.

Selection response was measured in relation to the unselected controls for both traits. This adjustment was made as follows:

$$\overline{P}_1 \text{ adj.} = \overline{P}_1 - \overline{P}_{c1}$$

$\overline{P}_1$ adj. = adjusted mean for generation one.

$\overline{P}_1$ = mean for generation one.

$\overline{P}_{c1}$ = mean of the two unselected control lines in generation one.

Thus, calculations of realized heritability could be made by the following equation:

$$h^2 = \frac{\overline{P}_{2\ adj.} - \overline{P}_{1\ adj.}}{\overline{P}_{s1} - \overline{P}_1}$$

Where $\overline{P}_{s1}$ is the weighted mean of the selected animals from generation one and $\overline{P}_1$ is the mean of the generation from which they were selected.

$$\overline{P}_{s1} = \frac{\overline{P}_f + \overline{P}_m}{2}$$

$\overline{P}_f$ = weighted mean of selected females from generation one.

$\overline{P}_m$ = weighted mean of selected males from generation one.

These values were weighted according to the number of offspring an individual contributed to the next generation. For example:

$$P_f = \frac{n_1}{n}\overline{P}_{f1} + \frac{n_2}{n}\overline{P}_{f2} \ldots \ldots \frac{n_i}{n}\overline{P}_{fi}$$

Where n_1 = the number of offspring contributed to the next generation by f_1.

And n = the total number of offspring in the next generation.

It is therefore possible to have a negative selection differential, $\overline{i} = \overline{P}_{s1} - \overline{P}_1/\sigma_p$, where σ_p = the standard deviation of the trait of interest for generation one, if some of the selected animals are

under the generation mean and these animals contribute heavily to the next generation.

Differences in treatment means within generations for both variables were tested by T-values obtained from pooled replicas. All comparisons were made at the five percent probability level.

All treated animals were exposed to x-rays from a General Electric Maxitron 300 x-ray therapy unit operating at 300 kvp and 20 ma with a dialed half-value layer of 2.0 mm Cu. With a full field and a target to tube distance of one meter, this machine produced 37 r per minute, the exposure rate used in all treatments. Exposure was measured with a standardized ionization chamber and Victoreen R-Meter. Pre-treatment experiments on the field distribution of the machine showed a noticeable "heel effect". Therefore, a masonite rotating table (14 r.p.m.) provided with neoprene tubes to hold the mice was used in treatment of the animals. The ionization chamber was placed in one of the tubes during the treatment to verify exposure.

In addition to comparing the response to selection of the treated and control lines, the effect of x-irradiation upon fertility, the induction of tumors, and the histology of certain endocrine glands was also studied. For this study all animals of the third generation were held for observation until they were ten months old. At this age, they were autopsied. Pituitaries, adrenals, thyroids, ovaries,

uteri, testicles, and seminal vesicles were weighed and a search was made for visible tumors. These tissues, with the exception of the pituitaries, were sectioned and stained with hematoxylin and eosin for fine-structure study. The relative sizes of the adrenal medulla and cortex, the height of the thyroid follicle cells and diameter of the three largest follicles in the thyroid, the size of the ovarian follicles and corpora lutea, and the height of the seminal vesicle epithelium were all measured with a calibrated ocular micrometer. A subjective appraisal of all cell types and a search for irregularities was made on all tissues. An analysis of variance was used to test for possible differences in the means of the five treatment groups and where differences were found the least significant difference was calculated to determine which means deviated significantly from control means.

A Nikon Microflex camera and meter on a Reichert Zetopan research scope was used for all photomicrography. Kodak Tri X-35 mm film was exposed at an original magnification of 50x. After printing, all photographs represented a 128x magnification of the original slides.

RESULTS AND DISCUSSION

Litter Size

The design of the experiment was such that all lines except the unselected controls should have received identical selection pressure. In practice, however, this was impossible because differential fertility provided some lines with an advantage by furnishing more animals from which to select. This was especially true in selecting for litter size where even the control lines received some selection automatically because the larger litters contributed more animals to the population and consequently there was a greater chance of desirable animals being chosen when selection was made at random. The selection intensities (standardized selection differentials expressed in standard deviations) for litter size are presented in Table 1.

Table 1. Selection intensity (expressed in standard deviations) for litter size during five generations of selection.

Line	Generation				Total	Average per generation
	1-2	2-3	3-4	4-5		
1A	0.21	0.36	-0.12	0.19	0.64	0.16
1B	0.17	0.41	0.08	0.24	0.90	0.23
2A	1.92	1.41	1.42	2.52	7.27	1.81
2B	1.27	1.40	0.89	1.12	4.68	1.17
3A	1.15	0.80	1.42	1.15	4.52	1.13
3B	0.68	0.59	1.00	1.39	3.66	0.92
4A	0.87	0.74	1.38		2.99	0.99
4B	0.63	1.03	0.97		2.63	0.88
5A	0.40	1.44	0.92		2.76	0.92
5B	1.02	0.99			2.01	1.01

Although slight, there was some automatic selection in the randomly selected control lines (1A and 1B). The non-irradiated lines (2A and 2B) showed the highest selection intensities, both averaging well above one standard deviation per generation. The lower selection intensity in the irradiated lines was a result of fertility problems which limited the number of animals from which selections were made. These problems will be discussed later.

The response to selection for litter size in each line for each generation is illustrated in Table 2. This response is shown graphically in Figure 1 and is probably best illustrated in Figure 2 which combines the two replicas of each treatment.

Table 2. Means and standard deviations for litter size during five generations of selection.

	Generation				
Line	1	2	3	4	5
1A	9.25±2.07	9.33±3.07	7.00±1.60	7.20±1.61	7.33±1.48
1B	8.40±1.98	5.50±1.40	7.17±0.90	7.50±1.13	7.60±1.53
2A	9.00±2.27	7.40±2.82	9.50±2.46	8.60±1.49	10.00±1.72
2B	8.50±2.00	7.50±1.09	8.27±2.08	6.57±1.91	7.75±0.91
3A	8.40±1.55	7.30±1.98	6.50±2.19	10.50±1.87	6.33±1.03
3B	7.75±1.58	7.33±1.87	9.00±2.54	5.00±2.24	4.00±1.70
4A	8.17±2.94	9.86±2.75	9.33±1.70	7.29±1.01	
4B	10.00±2.28	6.20±1.50	7.00±1.93	6.50±1.56	
5A	9.40±2.83	6.33±2.50	4.33±2.01	5.50±1.91	
5B	10.00±1.51	7.67±1.19	8.25±1.00		

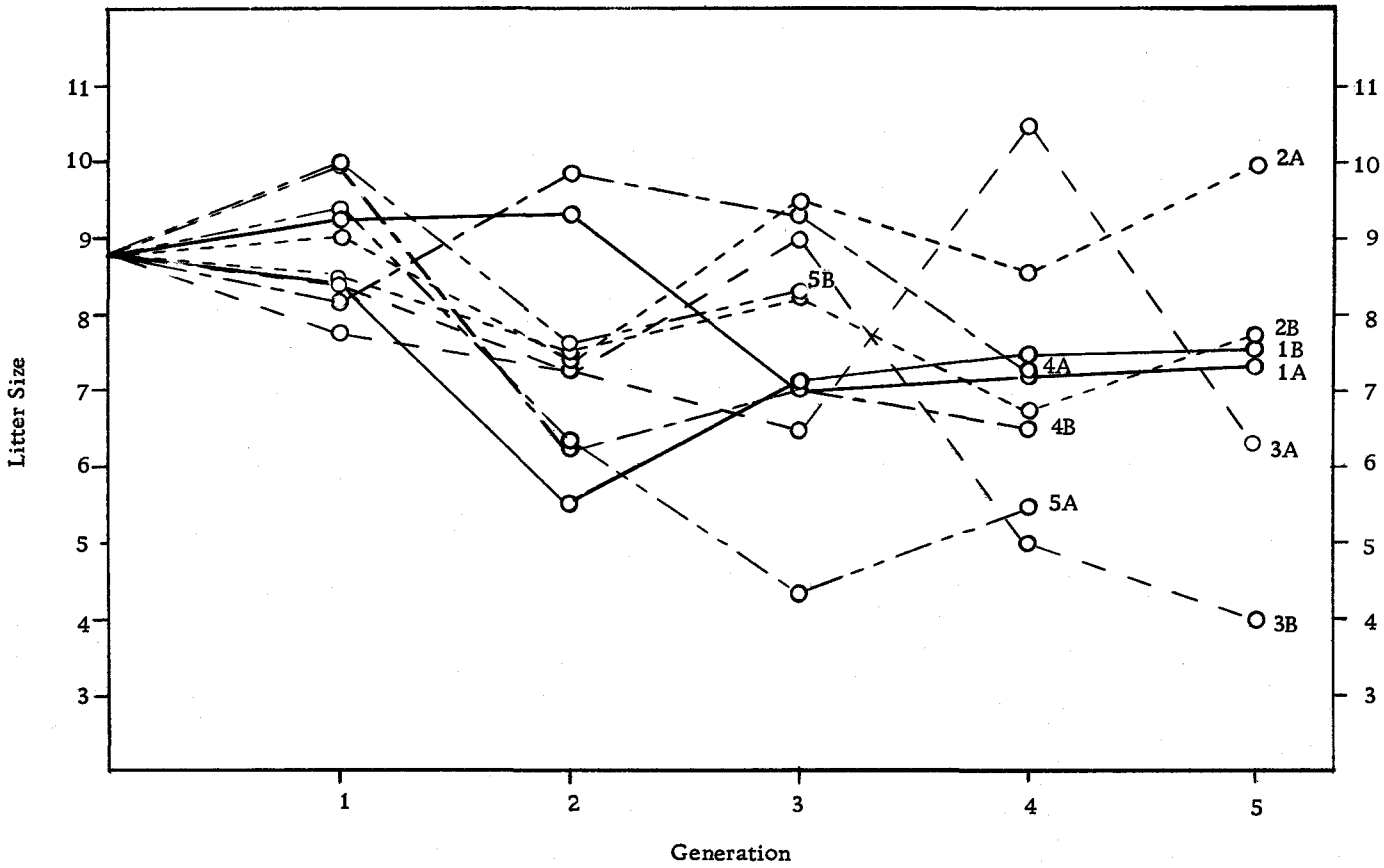
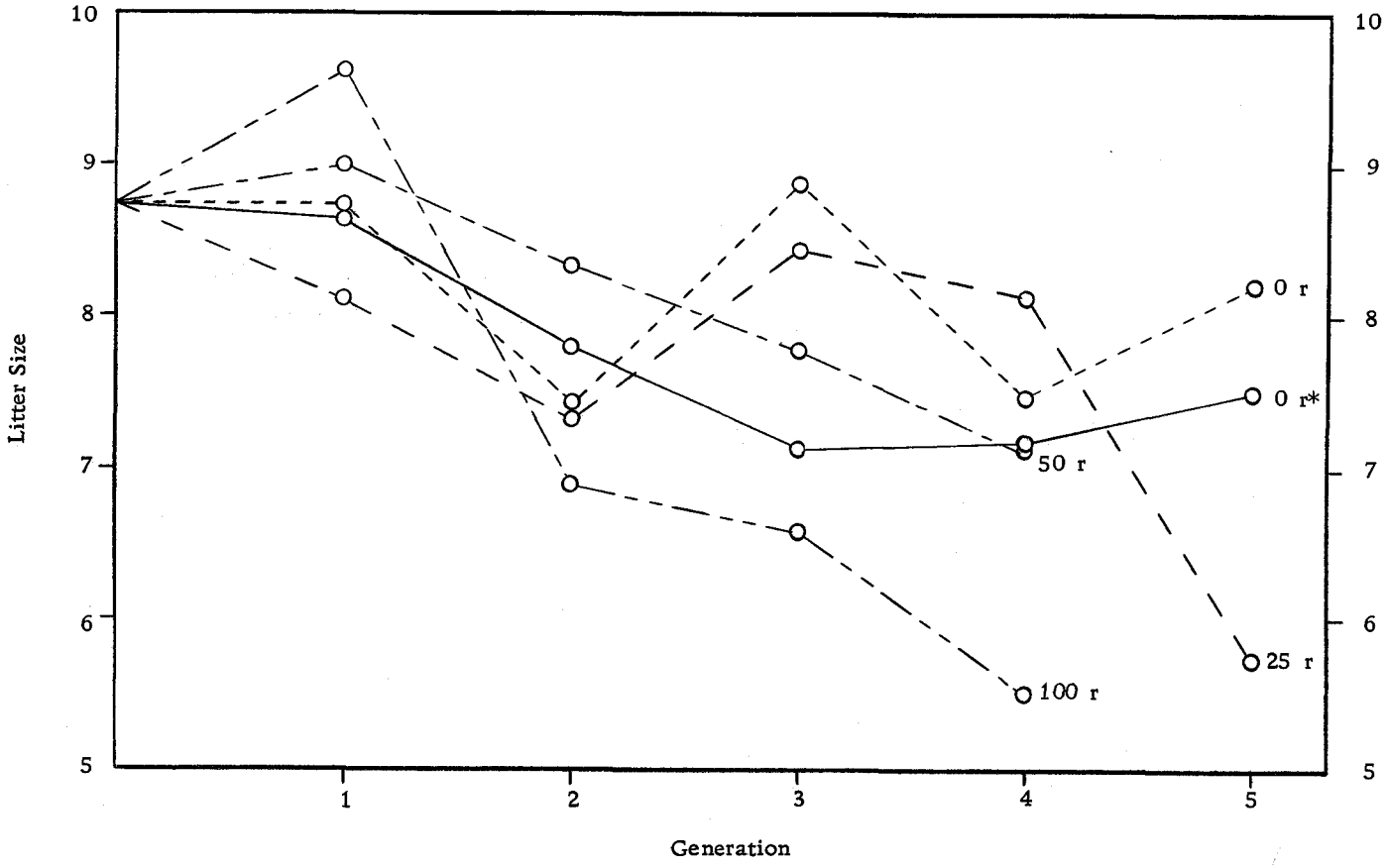


Figure 1. Response of individual lines to five generations of selection for large litters.



*non-selected control

Figure 2. Response of different treatment groups (replicated lines combined) to five generations of selection for large litters.

There was a steady decrease in litter size in the randomly selected controls even though there was some automatic selection for large litters. This is not surprising, however, when inbreeding is considered. As expected from small closed populations, inbreeding increased rapidly. The data in Figure 3 indicate that the randomly selected control lines in generation five had average inbreeding coefficients of 21.67 percent and 18.3 percent, somewhat lower than all selected lines except 2A which proved to be a highly fertile line and consistently produced large numbers of mice from which to select. Falconer (1960b) found litter size to decline about 0.5 young per ten percent increase in the inbreeding coefficient. This is quite in agreement with the present study where the mean litter size of the two randomly selected lines declined from 8.75 to 7.50 while the inbreeding increased from 0.0 percent to 20.0 percent.

As indicated by the data in Figure 1, all lines did not survive to generation five. Both 50 r lines (4A and 4B) and one 100 r line (5A) were lost in generation four. Although litter size was low in these lines, it was not the only factor involved in their failure to survive. Complete sterility of the irradiated females, to be discussed later, brought about the termination of these lines. It cannot be said, however, that litter size was not a factor because larger litters would have given more females from which to select and consequently a better chance of selecting fertile mice. The other 100 r

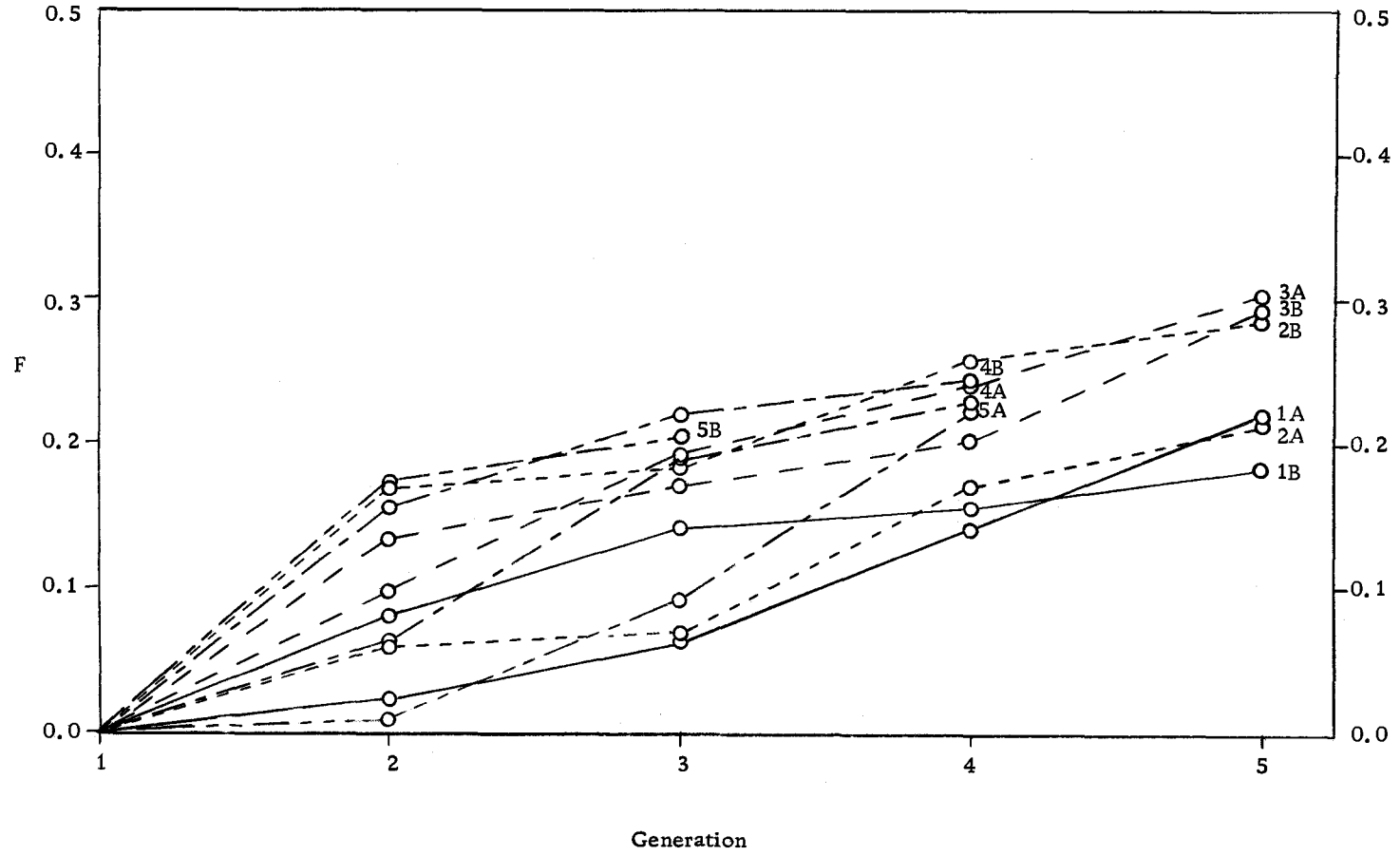


Figure 3. Increase in mean inbreeding coefficients for individual lines during five generations of selection.

line (5B) was lost in generation three while litter size was still at the relatively high value of 8.25. This line, however, reached a mean inbreeding coefficient of 20.5 percent in only three generations of selection and, once again, it was failure to produce litters rather than the size of the litters produced that led to its loss.

All lines declined in litter size during the selection period. Thus selection response was adjusted to the randomly selected controls so that a line whose decline due to inbreeding was less than that of the controls showed a positive selection response. The total selection response, mean selection response per generation and realized heritability over all generations of selection for litter size are illustrated in Table 3.

Table 3. Selection response and realized heritability for litter size during five generations of selection.

Line	Selection response		Realized heritability
	Total	Average per generation	
2A	2.14	0.54	0.13
2B	0.39	0.10	0.05
3A	-0.93	-0.23	-0.11
3B	-2.61	-0.65	-0.33
4A	0.59	0.20	0.09
4B	-2.03	-0.68	-0.42
5A	-5.37	-1.79	-0.81
5B	-0.22	-0.11	-0.08

Negative heritability values were considered as zero. Thus, the data in Table 3 indicate that irradiation as low as 25 r per

generation was sufficient to reduce heritability to zero as only one irradiated line (4A) out of six showed a positive value. These results are not surprising in view of the broad sense definition of heritability (Bogart, 1959):

$$h^2 = \frac{\text{genetic variance}}{\text{total variance}}$$

Since total variance includes variance due to environment, a situation where most of the variance was environmental could theoretically bring heritability down to almost zero. If the environmental effects were detrimental to reproduction, it is not unlikely that negative realized heritability values would be attained.

There are several ways in which irradiation could affect litter size:

a. By physiological action on the dam which alters the number of eggs ovulated.

b. By physiological action on the dam or sire which alters the number of eggs successfully fertilized.

c. By physiological action on the dam which alters embryo survival.

d. By genetic action on the dam or sire which alters ovulation, fertilization, or embryo survival.

e. By genetic-physiological interaction on the dam or sire which alters ovulation, fertilization, or embryo survival.

The first three possibilities would be expected to show their effect in the first generation, whereas genetic effects might well be accumulated over the generations of selection. Since generation one showed a slight increase in litter size in the irradiated lines, especially the 100 r lines (Table 2), the present study shows little physiological effects of irradiation up to 100 r on litter size and the small effects noticed were toward larger litters. Hahn and Ward (1967) showed a similar increase in litter size from female rats irradiated during metestrus and one day after metestrus of the cycle in which they were bred. Cox (1964) showed an increase in litters sired by irradiated Duroc boars. Thus, the litter sizes of generation one in the present study are not surprising.

The data in Figure 2 show that all three irradiated groups were below the randomly selected control when terminated. This indicates more than a total "masking" of genetic variance by environmental variance which would produce a selection response similar to the randomly selected controls and a heritability of zero. An accumulation of genes deleterious for litter size is strongly indicated. These might have been genes lethal to embryos or genes affecting the reproductive physiology of the parents. If genetic factors alone were responsible, the sudden sterility of females would not have been expected. On the other hand, physiological factors alone would have been expected to express themselves in the first generation. Thus,

an interaction of genetic and physiological factors is a logical explanation.

The response of the non-irradiated lines did not deviate from expected values. Falconer (1960 b) gave a heritability value of 0.08 for litter size in upward selected mice over 30 generations. The values of 0.13 and 0.05 found in the present study were for only five generations and should not be interpreted as being different from Falconer's value.

Response to selection can be predicted by the following equation (Bartlett et al., 1966):

$$R = i\sigma_p h^2,$$

where

R = response to selection.

i = selection intensity (standardized selection differential).

σ_p = phenotypic standard deviation.

h^2 = heritability of the selected trait.

Since irradiation reduced the selection intensity and heritability for litter size, the phenotypic standard deviation would have had to have been increased to keep selection response in the irradiated lines at a level equal to the non-irradiated lines. An examination of Table 2 reveals no such increase of phenotypic standard deviations in the

irradiated lines.

Twenty-eight-Day Weight

Some automatic selection for 28-day weight might be expected in the randomly selected controls since many of the smaller mice died and those surviving to an age of expected sexual maturity were often sexually immature and incapable of reproduction. Some selection was practiced in lines 1A and 1B (Table 4) although it was small--about 20 percent of the automatic selection for litter size.

As in selection for litter size, lines 2A and 2B received the greatest selection intensity for 28-day weight. This was attributed to higher fertility and consequently, more animals from which to select. All irradiated lines showed lower selection intensity although the 50 r and 100 r lines were higher than the 25 r lines. It should be noted however, that most of this difference occurred in selecting generation five--after the 50 r and 100 r lines had terminated.

Although the selection index used was designed to give equal emphasis to each trait, litter size was selected at a higher intensity than 28-day weight in all lines (Tables 1 and 4). This is not surprising, however, due to the greater automatic selection for litter size.

Table 4. Selection intensity (expressed in standard deviations) for 28-day weight during five generations of selection.

Line	1-2	2-3	3-4	4-5	Total	Average per generation
1A	0.03	-0.11	0.07	0.15	0.14	0.04
1B	0.07	-0.02	0.08	-0.03	0.10	0.03
2A	1.00	0.69	0.58	1.37	3.64	0.91
2B	0.60	0.71	0.42	0.47	2.20	0.55
3A	0.31	0.14	0.05	-0.18	0.32	0.08
3B	0.41	0.19	0.12	-0.08	0.64	0.16
4A	0.44	0.16	0.69		1.29	0.43
4B	0.40	-0.12	0.44		0.72	0.24
5A	0.61	0.30	0.68		1.56	0.52
5B	0.33	0.37			0.70	0.35

Response to selection for 28-day weight is illustrated in Table 5 by generation means. These means are graphed in Figure 4, and replicas are combined in Figure 5.

Table 5. Means and standard deviations for 28-day weight during five generations of selection.

	Generation				
Line	1	2	3	4	5
1A	13.66±3.41	14.64±3.91	12.12±0.87	13.69±2.33	13.22±1.66
1B	13.23±3.10	13.18±2.55	11.40±2.16	13.82±2.06	13.90±2.90
2A	12.18±2.76	12.96±3.67	10.80±2.71	13.13±1.68	14.10±2.60
2B	13.26±3.72	15.19±3.79	13.17±3.18	14.40±4.15	15.01±2.61
3A	13.20±2.97	13.68±4.40	12.69±3.54	12.84±3.40	13.71±3.60
3B	13.87±3.41	15.34±4.33	10.56±1.47	14.54±3.09	14.48±3.78
4A	13.86±4.06	12.54±2.49	10.81±2.55	13.93±3.63	
4B	13.96±4.27	15.60±2.80	9.26±2.41	14.84±3.60	
5A	12.17±4.61	13.42±3.83	11.85±4.20	13.90±3.54	
5B	14.07±1.95	14.66±3.82	12.20±1.33		

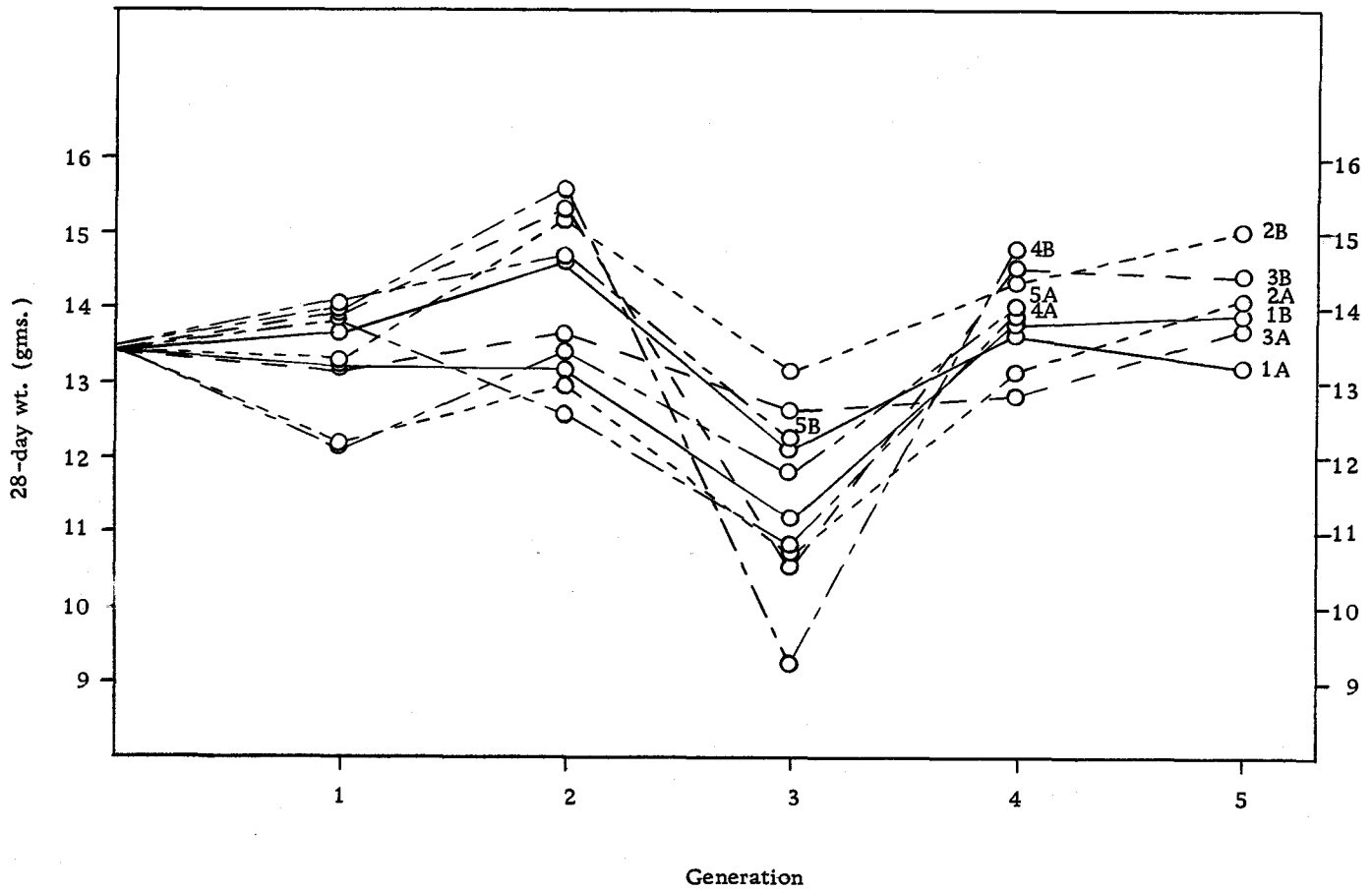


Figure 4. Response of individual lines to five generations of upward selection for 28-day weight.

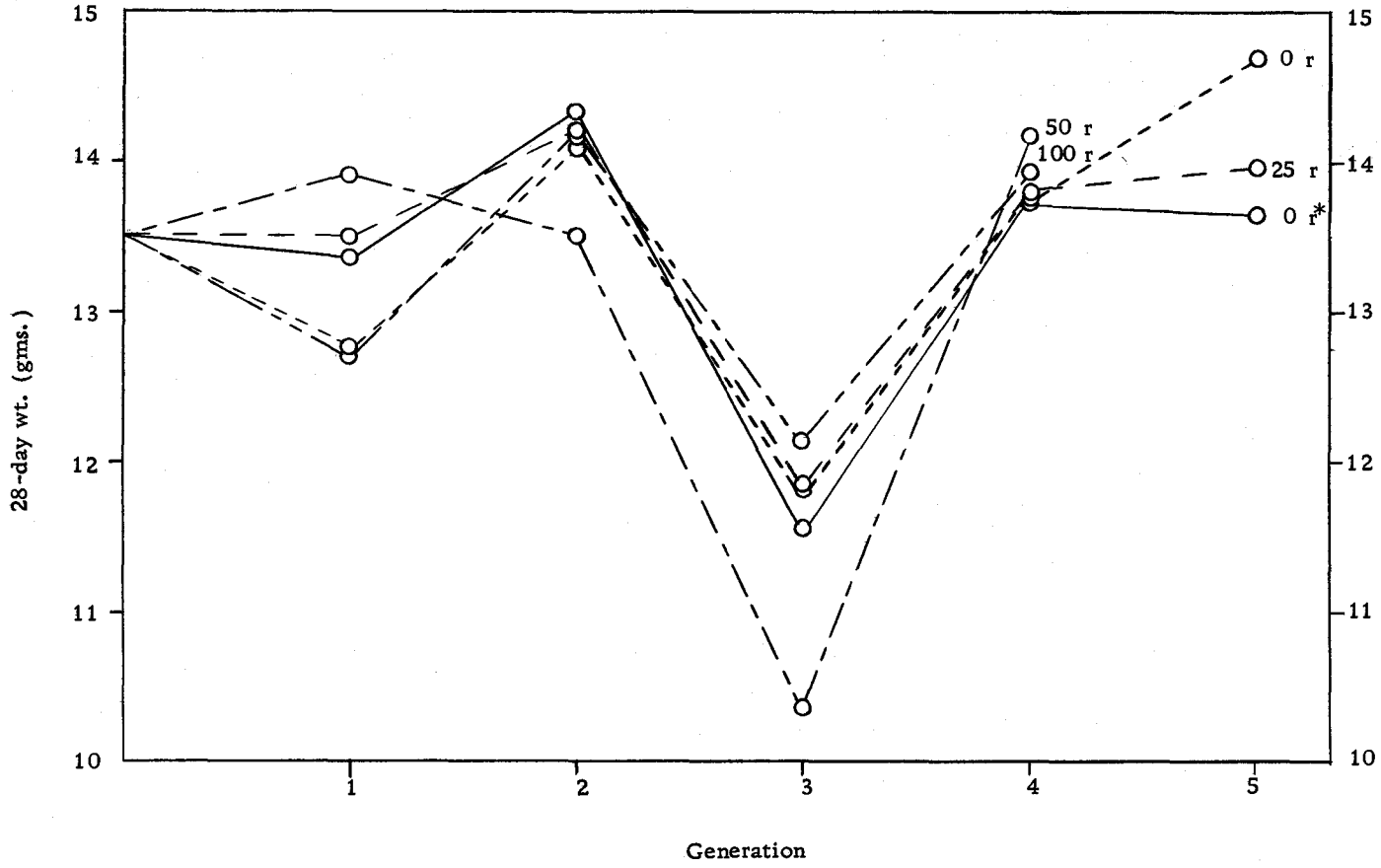


Figure 5. Response of different treatment groups (replicated lines combined) to five generations of upward selection for 28-day weight.

A severe environmental depression is noted in generation three where all lines fell considerably after which they resumed a healthy level. Generation three had a high rate of deaths before 28 days in all lines and those mice reaching 28 days were small and unhealthy. Diarrhea was common and examination at the Oregon State University diagnostic laboratory revealed the presence of Salmonella typhimurium in the infected animals. Careful isolation of infected animals and sterilization of cages resulted in a generation four that was apparently free of the organism.

Selection response was adjusted to the mean of the two randomly selected control lines. This response is shown along with realized heritability values in Table 6. The two non-irradiated lines were improved about 0.4 gm per generation and gave realized heritabilities of 16.8 percent and 18.2 percent. This is surprisingly high in that it compares with the realized heritability of 17.5 percent for 6-week weight in an experiment by Falconer (1955). Since weaning weights in most animals is lowly heritable (Bogart, 1959), this experiment indicates that much of the additive genetics for body size is expressed in the first week after weaning.

Although selection response was smaller in the irradiated lines (Table 6), heritability was often higher. Three of the irradiated lines had higher realized heritability values than either of the non-irradiated lines. Smaller average selection responses per

generation can be attributed to smaller selection intensities (Table 4) in all irradiated lines except 5A in which high selection differentials were maintained and 4A and 5B which showed a negative response. The inconsistency in the irradiated lines (from negative to 20 percent heritability values) illustrates the combined effect of irradiation and small population size. While three lines showed an increase in heritability and presumably an increase in usable genetic variance, two lines showed a complete loss of heritability. Any induction of usable genetic variance could have been expected to depend largely on chance in small populations since the nature of the induced variance (for large or small size) could not be controlled.

Table 6. Selection response and realized heritability for 28-day weight during five generations of selection.

Line	Selection response (gms)		Realized heritability
	Total	Average per generation	
2A	1.64	0.41	16.8%
2B	1.47	0.37	18.2%
3A	0.23	0.06	21.2%
3B	0.33	0.08	16.5%
4A	-0.32	-0.11	-12.3%
4B	0.49	0.16	21.7%
5A	1.34	0.45	20.3%
5B	-0.06	-0.03	-02.9%

It should be noted, however, that lines at every level of treatment (0 r, 25 r, 50 r, and 100 r) terminated above the mean 28-day weight of the control lines (Figure 5) even though some of the

irradiated lines were selected at low intensities. As mentioned previously, response to selection is predicted as $R = i \sigma_p h^2$. The phenotypic standard deviations, σ_p, are slightly, though not significantly, higher in the irradiated lines than in the non-irradiated lines. Heritability values, h^2, are higher in most irradiated lines. Thus, i, selection intensity appears to be the lone factor prohibiting accelerated response to selection for 28-day weight. Selection intensity is dependent upon the reproductive fitness of the population and several characteristics of reproductive fitness were observed in the present study.

Reproductive Fitness

Litter size, an important aspect of reproductive fitness has already been discussed. As pointed out, the irradiated lines have an initial increase in litter size in the first generation. This increase can be attributed to litters born 22, 23, and 24 days after their parents were treated. Litters born on these days represent the offspring of dams in metestrus or shortly thereafter at the time of irradiation. Litters born after day 26 in the irradiated group were noticeably smaller while in the control lines there was no such decline (Figure 6). This supports the findings of Hahn and Ward (1967) with rats but cannot be considered conclusive since these data come from only the first generation and represent only 44

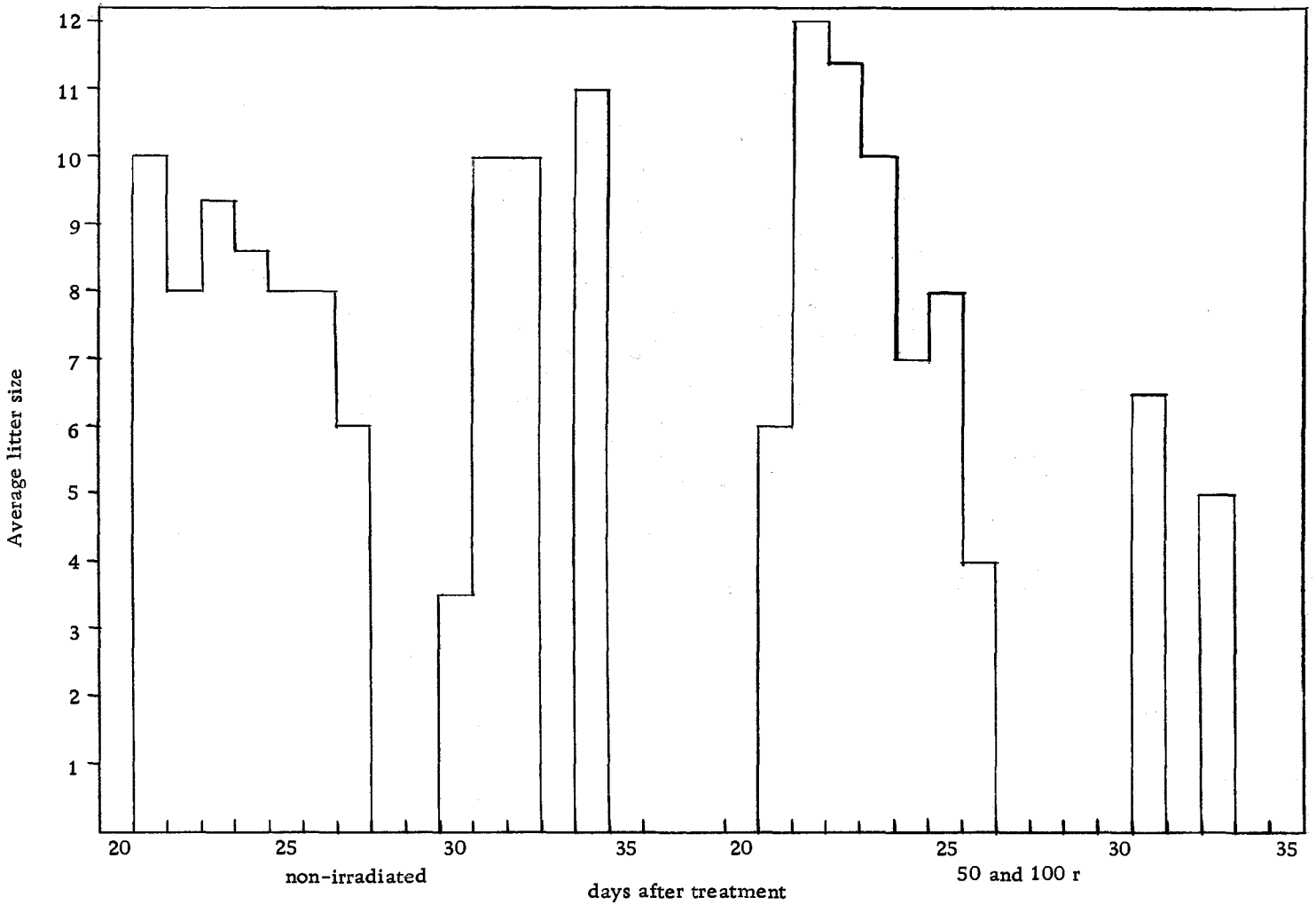


Figure 6. Average size of litters or lines receiving 0 r, 50 r and 100 r of x-irradiation born on various days after pairing of dam and sire.

litters.

After generation one, all irradiated lines decline rapidly in litter size despite attempted selection. This indicates an accumulation of genes deleterious to litter size.

Another factor contributing to the loss of some irradiated lines and reduced selection differentials in others was the failure of many females to produce a litter. As illustrated in Table 7, the number of litters born per generation in the irradiated lines decreased in the fourth and fifth generations.

Table 7. Number of litters born per generation in each of five treatment groups.

Treatment	Generation				
	1	2	3	4	5
Non-selected control	14	10	10	12	8
0 r	11	9	21	13	5
25 r	9	16	9	7	4
50 r	11	12	9	9	0
100 r	8	15	7	2	0

Line 5A produced only two litters in generation four while 5B produced no litters at all and was consequently lost. Line 5A was lost, as was 4A and 4B when all the generation four mice proved sterile by not producing a generation five. All attempts to breed females from the 50 r and 100 r lines after the initial two-week breeding term failed. This was not true for the 25 r lines, however, since lines 3A and 3B were maintained by extending the

breeding term another two weeks.

There was a noticeable decline in the number of litters born in all irradiated lines from females mating after the first six days of the breeding period. This is illustrated in Figure 7 by the relatively few litters born later than 26 days after pairing in the irradiated lines over all generations. In Figure 8 all non-irradiated lines are combined in one group and all irradiated lines in the other. Eighty percent of all litters born in the irradiated lines were born to females which mated during the first estrus cycle after treatment as compared to 69 percent in the non-irradiated lines. This indicates that the deleterious effect of radiation upon fertility becomes effective within one week after treatment. Although some reproductive difficulties with irradiated females were expected (Oakberg, 1958), a noticeable effect within the first two weeks of treatment was surprising and proved detrimental in the effectiveness of selection in these lines.

All selected animals of generation three were autopsied at ten months of age (eight months after irradiation). The relative weights of the pituitaries, thyroids and adrenals of different lines were not significantly different (Table 8). Neither selection nor irradiation significantly changed the relative weights of these glands in three generations. Histological examination revealed an increase, though not significant, in the width of the adrenal cortex layer, a decrease

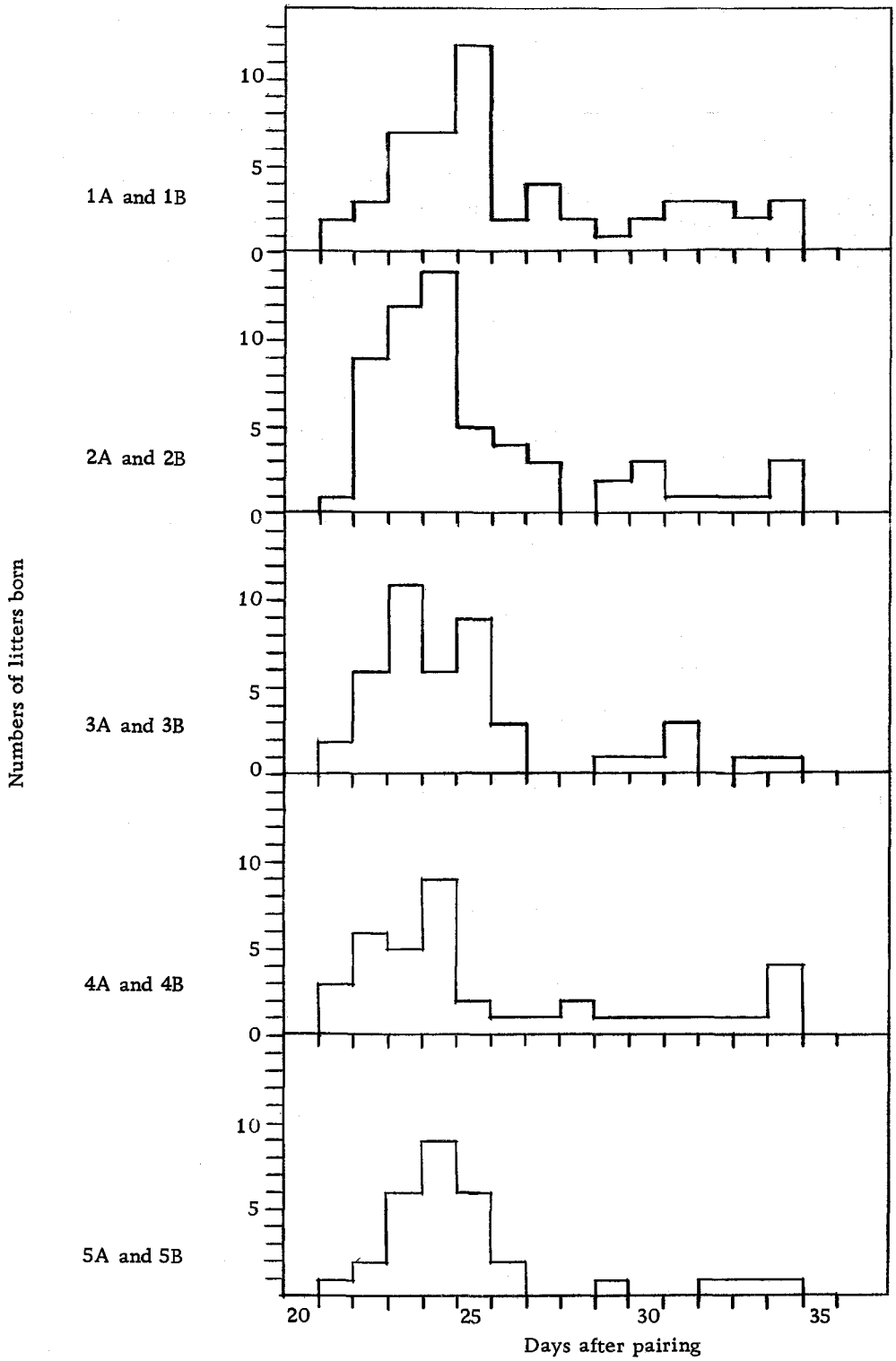


Figure 7. Number of litters born in different treatment groups (replicas combined) by days after pairing.

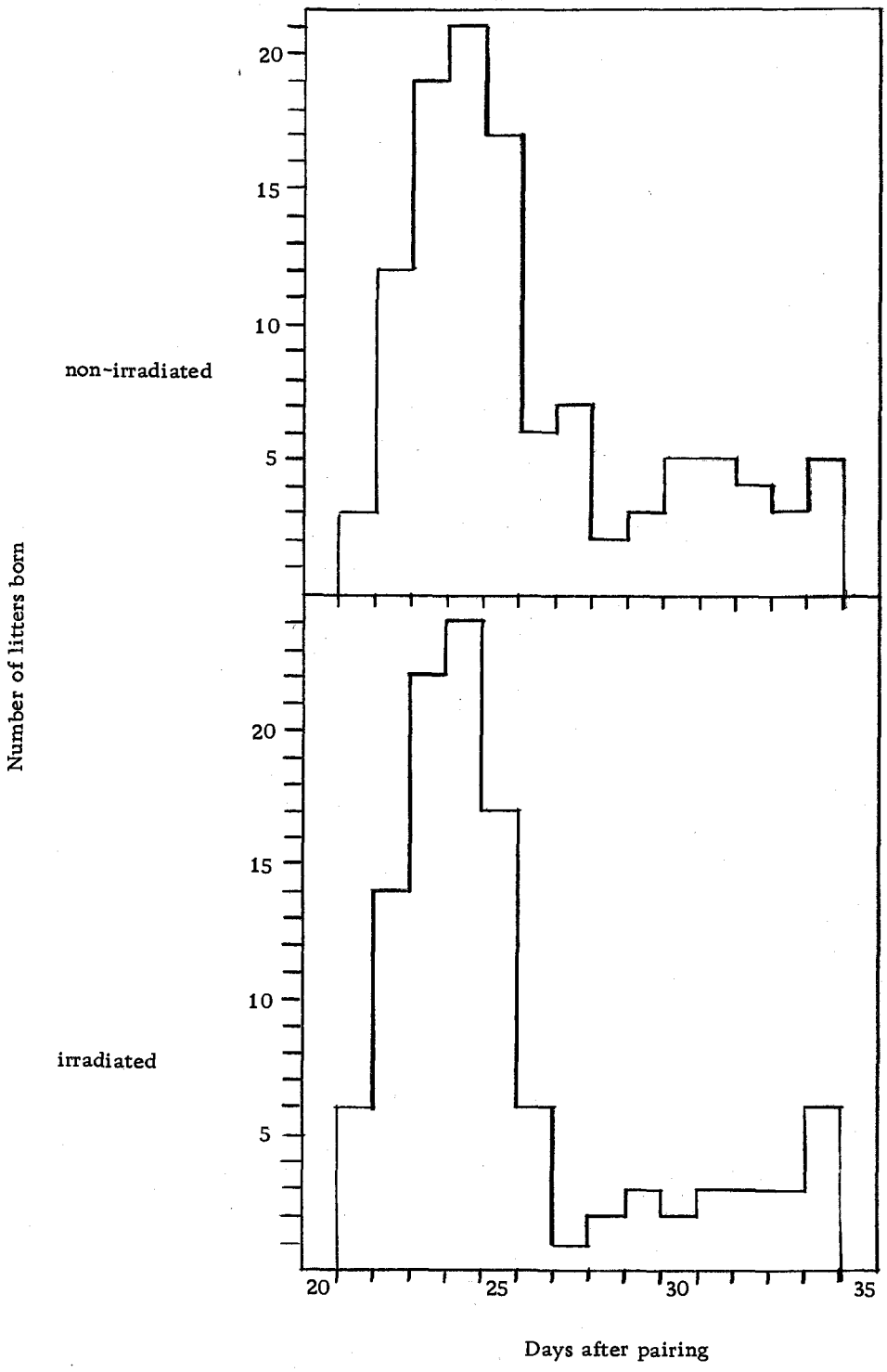


Figure 8. Number of litters born by days after pairing (non-irradiated lines combined and irradiated lines combined).

Table 8. Means and standard errors of pituitary, thyroid, and adrenal weights expressed in mgs. per gm. body weight.

Line	No. of animals	Weight in mgs. per gm. body weight		
		Pituitary	Thyroid	Adrenals
1A	16	0.080±0.009	0.258±0.019	0.451±0.024
1B	16	0.081±0.005	0.318±0.011	0.493±0.036
2A	16	0.074±0.011	0.301±0.015	0.533±0.030
2B	16	0.076±0.008	0.258±0.013	0.438±0.021
3A	16	0.084±0.007	0.304±0.020	0.430±0.026
3B	16	0.072±0.012	0.260±0.030	0.521±0.029
4A	16	0.079±0.010	0.291±0.027	0.480±0.034
4B	16	0.070±0.006	0.321±0.018	0.473±0.040
5A	16	0.080±0.009	0.268±0.019	0.521±0.030
5B	16	0.080±0.011	0.275±0.014	0.441±0.023

in the cross section diameter of the adrenal medulla, and a decrease in the height of the seminal vesicle epithelium in the irradiated lines (Table 9). If such trends do exist, they could be due to the direct effect of irradiation upon the organ or the effect of irradiation upon the pituitary or testicles in the case of seminal vesicle epithelium.

No between-treatment differences were found in the relative weights of the uteri, testes, and seminal vesicles (Table 10). Ovarian weight, on the other hand, was significantly reduced in the 50 r and 100 r lines and the 25 r line showed some reduction, though not significantly lower than the controls (Table 10).

Photomicrographs showed active spermatogenesis in males from all irradiated lines (Figure 9) indicating no permanent damage to male gametogenesis with doses as high as 100 r. Seminal vesicle weights were not significantly reduced indicating that if there was any effect on the seminal vesicle epithelium at these doses it was not enough to affect the over all weight of the gland. Histological examination of the uteri revealed no differences in endometrium thickness or gland formation which could not be attributed to the various stages of the estrus cycle.

Histological differences in the ovaries, however, were obvious. All females receiving 50 or 100 r were histologically sterile eight months after irradiation (no developing follicles or corpora lutea) while follicular development in the 25 r lines was significantly

Table 9. Means and standard errors of the diameter of the three largest follicles, diameter of the adrenal medulla, width of the adrenal cortex, and height of the seminal vesicle epithelium in mice from each level of radiation treatment.

Treatment	No. of animals	Diam. thy. fol. (μ)	Diam. adr. med. (μ)	Width adr. cort. (μ)	Height sem. ves. ep. (μ)
0 r	32	216±22.1	503±41.6	369±35.0	13.2±1.5
25 r	32	226±19.7	504±39.4	386±30.5	13.2+2.4
50 r	32	242±24.8	467±49.8	396±28.6	12.4±3.1
100 r	32	180±20.3	456±50.1	423±33.4	11.9±2.9

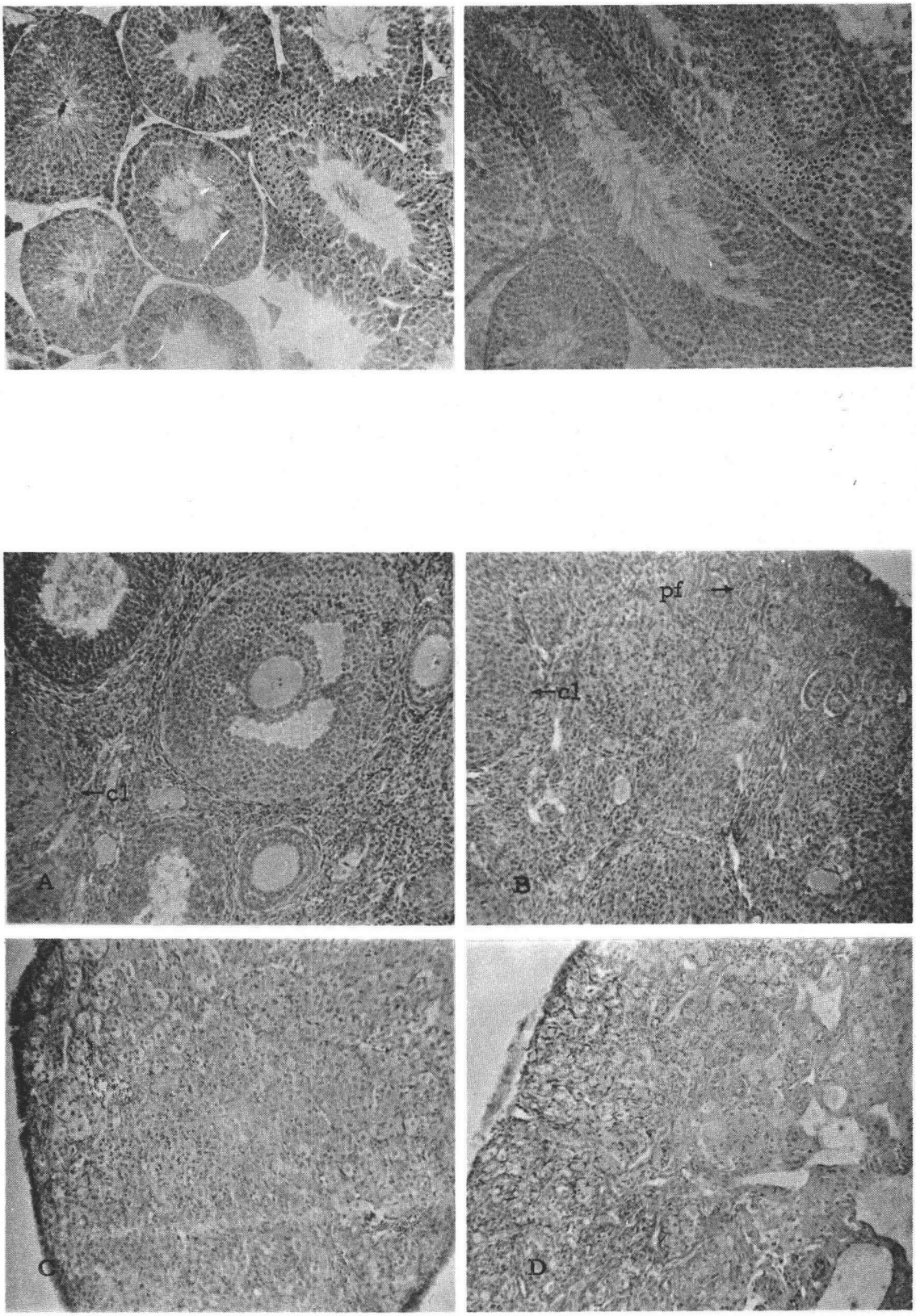
Table 10. Means and standard errors of ovary, uterus, testicle and seminal vesicle weights in mice from each level of radiation treatment.

Treatment	No. of animals	Weight in mgs. per gm. body weight			
		Ovaries	Uterus	Testes	Seminal vesicles
0 r	24 females	0.878±0.07	3.868±0.40	----	----
	8 males	---	---	7.103±0.81	6.050±0.69
25 r	24 females	0.745±0.08	4.396±0.45	---	---
	4 males	---	---	5.557±0.79	5.384±0.60
50 r	12 females	0.625*±0.05	3.368±0.39	---	---
	4 males	---	---	7.60±0.76	6.917±0.53
100 r	12 females	0.403*±0.05	3.926±0.41	---	---
	4 males	---	---	6.871±0.80	7.441±0.31

*Significantly different from control (0 r) at the five percent probability level.

Figure 9. Photomicrographs of seminiferous tubules from a non-irradiated male (left) and a male receiving 100 r x-irradiation (right) showing restoration of spermatogenesis eight months after irradiation. The apparent difference in size of the tubules is due to the plane of sectioning. Magnification, 128x.

Figure 10. Photomicrographs of ovaries from mice receiving 0 r (A), 25 r (B), 50 r (C) and 100 r (D) of x-irradiation. The non-irradiated material (A) shows an abundance of maturing follicles and one corpus luteum. The reduction in maturing follicles following 25 r x-irradiation is evident in B although one corpus luteum and several primordial follicles are present. The absence of any indication of fertility after 50 r irradiation is illustrated in C. An even more atretic ovary, D, received 100 r x-irradiation and appears as a compact mass of connective tissue stroma with severe medullary vascularization. Magnification, 128x. (cl = corpus luteum, pf = primordial follicle)


cl
A
pf
cl
B
C
D

impaired (Table 11).

Many of the developing follicles in females receiving 25 r appeared disorganized and atretic. However, corpora lutea, normal in size (Table 12) and in appearance indicated some fertility. Rugh and Wolff (1956) found similar results in CF_1 females exposed to 4 r per week for six months.

No maturing follicles or corpora lutea were found in any of the females receiving 50 r or 100 r (Table 11). The primordial follicles found in ovaries receiving 25 r were noticeably absent in those receiving 50 r and 100 r (Figure 10). Ovaries from the two higher levels of radiation treatment were smaller than controls (Table 10), showed no evidence of fertility (Table 11) and appeared histologically as a compact mass of connective tissue stroma (Figure 10).

Table 11. Means and standard errors of the number of developing follicles and corpora lutea in ovaries from mice of different levels of radiation treatment.

Treatment	No. ovaries examined	Average number per ovary	
		developing follicles	corpora lutea
0 r	48	8.33 ±0.41	4.69 ±0.32
25 r	24	3.33*±0.30	2.67*±0.21
50 r	24	0.00*±0.00	0.00*±0.00
100 r	24	0.00*±0.00	0.00*±0.00

*Significantly different from control (0 r) at the five percent probability level.

Table 12. Means and standard errors of the size of the largest follicle and largest corpus luteum in ovaries from mice receiving 0 r and 25 r of x-irradiation.

Treatment	No. ovaries examined	Average size (μ) largest follicle	Average size (μ) largest corpus luteum
0 r	48	383.6±12.4	513.6±18.4
25 r	24	316.7±19.3	506.4±20.3

Tumor Incidence

The present study was not designed to explore radiation induced carcinogenesis. All mice were autopsied at ten months of age or earlier, whereas a proper study of carcinogenesis would have allowed the mice to live as long as possible in order to detect tumors appearing in the later stages of life. Also, the presence or absence of the milk factor (Lorenz et al., 1951) was unknown in this particular strain of mice.

Of the 120 female mice of generation three, 112 lived to ten months of age. Autopsies of the eight mice that died between three and ten months of age revealed no tumors. The 112 living animals were autopsied at ten months (eight months after irradiation) and the previously discussed histological data were obtained. Mammary gland tumors, diagnosed as grade two and grade three adenocarcinomas, were found in 14 animals. All of these were large, clearly

visible in the living animal, and were first noticed between the ages of eight and nine months. The incidence of mammary tumors (no other tumor types were found) was noticeably higher in the 50 r and 100 r lines (Table 13).

Table 13. Incidence of mammary gland tumors in ten month old mice treated at four levels of irradiation.

Treatment	No. animals	No. with tumors	percent
0 r	48	1	2.1
25 r	24	0	0.0
50 r	24	6	25.0
100 r	24	7	29.2

Of the six animals in the 50 r group developing tumors, four were of line 4A and two of line 4B. Similarly, four females from 5B and three from 5A developed tumors. This discounts genetic isolation of certain genes for mammary tumor susceptibility in particular lines and strongly hints at induction by radiation.

Hormonal stimulation (Furth and Lorenz, 1954) may also be a factor since all tumor bearing animals gave birth to litters between two and three months of age. No associated ovarian tumors were found, although severe atrophy of the ovaries was found in the two higher levels of irradiation. The possibility that ovarian tumors might have developed at later than ten months of age cannot be discounted.

SUMMARY AND CONCLUSIONS

An attempt was made to maintain ten closed lines of mice for five generations of selection. Two lines were randomly selected, two were selected for increased litter size and 28-day weight, two were selected for the same two traits and in addition both dams and sires were treated with 25 r of x-irradiation immediately before mating, two were treated similarly except 50 r of x-irradiation was given and two were treated similarly except 100 r of x-irradiation was given. All 50 and 100 r lines were lost before five generations due to reproductive problems. Production of fewer litters and smaller litters both played a role in the termination of these lines. None of the irradiated lines responded positively to selection for litter size. Small selection differentials and reduced heritability values were both involved in the lack of response. While the non-irradiated selected lines showed realized heritability values of 0.05 and 0.13, heritability in the irradiated lines was reduced to zero. A combination of physiological and genetic factors or an interaction of the two was assumed to be responsible for the reduced heritability in the irradiated lines. Physiological influence alone did produce a noticeable environmental effect as witnessed by the increased litter size in generation one mice irradiated during metestrus. Accumulative deleterious genetic effects were also implicated by the gradual

decrease in litter size that occurred during selection.

Selection for 28-day weight was also more intense in the non-irradiated lines due to higher fertility in these lines. All but two of the irradiated lines showed a positive response to selection, however. Heritability values were generally increased in the irradiated lines except for the two lines which showed a negative selection response. Poor selection response and lack of heritability in these two lines was attributed to induction of genes for low 28-day weight in small closed populations. Heritability values higher than controls in the other irradiated lines indicated induction of genetic variance, at least some of which was toward greater body weight and was consequently usable in selection. Lower selection differentials appeared to be the lone factor in suppressing selection response for 28-day weight in most of the irradiated lines.

In analyzing reproductive fitness, it was discovered that fertility was impaired in the irradiated lines as early as six days after irradiation and the females given 50 r and 100 r irradiation were completely sterile two weeks after irradiation.

Dramatic histological damage was observed in ovaries of irradiated mice autopsied eight months after irradiation. Females receiving 25 r showed a decline in ovarian weight and a significant reduction in developing follicles and corpora lutea. Those receiving 50 r and 100 r showed a significant reduction in ovarian weight and

were histologically sterile in that no developing follicles or corpora lutea could be found.

Little, if any, permanent testicular damage was found even in the males in the lines given 100 r irradiation. Spermatogenesis, if ever impaired, was restored in eight months. Some slight but non-significant changes were noted in the relative sizes of the adrenal medulla and cortex and also in the height of the seminal vesicle epithelium. It could not be determined from this experiment if these effects were due to the effect of irradiation upon the organ itself, its effect on the pituitary, or both.

Although no mice were raised beyond ten months of age and consequently a complete study of tumor induction could not be made, there was an indication of higher mammary tumor incidence at ten months in those females receiving 50 r and 100 r. This may be an indication of tumor induction or merely an induction of earlier expression of a tendency to develop spontaneous tumors. Since this experiment did not allow non-irradiated mice to live long enough to develop spontaneous tumors, the distinction could not be made.

In conclusion, the use of x-ray induced variation in selection for litter size was totally ineffective. Heritability was reduced to almost zero and reproductive difficulties limited selection intensity. Although lower selection differentials limited selection response for 28-day weight, several irradiated lines showed an increase in

heritability, indicating that x-irradiation might be a useful tool in creating usable genetic variance for body weight in mammals if high selection intensity could be maintained. The present study indicated the high degree of radio-sensitivity in the female mouse ovary and suggests that some reproductive problems could be circumvented by using only irradiated males. The need for a similar study using males irradiated at various intervals of time before mating is evident since it is not unlikely that genes affecting body weight could best be altered by irradiation at a particular stage in gametogenesis.

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