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Effects of fractional doses of total body irradiation on semen of boars

Barry T. Ladd

To the Graduate Council:

I am submitting herewith a thesis written by Barry T. Ladd entitled "Effects of fractional doses of total body irradiation on semen of boars." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

R.L. Murphree, Major Professor

We have read this thesis and recommend its acceptance:

A.F. McFee, H.J. Smith, Henry Andrews

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 14, 1965

To the Graduate Council:

I am submitting herewith a thesis written by Barry T. Ladd entitled "Effects of Fractionated Doses of Total-body Irradiation on Semen of Boars." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

R. L. Murphree
Major Professor

We have read this thesis and recommend its acceptance:

Alfred F. McGee

Henry Andrews

Harold J. Smith

Accepted for the Council:

Hilton A. Smith
Dean of the Graduate School

EFFECTS OF FRACTIONATED DOSES OF TOTAL-BODY IRRADIATION ON SEMEN OF BOARS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Barry T. Ladd

June 1965

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CHAPTER I

INTRODUCTION

The effects of ionizing radiation on biological systems are being investigated more each day due to the increasing industrial uses of atomic energy. The potential hazards to both prenatal and postnatal animals have not been completely determined. These effects on man and farm animals are of prime importance, but intentional experimental exposures of humans are not possible except in special cases. Most of the present day knowledge about human reactions to radiation has come from extrapolation of data derived from irradiation of lower animals and accidental exposures of humans. Therefore, the more information that can be obtained from every available species, the more valid are the extrapolations to man likely to be.

One of the most pressing questions has been the effect of radiation on reproduction in farm animals. Does radiation permanently impair the reproductive ability of the male? Will fractionated doses be more deleterious than acute exposures? These are but two of the many questions which must be answered.

This study was initiated to determine the effects of fractionated doses of gamma radiation on sperm production and hematological elements in boars.

CHAPTER II

LITERATURE REVIEW

The effects of roentgen radiation on the testis were first described by Albers-Schönberg (1903) when he reported oligospermia and azoospermia in rabbits and guinea pigs without impairment of sexual potency.

Since Warren (1943) has published a comprehensive review of the literature concerning the effects of radiation on the gonads and Casarett (1956) on the effects of radiation on gametogenesis and fertility in mammals, only limited reference will be made in this thesis to work prior to these dates.

The effects of radiation on spermatogenic cells of the mouse have been studied by Oakberg (1955a,b), Harding (1961) and Monesi (1962). These workers concluded that the reduction in number of spermatogonia after irradiation was due to cell death, especially of intermediate and type B spermatogonia. Monesi (1962) injected male mice with tritiated thymidine, irradiated them one hour later with doses of 20, 50, 100, 200 or 300 r (X-rays), and sacrificed them at varying intervals thereafter. Counts were made of the numbers of normal and necrotic, unlabeled and labeled, type A, intermediate and type B spermatogonia from autoradiograms of the testes. The results indicated that damaged spermatogonia die as they reach a definite critical stage in the cell cycle, regardless of the stage at which they were irradiated. He found that cell death

occurred at late interphase or early prophase in type A and intermediate spermatogonia and at anaphase or telophase in type B cells. Type B spermatogonia were relatively resistant to cell killing for about 5 to 7 hours after completion of DNA synthesis. Monesi concluded that the much longer duration of the X-ray sensitive period (interphase) and the much shorter duration of the X-ray-resistant period (prophase) of the cell cycle in intermediate and type B spermatogonia in comparison to type A spermatogonia may account for the greater X-ray sensitivity of intermediate and type B cells.

Oakberg (1955b) exposed 12-week-old male mice to either partial or whole-body doses of 600 r of X-rays and sacrificed them at intervals ranging from 1 hour to 28 days postirradiation. The ratio of experimental to control counts of type A spermatogonia declined to 50 per cent by 12 hours. A minimum of 1.9 and 1.8 per cent of normal was reached at 5 and 7 days after irradiation; by 10 days repopulation was under way and by 28 days the number of type A spermatogonia had returned to 96 per cent of normal. Type B spermatogonia were reduced to one-third of control levels by 12 hours and to 0.2 per cent of normal by 24 hours after irradiation. With the exception of only two cells in the entire sample of testicular cross-sections, the type B spermatogonia had disappeared completely by 7 days and did not reappear until 15 days after irradiation. The number of type B cells increased very rapidly, but always lagged behind the type A since type B cells are formed from type A spermatogonia. Oakberg (1957b) determined the LD_{50} for late type A and early type B spermatogonia to be 20-24 r in the mouse; and

Partington, Craig, and Jackson (1962) confirmed the LD_{50} for type B to be 20-25 r, but found that 100 to 300 r of X-rays had no direct action on type B spermatogonia in the rat.

Bryan and Gowen (1956) disagreed with Oakberg's conclusion that depletion of spermatogonia is due to cell death. They concluded that depletion was due to inhibition of spermatogonial mitosis rather than irradiation-induced spermatogonial necrosis. After localized irradiation of the testes of adult rats with doses ranging from 81-3000 r of X-rays, Jones (1960) enumerated the numbers of type A, intermediate, and type B spermatogonia, mitotic figures and degenerating cells in cross sections of 50 tubules from each testis. The stage of spermatogenesis was recorded for each tubule according to the method of Leblond and Clermont (1952) as well as the number of resting primary spermatocytes in tubules at stages VI and VII. He noted a reduction in the number of type A and B spermatogonia at 24 hours; at 8 days some type A spermatogonia had survived 3000 r. No intermediate or type B cells survived 1098 r and none were seen in stages I-V after 730 r; at 21 days postirradiation type A's were absent from all tubules after 730 r and from tubules in stages I-V after 454 r.

Casarett and Casarett (1957a,b) concluded that both mitotic inhibition and premature differentiation of spermatogonia into spermatocytes are important causes of spermatogonial depletion in rats. The apparent differences in conclusions between Oakberg and Casarett and Casarett may be due to the methods of evaluation. Oakberg used a quantitative approach in which the tubules were classified on the basis

of acrosome development of spermatids as described by Leblond and Clermont (1952). Casarett and Casarett used a more subjective approach in classifying the seminiferous tubules on a scale from 0 to 4 with 0 representing a normal tubule or no change in the tubule, and 4 indicating an extreme change such that no cells remained or no mitotic figures were seen.

Oakberg (1959) extended his earlier observations over a wider dose range by exposing male mice to doses of 20 r of gamma rays, or 100, 300 or 600 r of X-rays. He confirmed his earlier report that cell death is the primary factor in radiation-induced depletion of spermatogonia following acute exposures. This conclusion was based on the finding of numerous necrotic spermatogonia coincident in time with a decrease in the number of normal cells. He postulated that one of the reasons why depletion of spermatogonia had been overlooked was that observations had not been timed to detect the peak incidence of degeneration at 12 to 15 hours postirradiation. He found that the rate at which the number of normal type A spermatogonia returns to control levels is dose-dependent and begins by 24 hours after 20 r of gamma rays, 3 days after 100 r, 7 days after 300 r, and 10 days after 600 r of X-rays. Nebel and Murphy (1960) partially confirmed Oakberg's observation that depletion of spermatogonia is due to cell death. They observed a drastic reduction in spermatogonia such that by 24 hours postirradiation there was an average of only 0.5 spermatogonia per tubular cross section and by the eighth day only 0.007 per cross section. They concluded that this was due largely to cell death, but the premise that mitotic inhibition may

be involved remains a distinct possibility. They postulated the formation of new cells, which they called "restitution cells," arising as a result of a metaphase block. If such cells are formed they may have been considered by some workers as Sertoli cells; if this were true then it could account for excessive numbers of Sertoli cells reported by some workers. This phenomenon may have been involved in the work of Deschner, Rugh and Grupp (1960), who noted that when human testes were irradiated with 1200 r the number of Sertoli cells increased until after 5 days there was a two-fold change.

According to some workers, cell death is not limited to spermatogonia, but after 320 r there is some loss of spermatocytes and a severe loss of spermatids over and beyond the loss resulting from diminished recruitment (Nebel, Murphy and Linder, 1960). This is contrary to the views of Oakberg (1955a) in which he saw no visible changes in spermatids and sperm with doses up to 1500 r.

The effects of irradiation on fertility of the male rodents have been studied by many researchers. Craig, Fox and Jackson (1959) exposed male rats to whole-body doses of 200, 300, or 500 r X-rays. Fertility of the rats was determined by pairing the irradiated subjects with one normal female each week. Males which received 200 r did not become sterile, while 300 r caused sterility associated with oligospermia during the ninth and tenth weeks after treatment. Animals which received 500 r were subfertile soon after treatment, sterile 40 to 45 days postirradiation and remained sterile for a minimum of 3 months. Craig, Fox and Jackson (1961) confirmed the above findings in both the

rat and mouse and also found that 300 r (whole-body) produced a short period of sterility associated with oligospermia about 45 days post-irradiation in the mouse and 65 days in the rat. Thus there were obvious differences in the time of onset in the two species. Murphree et al. (1952) exposed 8 male rabbits of known fertility to total-body doses of either 100, 200, or 300 r of x-radiation. Experimental matings to nonirradiated females were started during the first week after exposure. They found that irradiation, within the levels used, did not significantly affect the ability of sperm to fertilize the ova in normal females during the first two weeks of the postirradiation period. However, there was a significant increase in fetal mortality in females bred to the irradiated males. A highly significant difference in the number of stillborn young was also noticed (4.3 per cent in the controls and 11.1 per cent among the progeny of irradiated males). The above data suggest a deleterious effect on the prenatal and neonatal viability of the offspring when the male rabbit has been irradiated prior to mating.

The first suggestion that an arrest in the development of the gonads could be brought about by irradiation during fetal life was made by Bagg (1922). Erickson, Murphree and Andrews (1963) exposed gilts at various stages of gestation to a single 400 r dose of gamma rays to study the effects of radiation on postnatal development of the porcine testis. They found the first evidence of radiosensitivity in the prenatal testis at 35 days but a marked effect was not evident until the 50th day of gestation. Although a relatively high level of germ-cell

radiosensitivity continued for the remaining portion of gestation studied (to 90 days), none of the male progeny were completely sterile. The initiation of pronounced gonocyte sensitivity at day 50 of gestation was shown to be correlated with the definitive organization of the sex cords. Rugh and Jackson (1958) reported that 200 r of x-radiation delivered to mice on day 15.5 or 16.5 of gestation reduced fertility of their male progeny to 24 and 32 per cent, respectively. Of the progeny affected, approximately one-third were completely sterile. They concluded that the fetal testis is more sensitive than the adult testis.

Ershoff (1959) exposed gravid rats of the Long-Evans strain to a single dose of 150 r X-rays on the 10th, 14th, or 18th day of gestation; another group received a single exposure of 300 r on the 18th day of gestation. Mature male offspring from each group were caged with normal females for a period of one week. No litters were sired by any of the males which received either 150 or 300 r on the 18th day in utero. Testicular injury was evident in the offspring of rats irradiated on the 14th day of pregnancy, but was less severe than in those from mothers irradiated on the 18th day of pregnancy. No abnormalities in testicular function or morphology were observed in rats irradiated on the 10th day in utero.

Hupp et al. (1960) exposed female rats to 150 r (air-dose) of gamma radiation on the 17th, 18th, 19th, 20th, 21st, or 22nd days of gestation. At 160 days of age, testis weights of irradiated male progeny were only 60, 33, and 20 per cent, respectively, of control testes weights on the 17th, 18th, and 19th through 22nd day.

Murphree and Pace (1960) exposed female rats at 12 to 20 days of gestation to doses of 110, 150, or 220 r of gamma radiation. They found that the degree of retardation in testis weights among the progeny varied directly with the level of radiation and stage of gestation at the time of irradiation.

Parish, Murphree and Hupp (1962) reported that testis weights of calves irradiated during the last half of gestation (400 r to the dam) were approximately 50 per cent less than testis weights of control calves at 187 days of age. They observed that sperm production of bulls irradiated in the 5th or 8th month of fetal life was 42 and 52 per cent, respectively, less than that of control bulls. Murphree and Hupp (1961) exposed gilts, 54 to 64 days pregnant, to 400 r of gamma radiation and studied the effect on the male offspring. Testis weights of the males were approximately 30 per cent less than control testis weights. The sperm concentration for irradiated and control males was similar; however, the treated boars produced approximately 22 per cent less semen and 35 per cent less sperm per ejaculate than control animals.

The changes in semen production following exposure of mature males to single, acute doses of radiation have been reasonably well established in several species of mammals. The characteristic response observed in most species is a period of normal semen production for a few weeks followed by a sharp decrease in sperm numbers or sterility and eventual recovery in sperm production. The length of each phase may vary with the species and the level of radiation. Cox and Willham (1961) suggested that the interval from irradiation to minimum sperm numbers

can be shortened by frequent collection of semen. However, Welch (1965) (unpublished data in this laboratory) failed to find evidence in rabbits that frequency of collection exerted any effect on the postirradiation fertile period.

Parish (1958) exposed bulls to acute doses of 100, 200, 300, or 400 r of gamma radiation. Maximum reduction in numbers of sperm, per cent of live, motile, and normal sperm occurred during the 12th week postirradiation. Sixteen weeks following exposure some recovery in sperm numbers became evident. When the study was terminated at the 24th week postirradiation, the average production of the three irradiated groups was only about 70 per cent of the control level.

Gillette et al. (1964) observed similar responses in bulls following doses of 50 to 800 r of X-rays directed to the testes only. Histological examination of the testes at one year postirradiation revealed that the 800 r exposure had effected an approximate 20 per cent reduction in normal germinal tissue. Normal germinal tissue in the testes of bulls exposed to 400 r was diminished by only 10 per cent.

Erickson (1964) exposed prepubertal boars (1 to 30 days of age) to a total-body dose of 200 r of gamma rays. By 30 days postirradiation the number of gonocytes declined to 10 per cent of the control values, but some recovery was evident by day 60. Presumably permanent radiation damage was exhibited by 23 per cent of the seminiferous tubules when the testes were examined postpubertally. Their average sperm production at maturity was only 47 per cent of the control value.

Pace, Hupp and Murphree (1962) followed the semen production of mature boars which had been exposed to acute, whole-body doses of 200 or 400 r of gamma radiation. Single ejaculates were obtained weekly for 26 weeks postirradiation. There was no difference between the irradiated and control groups during the first 6 weeks in sperm production. Sperm numbers declined; however, very rapidly during the seventh through the ninth weeks, increased slightly during the 10th week, and returned to about 75 per cent of the control level by the 12th week after irradiation with a gradual increase during the remaining 14 weeks of the experimental period.

A similar response was observed by Willham and Cox (1961) in the semen production of boars which had been exposed to 300, 600, or 900 r (mid-testis dose) of localized x-radiation at 6 months of age. However, the rate of recovery in sperm production was considerably slower in their boars, presumably due to the fact that higher dose levels were used.

When a large dose of radiation is divided into several smaller but closely spaced exposures (fractionated) or is given continuously at low levels over long periods of time (chronic) the resulting effects may be quantitatively quite different from those produced by an acute dose of the same total size. Chronic irradiation at low dose rates seems to be much less effective in the production of semisterility. Casarett (1956) thinks the possible explanation for this is that low doses are delivered to cell populations which are continually renewed in the gonad,

so that relatively fewer cells are subjected to doses large enough to induce the chromosomal defects involved in semisterility.

Kohn and Kallman (1955) found only small differences in response between fractionated and single doses. They exposed mice to doses of x-radiation ranging from 80 to 240 r, given in various combinations of fractionation and different time intervals between fractions. On the other hand, Ferroux, Regaud and Samssonow (1938) reported that 2,045 r of X-rays to the testes permanently sterilized rabbits when the total dose was given in 4, 5, or 6 equal daily fractions, but when given in 2 or 3 equal daily fractions permanent sterility did not occur. Pitcock (1961) did not note any definite difference between acute doses and divided doses in producing permanent sterility in monkeys.

Although there have been many studies conducted to determine the effects of chronic exposures, the maximum level which will not impair reproductive functions has not yet been established (Rugh, 1960). Casarett (1950, 1953) and Casarett and Hursh (1956) studied the effects of low-level daily exposures over extended periods of time. Dogs exposed to 0.06 or 0.12 r per day (X-rays, total-body) for 5 consecutive days each week showed no changes in sperm counts or fertility due to radiation after total exposure times as long as 4 years. However, those exposed to 0.6 r daily showed a progressive decline in sperm counts after 20 to 30 weeks of exposure and reached levels less than 10 per cent of control values after 40 to 60 weeks. This decrease was accompanied by increases in per cent of abnormal, immotile, and dead sperm at sustained levels or further increases thereafter. Casarett and Casarett (1957a,b)

exposed rats, to an acute dose of 324 r or a chronic dose of 2.26 r per day, 5 days per week, to a total of 326 r of X-rays and found that spermatogonial depletion was produced by both of the irradiation procedures. They postulated that the major mechanisms responsible for the depletion in both acute and chronic exposure were inhibition of mitoses of type A spermatogonia and normal spermatogonial differentiation to produce sperm and a small amount of spermatogonial degeneration. The acute radiation also caused some mitotic inhibition of intermediate and type B spermatogonia, and possibly some premature differentiation of some of these spermatogonia into prespermatocytes. They found chronic exposure to be about twice as "efficient" as acute exposure in producing a reduction in prespermatocyte formation although there were suggestions that acute irradiation was more effective in producing a permanent effect on the spermatogonia. This became evident during the "post recovery" period in which the reduction in number of prespermatocytes was about twice as great with the acute as with the chronic irradiation.

There seems to be some difference of opinion as to the effects on the testis when the intensity of the dose is increased. Lorenz et al. (1947) found the testis weights of mice to be 50 per cent lower when whole-body exposures of 8.8 r per day were given in 1.5 minutes than when the same dose was given over a period of 8 hours.

Carter, Lyon and Phillips (1954) also using mice, found a decrease in fertility when gamma rays from radium were administered at a dose rate of 33.3 r per week. The first onset of sterility was noticed at an accumulated dose of 330 r; the median dose was found to be 430 r, and

100 per cent of the animals were sterile at 550 r. When the dose rate was lowered to 8 r per week an accumulated dose of 200 r induced sterility in 39 per cent of the mice. When the dose rate was further reduced to 1.64 r per week, an accumulated dose of 40 r caused 14 per cent sterility. Although these data suggest that as the rate of exposure is decreased, the damage resulting from a given dose tends to increase, these findings do not agree with any others found in this literature review.

Eschenbrenner, Miller and Lorenz (1948) exposed mice to either 8.8, 4.4, or 1.1 r of whole-body gamma radiation daily over an 8 hour period and found that in the mice exposed to 4.4 and 8.8 r there was a sharp decrease in testis weight after 2 and 4 months of irradiation. Those receiving 4.4 r per day declined to 50-60 per cent of normal at which time they leveled off, while those receiving 8.8 r per day declined to 20-25 per cent of normal. Eschenbrenner and Miller (1946) also noted sharp losses in testicular weight when 8.8 r per day was administered with an accumulated dose of 600 r.

Craig, Fox and Jackson (1959) found that rats became sterile about 40 days after the first of 5 consecutive exposures of 100 r per day. Brown et al. (1961) irradiated mice at the level of 2 r per day (continuous exposure) until they had accumulated a total dose of 200 r. Histological evaluations revealed no difference between these and control testes in the percentage of tubules in the various stages of spermatogenesis. The damage inflicted by this low, chronic exposure therefore appeared to be less than the capacity of the seminiferous epithelium to repair.

This work points up the fact that there seems to be a level of chronic irradiation at which the damage done is offset by the repair which takes place. There is a great need for more extensive research in this area since this point of equal damage and repair has not been satisfactorily determined. The intensity or dose rate and the time for chronic exposures may be as important as the total accumulated dose.

Rugh (1960) believes that for producing sterility, the dose rate is more important than the total dose.

The following study was undertaken to assess the effects of fractionated irradiation on the response of the boar testes.

CHAPTER III

MATERIALS AND METHODS

Sixteen boars from sows of mixed breeding and sired by purebred Yorkshire boars were selected on the basis of their willingness to serve an artificial vagina mounted in a dummy sow. They were assigned to three comparable groups on the basis of their average sperm production during a 3-week pretreatment characterization semen collection period. The three radiation treatments were: (1) 4 boars, 0 r; (2) 6 boars, 50 r every 7 days; and (3) 6 boars, 100 r every 14 days to a total dose of 600 r for the two latter groups. These doses were calculated as skin doses in air, and were delivered at a rate of .57 r per minute. The boars were maintained in individual pens, except during the periods of irradiation, to prevent fighting.

At 12 months of age the boars in Groups 2 and 3 were first exposed to doses of gamma radiation from the multi-curie cobalt-60 source (Figure 1) described by Wilding, Simons and Rust (1962) and by Trum et al. (1959). Since the publication of these articles, a semi-circular concrete wall 18 feet high and 2 feet thick has been constructed around a portion of the field. This necessitated further dosimetry measurements to account for the additional backscatter from the wall.

During the time of irradiation the boars were allowed to move about untethered on the exposure field. There was considerable fighting

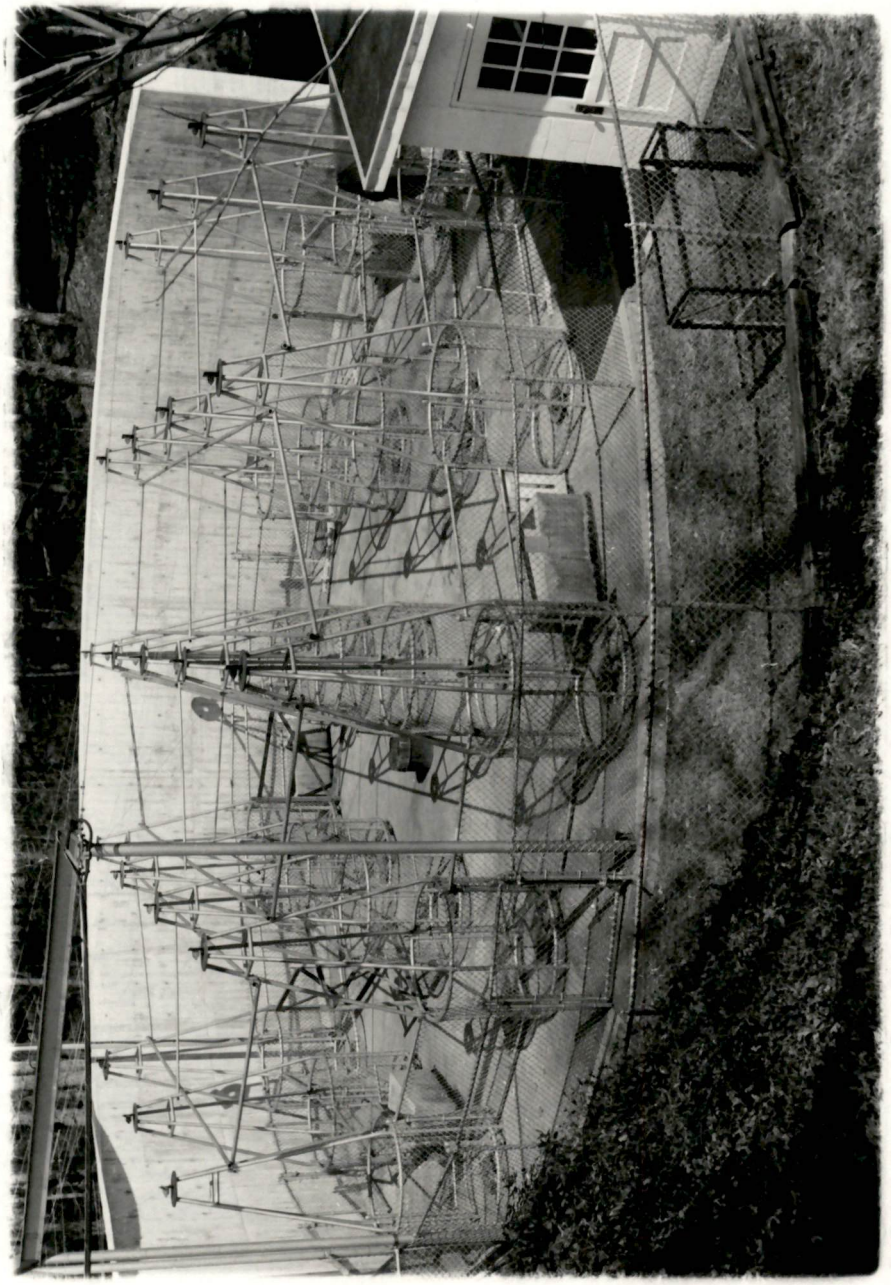

Figure 1. Large animal irradiation facility.

during the irradiation and in order to keep the groups as comparable as possible the control boars were placed together for a period of 3 hours every 14 days.

Beginning on the sixth day after the initial exposure, semen was collected at weekly intervals for 31 consecutive weeks. Collections were discontinued at this time and resumed on the 44th week for 5 consecutive weeks. A third collection period began on the 70th week following the initial exposure and continued for 6 consecutive weeks. Semen was collected in a plastic bag affixed to an artificial vagina. Immediately after collection the semen was strained through layers of cheese cloth to remove the gelatinous fraction. Semen samples were evaluated on the basis of strained semen volume, total sperm per ejaculate, rate of motility and per cent motile sperm. The volume to the nearest milliliter was recorded for each ejaculate. Sperm motility estimates were made by placing a drop of semen on a warm glass slide, covering with a coverslip, and examining it under a microscope at 100 X magnification. An estimate of the percentage of motile sperm was made for each ejaculate; the ratings ranged from a maximum of 80 per cent to 0 if no sperm or only dead sperm were observed. The rate of motility was also estimated at this time. This qualitative estimate was a rating from 1 to 4 based on the degree of movement of the sperm. Samples showing the highest degree of sperm motility were assigned the rating of 4 and those showing lower rates of motility were assigned progressively lower numbers.

The sperm concentration values were obtained through the use of a Cenco photelometer calibrated for the estimation of sperm concentration in boar semen (Pace, Murphree and Hupp, 1961). If the sample when examined under the microscope for motility showed no sperm a photelometer reading was not made. The average number of sperm per ejaculate was calculated from the semen volume and sperm concentration data.

Blood samples were obtained by jugular venipuncture at weekly intervals for 18 consecutive weeks beginning 2 weeks prior to irradiation and periodically thereafter to 75 weeks after the first exposure. Erythrocytes, leukocytes, differential cell counts and platelet numbers as well as hemoglobin and hematocrit values were determined by standard procedures employed in this laboratory (Brown et al., 1961).

Forty-nine weeks following the initial exposure the left testis from each animal was excised, the epididymis removed, and the testis weighed. Transverse segments for histological analysis were taken from the center and near the poles of the testis. Tissues were fixed in Zenker-formol, sectioned at 5 μ, and stained with hematoxylin and eosin. Following the last semen collection (76 weeks after the initial exposure) the right testis from each boar was excised and similarly processed for histological analysis.

The histological analysis was conducted on 600 randomly selected seminiferous tubule cross sections from each testis (1200 tubules per animal); each tubule was classified in one of the following categories: Type I: normal tubule, Type II: similar to Type I except for a reduction in number of cells, Type III: one or more stages absent and

a reduction in number of other cells, Type IV: sterile tubules that contain only Sertoli cells. An example of each of the classifications is presented in Figures 2 and 3.

Due to the death of 4 boars from heat prostration soon after the 49th week of the experimental period and the refusal of one boar in the 100 r group to serve the artificial vagina, semen data for the last collection period (70th-75th weeks) is based on 3, 5, and 3 boars, respectively in Groups 1, 2, and 3. Histological data from the testis of the boar refusing to serve the artificial vagina was obtained.

Analysis of variance (Snedecor, 1956) was used to test for differences between treatments and Kramer's Modification of Duncan's Multiple Range (1956) was conducted to test the significance of differences between individual means.

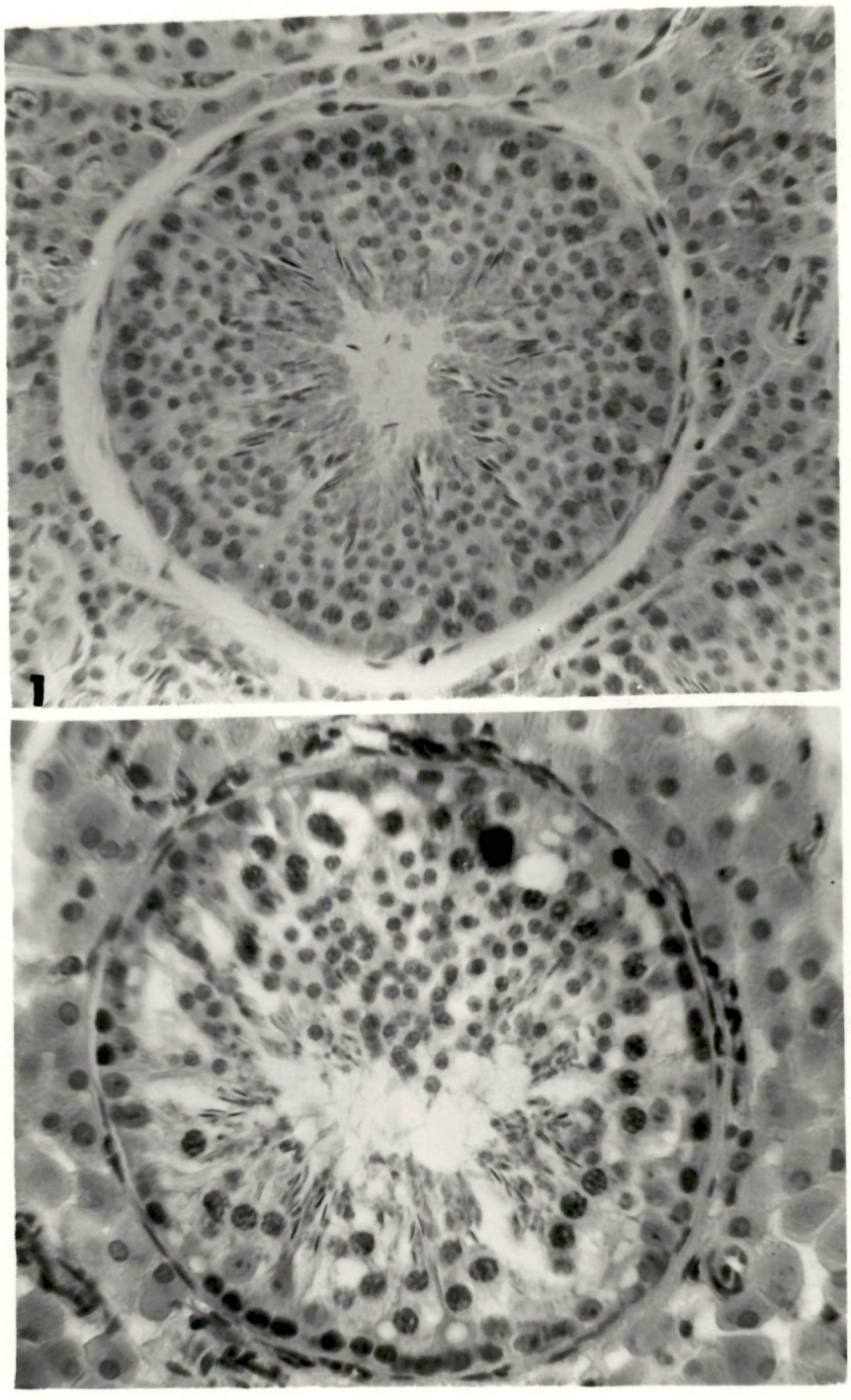

Figure 2. Histological classification of seminiferous tubules: (1) Type I and (2) Type II tubules. (X415)

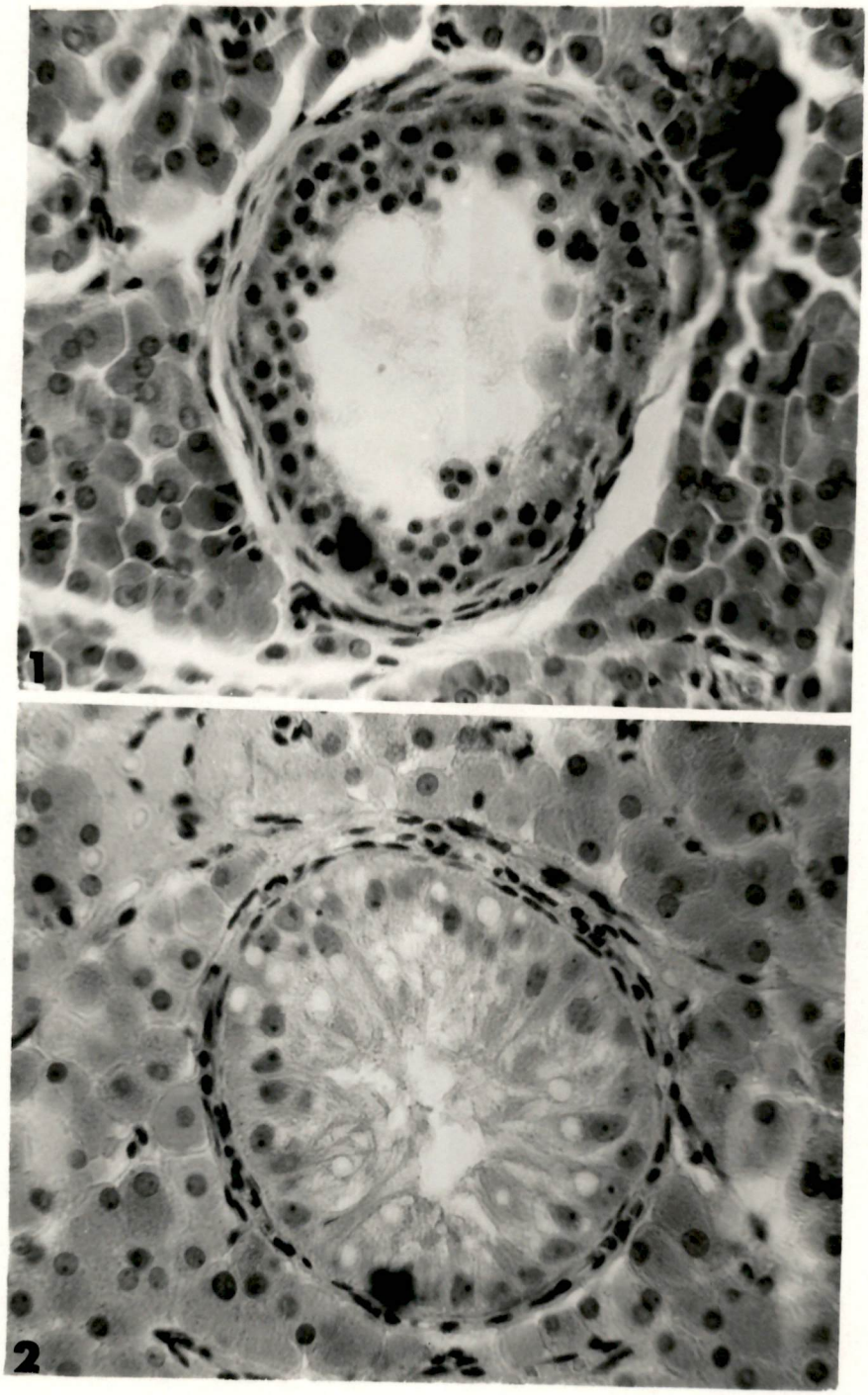

Figure 3. Histological classification of seminiferous tubules: (1) Type III and (2) Type IV tubules. (X415)

CHAPTER IV

RESULTS AND DISCUSSION

Sperm Production

The average number of sperm per collection for the boars in each treatment group is presented in Figure 4. There were no significant differences in sperm production between the control and either of the irradiated groups during the first 6 weeks following the initial exposure ($P > .05$). Sperm numbers of both irradiated groups were depressed ($P < .05$) during the seventh week following the initial exposure. The decline in sperm numbers at 7 weeks after the first exposure agrees with the report of Pace, Hupp and Murphree (1962) after acute doses of 200 and 400 r. This suggests that the initial decline in sperm numbers obtained from boars in this study was due to the first exposure of 50 or 100 r. Sperm production by the irradiated animals was even more severely depressed ($P < .01$) during the eighth week and a further decline in sperm numbers occurred until a low point was reached at 14 weeks following the initial exposure. From this point throughout the remainder of the study at no time did either of the irradiated groups regain a level of sperm production comparable to that of the controls. At the time of the second semen characterization period, 44 weeks after the first exposure, there was still a highly significant difference ($P < .01$) between both of the irradiated groups and the controls. This does not agree with Pace, Hupp and Murphree (1962) in

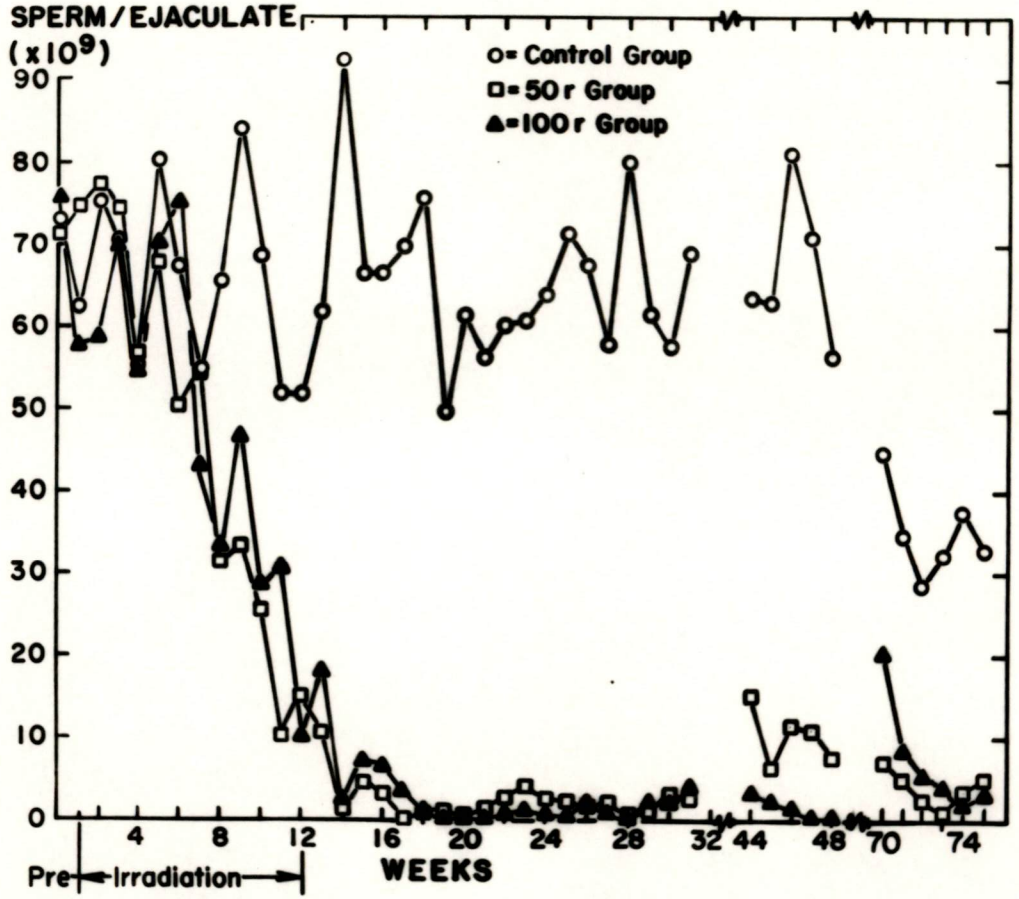

Figure 4. Sperm production of boars during and following irradiation.

that sperm numbers remained suppressed in their boars for only about 2 weeks before recovery began. It does, however, more closely approximate the sperm numbers produced by boars exposed by Willham and Cox (1961) to either 600 or 900 r in an acute dose. Their animals began to show some recovery at 20 weeks after exposure, but did not reach the control level during the remainder of the experiment. The most plausible explanation why sperm numbers were not suppressed longer in the experiment of Pace, Hupp and Murphree (1962) is that their acute dose of 400 r was high enough to reduce the number of spermatogonia, but at the same time was not high enough to cause a marked delay in repopulation of germ cells. This effect can be seen in the results of Willham and Cox (1961) in which semen production by boars which received 600 or 900 r remained suppressed for about 20 weeks following the acute exposure while that of boars which received 300 r began recovery at 12 weeks postexposure. The probable reason for this is that the higher levels of radiation killed large numbers of the spermatogonia and repopulation of the tubules was much slower, if it ever would have occurred.

The first evidence of recovery in either irradiated group was seen during the second collection period (44-48 weeks after the first exposure). Although the 50 r group produced a greater total number of sperm than did the 100 r group, the difference was primarily due to the relatively high sperm production of one boar which accounted for 48 per cent of the total sperm output by the 50 r group.

There was no difference ($P > .05$) in sperm numbers between the two irradiated groups during the third collection period (70-75 weeks after the first exposure).

The levels of radiation used in this study were so damaging that neither group showed sufficient recovery in sperm production for one to be able to differentiate between the two frequencies of exposure.

Sperm Motility

In general the per cent of motile sperm (Figure 5) and the rate of sperm motility followed the same pattern as sperm numbers, but neither measure of sperm motility declined as early as did sperm production. After reaching a low point at about 16 weeks after the first exposure both estimates of the motility tended to remain relatively constant through the remainder of the second collection period. This agrees with the report of Welch and Murphree (1964) in which bulls exposed to 100 r weekly to a total of 600 r failed to return within the control range of sperm motility during the 75 weeks following the initial exposure. It is not, however, the same pattern seen in the boar by Pace, Hupp and Murphree (1962) and in the bull by Parish (1958) and Gillette et al. (1964). In the aforementioned studies the pattern of sperm motility varied almost directly with sperm numbers. In the present study sperm motility of both irradiated groups was lower than in the control group during the third collection period ($P < .05$). Sperm motility of the 50 r group was also less than that of the 100 r group ($P < .05$).

Semen Volume

There was no evidence of a radiation effect on semen volume at anytime during the experimental period (Figure 6). The average volume

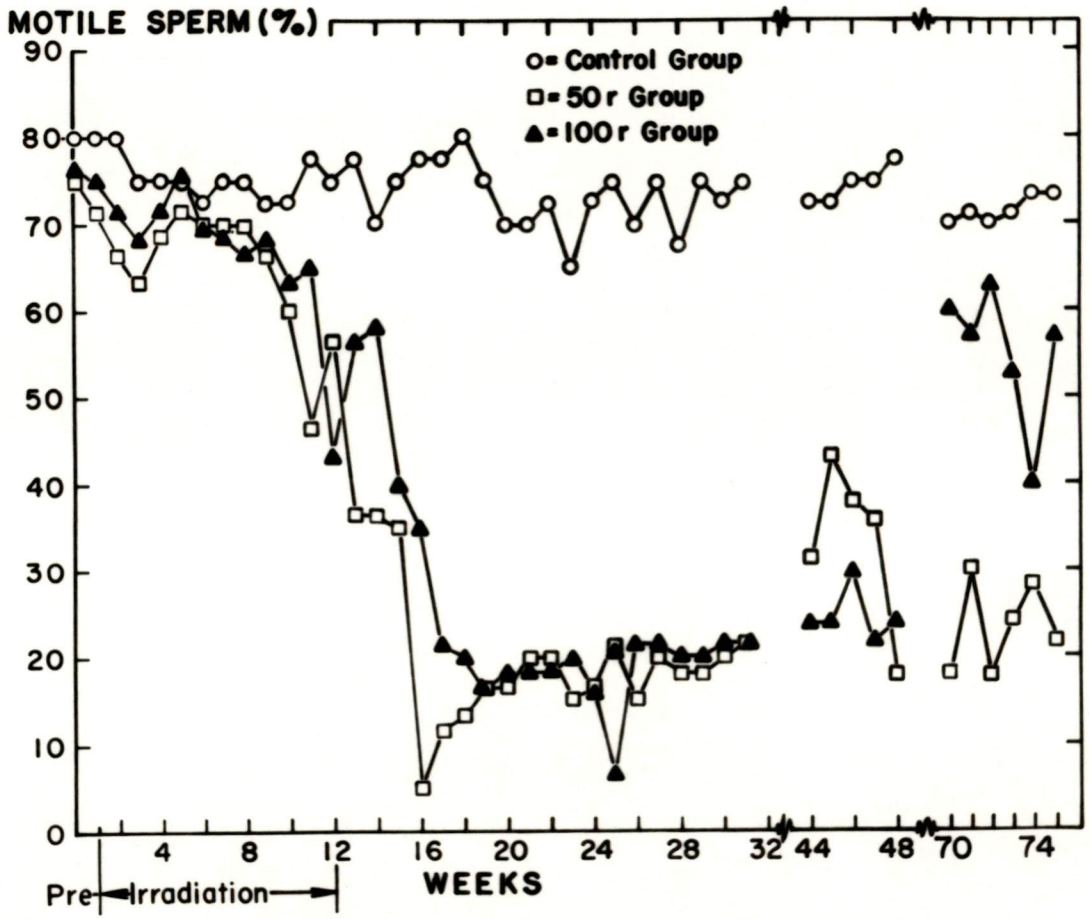

Figure 5. Per cent of motile sperm in the semen of boars during and following irradiation.

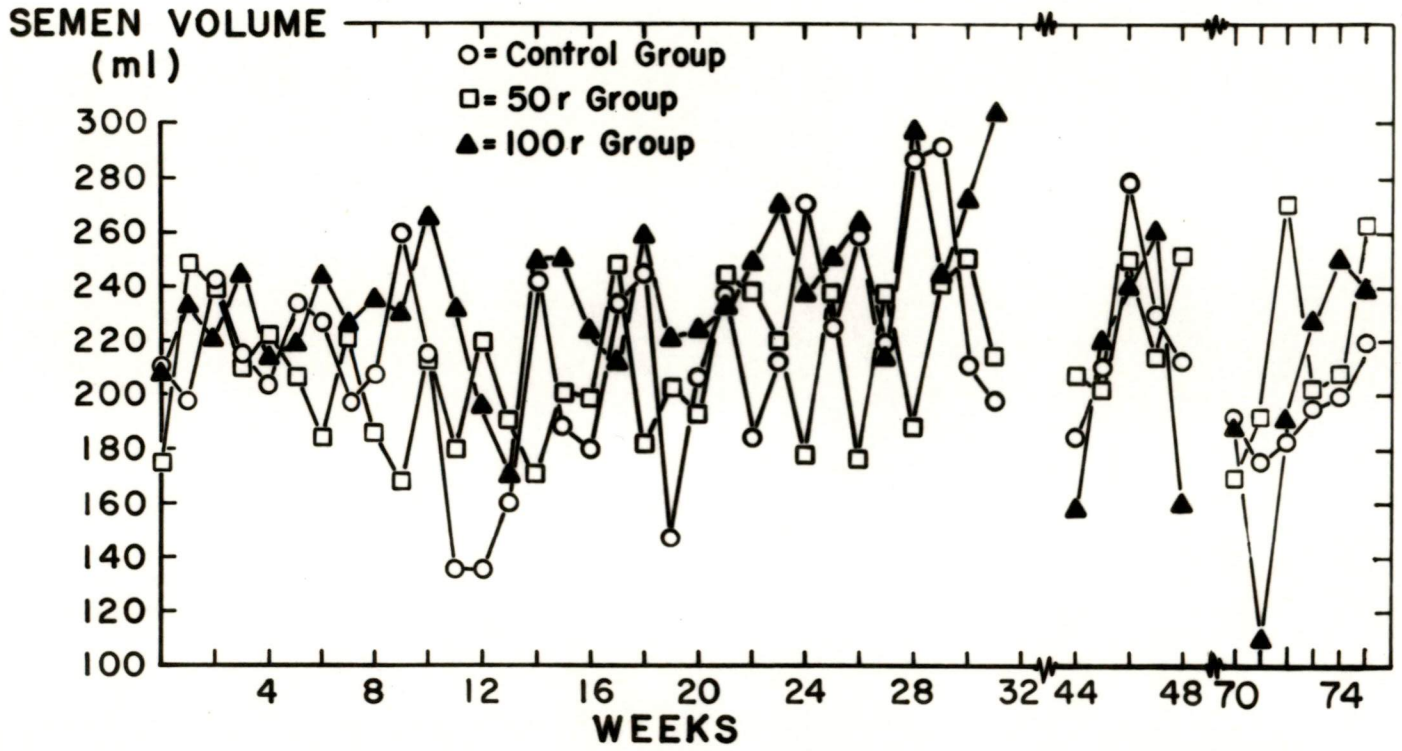

Figure 6. Semen volume during and following irradiation in boars.

increased only slightly from the preirradiation collections to the end of the second collection period. The lowered semen volume during the third collection period may have been a function of the boars having to become accustomed to being collected in the artificial vagina again after not having been collected for a period of 22 weeks. This lack of an effect of radiation on semen volume agrees with the results of Pace, Hupp and Murphree (1962) in the boar.

Testis Weights

When all animals were unilaterally castrated (left testis) at 49 weeks following the initial exposure, the control testes were significantly heavier than testes from either of the irradiated groups ($P < .05$, Table I). There was no difference in testes weights between the two irradiated groups, but they weighed only 55 per cent as much as those from control boars.

At the second castration (right testis) the control testes were again significantly heavier than testes from either of the two irradiated groups ($P < .05$, Table I). Testes from the exposed groups weighed approximately 58 per cent as much as the controls, but did not differ from each other ($P > .05$). A reduction in testis weight following irradiation has been noted in laboratory animals by Kohn and Kallman (1954) and in bulls by Welch and Murphree (1964) and Welch (1965). The mechanism involved is probably one of shrinkage of the seminiferous tubules as observed by Eschenbrenner and Miller (1962) following whole-body x-irradiation. The major part of the reduction in testis weight

TABLE I

TESTIS WEIGHTS OF CHRONICALLY IRRADIATED BOARS

Treatment	Testis[a]	Av. Testis Weight (gm.)
Controls	L	391 ± 46[b]*
	R	678 ± 149*
50 r weekly	L	217 ± 18
	R	419 ± 13
100 r biweekly	L	217 ± 12
	R	362 ± 72

[a]Left and right testes removed 49 and 76 weeks, respectively, following the first radiation exposure.

[b]Standard error.

*Different from testes of both irradiated groups ($P < .05$).

can be attributed to the reduction in numbers of spermatogenic elements in the tubules herein classified as Type III and to their absence in Type IV tubules. Other mechanisms, possibly hormonal in nature, may also contribute to the reduction in testis weights.

Testis Histology

Testes from both groups of irradiated boars contained fewer Type I and more Type IV seminiferous tubules than the controls ($P < .01$, Table II). Within the classification of Types II and III there was no difference between the controls and either of the irradiated groups ($P > .05$) and there was no difference between the two irradiated groups in any of the tubule classifications. The same boar in the 50 r group which had an unusually high sperm output contributed a disproportionately large number of Type I (57 per cent) and fewer Type IV (8 per cent) tubules. Even when the data for this animal are removed the differences between the 50 and 100 r groups are still not significant. Thus, both histology and sperm production data indicate there was either less permanent damage in the 100 r group or an earlier recovery in the 50 r group. The evidence of permanent damage seen in the present study does not agree with findings in the bull by Welch (1965) in which the same radiation regime was employed. The bulls had returned to near control levels of sperm production by 46 weeks following the initial exposure, whereas the boars never returned to more than 20 per cent of their preirradiation level of sperm production.

TABLE II

HISTOLOGICAL CLASSIFICATION OF SEMINIFEROUS TUBULES IN TESTES OF CHRONICALLY IRRADIATED BOARS

Treatment	Testis[b]	Per Cent of Seminiferous Tubules Classified as:[a]			
		Type I	Type II	Type III	Type IV
Controls		100.0**	0.0	0.0	0.0**
50 r weekly	L	16.8 ± 8.8[c]	2.3 ± 1.3	3.3 ± 4.4	77.6 ± 11.2
	R	18.2 ± 10.9	1.8 ± 0.6	2.6 ± 1.0	77.3 ± 12.1
100 r biweekly	L	4.9 ± 1.7	1.1 ± 0.5	1.6 ± 1.3	92.4 ± 2.7
	R	26.5 ± 5.3	3.7 ± 1.0	4.3 ± 1.1	65.5 ± 1.8

[a]Type I: Normal tubules.
Type II: Similar to Type I except for a reduction in number of cells.
Type III: One or more stages absent and a reduction in numbers of other cell types.
Type IV: Sterile tubules that contain only Sertoli cells.

[b]Left and right testes removed 49 and 76 weeks, respectively, following the first irradiation exposure.

[c]Standard error.

**Different from each of the other groups ($P < .01$).

Hematology Studies

The data on erythrocytes are presented in Figure 7. There were no significant differences at any time during the study between the three groups. This agrees with studies in the bull by Welch (1965) in which he employed the same radiation regime used in the present study. The hemoglobin and hematocrit values are shown in Figure 7. These two blood elements in the irradiated boars did not differ from the control values at any time during the experimental period. This is similar to the response seen in the boar by Pace, Hupp and Murphree (1962).

Platelet concentration (Figure 8) remained relatively constant in the irradiated animals during the entire period of observation, whereas platelet numbers in the control animals increased to a level significantly above that of the irradiated groups by 3 weeks after the first exposure. When platelet data for the period 3 through 20 weeks were analyzed by Kramer's modification of Duncan's Multiple Range test (1956), the irradiated groups were significantly lower than the controls. Twenty-eight weeks following the initial exposure there was no difference between any of the groups; this was not due to an increase in either of the exposed groups, but rather to a decrease in platelet values in the control boars during that sample period. Other observations at 35, 74, and 75 weeks following the initial exposure indicate an increase in the 100 r group while the 50 r group remained constant. This deviation at the last two observations may have been due in large part to fewer animals being tested.

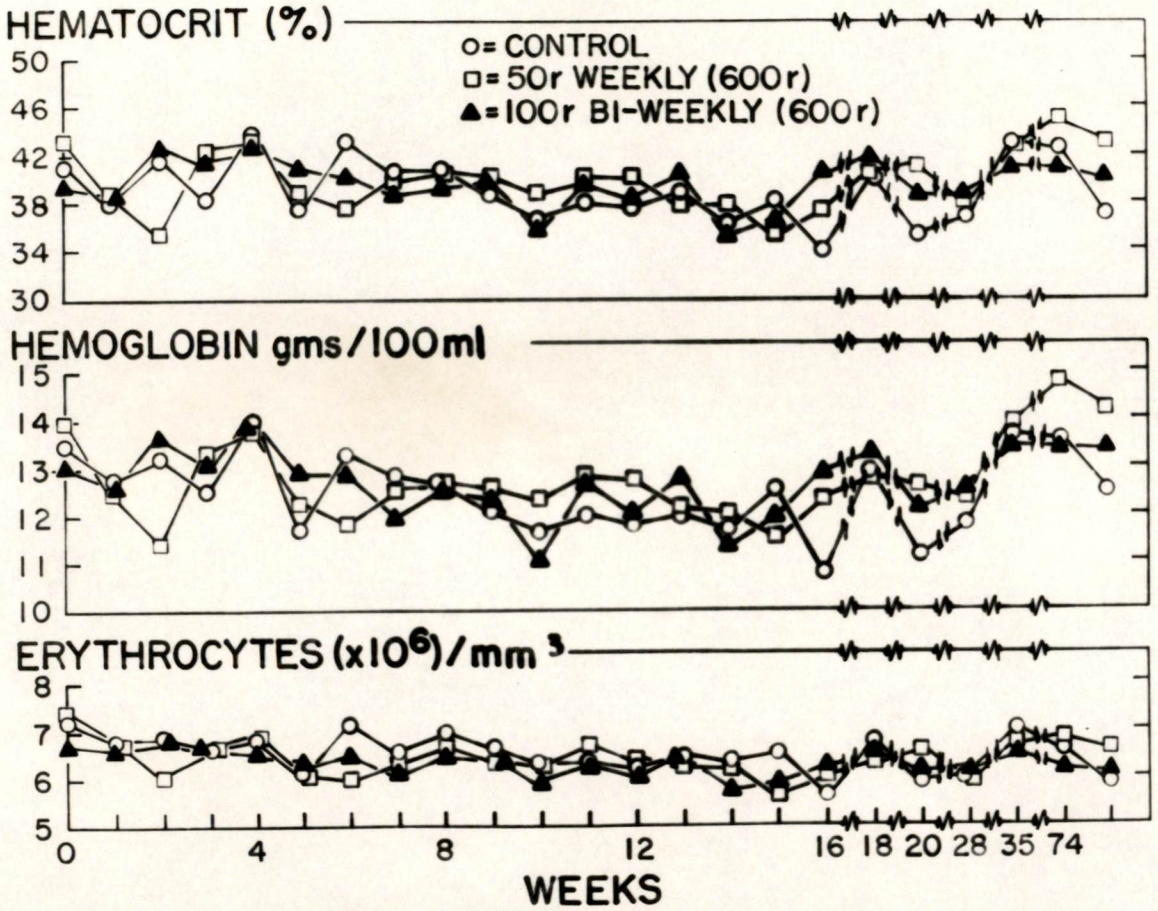

Figure 7. Concentration of erythrocytes (RBC) in the blood of boars during and following irradiation.

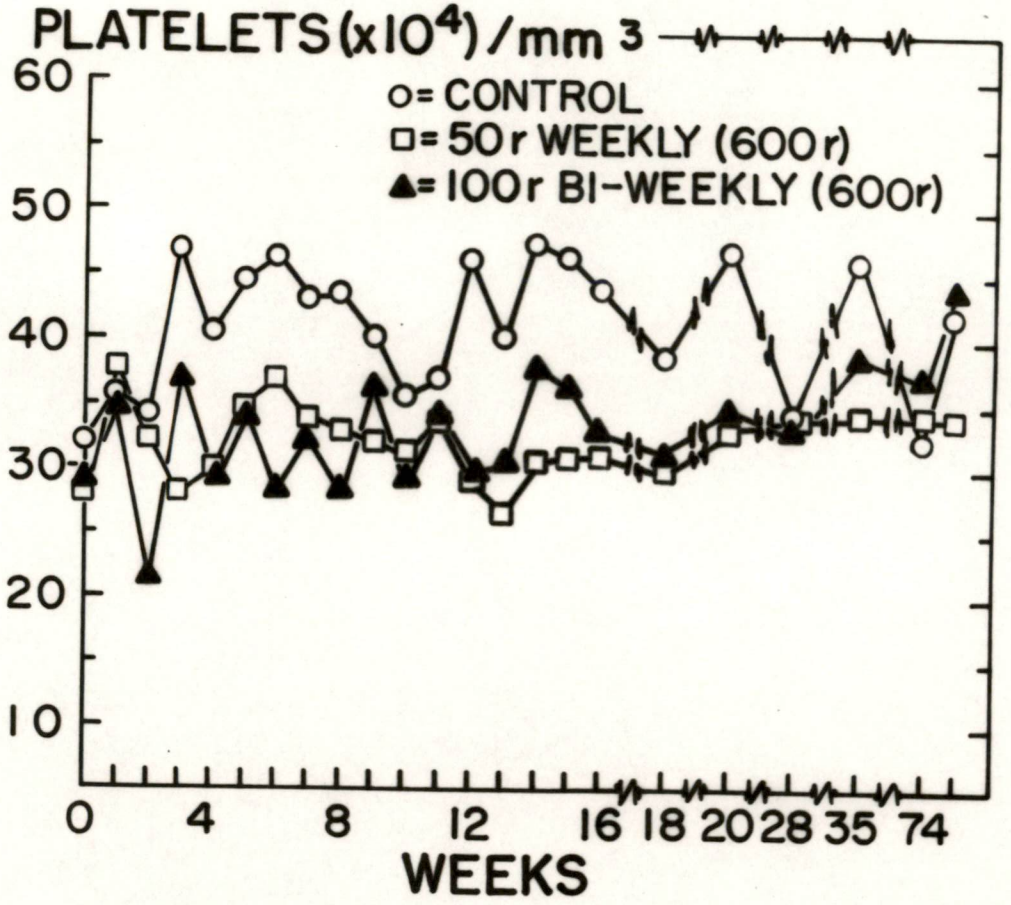

Figure 8. Platelet concentration in the blood of boars during and following irradiation.

One possible explanation for the increase in platelets among the control animals is that they were placed together for a period of 3 hours biweekly (during the first 12 weeks of the experiment) and allowed to fight. This increased stress may have been responsible for the platelet increase. At the same time fighting between animals could have caused a similar increase in platelet numbers in the two irradiated groups which was counteracted by the levels of radiation administered. This may also have been due to unidentified factors which caused an increase in the controls, while the radiation to the 50 and 100 r groups so affected them that they could not respond to these factors. This does not agree with the report by Pace, Hupp and Murphree (1962) in the boar. Following acute doses of 200 or 400 r they found a significant depression in platelet numbers which lasted for only 2 weeks before recovery became evident.

The concentration of leukocytes (Figure 9) was significantly reduced ($P < .05$) in both irradiated groups when compared to the control group. This reduction was seen 1 week following the initial exposure, and continued until the fourth week following the initial exposure. The difference between the irradiated and control groups persisted until the 35th week following the initial exposure at which time all three groups were similar. During the 74th and 75th weeks the controls were significantly higher ($P < .05$) as compared to the irradiated groups. This was due mainly to an increase in leukocyte numbers within the control group while the irradiated groups remained relatively constant. These are the approximate findings of Pace, Hupp and Murphree (1962) except that the

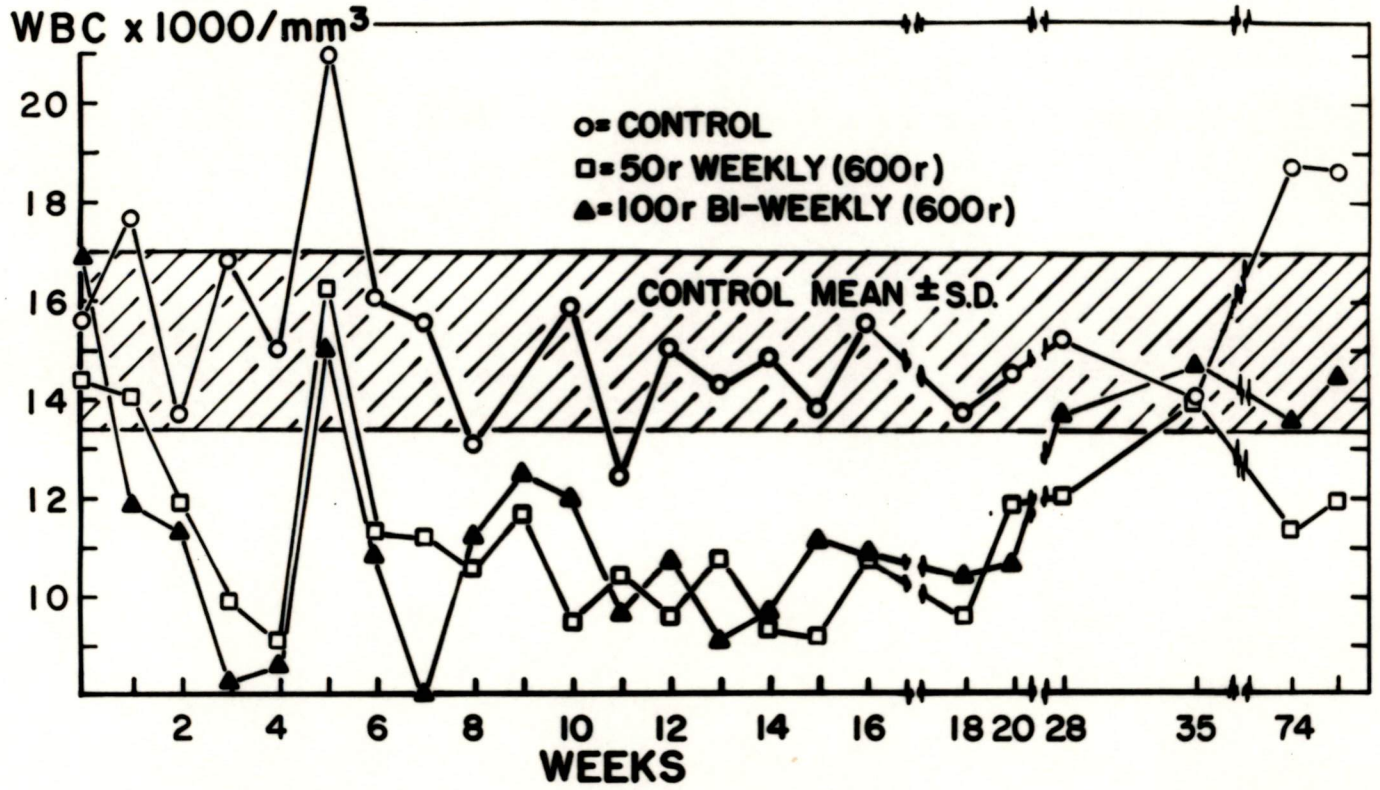

Figure 9. Concentration of leukocytes (WBC) in the blood of boars during and following irradiation.

irradiated animals in their study were slightly below the control levels at 200 days after the initial exposure. At no time in the present study were there any significant differences between the two irradiated groups in the number of leukocytes. The deficiency of leukocytes was due almost wholly to a reduced number of lymphocytes; segmented cells showed only occasional periods of depression (Figure 10).

The concentration of lymphocytes in both irradiated groups was significantly reduced ($P < .01$) below the control levels until the 35th week following the initial exposure. For the remainder of the experimental period there was no difference between either of the three groups, although there was considerable variation in the last two observation periods due to smaller numbers of animals in each group. The segmented cells were affected to a lesser degree than the lymphocytes although both irradiated groups were significantly reduced ($P < .05$) below the control levels at the third and fourth weeks after the initial exposure. Except for this 2-week period there was no difference between the three groups until the last 2 weeks of the trial when all three groups differed from each other ($P < .05$). This significance could be attributed to high control values and a slight reduction in the 50 r group. Again, these values were influenced by the small numbers in each group during the last 2 weeks of the trial. This does not agree with the report by Welch and Murphree (1964) in which bulls were exposed to 100 r weekly to a total of 600 r. They found a significant reduction not only in lymphocytes but also in segmented cells. Their irradiated groups had not returned to within the control range by

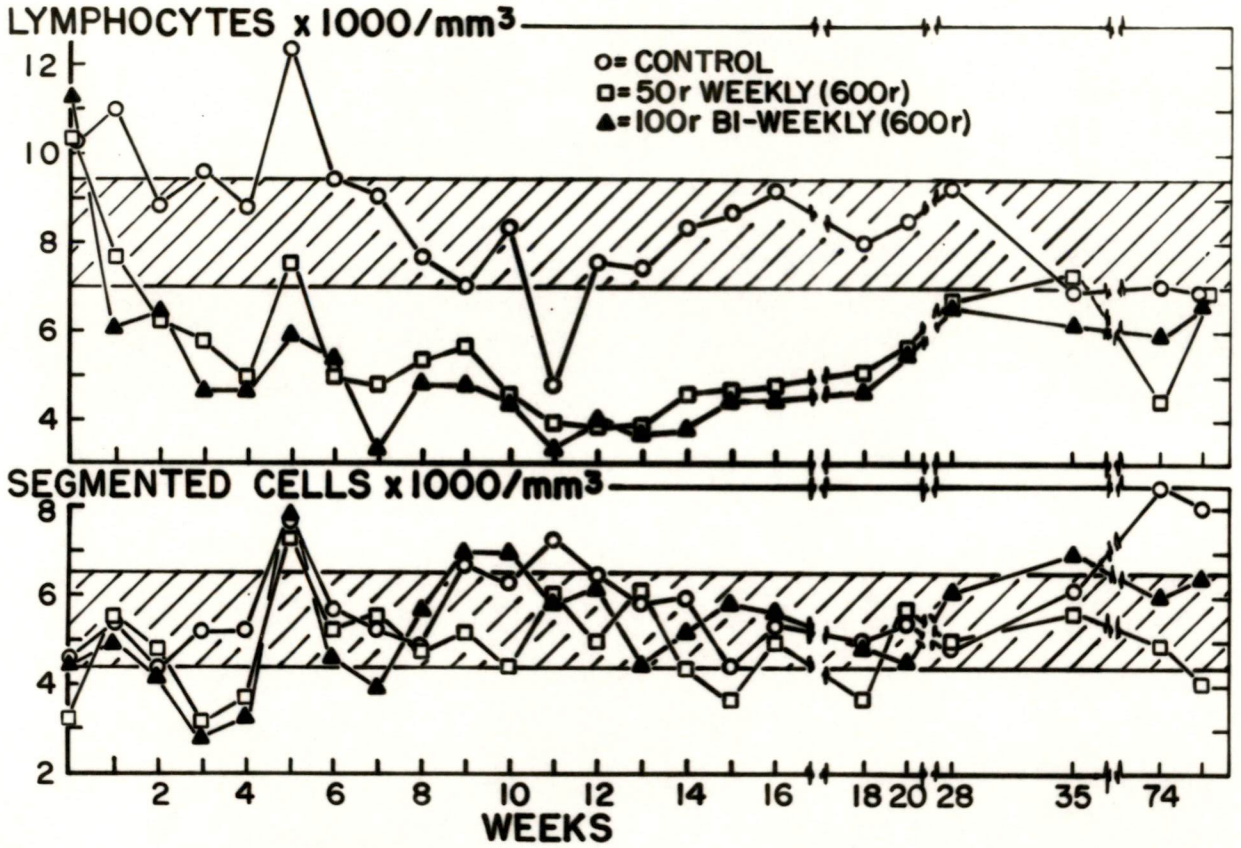

Figure 10. Concentration of lymphocytes and segmented cells in the blood of boars during and following irradiation.

2 years after the first exposure. The remaining constituents of the white-blood-cell complex consisting of the eosinophils, basophils, neutrophils and banded cells did not differ significantly among the three groups throughout the study.

CHAPTER V

SUMMARY

The effects of fractionated doses of whole-body gamma radiation on semen characteristics and blood constituents were studied in mature boars. On the basis of their pretreatment sperm production, 16 mature boars were assigned to three comparable groups which received 0, 50 r weekly or 100 r biweekly to an accumulated dose of 600 r at the rate of .57 r per minute. There were four, six, and six animals, respectively, per group.

Sperm output of irradiated boars began to decline 7 weeks after the first exposure and most of them were aspermic by the 17th week. Sperm output of both groups was about 3 per cent of the control level from the 14th through the 31st week. Although an increase in sperm production was evident during the 44th-48th weeks, both irradiated groups were still significantly below the control level. During this period sperm output by boars receiving 50 r weekly was greater than than of boars which received 100 r biweekly. At the 75th week there was still no significant difference between the exposed groups, but a significant difference existed between each of them and the control group. Decline in both rate and per cent of motile sperm followed the decline in sperm numbers but occurred 3 to 6 weeks later. There was no significant difference between the two irradiated groups in either the rate or per cent of motility through the second collection period.

There was a difference between all treatment groups during the third collection period (70-75 weeks following the initial exposure).

Analysis of the testicular histology indicated permanent damage as a result of irradiation. Data from the group receiving 50 r weekly indicated that most of the repairable damage had taken place by 49 weeks after the first exposure, while in the group receiving 100 r biweekly the repair took place between 49 and 76 weeks.

The leukocyte concentration was significantly reduced in both irradiated groups with this reduction being accounted for primarily by a reduction in lymphocytes. Platelet concentration was not significantly depressed in the irradiated groups. There were no significant changes in the other blood constituents.

It was concluded that the dose levels and frequencies of exposure used closely approach the minimum level required to produce permanent testicular damage. It was also concluded that permanent sterility resulted in some of the irradiated animals, while certain others would have been fertile in limited service.

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