December 11, 1957

To the Graduate Council:

I am submitting herewith a thesis written by Henry B. Pace entitled "Effects of External Irradiation of Pregnant Female Rats On Viability, Growth and Fertility Of The Offspring". 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:

M. C. Bell

Charles S. Hobbs

Accepted for the council:

Dale Trantham
Dean of the Graduate School

EFFECTS OF EXTERNAL IRRADIATION OF PREGNANT FEMALE RATS ON VIABILITY, GROWTH AND FERTILITY OF THE OFFSPRING

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

---

by

Henry B. Pace

December 1957

21.
33

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. R. L. Murphree for his guidance and assistance in helping to conduct this study, and to Dr. C. S. Hobbs for his kindness in awarding an assistantship which made it possible for the author to pursue an advance degree. Great appreciation is due to the UT-AEC Agricultural Research Program, Oak Ridge, Tennessee, for making available animals and facilities used in this study and also appreciation is due to colleagues for their kind assistance and cooperation and especially to Mrs. Ina T. Freshour for her endeavors. The author is deeply indebted to John Mitchell for his interest, help and care in collecting the data for this thesis.

385515

TABLE OF CONTENTS

CHAPTER		PAGE
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	3
III	MATERIALS AND METHODS	10
IV	RESULTS AND DISCUSSION	15
V	SUMMARY	28
IV	BIBLIOGRAPHY	31

CRANES CREST

LIST OF TABLES

TABLE		PAGE
I	Effect of Stage of Pregnancy at Time of Irradiation of Female Rats on Postnatal Viability and Growth of the Offspring	16
II	Growth of Control and Irradiated Rats Nursing Control or Irradiated Dams (dams received 150 r on 17th day of gestation)	18
III	The Effect of Prenatal Irradiation on Subsequent Fertility of Female Rats (150 r on the 17th day of gestation)	20
IV	The Effect of Prenatal Irradiation on the Sub-Sequent Fertility of Female Rats (150 r on the 19th day of gestation)	22
V	Average Body and Testes Weights of Control and Prenatally Irradiated Rats (dams received 150 r on the 17th day of gestation)	24
VI	Average Body and Testes Weight of Control and Prenatally Irradiated Male Rats (dams received 150 r on the 19th day of gestation)	26

CHAPTER I

INTRODUCTION

Knowledge of the effects of gamma radiation exposure of the mother on pre- and postnatal development of the offspring is of special interest because of its implication for humans, and also because of possible contributions to the field of mammalian embryology. Work in other laboratories, primarily with mice, using x-radiation has shown that radiation exposure of the pregnant female may result in death, anatomical defects, or lowered viability of the offspring. The type of response is governed, at least in part, by the level of radiation and the stage of gestation during which exposure occurs.

Ionization within individual cells is produced when tissues are exposed to high levels of radiation; this may lead to cell damage or death of the cells. Both x-rays and gamma rays are electromagnetic radiations which are thought of as being physically identical, the difference in name indicating only a difference in origin. The interaction of electromagnetic radiation with matter changes in character as the energy increases. In the low energy region, the interaction is chiefly by the photoelectron process in which each individual photon loses its entire energy in a single collision with an electron. In the intermediate energy region, both the photoelectric effect and Compton scattering are significant, the latter involving multiple, degrading collision of a single photon with several electrons. The Compton effect becomes more significant, and the photoelectric effect less significant,

as the energy increases. Above 1.02 mev* a third process is observed; this is the "pair production" process in which a single photon produces a positron and a negatron at the same time. The electrons produced by these three processes are responsible for ionization within the individual cells. Ordinarily, x-rays are in the low energy region, and interact chiefly by the photoelectric process, whereas gamma rays from cobalt-60 are in the high energy region, where a considerable amount of the initial energy loss is by the Compton process.

This study was initiated to determine the effects of gamma ray exposure of pregnant female rats on pre- and postnatal viability, growth and fertility of the irradiated offspring. It was hoped that the data obtained in this study would aid in designing further comparable type studies currently in progress with cattle and swine.

---

*Million electron volts.

CHAPTER II

REVIEW OF LITERATURE

The reports in the literature concerning the effects of radiation on embryonic and/or fetal development deal almost entirely with studies on mice, rats, guinea pigs and rabbits. The major part of the research published since 1935 is based upon studies with mice and rats. In all papers reviewed in this thesis, x-rays were used as the radiation source.

Russell (1954) has presented an excellent review of the literature concerning the effects of radiation on mammalian prenatal development up to 1952. Only limited references will be made in this thesis to work reported prior to 1952. For purposes of delineating the effects of radiation exposure at different stages of prenatal development, landmarks in development (e.g., blastocyst, first mesoderm, neural plate, first somite, etc.) have been plotted against days of gestation. Using such a system, it has been possible to arrive at approximate divisions of time for: (1) the pre-implantation period, (2) the period of major organogenesis and (3) the period of the fetus. For the mouse and rat these periods are: mouse 0-5, 6-13, 14 to term (19 to 20 days); rat 0-7, 8-15, 16 to term (21 to 22 days).

Russell and Russell (1950a, 1950b) irradiated female mice on .5, 1.5, 2.5, 3.5 and 4.5 days after mating in an attempt to determine the stage at which preimplantation deaths or abnormalities occurred. It was found upon examination of the uteri 10.5 or on 13.5 days after

mating that radiation induced embryonic deaths were considerable with the earliest stages being the more sensitive. Mortality between day 10.5 and term was the same as noted in the controls indicating that the radiation induced deaths occurred entirely before day 10.5 of gestation. Three types of radiation induced deaths were recognized during the preimplantation period: (1) preimplantation death of entire litters, (2) death of individuals within surviving litters, and (3) deaths of individuals after implantation. It was suggested that since implantation deaths involving whole litters were of equal importance in all three groups, the majority of deaths may not have been caused by direct radiation injury to the developing embryo, but by some effect on the implantation process. Individual preimplantation deaths were high only as a result of radiation prior to cleavage or in very early cleavage states. Postimplantation deaths were greatly increased when irradiation was applied before actual implantation. Since virtually all survivors which were irradiated during the cleavage stage were normal, this was interpreted as indicating a considerable degree of totipotency in the blastomers of the developing embryos.

The majority of work on the effects of radiation and the mammalian embryo deals with the period of major organogenesis, with the most of the more recent and extensive studies falling in this group.

Russell (1950) showed that complete interruption of pregnancy, which often followed irradiation during the preimplantation stages in mice, rarely occurred as a result of irradiation during later stages of pregnancy. This was obvious from the fact that a high percent of

the female mice that received 200, 300 or 400 r in stages of pregnancies which could be easily diagnosed by abdominal palpation, cast litters at the normal time. Doses of 100 r or less during the post-implantation stages apparently exerted no effect on prenatal viability. A dose of 200 r, which caused 80 per cent prenatal deaths when exposure was made on .5 to 2.5 days after fertilization, caused a decrease of only 11 per cent in litter size when administered between days 5.5 to 12.5. The major part of this depression was due primarily to reduced litter sizes of females which were irradiated near the beginning of the period of organogenesis since there was a 27 per cent reduction in litter size from females exposed on days 6.5, 7.5 or 8.5 of pregnancy as compared with the controls.

According to Russell (1954) when the data of several series of experiments (involving different genetic strains of mice) were pooled it was found that while 100 r and below had no effect on survival at birth, 200 r applied on any one of days 7.5 through 11.5 inclusive, caused an increased neonatal mortality, with a sharp peak for days 9.5 and 10.5 (75 and 67 per cent, respectively, as compared with the control group). Dose of 300 r increased neonatal mortality with the death rate paralelling the 200 r curve with the peak reaching 100 per cent on days 9.5 and 10.5.

The above data suggest that the mortality is markedly dependent on the stage of gestation at the time of irradiation even though the stages are separated by only 24 hour intervals, e.g., for irradiation on days .5 through 8.5 postconception, the LD 50 is less than 200 r;

for irradiation on days 9.5 and 10.5 postconception the LD 50 is greater than 200 r; for irradiation on day 11.5 postconception, the LD 50 is greater than 200 r but less than 300 r; for irradiation on days 12.5 to 15.5 postconception, the LD 50 is greater than 300 r.

In an experiment (Russell, 1950) in which several variables known to affect birth weights were controlled, it was found that mean birth weights for the 8.5 through 13.5 day irradiated mice fell considerably below the controls. In comparison, the 200 and 300 r curves were, in general, parallel with a mean difference of 0.2 grams. Minima for both curves lay between the 10.5 and 11.5 day stages. Holding the time of irradiation consistent, Russell et al. (1951) obtained a more complete dose weight series for day 11.5 which was found to be close to the stage of maximum susceptibility to growth retardation. The amount of retardation was governed by the level of radiation given as the smaller dose produced a less effect when compared to the high dose level of 300 r.

Since mortality resulting from irradiation of fetal stages has been found to occur mostly in the period between birth and two weeks of age, very few of the more recent investigators have concentrated their attention on irradiation during the period of the fetus.

Russell (1950) reported that irradiation during the period of the fetus did not lead to the striking differences in newborn which were produced by irradiation with comparable or even lower doses during the period of major organogenesis. She postulated that there was less chance for damage to be expressed as a gross change prior to

parturition as the interval between exposure and birth was shorter, but that there was a possibility that gross changes would be expressed later in life.

Rugh and Wolff (1956) state that 300 r is the minimal lethal dose which a 13.5 day mouse fetus will not survive beyond the first day of life.

Rugh (1956) concluded that increasing levels of x-radiation of nursing female mice caused a decreased growth of the suckling young. There was some growth depression from 50 r exposure of the dam on the 3rd day postparturition; there was pronounced growth depression at levels of 200 r or higher. New born mice starved by an irradiated mother recovered rapidly if given to a normal lactating mother 15 days postparturition and by 30 days of age were essentially comparable to the controls with respect to body weight.

The studies cited above have dealt entirely with mice as the experimental animal. The following reports are based on studies using rats as the experimental animals.

Wilson (1949) laparotomized 10-day pregnant female rats and used lead plates in such a manner as to permit direct irradiation of 2 to 5 embryos in each female without exposure of the remaining embryos in litter. Beginning 24 hours after irradiation, irradiated and control embryos were removed at 24 hour intervals for 5 consecutive days. At the time of recovery, the embryos were weighed and prepared for histological study. Exposure of embryos to 50 r had no effect on embryonic development, while 100 r slightly increased the incidence of intrauterine death, temporarily reduced growth rate, and caused retarded

or anomolous eye development in 60 per cent of the treated embryos. Exposure to 200 r caused similar developmental defects which were more pronounced as the death rate and developmental abnormalities increased. Exposure of the embryos to 400 r always caused embryonic death within 24 hours after exposure.

Wilson and Karr (1950) state that exposure to 200 r on the 9th day of gestation resulted in the death of all rat embryos.

Wilson et al. (1953) in an experiment using differentiation as a determinant of the reaction of rat embryos to irradiation, exposed the embryos on days 8, 9 or 10. Irradiation of embryos on the 8th day of gestation had a more limited effect on subsequent development than did similar treatment on the 9th and 10th days. The effect of exposure to 12.5 to 100 r on the 8th day was retardation of growth. Exposure to comparable doses on the 9th and 10th days caused an increase in mortality as well as growth retardation. The difference in effect between the 8th as compared to the 9th or the 10th day embryos appeared to be dependent upon the stage of cellular differentiation within the embryo at time of irradiation.

Wilson (1954) exposed rat embryos directly through an abdominal incision, to doses of 12.5 to 600 r. The only measurable effect after doses less than the total lethal dose (200 r) on the 8th day of gestation was growth retardation. With increasing levels of radiation on the 9th through the 11th days of gestation there was an increase in the number of anatomical malformations, mortality and growth retardation. This difference in pattern of response was attributed to a

change in susceptibility of the cells as the embryo passed from the undifferentiated state of the 8th day into the early stages of differentiation on the 9th day. Embryos exposed on the 9th, 10th or 11th day all displayed a similar pattern of response, but sensitivity to the various radiation-induced effects diminished with increasing age at the time of exposure.

CHAPTER III

MATERIALS AND METHODS

All irradiation of rats in this study was accomplished by exposure of the animals to a 100 curie cobalt 60 (gamma ray) source. During exposure, the rats were confined in 3 x 5 x 8 inch perforated plexiglas cages and the cages arranged in circular pattern on an isodose curve approximately 24 inches from the radiation source. The intensity of radiation for the various trials varied from 4.14 to 4.10 r per minute (air dose) as measured by Victoreen dosimeters. This decrease in rate was due to radioactive decay of the cobalt 60. The total radiation dose received by the animals was controlled by the length of exposure to the radiation source.

All rats used in this study were of the Sprague-Dawley strain and each animal was individually numbered by ear punch for identification purposes. Rockland rat feed and water were supplied ad libitum throughout the study. Every effort was made to maintain uniform environmental conditions for the control and irradiated groups. A constant temperature of 80° F was maintained in the colony room. Within trials, females of comparable age and parity were used.

In classifying the females as to stage of gestation, at the time of the treatment, the day following observed mating during the previous night was counted as day zero. Following this system of counting, the gestation period in this strain of rats was found to be 22 days. In those females where the date of breeding was not known, the stage of

pregnancy at the time of treatment was calculated by counting back from the date of littering to the treatment date. Examination for pregnancy, by abdominal palpation, was routinely started on the 10th day after the males were placed with any group of females.

A. The Effect of Irradiation of Pregnant Females on the Postnatal Viability and Growth of The Offspring

1. Viability and Growth of the Offspring.

Sixty-five nulliparous female rats (10 to 19 day pregnant) were used in the first phase of this study of the effect of irradiation of the dam on the viability, growth and fertility of the prenatally irradiated offspring. The pregnant females were randomly divided into three treatment groups: (1) control, 19 females; (2) 110 r, 25 females; and (3) 220 r, 21 females. The stage of gestation at the time of irradiation of the females was calculated by counting back from the date of parturition to the treatment date. As soon as possible after parturition, the total number of young born in each litter was recorded. At this time and at daily intervals to weaning at 21 days of age, the number of dead offspring was recorded. Litter weights were recorded on the 7th and 14th days and individual weights were obtained at weaning time.

Subsequently, thirty-three primiparous pregnant females were randomly divided into three treatment groups: (1) controls, 10 females; (2) 150 r on the 13th day of gestation, 13 females; and (3) 150 r on the nineteenth day of gestation, 10 females. Management and data obtained

were as described for the previous group. Randomly selected offspring from the control and 150 r (19-day) irradiated litters were retained for fertility tests.

2. Comparison of Radiation Effects on Dams vs. Offspring.

This phase was conducted to determine if the growth depression of the young noted in the earlier phase was due to the direct effects of radiation on the offspring or due to a reduced milk supply by the irradiated dams. Twenty-eight primiparious pregnant females were paired on the basis of time of mating. On the 17th day of gestation, one member of each pair was exposed to 150 r. On the fifth day after parturition each litter was weighed, the litters standardized to 10 young each and approximately equal numbers of offspring exchanged between pairs of dams. The reciprocal exchange of young between the control and irradiated dams was purposely delayed until the fifth day, postparturition, to avoid unequal litter sizes due to a possibly higher postnatal death loss among the irradiated offspring. As far as possible, equal number of each sex within litters within treatments were retained. Individual weights were obtained on the 14th and 21st days of age. Randomly selected offspring from the control and irradiated litters were retained for fertility tests.

CRANESHOT CREST

B. Effects of Irradiation of Pregnant Females on Sexual Development and Fertility of The Offspring

1. Fertility of Prenatally Irradiated Females (150 r on 17th Day of Gestation).

Nineteen control and 35 prenatally irradiated females (from litters that received 150 r on the 17th day of gestation) were retained for fertility studies. At 102 days of age both groups of females were placed with known fertile males for seven consecutive nights and all matings during a four-hour period (7 to 11 P. M.) were recorded. All females in both groups mated during this period and were autopsied 15, 16 or 17 days later. At autopsy the number of corpora lutea, fetuses, and fetal weights were recorded.

2. Fertility of Prenatally Irradiated Females (150 r on 19th Day of Gestation).

Twenty-five control and 52 prenatally irradiated females (from litters that received 150 r on the 19th day of gestation) were retained for fertility studies. At 105 days of age both groups of females were placed with known fertile males and the females mating during five consecutive nights were identified and recorded. The number of young born in each litter (alive or dead) and average birth weights were recorded. The number of living young was checked daily to weaning. Individual weights were recorded at weaning age (21 days).

CRANESEAT CREST

3. Testicular Development In Prenatally Irradiated Males (150 r On 17th Day of Gestation).

Thirty control and 21 prenatally irradiated males (from litters that received 150 r on the 17th day of gestation) furnished data on comparative body and testicular growth rates. Randomly chosen males from each treatment group were autopsied at intervals from 34 to 118 day of age. Body and testes weights were recorded for each group at autopsy.

4. Testicular Development and Fertility in Prenatally Irradiated Males (150 r on 19th Day of Gestation).

Twenty-six control and 47 prenatally irradiated males (from litters that received 150 r on the 19th day of gestation) furnished data on testicular growth rates and fertility. Randomly chosen animals from each group were autopsied at intervals from 64 to 153 days of age. Body and testes weights were recorded at autopsy. At 119 days of age five control and 12 irradiated males from the above groups were placed with known fertile females for a three week period to test the fertility of both groups of males. Each male was caged with two females for this test. Body and testes weights were recorded for this group of males at autopsy (153 days of age).

Analysis of variance, chi-square and the t-test as described by Snedecor (1956) were the statistical procedures used for the analysis of the data.

CHAPTER IV

RESULTS AND DISCUSSION

A. The Effects of Irradiation of Pregnant Females on the Postnatal Viability and Growth of The Offspring

1. Viability and Growth of the Offspring.

The levels of radiation, number of litters, stage of pregnancy at time of exposure, average litter size at birth, average viability at 7 and 21 days of age and the average weaning weights are shown in Table I. Exposure of the pregnant females to gamma irradiation did not reduce the litter size at birth in this study. However, the viability of the young at seven days of age was markedly reduced in the litters of the 110 r females exposed on the 12-14th days, the 150 r females exposed on the 13th day and the 220 r females exposed on the 11th to 15th day of gestation as compared to the control females (17, 0, 0 and 88 per cent, respectively). The differences are highly significant ($P < 0.001$). Virtually all deaths occurred during the first seven days, post birth (Table I).

The average individual weaning weights (last column of Table I), show that growth rates were depressed in all irradiated groups; the greatest depression occurred in the 110 r litters irradiated on the 12th to 14th day and the 220 r litters irradiated on the 16th to 19th day of gestation.

TABLE I

EFFECT OF STAGE OF PREGNANCY AT TIME OF IRRADIATION OF FEMALE RATS ON POSTNATAL VIABILITY AND GROWTH OF THE OFFSPRING

Treatment	Number of litters	Stage of pregnancy irradiated (day)	Average no.born	Average % alive (7 days)	Average % alive (21 days)	Average 21 day wts.(gm)[a]
Control	29	--	10.7	87.6	87.0	38.4 ± 6.4
110 r	2	10-11	12.5	84.0	84.0	33.5 ± 2.8
	11	12-14	12.5	17.4***	14.49***	27.6 ± 6.2**
	12	15-17	10.4	83.2	81.6	31.4 ± 5.0**
150 r	13	13	11.7	0***	0***	--
	10	19	12.1	88.4	84.8	34.6 ± 4.1
220 r	17	11-15	11.02	0***	0***	--
	4	16-19	10.3	92.7	91.0	26.4 ± 2.4***

[a]Mean ± standard deviation

**Significant at the 1% level of probability, respectively, in comparison with the control.

***Significant at the 0.1% level of probability, respectively, in comparison with the control.

With the exception of the litters from two 220 r females irradiated on the 11th day of gestation, no evidence of gross anatomical defects were observed in any of the irradiated offspring. All young in the two litters were born dead and had hydrencephalocele.

2. Comparison of Radiation Effects on Dams vs. Offsprings.

The average litter size at birth in the 14 control and 14 irradiated litters (150 r on the 17th day of gestation) was 11.3 and 11.2 young, respectively. Five and eight young, respectively, in the two groups died prior to the fifth day of age. The average weights at five and 21 days of age for the retained offspring were 9.98 vs 8.42 and 42.0 vs 33.4 gms, respectively, for the control and irradiated individuals (Table II). These differences were highly significant ($P < .01$). The difference of 1.4 gms (38.4 vs 37.0 gms) in weaning weights between the young (control and irradiated) sucking control dams or irradiated dams was significant ($P < .05$). However, this growth reduction of approximately four per cent is minor when compared to the reduction of approximately 20 per cent in the irradiated offspring. Thus, the evidence would strongly suggest that the major part of the growth retardation in the prenatally irradiated offspring was due to direct radiation effects on the developing fetuses rather than a depressed milk supply from the irradiated dams. That this growth retardation was permanent is indicated from a comparison of the mature weights (90 days of age) of five control and five irradiated males, 373 vs 278 gms, respectively. Likewise, the weights of 19 control and 34 irradiated females at 102 days of age were 296 and 233 gm, respectively.

TABLE II

GROWTH OF CONTROL AND IRRADIATED RATS NURSING CONTROL OR IRRADIATED DAMS (dams received 150 r on 17th day of gestation)

Treatment	Control female	Irradiated females
Av. 5 day wt. (gm.)[a]		
Control offspring on	10.01 ± 0.73 (65)[b]	---
Control offspring transferred to	---	9.96 ± 0.79
Irrad. offspring transferred to	8.43 ± 0.92 (75)	---
Irrad. offspring on	---	8.42 ± 0.96 (65)
Av. 21 day wt. (gm.)		
Control offspring on	42.9 ± 3.0 (65)	---
Control offspring transferred to	---	41.1 ± 2.8 (75)
Irrad. offspring transferred to	33.8 ± 2.1** (75)	---
Irrad. offspring on	---	32.9 ± 2.3** (64)
Av. 21 day wt. (gm.)		
Control + irrad. offspring	38.4 ± 5.2 (140)	37.0 ± 4.9* (139)

[a]Mean ± standard deviation.

[b]Indicates the number of individuals in each group

*Significant at the 5% level of probability, respectively, in comparison with the control.

**Significant at the 1% level of probability, respectively, in comparison with the control.

B. Effects of Irradiation of Pregnant Females on Sexual Development And Fertility Of The Offspring

1. Fertility of Prenatally Irradiated Females (150 r on 17th Day of Gestation).

Seventeen of the 19 (89 per cent) control females and 27 of the 35 (77 per cent) prenatally irradiated females (150 r on 17th day of gestation) conceived during a seven night controlled breeding period. This difference in conception rate between the two groups was not significant ($P > .05$). The average weight at autopsy (Table III) includes the fetuses, fetal fluids and membranes. However, when corrections were made for the difference in numbers of fetuses between groups, the weight difference between females was still highly significant ($P < .01$). At autopsy on the 15th, 16th or 17th day of gestation, the average number of corpora lutea and living fetuses was 12.6 and 12.4 vs 10.7 and 8.7, respectively, in the control and irradiated females (Table III).

The difference in average litter size (12.4 vs 8.7) suggests either a lowered fertilization rate, or an increased embryonic or fetal death rate. However, the average number of placental sites not represented by living fetuses was only 0.29 and 0.33 in the two groups of females. If a higher prenatal death rate occurred among the litters of the prenatally irradiated females, then it must have occurred prior to implantation.

The differences in fetal weights, at comparable stages of pregnancy, were not significant ($P > .05$).

TABLE III

THE EFFECT OF PRENATAL IRRADIATION ON SUBSEQUENT FERTILITY OF FEMALE RATS (150 r on the 17th day of gestation)

	Control females	Irradiated females
Av. female wt. (gm.) at autopsy	296 ± 9.6[a]	233 ± 16.9**
No. litters	17	17
Av. no. corpora lutea	12.7 ± 1.8	10.7 ± 2.8*
Av. no. fetuses	12.4 ± 1.6	8.7 ± 2.9***
Av. fetal wt. (gm.)		
15th day gestation	0.27 (61)[b]	0.27 (62)
16th day gestation	0.75 (87)	0.88 (58)
17th day gestation	1.34 (67)	1.05 (114)

[a]Mean ± standard deviation

[b]Indicates the number of individuals in each group.

*Significant at the 5% level of probability, respectively, in comparison with the control.

**Significant at the 1% level of probability, respectively, in comparison with the control.

***Significant at the 0.1% level of probability, respectively, in comparison with the control.

2. Fertility of Prenatally Irradiated Females (150 r on 19th Day of Gestation).

The average weights of the control and prenatally irradiated females (150 r on 19th day of gestation) at the time breeding was started were 238 and 212 gms, respectively.

Seventeen of the 25 (68 per cent) control females conceived during the five night controlled breeding period and five of the remaining eight females subsequently became pregnant during the following seven night period. Twenty-two of the 52 (42 per cent) prenatally irradiated females conceived during the first five nights and 22 of the remaining females conceived during the following seven night period. The overall conception rate during the 12 day breeding period of 88 and 85 per cent, respectively, for the two groups did not differ significantly ($P > .05$).

The average litter size, birth weight, per cent viability at birth, weaning weights and viability to weanling age for both groups of females are shown in Table IV. The average litter size (12.2 vs 10.4) in the control females as compared to the prenatally irradiated females was significantly higher ($P < .05$) while there was no difference in average birth weight, the viability at birth of the young from prenatally irradiated females was markedly reduced ($P < .01$). The growth to weaning age was not affected (Table IV). However, it should be pointed out that there was a difference in average litter size between the two groups (10.6 vs 7.4) in the control and irradiated litters, respectively. With such a difference in numbers of young per litter (3.2) a priori reasoning might lead one to expect that the young from the small sized litters would be heavier at weaning. This then raises a question as to

TABLE IV

THE EFFECT OF PRENATAL IRRADIATION ON THE SUBSEQUENT FERTILITY OF FEMALE RATS (150 r on the 19th day of gestation)

	Control females	Irradiated females
Av. female wt. before breeding (gm.)	238 ± 9.6[a]	212 ± 16.9**
No. litters	17	22
Av. no. young born	12.2 ± 3.2	10.4 ± 2.2*
Av. % alive at birth	94.2	80.7**
Av. % survival to 21 days	87.9	72.4**
Av. birth wt. (gm.)	6.2 ± 0.6 (195)[b]	6.0 ± 0.5 (184)
Av. weaning wt. (gm.)	36.8 ± 6.4 (181)	36.9 ± 5.0 (163)
Av. litter wt. at 21 days	377 ± 69	284 ± 90**

[a]Mean ± standard deviation.

[b]Indicates the number of individuals in each group.

*Significant at the 5% level of probability, respectively, in comparison with the control.

**Significant at the 1% level of probability, respectively, in comparison with the control.

whether or not the young from the prenatally irradiated dams were in any way affected. These data do not furnish a satisfactory basis for answering this question.

3. Testicular Development in Prenatally Irradiated Males (150 r on 17th Day of Gestation).

The average body and testes weights of the prenatally irradiated males (150 r on 17th day of gestation) are shown in Table V. The average difference in body weights of the two groups autopsied at 48, 68, 77 or 90 days of age, were 28, 62, 56 and 95 gm, respectively, in favor of the control males. The average difference in testes weights at the same ages, listed above, were 1.2, 1.2, 1.9 and 1.7 gm, respectively. The differences in body and testes weights at each autopsy age were highly significantly in favor of the control males ($P < .01$).

4. Testicular Development and Fertility in Prenatally Irradiated Males (150 r on 19th Day of Gestation).

The average body and testes weights of the control and irradiated males (150 r on the 19th day of gestation) in this trial are shown in Table VI. The average difference in body weights of the two groups autopsied at 64, 76, 90, 105 and 153 days of age were 36, 53, 47, 57 and 47 gm, respectively. The average difference in testes weights at the same ages were 2.9, 2.9, 3.1, 2.8 and 2.9 gm, respectively, in favor of the control males.

It would appear from these data that body and testicular growth rates were depressed at all ages in both groups of irradiated males. The major difference in response between the males irradiated on the

TABLE V

AVERAGE BODY AND TESTES WEIGHTS OF CONTROL AND PRENATALLY IRRADIATED RATS (dams received 150 r on the 17th day of gestation)

Age at autopsy (days)	No. of animals	Control	No. of animals	Irradiated
		Body weight (gm)[a]		
48	5	186 ± 6.9	5	158 ± 13.8**
68	5	282 ± 7.0	5	220 ± 14.1***
77	5	318 ± 13.9	3	262 ± 5.9***
90	5	373 ± 13.9	5	278 ± 21.4***
104	6	350 ± 20.5	3	279 ± 3.0**
		Testes weight (gm.)		
48	5	2.3 ± 0.18	5	1.1 ± 0.12***
68	5	3.2 ± 0.27	5	2.0 ± 0.25***
77	5	3.4 ± 0.09	3	1.5 ± 0.12***
90	5	3.4 ± 0.24	5	1.7 ± 0.36***
104	6	3.5 ± 0.46	3	2.0 ± 0.32**

[a]Mean ± standard deviation.

**Significant at the 1% level of probability, respectively, in comparison with the control.

***Significant at the 0.1% level of probability, respectively, in comparison with the control.

17th as compared to the 19th day of gestation was the difference in testes weights; the testicular weights of the 19th day males averaged approximately 31 per cent as heavy as those of the 17 day males. As compared to their respective control groups, the average testicular weights of the 17- and 19-day irradiated males were only approximately 53 and 13 per cent, respectively, as heavy as those of the control males.

Prior to autopsy at 153 days of age the five control and 12 irradiated males (19 day) were test mated at 119 days of age. During the succeeding three-week fertility test period the five control males were all fertile and the 12 (19-day) irradiated males were all sterile.

The results presented here on irradiation effects on the viability, growth and fertility of rat offspring which were treated (10-19 days) prenatally with three levels of radiation (110 r, 150 r and 220 r) show that gamma irradiation (Co-60) has a harmful effect on each of the above factors. The viability at the three levels of irradiation used was affected only at certain stages of gestation. That the fetuses at certain stages of development are less tolerant to irradiation than at other stages are in agreement with the findings of other investigators (Rugh and Wolf, 1956; Russell, 1950; Russell and Russell, 1050a, 1950b; Russell and Russell, 1952; Russell and Russell, 1954; Wilson, 1949; Wilson and Karr, 1950; Wilson et al., 1953; Wilson, 1954). The data on viability is in agreement with the work of Russell (1950) in that most of the irradiation deaths occurred during the first seven days postparturition; the remainder of the deaths could be attributed to factors other than irradiation since the death loss after seven days was not excessive in comparison with the controls.

TABLE VI

AVERAGE BODY AND TESTES WEIGHT OF CONTROL AND PRENATALLY IRRADIATED MALE RATS (dams received 150 r on the 19th day of gestation)

Age at autopsy (days)	No. of animals	Control	No. of animals	Irradiated
		Body weight (gm.)[a]		
64	5	279 ± 9.3	5	243 ± 15.4**
76	3	316 ± 10.0	5	263 ± 49.0
90	8	353 ± 16.9	15	306 ± 16.9***
105	5	334 ± 14.8	10	277 ± 53.9*
153	5	429 ± 40.8	12	382 ± 23.6*
		Testes weight (gm.)		
64	5	3.4 ± .16	5	0.5 ± .047***
76	3	3.5 ± .12	5	0.6 ± .07***
90	8	3.6 ± .23	15	0.5 ± .07***
105	5	3.4 ± .17	10	0.6 ± .06***
153	5	3.5 ± .04	12	0.6 ± .005***

[a]Mean ± standard deviation.

*Significant at the 5% level of probability, respectively, in comparison with the control.

**Significant at the 1% level of probability, respectively, in comparison with the control.

***Significant at the 0.1% level of probability, respectively, in comparison with the control.

Radiation exposure of the pregnant females did not reduce the litter size at birth. However, the viability of the young at seven days was markedly reduced in the litters of the 110 r females exposed on days 12-14, the 150 r females exposed on day 13 and the 220 r females exposed on days 11-15 as compared to the control females (17, 0, 0 and 87.6 per cent, respectively. The differences are highly significant ($P < 0.001$). This finding of a high postnatal mortality rate following irradiation during the 11th to 15th day of gestation is not in agreement with the reports of Rugh and Wolff (1956), and Russell (1954). The former authors state that 300 r was the minimal level of radiation which would kill all 13.5 day mouse fetuses. The latter author stated that following exposure on days 12.5 to 15.5 of gestation the LD 50 for mouse fetuses was greater than 300 r. This lack of agreement possibly can be accounted for by (1) a difference in sensitivity between the rat and mouse, or (2) slight differences in stage of development in the two species for a given day of gestation, or (3) a difference in intensity of radiation from the cobalt 60 source as compared to x-rays. There was growth retardation at all levels and at all stages of development studied. This retardation was permanent as indicated by body weights taken at intervals through 153 days of age.

The greatest depression was in the 110 litters which were irradiated on days 12-14 and the 220 r litters which were irradiated on days 16-19 of gestation.

Comparison of the radiation effects on dams vs their offspring tends to prove the theory that the damage to the developing fetus was

almost entirely due to direct radiation effects during the developmental stage, with only a minor part caused by the radiation effect (lowered milk production) on the dam after parturition. This is not in agreement with the report of Rugh (1956) in which he stated that 50 r had some effect on the lactating ability of the females which were irradiated postparturition. However, he stated that there was a pronounced effect using 200 r or higher.

Fertility was measurably affected in both male and female offspring which were prenatally irradiated (150 r) on the 17th or 19th day of gestation. It is suggested that the ovaries and testes were permanently affected since there was a lowered ovulation rate in the females and complete sterility in the males. More data regarding these effects are needed.

Most of the work on fertility, as affected by radiation, has been concerned with postnatal irradiation of the males or females, whereas the experimental findings in this thesis are concerned with the effects of prenatal irradiation.

In general, the findings in this study, using gamma rays from Co-60, parallels the findings of other investigators using x-rays.

CHAPTER V

SUMMARY

Pregnant female rats were used in a study of the effects of gamma irradiation on the viability, growth and fertility of the offspring. The intensity of the Co-60 source was 4.14 r/min. to 4.10 r/min. at a distance of 24 inches as measured by a Victoreen dosimeter. Twenty-nine randomly selected females were not exposed (control), 25 received 110 r, 23 received 150 r and 21 received 220 r. The stage of pregnancy at time of exposure varied from 10 to 19 days and all females littered. The average litter sizes at birth were 10.7, 11.1, 11.9 and 11.3, for the control, 110 r, 150 r and 220 r females respectively. The average per cent of young alive at seven days of age were 87.6, 61.5, 40.2 and 17.3, and at 21 days of age were 87.0, 54.7, 39.4 and 15.6 for the four groups, respectively. Only four of 21 litters in the 220 r group survived longer than 24 hours after birth. The average weaning weights of the offspring were 38.4, 30.4, 34.0 and 26.3 gm., for the control, 110 r, 150 r and 220 r females, respectively. Twenty-eight pregnant rats were paired on the basis of breeding dates with one female of each pair being irradiated (150 r) on the 17th day of gestation. On the fifth day postparturition, all litters were standardized at 10 young with equal numbers of offspring being exchanged between the paired control and irradiated dams. The average weights of the control and irradiated offspring at the time of exchange were 9.9 and 8.4 gm., respectively. The average weaning weights of the control offspring nursing control dams, control offspring nursing

irradiated dams, irradiated offspring nursing control dams and irradiated offspring nursing irradiated dams were 42.9, 41.1, 33.8 and 32.9 gm., respectively. The difference in growth between the young suckling control vs irradiated dams was significant ($P < .05$). The difference in growth between control and irradiated offspring was highly significant ($P < .01$). This would suggest that a small part of the observed growth retardation was due to the irradiation of the dam but that the major portion of the growth retardation was due to direct irradiation of the developing fetus.

Fertility and growth were affected in the surviving offspring of all irradiated groups as compared with the controls. The level of radiation and the period of gestation when radiation was applied, are two factors which seem to govern the damage that can be detected grossly.

The female offspring irradiated in utero and bred to normal males had a reduced ovulation rate, but a high per cent of the females cast litters. The males that were irradiated in utero were tested and were found to be completely sterile at 152 days of age. The gross anatomical and physical defects visually noted in the first generation were: reduced size (skeletal structure), growth retardation, hydrencephalocele (2 litters), nervousness and depressed testes development accompanied by sterility.

CRANES CREST

BIBLIOGRAPHY

CHAPTER VI

BIBLIOGRAPHY

Rugh, R. 1956. Effect on suckling young of x-irradiation of lactating mothers. Proc. Soc. Exp. Biol. and Med. 93(3): 523-524.

Rugh, R. and J. Wolff. 1956. Neurological corollaries of neonatal death following x-radiation of the mouse fetus on gestation day 13.5. Military Med. 118(6): 534-561.

Russell, L. B. 1950. X-ray induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. I. External and gross viscerol changes. J. Exptl. Zool. 114:545-602.

Russell, L. B. and W. L. Russell. 1950a. The effects of radiation on the preimplantation stages of the mouse embryo. Anat. Record, 108: 521.

Russell, L. B. and W. L. Russell. 1950b. Changes in the relative proportions of different axial skeletal types within inbred strains of mice brought about by x-irradiation at critical stages in embryonic development. Genetics, 35:689.

Russell, L. B., W. L. Russell and M. H. Majors. 1951. The effect of hypoxia on the radiation induction of developmental abnormalities in the mouse. Anat. Record, 111:455.

Russell, L. B. and W. L. Russell. 1952. Radiation hazards to the embryo and fetus. Radiology, 58:369-376.

Russell, L. B. 1954. The effects of radiation on mammalian prenatal development. Radiation Biology, 1:861-918. McGraw Hill Book Company.

Russell, L. B. and W. L. Russell. 1954. An analysis of the changing radiation response of the developing mouse embryo. Jour. Cell. and Comp. Physiol. 43 (Suppl. 1):103-149.

Snedecor, W. 1956. Statistical Methods. 5th Edition. Iowa State College Press, Ames, Iowa.

Wilson, J. G. 1949. Effects of x-irradiation on embryonic development in the rat. Anat. Record, 103:520.

Wilson, J. G. and J. W. Karr. 1950. Differences in the effects of x-irradiation in rat embryos of different ages. Anat. Record, 106:259-260.

Wilson, J. G., R. L. Brent and H. C. Jordan. 1953. Differentiation as a determinant of the reaction of rat embryos to x-irradiation. Proc. Soc. Exptl. Biol. and Med., 82:67.

Wilson, J. G. 1954. Differentiation and the reaction of rat embryos to radiation. Jour. Cell. and Comp. Physiol. 43 (Suppl. 1): 11-37.