# MORPHOLOGY AND FUNCTION OF THE GONADS OF RATS EXPOSED TO ACUTE PREPUBERTAL POSTNATAL IRRADIATION

---

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN RADIATION BIOLOGY IN THE GRADUATE SCHOOL OF THE TEXAS WOMAN'S UNIVERSITY

COLLEGE OF

ARTS AND SCIENCES

BY

MARGARET DAVIS TEAGUE, B.A., M.S.

---

DENTON, TEXAS

MAY, 1971

# Texas Woman's University

Denton, Texas

May, 19 71

We hereby recommend that the dissertation prepared under our supervision by Margaret Davis Teague entitled "Morphology and Function of the Gonads of Rats Exposed to Acute Prepubertal Postnatal Irradiation"

be accepted as fulfilling this part of the requirements for the Degree of Doctor of Philosophy.

Committee:

Eugene W. Hayes
Chairman

M. M. Aboul-Ela

Robert Juers

Kenneth A. Fry

Ray D. Barker

Accepted: S. L. Morrison
Dean of Graduate Studies

## ACKNOWLEDGMENTS

The author is sincerely grateful to Dr. Eugene W. Hupp for his guidance, patience, and encouragement during the writing of this dissertation, and his supervision of her graduate program.

Sincerest appreciation is expressed to Dr. Mohamed Aboul-Ela, Dr. Ray Burkett, Dr. Kenneth A. Fry, and Dr. Robert Fuerst for their helpful suggestions and criticisms.

The author expresses appreciation to the National Science Foundation and to the Texas Woman's University for their financial support.

The author is especially grateful for the encouragement and help of her children for without it this dissertation would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

# CHAPTER I

## INTRODUCTION

The deleterious effects of ionizing radiation on the gonads has long been known. As early as 1903 Albers-Schönberg found that irradiation was especially damaging to the testes, and subsequently particular attention was given to this area of study. Although a voluminous amount of material on the effects of radiation on the reproductive system has been obtained, there is a need for further study in this field.

An understanding of normal gonad development and germ cell formation is essential to an understanding of the changing sensitivity of the gonads during development and of the basis for differences of response to irradiation between the sexes.

The migration of the primordial germ cell from extra-gonadal origin to their germinal ridges in human embryos was observed by Witsche (1948). Later Chiquoine (1954) was able to trace their migration from their origin in the region of the caudal end of the primitive streak, root of the allantoic mesoderm, and yolk sac splanchnopleure in the 8-day mouse embryo. Mintz (1960) reported that sterility and reduced fertility associated with certain genotypes of the mouse can be correlated with a reduced number of primordial germ cells.

The conclusion from this evidence was that these are the stem cells of the definitive gametes. Primordial germ cells transform directly into oocytes in the female (Mintz, 1960; Baker, 1963), and in males they give rise to Type A spermatogonia (Clermont, 1957).

The same basic process of Type A spermatogonia renewal has been observed in rat, guinea pig, monkey, and man (Oakberg, 1968). These stem cells maintain a steady state while giving rise to an unlimited number of differential spermatogonial types which are eventually differentiated into spermatids. This process begins shortly after birth of the rat (Oakberg, 1956) and by 10 years in man (Clermont, 1962). Type A spermatogonia undergo four divisions, with an intermediate spermatogonia as the result. The intermediate spermatogonia divide to form spermatogonia of Type B, which in turn divide to form primary spermatocytes. In the mouse and rat, either 4 or 5 cell generations occur in a given tubule section (Oakberg, 1968), depending upon the stage of the cycle of the seminiferous epithelium. Quantitation of radiation response of specific cell types is possible for the cellular association and the number of cells are characteristic for each stage of the cycle. In man, the process is slightly different as two morphologically different types of A cell, Ad and Ap, occurs (Clermont, 1963). Ad spermatogonia develop into Ap, and Ap divide to form spermatogonia B. The time required for spermatozoa to develop

varies with each species; in the mouse, the duration of spermatogenesis has been estimated as 35 days (Oakberg, 1956); 48 days in the rat (Clermont, Leblond, and Messier, 1955); 49 days in the ram (Ortavant, 1956) and 73 days in man (Heller and Clermont, 1964).

The sex-life of the male begins at puberty when the adenohypophysis begins to secrete follicle stimulating hormone, FSH, and luteinizing hormone, LH. These cause an upsurge of testicular growth (Guyton, 1969). FSH causes proliferation of the cells in the germinal epithelium, resulting in the formation of sperm. As estrogen is also produced at this time by the sustentacular cells, it is thought by some that FSH is the stimulant that acts on the sustentacular cells to cause the production of estrogen. The estrogen stimulates the germinal epithelium cells to make them proliferate. The luteinizing hormone causes the production of testosterone by the interstitial cells of the testes. Testosterone stimulates the production of the male sexual characteristics.

Oogonia are not present in the adult ovary while spermatogonia are present in adult testes, and as a result the radiation response of males differs from that of females. In the rat, the process of oogenesis ceases 4 days before birth (Beaumont and Mandl, 1961). The population of normal germ cells at birth is approximately 39,000. By the end of the second day after birth degeneration and atresia of oocytes decrease the germ cells to 19,000.

Growth of these primary oocytes is stimulated by the secretion of the follicle stimulating hormone produced by the adenohypophysis. The primary follicles begin the developing process by the follicular epithelial cells becoming first cuboidal, then columnar and finally stratified, to form secondary follicles. Fluid begins to accumulate between the cells of the thick covering of collicular epithelial cells. The little pools of fluid fuse into one pool and a cavity called an antrum is formed. The FSH stimulates the ovary to secrete estrogen, which in turn stimulates the adenohypophysis to secrete LH, the luteinizing hormone (Guyton, 1969).

This hormone increases the rate of fluid secretion in the follicle until it bursts. The secondary oocyte is liberated into or close to the open end of the oviduct. Simultaneously the LH causes the follicle cells to increase in size. In the rat, a third hormone, luteotrophin, continues to stimulate the growth of the follicle cells to form a functional corpus luteum. Under the continued stimulus of luteotrophin, the follicular epithelial cells enlarge greatly to become what are called follicular or ganulosa lutein cells (Guyton, 1969).

The corpora lutea secretes progesterone to maintain the endometrium in a luxuriant condition as long as LH is secreted. The failure of the corpora lutea precipitates the disintegration of the endometrium and stops the secretion of progesterone which has up until this time prevented the

production of FSH by the adenophypophysis. With the production of FSH again stimulated in the adenohypophysis, the primary follicles once again begin growth in the ovary (Ham, 1965).

It is becoming increasingly evident that at certain stages in their development oocytes may be the most sensitive cells to lethal effects of radiation. The speed with which degeneration sets in seems not to depend on the dose administered; the latter mainly influencing the proportion of oocytes showing degenerative changes. Doses sufficient to destroy most of the primordial follicles exert relatively little effect on oocytes which have entered upon the rapid phase of growth (Parson, 1962; Mandl, 1959).

Mandl (1963) observed that in general, the first sign of radiation induced damage to growing mammalian follicles occurs in the granulosa cells rather than in the oocyte. The granulosa cells become pyknotic within a very few hours of exposure. Damage to the follicular envelope appears to be reparable presumably due to the mitotic activity of the surviving granulosa cells.

Erickson (1967) states that the primordial follicle is affected by doses within the sublethal range as evidenced by both increases in signs of atresia among primordial follicles and elevated levels of growth and atresia among the more advanced follicle classes.

Udgaonkar and Batra (1968) observed that the sensitivity pattern of mammalian female germ cells has two peaks. In

rats, the first is at 15.5 days post coitum when the mitotic activity is highest, and the second is about 3 days post partum with the onset of the "dictyate phase of meiotic prophase." Before or shortly after birth, there occurs in the ovaries a significantly greater number of oocytes in the diplotene stage (Mandl, 1963; Kochar and Batra, 1967).

Peters and Levy (1963) observed oocytes of the mouse in pachytene, diplotene, and dictyate stages of meiosis at birth (Borum, 1961) and reported that they reached the dictyate stage 3 to 4 days after birth. Erickson (1967) states that bovine oocyte in a primordial follicle progresses no further than pachytene of the meiotic prophase. This is probably the reason for the difference in the radio-response of primordial follicles seen in the rodent and bovine.

The primordial follicle in the guinea pig (Lacassagne et al., 1962; Oakberg and Clark, 1964) and the monkey (Van Eck-Vermande, 1959) is said to be less easily damaged by irradiation than are oocytes in growing follicles. The discrepancy between these observations and those made on the mouse, rat, and rabbit may well be due to the fact that the chromosonal configuration of oocytes in primordial follicles is not the same (Mandl, 1959). That oocytes in primordial follicles in the monkey differ from those of the rat has already been substantiated by Baker (1963).

Oakberg (1968) states the "nuclear morphology of the diplotene stage varies between species; a 'typical' diplotene is characteristic of the human, goat, and dog; a synizesis-like diplotene is present in the mouse and rat" (Chiquoine, 1954). In mammals, the size of these primordial follicles containing oocytes at diplotene apparently remains stationary for prolonged periods. These germ cells represent 90% of the total stock of oocytes (Franchi, Mandle and Zuckerman, 1962). Following radiation, they rapidly become pyknotic (Parson, 1962) and are eliminated from the ovary within 1/2 to 2 days, depending on the species (Oakberg, 1962).

An observation on the effect of radiation on reproduction was that irradiation of the postpuberal mouse or rat induced a superfollicular response (Erickson, 1967). This response has been measured histologically and functionally as evidenced by both superovulation and superfetation. Heretofore, a superfollicular response has not been reported in prepuberal mammals, but this is probably due to species differences in ovarian development.

Exposure to ionizing radiation accelerates the rate of loss of oocytes from the ovary (Krohn, 1967). In the mouse, radiation sensitivity of oocytes changes greatly between birth and sexual maturity. Mice had low sensitivity when irradiated at birth with 300 R of X-rays (Russell, Russell, Steele and Phipps, 1959). Peters and Levy's (1961) studies on newborn female mice exposed to 29 R of X-ray showed that pachytene and late

diplotene were sensitive to immediate cell killing; that the cells were extremely sensitive was evidenced by the fact that at certain stages a dose of 20 R killed 99% of these small oocytes.

In comparing radiation-induced sterility with that caused by aging, Oakberg (1966) noted that irradiated female mice must somehow utilize their oocyte supply more efficiently than non-irradiated females. He stated that the effect for all oocyte stages is a significantly higher rate of decrease in oocyte numbers with age in irradiated females. The surprisingly good reproductive performance of the irradiated female, therefore, appears to stem from the fact that enough oocytes are left to give several litters during the peak physiological age for reproductive performance. This suggested to Oakberg that the female is able to ovulate nearly the normal number of eggs even though the total oocyte number is very low. Controls become sterile with an ample oocyte supply in the ovary, whereas the irradiated mice become sterile because the oocyte supply is exhausted.

Mole (1959) showed that exposure of young mice to 25 R whole body irradiation reduced total reproductive capacity to one half, though the number of young produced within 3 months of irradiation was comparable to that born to unirradiated litter mates. From the 4th month there was a reduction, and in the last third of the reproductive life span the irradiated mice were sterile. Mole comments that the practice

of counting a female as fertile if she produces just one litter, however small, may give a misleading impression; he also points out that in studies involving chronic irradiation, the age of the animals must be taken into account.

Nash and Gowen (1961) irradiated fetal mice at different stages of intra-uterine life and reported that subsequent reproductive capacity in both sexes is dependent both on the dose and the age at irradiation.

Rugh and Jackson (1958) reported that the total number of young produced over a period of 9 months was halved in mice that had been exposed to 100 R at 16.5 days p.c. Unless the strain of mice used by Rugh and Jackson differs from those documented cytologically (Borum, 1961), it would appear that maximum sensitivity coincided with the onset of meiotic prophase in the oocytes. Russell, Bagett and Saylors (1960) irradiated mouse fetuses aged 0.5-13.5 days p.c. and observed that irradiation on days 11.5 and 13.5 exerted the greatest effect on the reproductive capacity of the females; at this stage, the ovaries would be expected to contain mitotically active oogonia (Peters et al., 1962). This finding is in agreement with Beaumont's (1961, 1962) conclusion that maximum radiosensitivity of germ cells in the female fetal rat is associated with mitotic activity.

Peters and Levy (1963) found an increase in radiosensitivity between birth and the age of 2 weeks in mice. Their study further demonstrated that maximal reduction in

breeding performance occurred following irradiation at the age of 3 weeks, when a dose of 20 R reduced the number of young born (over one year) to 1.4% of the control level.

There is a difference in the degree of sensitivity of oocytes in mice and rats. Kitaeva (1960) found that mice exposed to 15 R showed the same effect as that obtained in rats exposed to 100 R. Similarly Mandl's (1959) studies indicate that although the primary follicles of the rat are very sensitive to radiation-induced cell killing, they are certainly less sensitive than those of the mouse.

Beaumont (1960) did a preliminary quantitative study of the number of oocytes of rats and found indication that the maximum radio-sensitivity occurs at 15 days when the primordial germ cells are dividing mitotically, with radio-sensitivity decreasing as the cells enter the early stages of meiotic prophase.

Leonard and Maisin (1963) noted a marked difference between 8 and 17 days old rats, as judged by reproductive capacity over 200 days in contrast to the mouse, whose sensitivity decreases between 8 and 17 days. Chu and Hsung (1962) also observed that some rats given relatively low doses of X-irradiation on the 7th day after birth were sterile, while others gave birth to small litters.

The majority of oocytes in the rat and monkey were destroyed following X-ray doses of 300 R or 700 R, respectively (Baker, 1969). These doses are slightly less in vitro

than they are in vivo, possibly owing to absence of pituitary hormones in the nutrient medium.

Hupp, Brown and Austin (1969) reported that a single exposure of female rats to 150 R of gamma radiation during the period from the 14th day of gestation to the 4th day after birth caused little or no change in the incidence of fertile animals, but exposure during the period from 5 to 13 days after birth caused significant reductions in the incidence of fertile animals.

The study of the effects of irradiation on male rats was begun as early as 1904 when Bergonie and Tribondeau reported that seminiferous tubules were partially or completely devoid of epithelium in rat testes that were exposed to X-rays. It was found by Nebel (1958) that the Sertoli cells of the mouse are highly resistant to irradiation. Similar results were seen by Lacy (1964) in the rat. Type A spermatogonia were observed by Clermont (1962) and Monesi (1962) to be more radioresistant than subsequent spermatogonial generations.

An experiment examining the fertility of male rats irradiated with 200 R X-rays on days 15.5 and 16.5 pernatal was conducted by Rugh and Jackson (1958). They discovered that fetal male rats exhibit a reduction in subsequent fertility in the 36% of those animals irradiated on day 15.5, and 37% of those irradiated on day 16.5 were completely sterile. The animals were killed at 270 days

postnatal and it was noted that the testes weight showed great variation, averaging about 30% of that of controls. Beaumont (1960) concluded from animals autopsied at 25 days of postnatal life that the male rat exhibits greatest radiosensitivity during days 19.5 to 21.5 of prenatal life.

It was noted by Kohn (1955) that irradiation between 180 and 210 R resulted in marked decrease in testes weight with comparable marked depopulation of the germinal epithelium. Murphee and Pace (1960) reported a reduction in the weight of testes following irradiation on the 17, 18, and 20 days of gestation using 150 R $^{60}Co$ gamma irradiation. In a later report, Hupp, Pace, Furchtgott and Murphree (1960) stated that 150 R of gamma irradiation from a $^{60}Co$ source, on 18, 19, 20, 21, and 22 day of gestation, caused permanent sterility with marked reduction in size of testes. According to these investigators, males irradiated on the 17 day of gestation exhibited a different effect, since 66% were sterile, while 100% of the testes were reduced in size as compared to normal controls.

Harding (1961) counted the number of germ-cells which survived exposure to doses up to 430 R given 4-8 days after the birth of rats. Even the highest dose was found to cause no consistent reduction in the numbers of gonocytes and spermatogonia type A within 3 days of treatment. Harding concluded that the response may be due either to an intrinsic alteration in the radiosensitivity of the testes between the

21st day of gestation and 4 days post partum, or to prolonged inhibition of mitosis after irradiation. The short interval between irradiation and evaluation may have been responsible for the failure to detect a radiation effect.

Ricks and Hupp (1964) and Ricks (1964) irradiated male rats with 150 R gamma rays on gestation days 14, 16, 17, 20, and days 1 and 2 postnatal. Testis weights were suppressed in all irradiation groups. At 185 days of age, animals irradiated on days 14, 16, and 17 post coitum had an average testis weight of 78.5% of controls; those irradiated at 18 p.c. were 67.1% of controls, while those irradiated at 20 p.c. and 1 and 2 days postnatal were 25.3% of controls. When these investigators compared the percentages of active tubules at 185 days post partuition, the animals irradiated on days 14, 16, and 17 p.c. had 98% fully active tubules; day 18 animals, 71.5%; day 20 animals, 1.2%; and days 1 and 2 animals, 27.4% fully active tubules. It was concluded that there is an increasing radiosensitivity seen in fetal and neonatal rats from days 14 to 20 with 20 prenatal through day 2 postnatal showing the most damage.

Hwang, Pace and Hupp (1964) reported the results of irradiating rats with 150 R. Ten of the 15 males irradiated on day 17 of prenatal life were fertile, all of the other irradiated males were sterile. The percentage of active tubules in fertile 17 days; sterile 17th, 18th, 19th, through 21st and 22nd day was 93, 14, 10, 2, and 6% respectively of

the controls. The diameter of active tubules in the irradiated rats did not differ from controls; the mean diameter of inactive tubules was 63% of the active tubules.

Hupp, Brown and Austin (1969) noted that suppression in the number of active seminferous tubules was observed in rats irradiated on the 14th through the 22nd day of prenatal life and on various days of postnatal life from days 1 through 21, with the maximum effect coinciding with the maximum reduction in testis weight. All males irradiated with 150 R on days 18 through 22 were sterile; fertility was reduced in the males irradiated on days 17 prenatal and day 1 postnatal.

The preceding survey of the literature indicates that numerous studies have been conducted in which germ cells of many species have been irradiated at all stages of development. However, these studies have not included extensive series of experiments in which the relative sensitivity of various stages of postnatal prepubertal development in the same strain have been investigated in detail. Therefore, this study was initiated for the purpose of investigating the effects of acute X-irradiation of 150 R delivered on the whole body on either day 3, 5, 7, 9, 13, 17, 21, 28, 35, or 42 of postnatal life, on the gonads of Sprague Dawley rats. This is a report of the effects of irradiation on the age of puberty, estrus cycle, fertility, the number of oocytes in the ovaries, and the number of tubules in the testes, correlated with ovarian, uterine, and testes weights.

## CHAPTER II

## PROCEDURE

Sixty-five pregnant female Sprague Dawley rats were kept in clean wire mesh cages until approximately three days before littering. At this time they were placed in individual cages measuring 7" high by 7" wide and 10" deep. The bottom of each cage was lined with a fine wire mesh and a generous portion of shredded paper. The rats were housed in a clean animal room maintained under artificial lighting with a 12 hour photoperiod and a mean temperature of 23 C. The animals were fed Purina Laboratory Chow and water ad libitum.

Each pregnant female was observed each day to see if she had littered. All litters born before 1:00 P.M. were considered to have been born that day. The litters were irradiated between 1:00 and 4:00 P.M. on either day 3, 5, 7, 9, 13, 17, 21, 28, 35, or 42 of age. Enough litters were irradiated on each day to assure at least 20 females and 20 males for each day to be irradiated. Rats that had previously been irradiated on day 1 were also evaluated in this study.

Each litter to be irradiated was transported from the animal room to the irradiation room immediately before irradiation. The litters irradiated on day 3, 5, and 7 were placed in shallow plastic petri dishes with tops taped

securely to assure that the animals would not crawl out. At later days of age the rats were placed in plexiglass rat holding devices. All litters were subjected to 150 R of X-irradiation as determined by Victoreen ionization chambers. The source of X-rays was a 250 kVp General Electric Therapy Unit operated at 15 ma, with 0.5 mm copper and 1.0 mm aluminum filtration. The target to subject distance was 57 cm to obtain a dose rate of approximately 55 R per minute. Immediately following irradiation the litters were returned to the animal room and each litter was returned to the mother's cage and remained until weaning age. The control animals were handled in the same manner as the irradiated except they were sham irradiated.

As the young rats reached 22 days of age, they were placed in wire mesh cages measuring 7" high by 16" wide and 10" deep. The female rats were observed daily for puberty as indicated by vaginal opening. At approximately five weeks of age the animals were sexed and placed in cages 7" high by 16" wide and 10" deep for six animals, or 7" high by 24" wide and 10" deep for ten animals. Each animal was marked by the use of ear notches in a number sequence according to number in the litter.

When the animals reached 65 days of age, ten females of each day irradiated were killed and ten were kept until they reached 90 days of age at which time they were placed in cages with males of known fertility. As the females

were palpated pregnant they were placed in wire mesh cages prepared for littering. As each animal littered, the young were counted and the number and date recorded. The litter was killed by overanesthetizing with ether. The female was returned to the mating cage.

Those females that appeared sterile after being caged with several males of known fertility for 8 weeks were removed from the mating cages and vaginal smears were made daily until an estrus cycle was determined, or 21 days had passed. Fertile males were placed with the females in individual cages and they were observed daily for vaginal plugs on the papers beneath the cages. When plugs were present, vaginal smears were made to observe for the presence of sperm. At 185 days of age all animals were killed.

Thirty male rats that were irradiated on either day 28, 35, or 42 were killed at 65 days of age to extend information that had been obtained in a previous study. Ten male rats for each day irradiated were placed individually in wire cages 7" high by 7" wide and 10" deep with a female of proven fertility. The males were left in the cage until the female was determined to be pregnant by abdominal palpation. At the age of 185 days the males were killed.

Ten control animals of each sex were killed at 65 days of age. After testing for fertility, ten control animals were killed at 185 days of age.

On each necropsy day the animals were killed by an overdose of phenobarbital or overanesthetizing with ether. Body

weights were taken, and the testes, uterus, and ovaries were removed and weighed. The tissue was placed in Bouins solution consisting of 85% saturated picric acid, 10% formalin and 5% acetic acid. After 24 hours the ovaries and uterus were transferred to 70% ethyl alcohol. The testes were placed in the alcohol after 48 hours in the fixative. Histological sections of the testes and ovaries were prepared by the paraffin technique employing standard procedure. In order to obtain uniformity, the largest cross-section of each testis and ovary were taken. The testes were sectioned at seven or eight microns in thickness and the ovaries at four or five microns. The prepared tissues were stained using hemotoxylin and eosin.

The largest, most complete cross-section from each testis was placed on a Leitz Prado 500 Microscope Projector or a Master Model D Bioscope and projected onto a piece of white paper. Each seminiferous tubule was drawn on the paper. The total number of seminiferous tubules and the total number of tubules in each class were then counted and recorded.

The criterion for classification of seminiferous tubules into the six histological classes was the modified classification reported by Ricks and Hupp (1964) and used by Gates and Hupp (1967) and Partlow (1969), who stated that the criterion in the following manner:

> . the classification of seminiferous tubules into six histological classes, numbered 0 through 5 with 0

showing the most damage and 5 appearing to be normally active. Classes 0 through 2 are inactive with varying degrees of vacuolation, with Class 0 having large vacuoles and a disorganized arrangement of cell nuclei; Class 1 is a transitional stage between Classes 0 and 2; Class 2 contains no or few vacuoles and the cell nuclei lying in orderly arrangement at the periphery of the tubule. Class 3 tubules may have some circular spermatocytes present, but no terminal elements which, in this case, are elongated spermatids; and Class 5 tubules appear to be completely normal with a complete spectrum of cell types, including many sperm or spermatids throughout the lumen.

Cross sections of the ovaries were evaluated by observing each section with a biocular microscope and counting and recording the number of primary, growing, vesicular, luteinized, empty, and atretic follicles. The primary oocyte had one layer of flattened follicular epithelial cells and a relatively large oogonium; the growing follicle contained several layers of epithelial cells surrounding the ovum; vesicular follicles contained follicular fluid in varying amounts; luteinized follicles had granulosa lutein cells; empty follicles contained a single layer of epithelial cells without an oogonium; and atretic follicles were growing follicles size with cells which appeared as granulosa lutein cells. The average diameters of the empty follicles and atretic follicles were obtained by placing the section of an ovary on a scanning scope and measuring the size of the follicles with a scale that had been calibrated with a stage micrometer.

The data were subjected to appropriate statistical analyses, which included analysis of variance, followed by

the studentized range tests, or Chi-square analysis to determine the significance of differences between various means (Snedecor, 1956).

Using the information obtained in this study, the relationship of the changes in irradiated animals' weight, fertility, the histological patterns of the seminiferous tubules, the differences in the follicle number and kind, and the effect on the age of puberty and estrus cycle were noted and evaluated.

# CHAPTER III

# RESULTS

## Results on Males

The following results were obtained on male rats that were irradiated with 150 R of X-radiation on either day 1, 3, 5, 7, 9, 13, 17, 21, 28, 35, or 42 of life and killed at the age of 65 or 185 days.

### Animals Killed at 65 Days of Age.

Body Weight: Animals irradiated on day 1, 3, 5, 28, and 35 had body weights that were less than the control; however, only the day 1 group animals was significantly less than the control at the 5% level (Table 1). Day 1 animals averaged 82.7% of the control weight. The groups irradiated on days 7, 9, 13, 21, and 42 had heavier average body weights than the control, but none were significantly greater at the 5% level.

Testes Weight: There was a significant depression of weights of the irradiated animals killed at 65 days of age compared to control. Animals irradiated on day 1, 3, and 28 had testes weights that averaged 26.0, 35.4, and 66.6%, respectively of control. Animals irradiated on day 5, 7, 9, 13, 17, 21, 35, and 42 had similar mean testes weights that were 72 to 84% of control (Table II).

TABLE I

BODY WEIGHT OF 65 AND 185 DAY OLD MALE RATS IRRADIATED WITH 150 R

| Treatment | 65 Day (g) | 185 Day (g) |
|---|---|---|
| Control | 247 | 407 |
| Day Irradiated | | |
| 1 | 204[a] | 399 |
| 3 | 219 | 387 |
| 5 | 241 | 389 |
| 7 | 269 | 374 |
| 9 | 261 | 399 |
| 13 | 256 | 388 |
| 17 | 247 | 383 |
| 21 | 273 | 385 |
| 28 | 232 | 382 |
| 35 | 230 | 416 |
| 42 | 270 | 406 |

[a]Significantly different from control at the 1% level.

TABLE II

TESTES WEIGHT AND MEAN LITTER SIZE OF RATS KILLED AT 65 AND 185 DAYS OF AGE

| Treatment | 65 Day | 185 Day | | |
|---|---|---|---|---|
| | Testes wt, g | Testes wt, g | Fertile % | Ave.no.in litter |
| Control | 2.902 | 3.614[a] | 90 | 10.3 |
| Day Irradiated | | | | |
| 1 | 0.749 | 1.171 | 20 | 3.0 |
| 3 | 1.318 | 1.817 | 100 | 9.0 |
| 5 | 2.097[bc] | 2.245[e] | 90 | 10.8 |
| 7 | 2.434[a] | 2.647[cd] | 100 | 9.1 |
| 9 | 2.126[abc] | 2.453[de] | 90 | 11.2 |
| 13 | 2.262[abc] | 2.554[de] | 100 | 12.3 |
| 17 | 2.102[bc] | 3.309[a] | 90 | 8.2 |
| 21 | 2.140[abc] | 2.979[bc] | 100 | 9.2 |
| 28 | 1.932[c] | 3.259[ab] | 100 | 10.2 |
| 35 | 2.308[ab] | 3.488[a] | 90 | 11.3 |
| 42 | 2.402[ab] | 3.470[a] | 100 | 10.0 |

[a,b,c,d,e] Testes weight with same superscript in the same column do not differ at the 5% level of significance.

Histological Evaluation: All of the animals killed at 65 days of age had an absence of Class 0 tubules (Table III). Only animals irradiated on day 3, 5, and 28 contained Class 1 tubules. Numerous Class 2 tubules were recorded for days 1 and 3; days 5, 21, and 28 which had approximately 5%, and day 17 had only 1.7%. Testes of the other irradiated animals did not contain tubules of Class 2.

Approximately 10% Class 3 tubules were recorded for animals irradiated on days 3, 5, 17, 21, and 28. Testes of all of the other groups of animals contained 6% or less of Class 3 tubules. With the exception of the day 1 and 3 groups, there was an increase in Class 4 tubules compared to the less active tubule types. There was a gradual increase in the percentage of tubules of this class with increasing age at irradiation to a maximum of 55% in the group irradiated at 21 days of age, then a sharp decline to 13 and 14% in the 35 and 42 day irradiated groups. Class 5 tubules were found to be the most numerous of the tubules in all irradiated animals with the exceptions of day 1, 3, and 21, which contained 15, 34, and 27%, respectively. Animals of day 7, 9, 35, and 42 had between 73 and 82% Class 5 tubules, and day 5, 13, 17, and 28 had approximately 60% as may be seen in Table III.

The mean number of seminiferous tubules was reduced in animals irradiated on days 1 through 17 as seen in Table IV.

TABLE III

PERCENT OF TUBULES PER CLASS FOR THE
65 AND 185 DAY IRRADIATED RATS

| Treatment | Percent of 65 day tubules class | | | | | | Percent of 185 day tubules class | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 | 3 | 4 | 5 |
| Control | 0 | 0 | 4.0 | 3.8 | 9.4 | 82.7 | 0 | 0 | 0 | 2.7 | 4.9 | 92.3 |
| Rad. Day | | | | | | | | | | | | |
| 1 | 0 | 0 | 76.4 | 5.9 | 3.1 | 14.7 | 3.7 | 8.0 | 52.7 | 5.2 | 3.4 | 27.0 |
| 3 | 0 | 5.7 | 38.7 | 10.4 | 10.8 | 34.3 | 1.2 | 5.1 | 7.5 | 4.7 | 5.6 | 75.9 |
| 5 | 0 | 0.4 | 5.5 | 8.3 | 22.3 | 63.5 | 0.1 | 0.4 | 2.5 | 3.4 | 4.6 | 89.1 |
| 7 | 0 | 0 | 0 | 2.9 | 23.4 | 73.7 | 0 | 0.1 | 0.8 | 1.9 | 6.6 | 90.2 |
| 9 | 0 | 0 | 0 | 1.6 | 24.5 | 73.8 | 0 | 0 | 0.3 | 1.2 | 6.2 | 92.4 |
| 13 | 0 | 0 | 0 | 3.9 | 31.7 | 64.3 | 0 | 0 | 0.5 | 2.7 | 5.2 | 91.6 |
| 17 | 0 | 0 | 1.7 | 7.8 | 29.1 | 61.4 | 0 | 0 | 0.2 | 1.3 | 4.4 | 93.9 |
| 21 | 0 | 0 | 5.7 | 11.7 | 55.3 | 27.4 | 0 | 0 | 0.1 | 1.4 | 4.9 | 93.5 |
| 28 | 0 | 0.6 | 5.3 | 12.3 | 21.6 | 60.2 | 0 | 0 | 1.5 | 3.3 | 3.6 | 91.7 |
| 35 | 0 | 0 | 0 | 6.8 | 13.0 | 80.2 | 0 | 0 | 1.4 | 3.5 | 4.5 | 91.6 |
| 42 | 0 | 0 | 0 | 3.2 | 14.5 | 82.2 | 0 | 0 | 0 | 2.5 | 4.9 | 92.2 |

TABLE IV

AVERAGE NUMBER OF TUBULES PER CROSS-SECTION IN 65 AND 185 DAY IRRADIATED RATS

| Treatment | 65 Days of age | | 185 Days of age | |
|---|---|---|---|---|
| | # of tubules | Percent of control | # of tubules | Percent of control |
| Control | 674 | | 711 | |
| Day Irradiated | | | | |
| 1 | 463[a] | 68.7 | 528[a] | 74.4 |
| 3 | 446[a] | 66.4 | 497[a] | 69.6 |
| 5 | 569[a] | 84.5 | 582[a] | 82.0 |
| 7 | 556[a] | 82.5 | 625[a] | 88.0 |
| 9 | 576[a] | 85.6 | 630[a] | 88.5 |
| 13 | 608[a] | 90.1 | 665 | 93.3 |
| 17 | 608[a] | 90.1 | 758 | 106.4 |
| 21 | 709 | 105.2 | 755 | 95.5 |
| 28 | 653 | 97.0 | 693 | 97.3 |
| 35 | 684 | 100.1 | 697 | 96.5 |
| 42 | 752 | 111.6 | 729 | 102.5 |

[a]The number of tubules differed from the control at the 1% level of significance.

The maximum effect was observed in testes of animals irradiated on days 1 and 3. Days 21, 35, and 42 animals had slightly greater means for the number of seminiferous tubules in the cross sections of testes than did control.

Animals Killed at 185 Days of Age.

Fertility Tests: Only 2 of 10 males irradiated on day 1 sired litters: one with a litter size of 1, and the other with a litter size of 5, yielding a mean litter size of 3 for the fertile animals which had been irradiated on day 1 (Table II). All of the males irradiated on day 3 that were fertility tested sired litters producing a mean litter size of 9. Males irradiated on either day 5, 9, 13, or 35 sired litters whose mean sizes were slightly but not significantly greater than that of the control. The fertile males irradiated on days 7, 17, 21, 28, and 42 sired litters with a mean size slightly less than controls. Only animals irradiated on day 1 differed significantly from the control (1% level) for either percent of the males that were fertile, or in mean litter size.

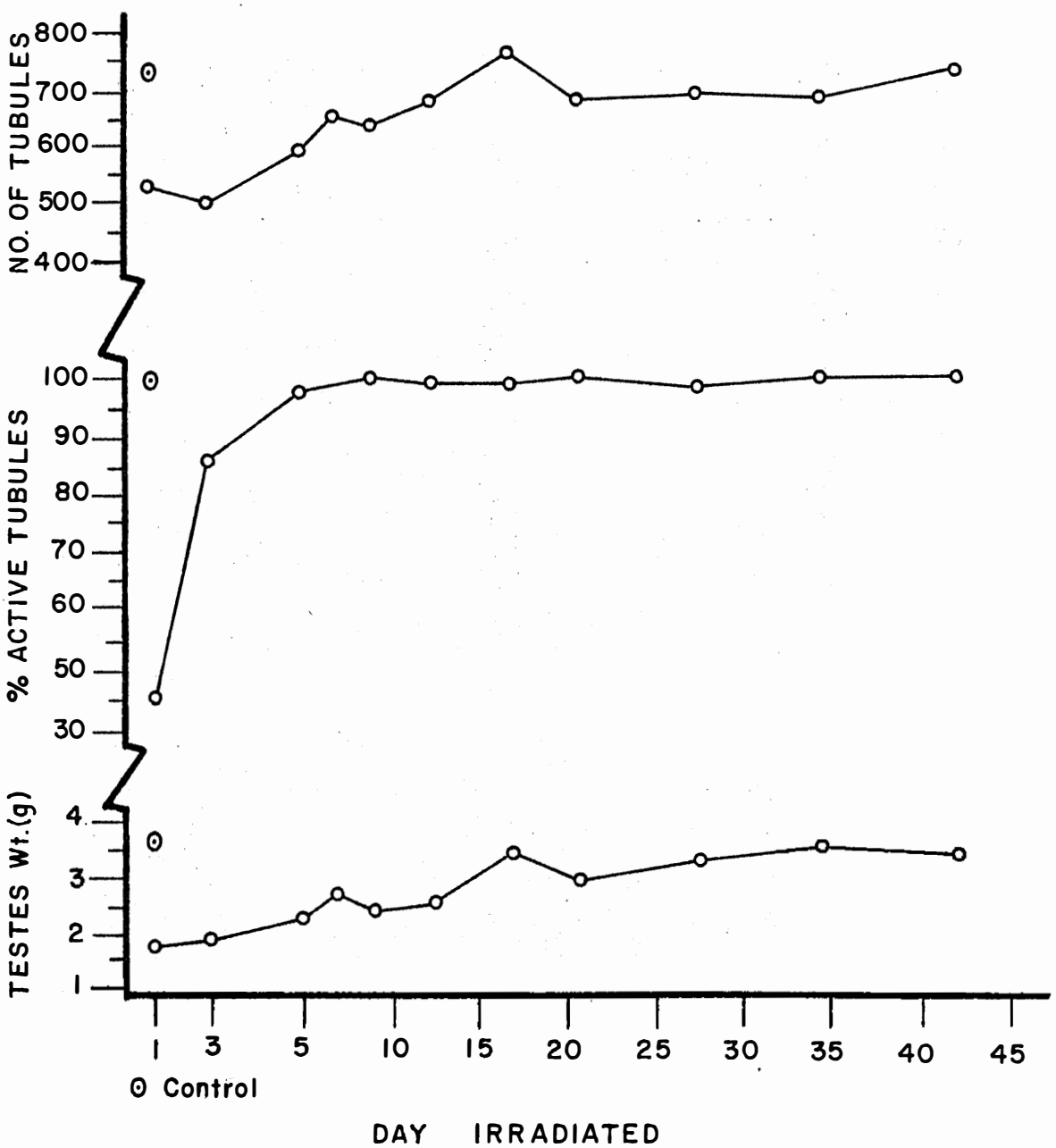
Body Weight: In the males killed at 185 days of age, the radiation effect on body weight was no longer evident as noted in the animals killed at 65 days of life. Although males irradiated on days 1 through 28 weighed slightly less than the other groups, there was no significant difference at the 5% level of probability as shown in Table I.

Apparently, the depression of body weight in the day 1 and 3 animals which was observed at 65 days was overcome by 185 days of age. While animals irradiated on days 7, 9, 13, and 42 had mean body weights slightly greater than control at 65 days of age, the mean body weights of these groups was slightly less than controls at 185 days of age.

Testes Weight: The testes weights of the animals killed at 185 days of age (Table II) showed a severe depression of testes weights in those animals irradiated on day 1; there was less depression in those animals irradiated on day 3. There was an increase in testes weight with increasing age at irradiation, beginning at day 3 and continuing through day 42. While mean testes weights of all groups were less than control, the groups irradiated on days 17, 28, 35, and 42 did not differ significantly from the control group ($P<0.05$). All of the testes weights of 65 days of age animals were less than those of 185 day animals, which shows a trend of increasing weight with age as seen in Figure 1.

Histological Evaluation: The testes of the animals irradiated on postnatal day 1 had 64% in Classes 0, 1 and 2 with 53% being in Class 2 (Table III). Animals irradiated on postnatal day 3 were observed to have 14% non-productive tubules (i.e. Class 0, 1, and 2), with 70% of the total number of seminiferous tubules found in the controls. Thus there was a decrease in the total number of tubules found in

Figure 1. Comparison of Mean Testes Weights, Percent of Active Tubules and Total Number of Tubules in Rats Irradiated with 150 R of X-rays and Killed at 185 Days of Age.


NO. OF TUBULES
800
700
600
500
400
% ACTIVE TUBULES
100
90
80
70
60
50
30
TESTES Wt.(g)
4
3
2
1
1
3
5
10
15
20
25
30
35
40
45
⊙ Control
DAY IRRADIATED

a cross-section of the testes, as well as a decrease in the number of inactive tubules.

Testes of animals irradiated on postnatal days 5, 7, 9, 13, 17, 21, 28, 35, and 42 show an absence of Class 0 tubules and few in Classes 1 and 2. The number of tubules in Classes 3, 4, and 5 in these groups was similar to the control (Table III).

The average number of tubules in each treatment group was approximately the same for both 65 and 185 days of age except for day 1, 7, 9, 13, and 17 where there were somewhat more tubules at 185 days of age than at 65 days of age. Testes of animals irradiated on all days except 17, 21, and 42 had a lower mean number of seminiferous tubules; however, the difference was significant ($P<0.05$) only on days 1, 3, 5, 7, and 9.

## Results on Females

Female rats that were exposed to 150 R of X-radiation on either postnatal day 3, 5, 7, 9, 13, 17, 21, 28, 35, or 42 were observed for effect on age at puberty, estrus cycle, fertility, body weight, ovarian and uterine weights, and the stage of development and number of follicles in a cross-section of an ovary.

### Animals Killed at 65 Days of Age.

Age of Puberty: The effect of irradiation on the age of puberty was determined by observing the animals daily and

recording the date of the vaginal opening which has been accepted as a guide for the presence of puberty in the female rats. There appeared to be no effect of irradiation upon the age of puberty for it was determined to fall between the ages of 34 and 39 days which is the accepted range for puberty in female rats.

Body Weights: The groups of irradiated females that were killed at 65 days of age had depressed body weights when compared to control with the exception of the animals irradiated on day 42. The animals irradiated on day 5 had the least body weight which averaged 84% of the control body weight.

Uterine Weight: The mean uterine weights of all irradiated groups were less than the control mean (Table V). Only those irradiated on days 3 and 9 differed at the 5% level of significance. There was an increase in weights as the animals increased in age before irradiation. There was much variability in weights within groups; a portion of which was due to varying fluid content of the uterus due to differing stages of the estrus cycle.

Ovarian Weight: Animals irradiated on day 5 had the smallest mean ovarian weight which was only 44% of the control; this differed from the control at the 1% level of significance. Day 7 animals had the next lowest weight which was only 47% of control. The mean ovarian weights of the

TABLE V

MEAN BODY, UTERINE, AND OVARIAN WEIGHT OF RATS IRRADIATED WITH 150 R AND KILLED AT 65 AND 185 DAYS OF AGE

| Treatment | 65 Days of age | | | 185 Days of age | | |
|---|---|---|---|---|---|---|
| | Body wt, g | Uterine mg | Ovarian mg | Body wt, g | Uterine mg | Ovarian mg |
| Control | 190.7[a] | 341.3[a] | 122.4[a] | 289.7 | 447.7[bc] | 165.3 |
| Day Irradiated | | | | | | |
| 3 | 175.5[ab] | 229.3[b] | 74.9[b] | 257.5[ab] | 410.8[bc] | 94.4[ab] |
| 5 | 162.7[b] | 283.3[a] | 54.2[c] | 228.2[c] | 375.4[e] | 66.1[bcd] |
| 7 | 180.0[ab] | 236.1[b] | 62.2[c] | 252.4[abc] | 378.0[de] | 49.1[d] |
| 9 | 180.5[ab] | 276.3[ab] | 79.0[b] | 241.1[bc] | 414.9[cd] | 53.4[d] |
| 13 | 181.2[ab] | 297.4[a] | 84.1[b] | 237.3[bc] | 478.8[b] | 58.1[cd] |
| 17 | 175.9[ab] | 294.1[a] | 88.4[b] | 250.6[abc] | 605.5[a] | 54.9[abc] |
| 21 | 180.1[ab] | 328.7[a] | 86.4[b] | 250.2[abc] | 447.3[bc] | 125.1[a] |
| 28 | 176.2[ab] | 299.1[a] | 88.6[b] | 243.5[abc] | 490.8[b] | 89.2[ab] |
| 35 | 173.6[ab] | 337.4[a] | 94.2[b] | 254.4[abd] | 422.0[cd] | 104.4[a] |
| 42 | 190.8[a] | 339.6[a] | 117.8[a] | 267.8[a] | 559.2[a] | 118.7[a] |

[a,b,c,d] Weights with the same superscript in the same column do not differ from each other at the 5% level of significance.

animals irradiated on these days did not differ from each other but did weigh significantly less than all other groups. Day 3 and 9 females' ovarian weights were approximately 62% of the control. The groups irradiated on days 13, 17, 21, 28, and 35 had ovarian weights ranging from 70 to 74% of control, while day 42 was only slightly less than that of the controls. The mean ovarian weight of all groups except those irradiated on day 42 were significantly different from control.

Histological Evaluation: All of the groups of animals killed at 65 days of age had follicles in all six stages of development, with a large number of atretic follicles in each group, as seen in Table VI. Animals irradiated on days 3, 7, and 9 had the largest number of empty follicles, with day 35 having an absence of empty follicles. Rats irradiated on day 21, 35, and 42 had a large number of corpora lutea which decreased to one-third of that number for day 5 animals. All of the cross-sections of ovaries of irradiated animals appeared to have a decrease in primordial, growing and vesicular follicles in comparison to the controls. A maximum effect was observed in the day 3, 5, 7, 9, and 13 groups, with less effect shown as age of irradiation increased from day 17 on, with the day 42 animals having mean numbers of primordial, growing and vesicular follicles approximately that of control (Figure 2).

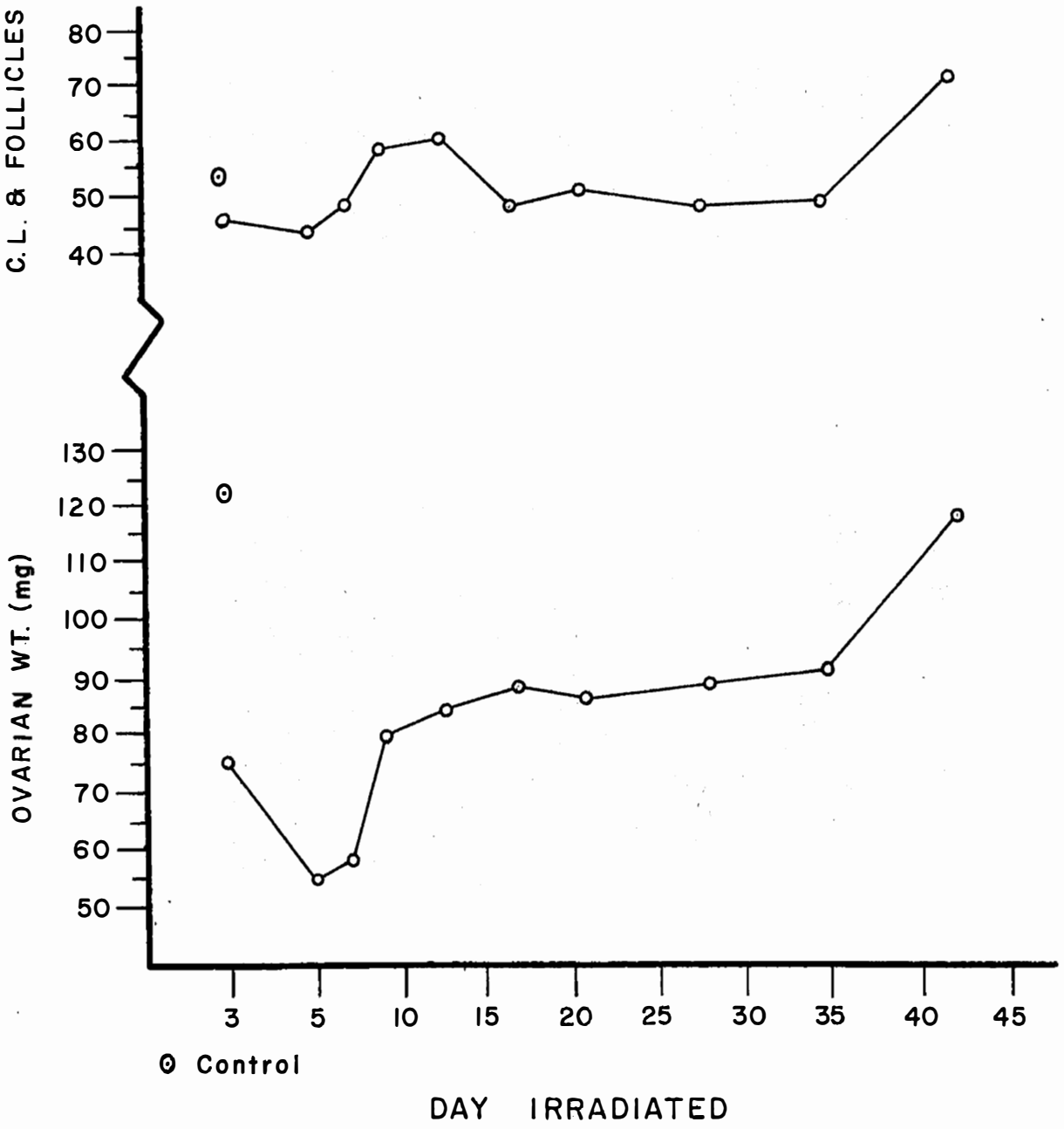
TABLE VI

MEAN OVARIAN WEIGHT AND STAGES OF FOLLICULAR DEVELOPMENT IN IRRADIATED RATS KILLED AT 65 DAYS OF AGE

| Treatment | Ovarian wt, mg | Mean number of follicles in each stage of development | | | | | |
|---|---|---|---|---|---|---|---|
| | | Primary | Growing | Vesicular | Corpora lutea | Empty | Atretic |
| Control (9)* | 122.4 | 8.6 | 6.9 | 9.6 | 7.9 | 0.4 | 21.0 |
| Day Irradiated | | | | | | | |
| 3 (8) | 74.9 | 0.5[a] | 1.9[a] | 1.5[a] | 5.0[a] | 13.3[a] | 23.9 |
| 5 (12) | 57.3 | 2.1[a] | 1.2[a] | 1.3[a] | 3.7[a] | 9.9[a] | 27.8[a] |
| 7 (11) | 58.5 | 3.0[a] | 1.6[a] | 2.1[a] | 6.2[a] | 11.0[a] | 38.8[a] |
| 9 (11) | 79.4 | 1.6[a] | 0.8[a] | 1.0[a] | 6.0[a] | 11.8[a] | 36.8[a] |
| 13 (12) | 84.1 | 3.8[a] | 0.8[a] | 1.1[a] | 8.5 | 8.3[a] | 38.8[a] |
| 17 (8) | 88.4 | 5.0[a] | 0.5[a] | 4.3[a] | 7.8 | 3.4[a] | 27.4[a] |
| 21 (8) | 86.4 | 4.4[a] | 0.6[a] | 2.3[a] | 11.3[a] | 3.4[a] | 30.6[a] |
| 28 (14) | 88.9 | 4.7[a] | 1.5[a] | 3.5[a] | 8.3 | 2.3[a] | 27.9[a] |
| 35 (10) | 87.5 | 3.7[a] | 3.7[a] | 5.9[a] | 10.1[b] | 0[b] | 25.3[a] |
| 42 (10) | 119.5 | 8.6 | 4.3[a] | 10.4 | 12.7[a] | 1.6[a] | 33.9[a] |

*Number in () is the number of animals analyzed in that group.
[a]Differs from the control at the 1% level of significance.
[b]Differs from the control at the 5% level of significance.

Figure 2. Comparison of Mean Ovarian Weight and Total Number of Corpora Lutea (C. L.) and Follicles of Rats Irradiated with 150 R of X-rays and Killed at 65 Days of Age.


C.L. & FOLLICLES
80
70
60
50
40
OVARIAN WT. (mg)
130
120
110
100
90
80
70
60
50
3
5
10
15
20
25
30
35
40
45
0 Control
DAY IRRADIATED

Animals Killed at 185 Days of Age.

Fertility Tests: Results of the fertility tests are presented in Table VII. Of the 11 females irradiated on day 3, eight littered a total of 11 times with a mean litter size of 4.5. Females irradiated on day 5 had four fertile animals out of nine with a mean litter size of 4.5. The 19 animals that were irradiated on day 7 exhibited the greatest irradiation effect in that they all proved to be sterile. The mean litter size of the females irradiated on days 9 through day 35 differed significantly from the control with less effect being shown as age of irradiation increased. All females irradiated on day 42 were fertile with mean litter size of 9.4. Of the 10 control animals, 9 were fertile with a mean litter size of 10.4. With the exception of animals irradiated on day 42, the mean litter sizes of the fertile animals were significantly different from control.

Vaginal Smears of Sterile Animals: The non-pregnant females that had been caged with males of known fertility for approximately 8 weeks were removed and after a week of rest for the females, vaginal smears were made daily. The smears contained epithelial cells, leucocytes, and cornified cells. The smears made daily from each animal were stained, analyzed, and the approximately percentage of each type of cells was recorded to obtain information on the estrus pattern.

TABLE VII

MEAN OVARIAN WEIGHT, LITTER SIZE, AND STAGES OF FOLLICULAR DEVELOPMENT OF FERTILE IRRADIATED RATS KILLED AT 185 DAYS OF AGE

| Treatment | Number | | Litter size | Ovarian wt, mg | Mean number in each stage of follicular develop. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Irrad. | Fertile | | | Primary | Growing | Vesicular | Corpora lutea | Empty | Atretic |
| Control | 10 | 9 | 10.4 | 171.1 | 2.6 | 1.6 | 3.6 | 9.1 | 2.4 | 21.1 |
| Irrad. Day | | | | | | | | | | |
| 3 | 11 | 8 | 4.5[a] | 92.4 | 0.9[a] | 0.9 | 0.8[a] | 6.4[b] | 5.4[a] | 0[a] |
| 5 | 9 | 4 | 4.5[a] | 66.9 | 1.0[b] | 0.8 | 0.6[a] | 5.0[a] | 9.2[a] | 1.8[a] |
| 7 | 19 | 0[a] | 0[a] | | | | | | | |
| 9 | 10 | 2[b] | 2.0[a] | 44.1 | 0[b] | 0 | 0[a] | 0[a] | 2.0 | 0[a] |
| 13 | 11 | 3 | 6.3[b] | 64.5 | 1.5 | 0[b] | 0[a] | 0.8[a] | 17.5[a] | 0[a] |
| 17 | 10 | 6 | 7.4[a] | 103.2 | 2.2 | 1.8 | 4.6 | 5.8[a] | 19.6[a] | 6.0[a] |
| 21 | 8 | 5 | 5.4[a] | 151.5 | 0[a] | 0.2[a] | 0.6[a] | 4.6[a] | 15.6[a] | 0[a] |
| 28 | 12 | 11 | 8.4[a] | 98.1 | 2.0 | 0.6[a] | 2.0[a] | 7.4 | 13.6[a] | 5.1[a] |
| 35 | 11 | 11 | 8.2[a] | 104.4 | 2.0 | 1.1[a] | 2.3[b] | 9.3 | 6.1[a] | 10.3[a] |
| 42 | 11 | 11 | 9.4 | 118.7 | 0.9[a] | 0.7[b] | 2.7 | 11.3[b] | 11.4[a] | 21.8 |

[a]Significantly different from the control at the 1% level.
[b]Significantly different from the control at the 5% level.

The results obtained on each animal are presented in Table VIII. There were many irregular estrus cycles ranging in length from 3 to 15 days, however, most of the animals did exhibit an identifiable cycle, with approximately half of them of the normal 4-6 day length. The smears contained varying amounts of cornified cells; from 5 to 90% cornified cells present in all smears of some animals, to 100% cornified cells for 10 successive days. Two of the females, one irradiated on day 9 and the other on day 17, had vaginal smears with continuous 100% cornified cells. Females irradiated on day 7 were the most sensitive to irradiation with 100% of the animals sterile and all were observed to have irregular estrus cycles.

All animals were returned to the mating cages and the service pans beneath the cages were observed each morning for vaginal plugs. Of the forty sterile animals treated in this manner, only 3 animals failed to exhibit vaginal plugs. These were the control and animals irradiated on day 21 and 28. Smears were made when possible when plugs were observed and sperm were found in the vaginal smears, thus mating did occur in almost all cases.

Body Weight: Body, uterine, and ovarian weights of the animals killed at 185 days of age are presented in Table V. Animals irradiated on day 5 had 78.8% of control body weight. The body weights of day 13 animals was 72.3% of control.

TABLE VIII

UTERINE WEIGHT, LENGTH OF ESTRUS CYCLE OF 185 DAY STERILE RATS

| Treatment | Uterine wt, mg | Estrus cycle # days | Treatment | Uterine wt, mg | Estrus cycle # days |
|---|---|---|---|---|---|
| Control | 44.8 | 5 | Irradiated | | |
| | | | Day 9 | | |
| Irradiated | | | 1 | 56.4 | +++ |
| Day 3 | | | 2 | 44.8 | +++ |
| 8* | 45.3 | 6 | 3 | 43.9 | 6 |
| Day 5 | | | 4 | 12.4 | 4++ |
| 1 | 77.6 | 10++ | 5 | 55.0 | 5 |
| 2 | 48.3 | 10++ | 6 | 44.6 | +++ |
| 3 | | 7+ | 7 | 37.4 | +++ |
| 5 | 45.5 | 6+ | 8 | 38.3 | 5 |
| 6 | 50.2 | 3+ | Day 13 | | |
| 7 | 47.7 | 4 | 1 | 45.4 | 3+ |
| Day 7 | | | 6 | 49.4 | +++ |
| 1 | 36.6 | 5 | 7 | 53.2 | 7 |
| 2 | 27.0 | 10+ | 9 | 41.0 | 4++ |
| 3 | 37.5 | 7+ | 10 | 47.4 | 6 |
| 4 | 39.9 | 15 | 11 | 64.8 | 9 |
| 5 | 45.2 | 4++ | Day 17 | | |
| 7 | 36.1 | 11 | 4 | 52.5 | 8 |
| 13 | 37.8 | 11 | 7 | 60.6 | 4 |
| 16 | 40.0 | 11 | 8 | 73.4 | +++ |
| 18 | 56.3 | 5+ | 9 | 65.6 | 5 |
| 19 | 45.9 | 5++ | 10 | 61.2 | 5 |
| Day 28 | | | Day 21 | | |
| 9 | 39.3 | 10+ | 3 | 52.0 | 5+ |
| | | | 4 | 53.2 | 7 |

*Animal number
+Some continuous cornification
++50% continuous cornification
+++90-100% continuous cornification

The animals irradiated on day 3, 7, 9, 17, 21, 28, 35, and 42 all weighed significantly less than control, but there was not a significant difference between the body weights of the animals irradiated on these days of age.

Uterine Weight: The uterine weights of the rats irradiated on days 5 and 7 were 83.4% of the control uterine weight, which was significantly different from the control and the animals of the other groups at the 5% level. Females irradiated on day 17 and 42 had larger uterine weights than control, with remaining animals of days 3, 9, 13, 21, 28, and 35 having the approximate weight of the controls with no significant difference between the uterine weights of the animals.

Ovarian Weight: All of the irradiated females killed at 185 days of age had ovarian weights which differed significantly from control, as presented in Table V. The animals irradiated on days 3, 21, 28, 35, and 42 had ovarian weights that were 57.1, 75.5, 63.2, and 71.8% of the control. The ovaries of animals irradiated on day 7 weighed only 29.7% of control. Days 5, 9, 13, and 17 irradiated animals had mean ovarian weights ranging from 32 to 40% of the control.

Histological Evaluation: For evaluation of the stages of follicular development observed in ovarian sections, the females killed at 185 days were separated into two groups:

those animals that littered and consequently were called fertile, and those animals that failed to litter and therefore were called sterile (Table VII and Table IX).

The primary follicles were somewhat more evenly distributed in fertile irradiated animals than in the sterile animals. The groups irradiated on days 3, 5, 9, 21, and 42 had significantly less primary follicles than control. Primary follicles were not observed in the ovarian sections of fertile animals irradiated on day 9 and 21. Fertile animals irradiated on days 3 and 42 had a mean of less than one primary follicle per ovarian cross section, while the remaining irradiated groups had means between one and two for the number of primary follicles.

The sterile animals irradiated on day 9 had the largest number of primary follicles of all the animals with a mean of 6.5 observed in the ovarian sections, while day 5 irradiated animal had a mean number of 3.4 which was only smaller than the sterile control which had 4.0 primary follicles per cross section. Ovarian sections of animals irradiated on days 13 and 21 were observed to have a mean of two primary follicles, while animals of day 3, 7, 17, and 28 were observed to have mean numbers of 0.2 to 1.5. There were no sterile animals among those animals irradiated on day 35 and 42. The groups of animals irradiated on days 7, 9, 13, and 17 differed significantly from the control at the 1% level.

TABLE IX

MEAN NUMBER OF FOLLICLES IN EACH STAGE OF FOLLICULAR DEVELOPMENT IN IRRADIATED RATS WHICH WERE STERILE

| Treatment | Number | | Ovarian wt, mg | Mean number in each stage of follicular develop. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Irrad. | Sterile | | Primary | Growing | Vesicular | Corpora lutea | Empty | Atretic |
| Control | 10 | 1 | 113.1 | 4.0 | 4.0 | 4.0 | 10.0 | 0 | 36.0 |
| Irrad. Day | | | | | | | | | |
| 3 | 11 | 1 | 108.4 | 1.0 | 1.0 | 3.0 | 10.0 | 7.0[a] | 0[a] |
| 5 | 9 | 5 | 52.3 | 3.4 | 0[a] | 0[a] | 2.6[a] | 9.2[a] | 0[a] |
| 7 | 19 | 19 | 49.1 | 1.5[a] | 0.2[a] | 0.2[a] | 1.2[a] | 22.9[a] | 0.2[a] |
| 9 | 10 | 8 | 58.2 | 6.5[a] | 1.1[a] | 1.6[a] | 4.4[a] | 20.1[a] | 1.6[a] |
| 13 | 11 | 7 | 58.7 | 2.0[a] | 0[a] | 0.3[a] | 2.3[a] | 19.1[a] | 0[a] |
| 17 | 10 | 5 | 72.2 | 0.2[a] | 0.8[a] | 0.6[a] | 1.6[a] | 17.4[a] | 0[a] |
| 21 | 8 | 3 | 87.9 | 2.0 | 0.3[a] | 2.3 | 4.0[a] | 11.0[a] | 0[a] |
| 28 | 12 | 1 | 73.9 | 1.0 | 0[a] | 0[a] | 4.0[a] | 6.0[a] | 13.0[a] |
| * | | | | | | | | | |

*Animals irradiated on days 35 and 42 were not sterile.
[a]Differed from control at the 1% level of significance.
[b]Differed from control at the 5% level of significance.

The largest numbers of growing follicles in fertile animals were found in the control and 17 day irradiated animals with a mean number of 1.6 and 1.8 per ovarian cross section respectively. The irradiated groups had varying mean numbers between 0.2 and 1.1, with the exception of animals irradiated on days 9 and 13, which had an absence of growing follicles. Fertile animals irradiated on days 13, 21, 28, 35, and 42 had mean numbers of growing follicles per ovarian section which varied significantly from the control. The sterile control animal had four growing follicles recorded for the ovarian section. There was a significant difference in the number of growing follicles observed in the ovarian cross section of the groups of irradiated animals and that of the control except for those animals irradiated on day 3.

The largest mean numbers of vesicular follicles in the fertile animals were found in the control, which had 3.6, and in the animals irradiated on day 17, which had a mean of 4.6, per ovarian cross section. Means of animals irradiated on days 3, 5, 9, 13, 21, 28, and 35 differed significantly from control. Animals irradiated on days 28, 35, and 42 had mean numbers of approximately two per ovarian cross section, while animals of days 3, 5, and 21 had mean numbers less than one per ovarian section. Vesicular follicles were not observed in ovarian sections of animals irradiated on days 9 and 13. The sterile control animal had an ovarian section

which contained four vesicular follicles. Sterile animals irradiated on day 9 and 21 had mean numbers of 1.6 and 2.3 respectively for vesicular follicles. Vesicular follicles were not observed in ovarian sections of animals irradiated on days 5 and 28, and a mean number less than one was observed for those animals irradiated on days 7, 13, and 17. All of the irradiated sterile animals had vesicular follicle means in the ovarian cross section which were significantly different from control except the means of the animals irradiated on days 3 and 21.

Corpora lutea were observed in ovarian sections of all animals except those fertile females irradiated on day 9; fertile animals irradiated on day 13 only had 0.8 corpora lutea per cross section. The greatest mean number was noted for fertile animals irradiated on day 42. All of the groups of animals had a significant difference in the mean number of corpora lutea per cross section when compared to the control except for those animals irradiated on days 28 and 35. Fertile control and day 35 animals had means of approximately nine per ovarian cross section, while day 28 animals had a mean number of 7.4 and animals of day 5 had a mean number of 6.4. Animals irradiated on days 5, 17, and 21 had similar mean numbers of five. The sterile control and day 3 animals had a mean of 10 corpora lutea. Animals irradiated on day 3 were the only animals which did not have a mean number of corpora lutea per ovarian cross section which differed

significantly from the control. Sterile animals irradiated on day 9, 21, and 28 had a mean number of four. Animals irradiated on days 4, 7, 13, and 17 had the lowest mean numbers of corpora lutea in the sterile animals with means of 1.6 to 2.6 per ovarian cross section.

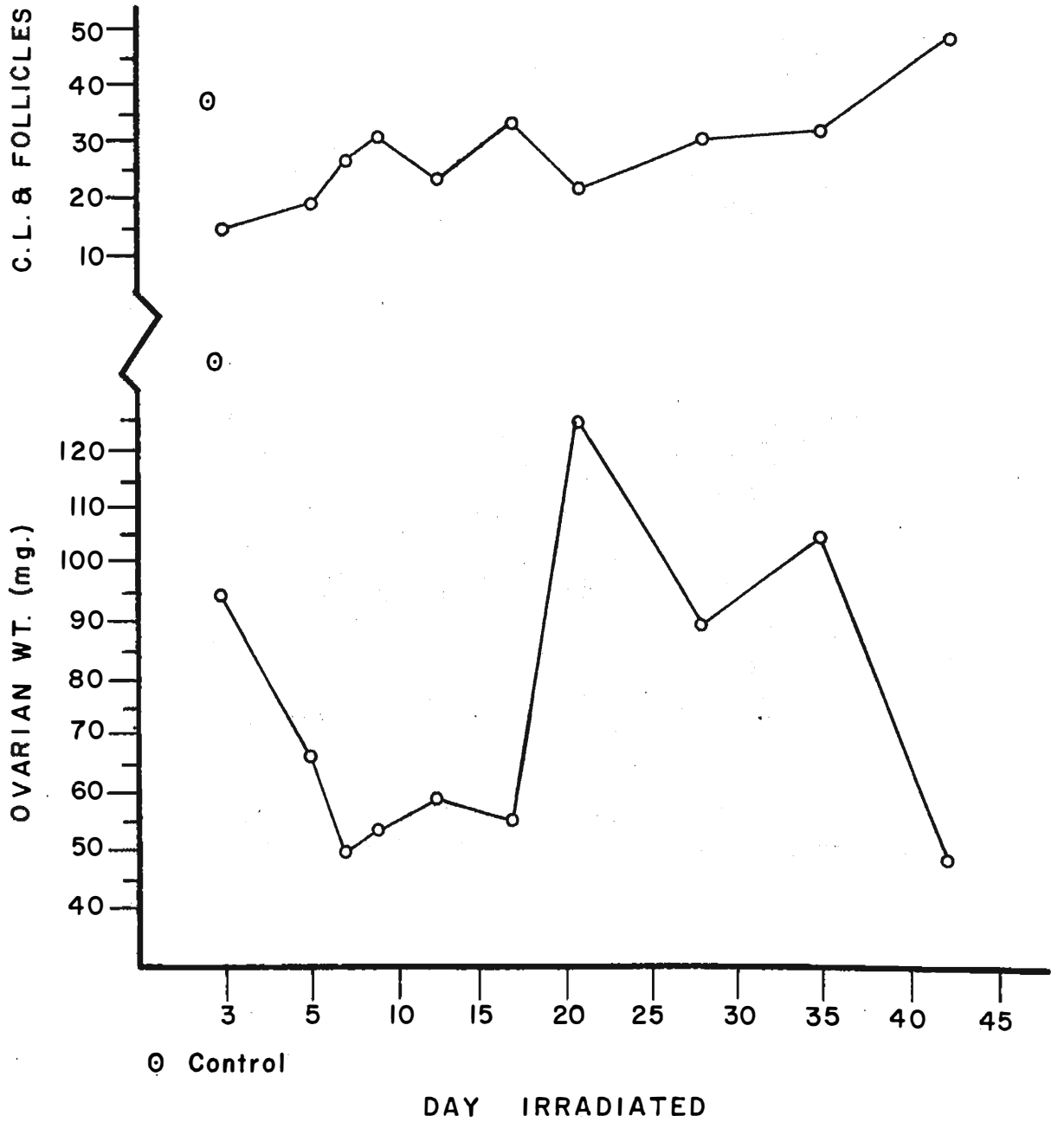
"Empty" follicles have only a single, irregular layer of granulosa cells with an absence of an oogonium and a mean diameter of 0.0091 micron. The empty follicles were found in all ovarian sections except the one sterile control. The animals irradiated on days 7, 9, 13, and 17 had the largest number of empty follicles. The fertile animals irradiated on day 9 had few empty follicles, but their ovaries did not contain any other germinal elements. Animals irradiated on days 21 and 28 also had a large number of atretic follicles. Animals that were irradiated at the earliest age, days 3 and 5, had small mean numbers of empty follicles, with sterile animals of day 28 and fertile animals of day 35 having the same mean number of six. Except for the fertile day 9 animals, all irradiated groups had more empty follicles than control.

Atretic follicles were found in the greater number in the fertile animals, while rats irradiated on day 3, 9, and 13 did not have atretic follicles observed in the ovarian sections. The control, and animals irradiated on day 35 and 42 had large numbers, with control and day 42 animals having the same mean number of 21. Days 5, 17, and 28 animals had low number of atretic follicles, ranging from two to six per

cross section. In the sterile animals, controls had an average of 36 atretic follicles counted per ovarian section. Sterile animals irradiated on days 28 and 9 had 13 and 1.6, respectively, observed atretic follicles. It is interesting to note that fertile animals irradiated on day 9 had only empty follicles observed in the ovarian sections. All of the irradiated animals differed significantly from control in the mean number of empty and atretic follicles per ovarian cross section except the two fertile animals irradiated on day 9.

When the mean number of follicles in each stage of development were compared between the sterile and fertile animals, it was noted that the sterile animals had a wider distribution of follicles in all stages of development except those in the growing follicular stage. Rats that were irradiated at 42 days of age had reached the age of puberty before being irradiated which could be a factor in the larger number of follicles per ovarian section observed in these animals as compared to the animals irradiated at a younger age. The control, fertile and sterile, had a larger number of follicles in all stages of follicular development except for the atretic and empty follicles observed in ovarian sections of rats killed at 65 days of age and empty follicles observed in the ovarian sections of those killed at 185 days of age. These two stages of follicular development are non-productive terminal elements of the ovaries and were prevalent in the irradiated animals (Figure 3).

Figure 3. Comparison of Mean Ovarian Weight and Total Number of Corpora Lutea (C. L.) and Follicles of Rats Irradiated with 150 R X-rays and Killed at 185 Days of Age.


C.L. & FOLLICLES
50
40
30
20
10
0
0
OVARIAN WT. (mg.)
120
110
100
90
80
70
60
50
40
3
5
10
15
20
25
30
35
40
45
0 Control
DAY IRRADIATED

# CHAPTER IV

## DISCUSSION

Animals irradiated on day 1 of postnatal life had a severe depression in testes weight (to an average of 26% of control); beginning with day 3 there was a recovery in testes weight, continuing through day 42. There was also a striking difference in fertility between males irradiated on day 1 and those of later dates. Only 20% of the males irradiated on day 1 sired litters with a mean litter size of 3, as compared to 90 to 100% fertility and a mean litter size of approximately 9 for animals irradiated at later ages.

Males irradiated on day 1 were recorded as having only 74.3% of the number of tubules of control. This could possibly be because of the damage done by irradiation to the cells which produce the elongation of the seminiferous tubules by their mitotic division, resulting in fewer cross-sections of tubules. As the rat's ages increase before irradiation, it appears there is an increase in the total number of tubules in the cross-sections of the testes. This would seem to be because of the delay before irradiation gave the seminiferous tubules more time to elongate before the deleterious effects of the radiation on cell proliferation.

This additional growth period of the seminiferous tubules would be partially responsible for the increase in testes weight.

It is interesting to note that animals irradiated on day 1 and killed at 65 days of age had testes weight 26% of control with approximately 53% Class 2 tubules while animals that were irradiated on day 1 and killed at 185 days of age had similar testes weight depression, but contained more of Class 2 tubules (76%). The animals that were irradiated on days 3 through 42 and killed at 185 days had increased numbers of Class 3, 4, and 5 with all of the animals having approximately 91% of Class 5 tubules. The increase in the number of the Class 5 tubules with the full complement of cellular elements would also add to the weight of the testes.

In general, mitotically active gonia are highly sensitive to cell killing (Mandl, 1963). Paradoxically, permanent sterilization in male rats is most readily achieved by irradiating mitotically inactive primordial germ cells before or shortly after birth. Sterilization was observed in 60% of the males irradiated on day 1 of postnatal life in this study. Permanent sterilization apparently was not produced by irradiating on days 3 or later. The surviving primordial germ cells repopulate the tubules with spermatogonia, restoring fertility, and nearly normal tubule histology by 185 days of age in animals irradiated on day 5 or later.

The response of females to irradiation is different than that of males. The germ cells of the female that are damaged or destroyed by irradiation cannot be replaced. Primordial follicles in the adult rat are very sensitive with only about 200-300 R being needed to destroy them within five days (Mandl, 1962). Smaller doses are needed for sterility in immature rats. All females irradiated on postnatal day 7 with 150 R of X-irradiation in this study were sterile. Eighty percent of the animals irradiated on the 9th day of life, and 70% of the animals irradiated on the 13th day of life were sterile. It is obvious that as the animal increases in age beyond day 13 that the sensitivity of the germinal cells decreases.

"Empty" follicles, which are large (presumably germinal) follicles containing a single irregular layer of granulosa cells and an absence of oogonium, were about 36% of the follicles found in those animals which were sterile. It was interesting to note that the animals irradiated on day 7 and killed at 65 days of age had approximately 50% atretic follicles, 32% empty follicles, 13% corpora lutea, and 3 to 5% of the other follicular types. The animals that were irradiated on day 7 and killed at 185 days of age contained 90% empty follicles, 5.7% primordial follicles, and 4.5% corpora lutea, and 1% of the other follicular types. It is apparent that irradiation damaged follicles degenerate as the animal increases in age. It is not clear, however, as to which of

the follicular types underwent degeneration to produce the empty follicle, if indeed, it was produced from a follicular type. There was no evidence of the empty follicle having contained an oogonium; therefore, perhaps it was the result of irradiation stimulated growth of a germ cell. The empty follicle, being markedly larger than a primordial follicle, indicates that if it is the result of degeneration of the oocyte in a primordial follicle, then there was continued growth of the primordial follicle after being exposed to irradiation.

Mandl (1962) noted the most sensitive stage of oocyte development is "at or near first meiotic metaphase," or diakinesis in the rat. It is at this stage of development that the oocyte remains until it undergoes maturation division immediately before ovulation. This prolonged diplotene phase is not a "resting" stage, for Mandl (1963) and Beaumont (1964) reported a possible division of the diplotene phase into three or four "subphases" in the rat. During these subphases there are changes occurring between the oocyte and its enveloping follicle cells. Ham (1965) stated that primary oocytes only complete their first meiotic division after puberty, which for the rat is 34-39 days of age. Animals irradiated at the age of an active "subphase" would have the effect observed at a later age of active cellular division. This delay in manifestation of the maximum effect

was seen in the higher proportion of empty follicles in the females killed at 185 days of age.

It is generally accepted that the vaginal smear technique with its various modifications is the most accurate method for detecting copulation in rodents. However, this technique leaves something to be desired. Frequent smearing may induce vaginal cornification, while cervical stimulation during the diestrus preceding ovulation may induce pseudopregnancy.

A copulation plug is formed immediately after mating. With respect to the presence of plugs in the service pan, and not in the female, the possibility of spontaneous ejaculation should be considered (Szabo et al., 1969). Smears made in the morning upon observing plugs in the service pan proved to have sperm; therefore, the plugs observed in this research were assumed to be formed from copulation.

Vaginal smears of the sterile animals revealed that the animals did undergo estrus, although the lengths of the estrus cycles were anomalous. Of the 40 animals considered sterile, 15 had estrus cycles of 4 or 5 days, but other animals had cycles which were as many as 15 days in length. The vaginal smears showed varying amounts of cornification. Some animals had from 90 to 100% continuous cornification, while others had 10 to 50% continuous cornification. It was noted by Parks (1927) that 11 mice which were irradiated at birth and were sterilized, mated several times. Four of the

animals did not mate during the periods in which vaginal cornification was present. Mandl and Zuckerman (1955) stated that X-irradiated rats either refused to mate or failed to attract the male during phases of vaginal cornification. In this research, animals which had 90 to 100% cornification mated, while the two sterile animals which failed to mate had less than 10% cornified cells present in each vaginal smear. All of the animals which were irradiated on postnatal day 7 were sterile, with 50% of the animals having some degree of continuous vaginal cornification. All of the animals were assumed to have mated, based upon copulation plugs and sperm in vaginal smears.

The ovarian weights obtained in this study were not completely accurate for inadvertently there were varying amounts of adipose tissue left on the ovaries. However, there was an increase in ovarian weights as the animals increased in age before irradiation. For day 7 animals, killed at 185 days of age, ovarian weights were 78.9% of the ovarian weights of the day 7 animals killed at 65 days of age. This was probably due to the fact that the 65 days of age animals had more vesicular, corpora lutea, and atretic follicles than those animals of 185 days of age which had a greater number of empty follicles. In the animals killed at 65 days of age the number of surviving primordial and growing follicles had not been depleted. At 185 days of age there was a decrease in follicles of all types except

those of the empty stage which increased in number. This was especially true of those animals whose age also increased before irradiation.

Considering the number and stages of follicular development found in the older animals, the continued mating of the sterile females and the irregular, but present, estrus cycle of the sterile animals, it must be concluded that the factors underlying the changing pattern in radiosensitivity of the female mammalian germ cell are not yet fully understood. It must be remembered that whole body irradiation affected the hormonal status of the pituitary, therefore this researcher suggests that endocrine changes should be also considered for further investigation of radiation effects on reproductivity in mammals.

# CHAPTER V

## SUMMARY

At least 20 male and 20 female rats were irradiated with 150 R of X-rays on each of the following days: 3, 5, 7, 9, 13, 17, 21, 28, 35, or 42. Ten animals of each sex for each day irradiated were killed at 65 days of life, and 10 animals of each sex for each day irradiated were tested for fertility and killed at 185 days of age. Gonads from all of the animals were evaluated histologically along with gonads from animals irradiated on postnatal day 1 in a previous study.

In the male animals the groups irradiated on day 1, 3, 5, 28, and 35 of age and killed at 65 days of life showed a depression of body and testes weights; all groups had depressed testes weight and groups irradiated on days 1 through 17 had a reduced number of seminiferous tubules in the testes. With the exception of animals irradiated on day 1 and 3, the animals killed at 185 days of age showed recovery of body and testes weight and number of seminiferous tubules. Day 1 animals killed at 65 and 185 days of age had approximately 25% of the testes weight of control, and 20% of the animals fertility-tested sired litters with a mean litter size of 3. Animals irradiated on day 3 showed some recovery when killed

at 185 days of age, with the body and testes weights and number of seminiferous tubules remaining less than control and the fertility being the same as control.

That the irradiated females were the most radiosensitive at postnatal day 7 was concluded from the fertility tests and ovarian and uterine weights. All of the animals killed on 65 days of age had a decrease in ovarian weights when compared to control. The ovarian weights of those animals irradiated on days 7, 9, 13, and 17 were larger for those animals killed at 65 days of age than those killed at 185 days of age. This observation can be accounted for by the decrease in the number of vesicular follicles and corpora lutea found in the older animals. The body and uterine weights in all of the animals killed at 65 days of age were smaller than those of the animals irradiated on the same day of life and killed at 185 days of age.

Females irradiated on day 7 were sterile, and females irradiated on days 3, 5, 9, 13, 17, and 21 had a decrease in the percent of fertile animals and mean litter size. Forty-four percent of the animals irradiated on day 5 were fertile with a mean litter size of 4.5; 20% of the animals of day 9 were fertile with a mean litter size of 2; day 13 had 27% fertile animals with a mean litter size of 6.3 and days 17 and 21 animals had approximately 60% fertility with a mean litter size of approximately 7.4 and 5.4 respectively.

Vaginal smears of the sterile animals indicated that 8 of the 40 animals had 90 to 100% continuous cornification. Thirty-two of the animals had estrus cycles, with 15 of them being anomalous when compared to the control. Thirty-eight of these animals mated as indicated by copulation plugs and sperm in vaginal smears.

The animals irradiated on days which proved to be the most deleterious to the ovaries of the animals as measured by reproductivity had a high percentage of "empty" follicles and a decrease in the number of vesicular follicles and corpora lutea. However, all of the animals had some corpora lutea, which indicated ovulation to some degree by all animals.

## LITERATURE CITED


Albers-Schönberg, H. E. 1903. Über eine bisher unbekannte Wirkung der Roentgenstrahlen auf den Organismus of Tiere. Münch. Med. Wschr. 50:1859-1860. Cited in: Oakberg, E. F. 1955. Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse. Rad. Res., 2:369-391.

Baker, T. G. 1963. A Quantitative and cytological study of germ cells in human ovaries. Proc. Roy. Soc. B. 158: 417-433. Cited in: Mandl, Anita M. 1964. The radiosensitivity of germ cells. Bio. Rev., 39:288-371.

Baker, T. G. 1969. The sensitivity of rat, monkey and human oocytes to X-radiation in organ culture. In Sikov, M. R. and D. D. Mahlum (eds.). Radiation Biology of the Fetal and Juvenile Mammal. U.S. AEC, Division of Technical Information Conf. 690501, pp. 955-961.

Beaumont, Heather M. 1960. Changes in the radiosensitivity of the testis during foetal development. Int. J. Rad. Biol., 2(3):247-256.

Beaumont, Heather M. 1961. Radiosensitivity of oogonia and oocytes in the foetal rat. Int. J. Rad. Biol., 3:59-72.

Beaumont, Heather M. 1962. The radiosensitivity of germ-cells at various stages of ovarian development. Int. J. Rad. Biol. 4:581-590.

Beaumont, Heather M. and Anita M. Mandl. 1961. A quantitative and cytological study of oogonia and oocytes in the foetal and neonatal rat. Proc. Roy. Soc. B. 155: 557-570.

Borum, K. 1961. Radiosensitivity of oogonia and oocytes in the foetal rat. Int. J. Rad. Biol. 3:59-72. Cited in: Krohn, P. L. 1967. Factors influencing the number of oocytes in the ovary. Archives D'Anatomie Micro. de Morph. Exper. 56:151-159.

Casarett, G. W. 1969. Chairman's Remarks. In: Sikov, M. R. and D. D. Mahlum (eds.). Radiation Biology of the Fetal and Juvenile Mammal. U.S. AEC, Division of Technical Information Conf. 690501, pp. 955-961.

Chiquoine, A. D. 1954. The identification, origin and migration of the primordial germ cells in the mouse embryo. Anat. Rec. 118:135.

Chu, E. H. Y. and C. Hsung. 1962. Effects of X-ray in the growth and maturation and fertility of rats irradiated by 7th day after birth. Acta. Biol. Exp. Sinico. 8: 54-95.

Clermont, Y. and B. Perey. 1957. Quantitative study of the cell population of the seminiferous tubules in immature rats. Am. J. Anat. 100:241-268.

Clermont, Y. C., P. Leblond, and B. Messier. 1955. Duree du cycle de l'epithelium seminal du rat. Archs. Anat. Microsc. Morph. Exp. 48 bis:37. Cited in: Oakberg, E. F. 1968. Mammalian gametogenesis and species comparisons in radiation response of the gonads. Report of a study group. Effects of Radiation on Meiotic Systems. IAEA. Vienna, pp. 3-15.

Clermont, Y. 1962. Quantitative analysis of spermatogenesis of the rat: A revised model for the renewal of spermatoogonia. Amer. J. Anat. 111:111-129.

Clermont, Y. 1963. The cycle of the seminiferous epithelium in man. Amer. J. Anat. 112:35.

Erickson, B. H. 1967. Effect of the gamma radiation on the prepuberal bovine ovary. Rad. Res. 31:441-451.

Franchi, L. L., Anita M. Mandl, and S. Zuckerman. 1962. The development of the ovary and the process of oogenesis. In: The Ovary, vol. 1:1-88. Ed. Zuckerman, S., Anita M. Mandl, and E. Ekstein. London: Academic Press, Inc.

Guyton, A. C. 1969. Function of the Human Body, pp. 441-447. W. B. Saunders Company, Philadelphia, London.

Ham, A. W. 1965. Histology, pp. 884-917. Lippincott Company. 5th edition. Philadelphia, Montreal.

Harding, L. K. 1961. The survival of germ cells after irradiation of the neonatal male rat. Int. J. Rad. Bio. 3:539-551. Cited in: Mandl, Anita M. 1963. The radiosensitivity of germ cells. Biol. Rev. 39:288-371.

Heller, C. G. and Y. Clermont. 1964. Kinetics of the germinal epithelium in man. Recent Prog. Horm. Res. 20:545. Cited in: Oakberg, E. F. 1968. Mammalian gametogenesis and species comparisons in radiation response of the gonads. Report of a Study Group. Effects of Radiation on Meiotic Systems. IAEA. Vienna.

Hupp, E. W., H. B. Pace, E. Furchtgott, and R. L. Murphree. 1960. Effect of fetal irradiation on mating activity in male rats. Phychol. Rep. 7:289-294.

Hupp, E. W., S. O. Brown, and J. W. Austin. 1969. Continuous and acute irradiation effects on developing animals. In: Sikov, M. R. and D. D. Mahlum (eds.). Radiation Biology of the Fetal and Juvenile Mammal. U.S. AEC, Division of Technical Information Conf. 690501, pp. 589-602.

Hwang, U. K., H. B. Pace, and E. W. Hupp. 1964. Postnatal testicular morphology of male rats. Texas J. of Sci. 16:484.

Kitaeva, O. N. 1960. The influence of X-ray irradiation on the ovaries of mice and rats. J. of Gen. Bio. 21: 439-446. Cited in: Mandl, Anita M. 1963. The radiosensitivity of germ cells. Biol. Rev. 39:288-371.

Kochar, K. and B. K. Batra. 1967. The effect of X-rays on the ovaries of the progeny of irradiated mice, with special reference to small oocytes. Rad. Res. 31:79-90.

Kohn, H. I. 1955. On the direct and indirect effects of X-irradiation on testes of rats. Rad. Res. 3:153-156.

Krohn, P. L. 1967. Factors influencing the number of oocytes in the ovary. Archives D'anatomie Micro. de Morph. Exper. 56:151-159. 1957.

LaCassagne, A., J. F. Duplan, H. Marcovich, and A. Raynaud. 1962. The action of ionizing radiation on the mammalian ovary. In: The Ovary, vol. 11:463-532. Ed. Zuckerman, S., Anita M. Mandl, and E. Ekstein. London: Academic Press, Inc.

Lacy, D. 1964. Comparison of effects produced by high dose of ionizing radiation and oestrogenic hormone on the seminiferous tubules of rat testes. In: Effects of Ionizing Radiation on the Reproductive System, pp. 189-212. Ed. D. W. Carlson and F. X. Gassner. Pergamon Press, New York.

Leonard, A. and J. R. Maisin. 1963. Influence des rayons X sur la fertile de jeunes rates irradies 8 ou 17 jours apres la naissance. Cr. Soc. Biol. Paris 157:1529-1533. Cited in: Mandl, Anita M. 1964. The radiosensitivity of germ cells. Bio. Res. 39:288-371.

Mandl, Anita M. and S. Zuckerman. 1955. The reactivity of the X-irradiated ovary of the rat. J. Endocrin. 13:243-261.

Mandl, Anita M. and Margaret Shelton. 1959. A quantitative study of oocytes in young and old nulliparous laboratory rats. J. Endocrin. 18:444-450.

Mandl, Anita M. 1962. The radiosensitivity of oocytes at different stages of maturation. Proc. Roy. Soc. B., 158:119-141.

Mandl, Anita M. 1963. The radiosensitivity of germ cells. Biol. Rev. 39:288-371.

Mintz, B. 1960. Embryological phases of mammalian gametogenesis and species comparisons in radiation response of the gonads. Study Group Report: Effects of Radiation on Meiotic Systems. IAEA. Vienna, pp. 3-15.

Mole, R. H. 1959. Impairment of fertility by whole-body irradiation of female mice. Inter. J. Rad. Bio. 1 (2): 107-144.

Monesi, V. 1962. Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. J. Cell. Biol. 14:1-18.

Murphree, R. L. and H. B. Pace. 1960. The effects of prenatal irradiation on postnatal development in rats. Rad. Res. 12:495-504.

Nash, D. J. and J. W. Gowen. 1961. Reproductive performance of mice exposed to embryonic X-irradiation. Amer. Zool. 1:377.

Nebel, B. R. 1958. Fine structure of chromosomes in man and other Metazoa and testicular recovery from X-rays in mammals. Proc. 2nd. UNIC. Peaceful Uses of Atomic Energy 22:308-312.

Oakberg, E. F. 1956. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am. J. Anat. 99:507.

Oakberg, E. F. 1962. Gamma ray sensitivity of oocytes in immature mice. Proc. Soc. Exp. Biol. Med. 109:763-767.

Oakberg, E. F. 1966. Effect of 25 R of X-rays at 10 days of age on oocyte numbers and fertility of female mice. In: Lindop, Patricia J. and G. A. Sacher (eds.). Radiation and Ageing. Proceedings of a Colloquium. Austria, pp. 293-306.

Oakberg, E. F. and E. Clark. 1964. Species comparisons of radiation response of the gonads. In: Carlson, W. D. and F. X. Gassner (eds.). Effects of Ionizing Radiation on the Reproductive System. Pergamon Press, Oxford, pp. 11-24.

Oakberg, E. F. 1968. Mammalian gametogenesis and species comparisons in radiation response of the gonads. In: Study Group Report. Effects of Radiation on Meiotic Systems. IAEA. Vienna, pp. 3-15.

Ortavant, R. 1956. Autoradiographic des cellules germinals du testicle de Beliert; duree des phenomenes spermatogenetiques. Archs. Anat. micro. Morph. exp. 45:1. Cited in: Oakberg, E. F. 1968. Mammalian gametogenesis and species comparison in radiation response of the gonads. Study Group Report. Effects of Radiation on Meiotic Systems. IAEA. Vienna, pp. 3-15.

Parks, A. S. 1927. Proc. Roy. Soc. B. 101:71. Cited in: Mandl, Anita M. and S. Zuckerman. 1955. The reactivity of X-irradiated ovary of the rat. J. Endocrin. 13: 242-261.

Parsons, D. F. 1962. An electron microscope study of radiation damage in mouse oocyte. J. Cell. Bio. 14: 31-48. Cited in: Mandl, Anita M. 1964. The radiosensitivity of germ cells. Biol. Res. 39:288-371.

Partlow, Nancy E. 1969. Testis microstructure and function in rats irradiated on the seventeenth or eighteenth day of prenatal life or on the first day of postnatal life. Master's Thesis. Dept. of Bio. Texas Woman's University.

Peters, Hannah and E. Levy. 1961. Radiation sensitivity of oocytes at different stages of development in this immature mouse. Rad. Res. 15:582-593.

Peters, Hannah, E. Levy, and M. Crone. 1962. Deoxyribonucleic acid synthesis in oocytes of mouse embryos. Nature, Lond. 195:915-916.

Peters, Hannah and E. Levy. 1963. Effect of irradiation in infancy on the fertility of female mice. Rad. Res. 18:421-428.

Ricks, R. C. 1964. The postnatal development of the sex organs in the prenatally and early postnatally irradiated male albino rats. Unpublished Master's Thesis. Dept. of Bio. Texas A & M University.

Ricks, R. C. and E. W. Hupp. 1964. The postnatal development of the sex organs in the prenatally and early postnatally irradiated male albino rats. Texas J. of Sci. 16:491.

Rugh, R. and S. Jackson. 1958. Effects of fetal X-irradiation upon the subsequent fertility of the offspring. J. Exp. Zool. 138:209-221.

Russell, L. B., W. L. Russell, M. H. Steele, and E. L. Phipps. 1959. Estreme sensitivity of an immature stage of the mouse ovary to sterilization by irradiation. Science 129:1288.

Russell, L. B., S. K. Badgett, and C. L. Saylors. 1960. Comparison of the effect of acute, continuous and fractionated irradiation during embryonic development. Cited in: Chi-Hsien Chen Teng. 1969. Relationship between gonodal development and radiation sensitivity in the golden hamster. Master's Thesis. Dept. of Bio. Texas Woman's University.

Snedecor, G. W. 1956. Statistical Methods. Iowa State University. Ames, Iowa.

Szabo, T., S. M. Free, Helen A. Birkhead, and Patricia E. Gay. 1969. Predictability of pregnancy from various signs of mating in mice and rats. Lab. Animal Care 19:822-825.

Udgaonkar, S. M. and B. K. Batra. 1968. A study of the mitotically active germ cells of the gonads of X-irradiated chick embryos. Rad. Res. 33:337-347.

Van Eck-Vermande, G. J. 1959. Effect of low dosage X-irradiation upon pituitary gland and ovaries of the rhesus monkey. Fertil. and Steril. 10:190-202.

Witschi, E. 1948. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Contr. Embryol. 32:67. Cited in: Oakberg, E. 1967. Mammalian gametogenesis and species comparisons in radiation response of the gonads. Study Group Report. Effects of Radiation on Meiotic Systems. IAEA. Vienna, pp. 3-15.

# VITA

Margaret Louise was born December 27, 1921 to Mr. and Mrs. Ben F. Davis in Snyder, Texas. She graduated from the Rotan High School, Rotan, Texas in 1939 and entered McMurry College, Abilene, Texas that fall. For her junior year she transferred to West Texas State College, Canyon, Texas and continued there until January, 1942 when she withdrew to travel with her husband, Lt. Harold A. Capelluto, a pilot of the United States Air Force. After Lt. Capelluto was killed during the invasion of Europe in 1944, she and her young son returned to Colorado City, Texas, where she was employed in a refinery of Standard Oil Company as a laboratory technician. In 1946 she was employed by the public schools of Rotan, Texas to teach Biology and Chemistry.

In 1947 Margaret married Elmo A. Teague. She entered Howard Payne College in January 1959 and graduated in August with a B. A. degree in Chemistry. She taught science in the schools of Coleman and Comanche, Texas until 1962 when she entered Texas Woman's University on the A. Y. I. program sponsored by the National Science Foundation. She earned a Master of Science Degree in Science Education in 1963.

Mrs. Teague was employed by the Public School System of Killeen, Texas to teach Chemistry in 1964. In June 1969, she

took a leave of absence to continue work toward the Ph. D. in Radiation Biology. She returned to Killeen with two of her five children in September 1970. She returned to Killeen High School as the Head of the Science Department and teacher of Chemistry.

She is an active member of Delta Kappa Gamma, American Association of University Women, Business and Professional Women's Association, Texas State Teachers Association, Texas Classroom Teachers Association, and National Education Association.