A Study on the Histological Effects of Gamma Radiation on the Gonads of Mice 24 Hours and One Week After Exposure

by

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I. A B S T R A C T

Fourty mice, twenty males and twenty females were divided into five groups and given whole-body exposure of different levels of gamma radiation : 0, 150, 300, 600 and 750 rads. Two males and two females from each level were sacrificed after 24 hours, while the rest were sacrificed after one week. The testes and ovaries were studied histologically. This study aims to compare the effects of different levels of gamma radiation after 24 hours and to determine whether a regeneration or repair has occured after one week or not.

It was found out that the spermatogonia were the most sensitive among the spermatogenic cells while the spermatozoa were the most resistant. At 150 and 300 rads, the spermatogonia were the most affected. At 600 rads, the spermatogonia were very few and some spermatocytes have disappeared. At 750 rads, only a few spermatocytes but much spermatids and spermatozoa were present. After one week, all levels showed a decrease in the number of cells, particularly the spermatogonia. At 600 rads, a new type of cell, having large, compact, dark-staining nucleus was observed.

In the ovaries, it was observed that the most sensitive were the primordial and primary follicles while the Graafian follicles were more resistant. In the 300 rads, a cyst surrounded by columnar epithelium was observed. Primordial and primary follicles decreased continuously as the dose increased. Likewise, many secondary follicles showed atresia. After one week, the ovaries showed many empty spaces within the organ, signifying great degeneration of the follicles. The follicles, especially the primordial and primary ones showed a decrease in number.

It was concluded that after one week, regeneration has not yet taken place in all the doses used here. But if regeneration were to occur after one week, it can be said that the gonads exposed to the higher doses, 600 and 750 would need longer time to recover because here, the stem cells were almost totally wiped out.

I. INTRODUCTION

The past 250 years has seen the full development of science and technology - a development that has totally changed the direction of the world. This development has been so rapid and so widespread that all people have been in one way or another affected by it. In fact, it has become so much ingrained in man's life that many products of science and technology are not any more considered as luxuries, but "necessities" . In many instances,these products have been tools in saving lives, in the success and failures of industries, and as determinants of the rise and fall of countries. Indeed, science and technology have produced innovations and many new equipment which have helped man live comfortably and longer.

With the invention of the atomic bomb in 1945, one part of science and technology came into heavy focus - that of radiation. Since then, it has been widely studied and widely used. Indeed, it has numerous applications in different fields such as medicine, agriculture and industry. Radioisotopes are now commonly used for the diagnosis of certain diseases; radionuclides like Iodine-131, Arsenic-74 or Phosphorus-32, Sodium chloride - 24 are used to test thyroid functions, presence of brain tumors, and to measure blood supply, respectively. Furthermore, other radionuclides like Cobalt-60 are used for therapy, especially in cancer. In agriculture, they are also used to

study plant-relationships, plant breeding and the metabolism of animals. Radiation has also been very useful in the industry. It has been used in food treatment, in controlling pests and in the process of sterilization. Radiation proves to be capable of preserving food by inhibiting or destroying the microorganisms. Exposure to radiation renders insects sterile, thus making them unable to perpetuate their own kind. It is also now used to sterilize medical products like syringes, absorbent cotton, gloves etcetera (Pahl, 1980). Furthermore, nuclear reactors are now so widely used in many industrialized and developing countries like Japan, America, France, England and Taiwan. These reactors have been sources of electrical energy, and have propelled ships. It can indeed be said that radiation has brought innumerable benefits to mankind.

However, radiation is a two-edged sword. While it has served its "master" well, it has also given numerous hazards to users. While it helps diagnose and treat diseases, it is in itself the cause or the perpetuator of some of these diseases. While it is a tool to provide basic services like electricity, accidents or improper and careless construction and handling of these equipments had resulted to deaths and casualties as shown by the Chernobyl accident in the Soviet Union. Especially susceptible are the personnel who work and operate these equipments. But the most alarming is the use of radioactive materials in making war weapons. Atomic bombs and now nuclear bombs are just a "push of

button" away. In fact, the survivors of the 1945 bombing of Japan are just manifestations of the great destruction and sufferings that are brought about by radiation. It is capable of inflicting serious damages on all parts of the human body, on all animals and plants and even the environment. However, it is impossible or very difficult at this stage to totally eradicate the use of radiation. What may be done is to minimize its harmful effects.

This paper aims to study the histological effects of radiation, specifically different levels of cobalt-60 gamma radiation on the gonads of mice. It is also the aim of this research to determine whether these organs , which might have suffered some damages would still be able to recover within one week.

However, the study would still be limited to the gonads of the mice. Although the other organ systems may be affected and may even be causes of death, the gonads will be the determining factor as to whether the individual would still be able to help in the perpetuation of species. Furthermore, only the histological parts will be studied. The physiology, gross anatomy and behavior would not be considered. Also, only gamma radiation would be used here. And lastly, the effects would be studied after twenty-four hours and after one week only due to time constraints. Other effects like regeneration/recoveries or degeneration after one week would not be studied anymore.

III. REVIEW OF RELATED LITERATURE

A. The Mouse

The mouse, scientifically named Mus musculus is a small, furry animal which is under the Order Rodentia. Rodents comprise the largest mammalian order and are distributed worldwide. Kent (1987) gave the characteristics of this order : they have a single pair of long, curved, upper and lower incisor teeth that are used for knawing. These teeth have enamel on their outer surface only, which provides a chisel-like edge as the softer dentin wears away. The teeth grow throughout life. Canine teeth are absent, so there is a stretch of toothless jaw or diastema between the incisors and the first grinding teeth. Rodents are generally cellulose eaters and at the beginning of the large intestine, there is a long, coiled cecum that houses commensal cellulose-digesting microorganisms. Hyman (1965) further added that rodents have a smooth or slightly convoluted brain, and there is the presence of clavicles. There are three suborders : Sciuromorpha, Myomorpha and Caviomorpha (Hystricomorpha). The mouse' place in the classification scheme is as follows :

PHYLUM	:	Chordata
SUBPHYLUM	:	Vertebrata
CLASS	:	Mammalia
SUBCLASS	:	Eutheria or Monodelphia or Placentalia
ORDER	:	Rodentia
SUBORDER	:	Myomorpha
GENUS	:	Mus
SPECIFIC EPITHET	:	musculus

The male reproductive organs consist of paired testes, urethra, penis, and associated ducts and glands. Female reproductive organs consist of paired ovaries and oviducts, uterus, cervix, vagina, clitoris and paired clitoral glands. External influences such as noise, diet, light and population density, play an important role in reproduction and directly or indirectly influence the hypothalmic-pituitary axis for hormonal control of ovarian and testicular function. Genotype also dramatically affects the reproductive performance of the mouse (Jacoby and Fox, 1984).

Follicle-stimulating hormone promotes gametogenesis in both sexes. Luteinizing hormones promote the secretion of estrogen and progesterone in the female and androgen in the male. Prolactin promotes lactation and development of the ovary during pregnancy. These gonadal hormones also ensure proper maintenance of the reproductive tract and modulate behavior to promote successful mating. The hypophysis is usually responsive to hormonal influence by day 6 in the male and by day 12 in the female. Ovarian follicle development begins at 3 weeks of age and matures by 30 days. Rising titers of gonadotropin evoke signs of sexual maturity at about the same age. In the female, estrogen-dependent changes such as cornification of vaginal epithelium at the vaginal opening can occur as early

as 24-28 days. Puberty is slightly later in the male (up to 2 weeks). Sexual maturation varies among strains and stocks of mice and is subject to seasonal and environmental influences. Mating behavior and ability to conceive and carry fetuses to parturition are under the complex hormonal balance mediated by the anterior pituitary (Jacoby and Fox, 1984).

The mouse is polyestrous and cycles every 4 to 5 days. In the first two phases (proestrus and estrus), active epithelial growth in the genital tract culminates in ovulation. Degenerative epithelial changes occur during the third phase, followed by diestrus, a period of quiescence or slow cell growth. The cycle can be followed by changes in the vaginal epithelium that are often used to determine optimum receptivity of the female for mating and fertilization. Patency of the vaginal orifice and swelling of the vulva are useful signs of proestrus and estrus. Irregularities of the estrous cycle occur during aging. Seasonal and dietary factors and genetic backgrounds also influence estrous cycles. Estrus is routinely observed in mice at about 14-24 hr after parturition (postpartum estrus). The cyclity of estrus and ovulation are controlled by the diurnal rhythm of the photoperiod. Mating, estrus and ovulation most

often occur during the dark phase of the photoperiod. Reversing the timing of the light-dark cycle reverses the time of estrus, ovulation and mating (Jacoby and Fox , 1984).

Gestation is usually 19-21 days. Because of postpartum estrus, lactation and gestation can occur simultaneously. Lactation can delay gestation because of delayed implantation. This may cause prolongation of gestation for up to 12-13 days in inbred strains. The effective reproductive life of some inbred strains approaches 2 years where optimum environmental conditions are maintained, but litter size usually decreases as the female ages. Therefore, females are usually retired by 1 year of age. Average litter size is strain dependent and commonly ranges from 1 to 12 pups (Jacoby and Fox, 1984).

Since this study is dependent on the sex of the mice, it is necessary to familiarize oneself in differentiating males from females. The following may apply both to mice and albino rats . The most dependable external characteristic by which to recognize sex at birth is the ano-genital distance. This distance is measured from the anal aperture to the base of the genital papilla (clitoris or penis). The ano-genital distance is always much greater in the male than in the female of the same age (Figure

1, Appendix B). Furthermore, the genital papilla of a male is much larger than the genital papilla of a female of the same age. These two characteristics apply at birth and at all subsequent ages (Greenman and Duhring, 1931).

In addition to these external characteristics, the following may be noted :

At sixteen or seventeen days of age, when the rat has acquired a coat of hair, the line between the anus and the genital papilla of the female remains relatively bare, while in the male this line or space is covered with hair except a small area just ventral to the anus. This small bare area corresponds to the dorsal part of the scrotal area where, after the sixth week, the testes may occasionally be found.

From eight to fifteen days of age the mammae (nipples) of the female, six pairs, are clearly visible. Later these are obscured by hair (Greenman and Duhring, 1931).

Table 1, Appendix A gives some normative data about the mice .

B. The GONADS

The testis is a compact ovoid compund tubular gland enclosed in a thick fibrous capsule called the tunica albuginea because of its white fibrous

appearance. Thin fibrous septa, called SEPTULA TESTIS, extend into the tunica albuginea, dividing the organ into pyramidal compartments, the lobuli testis. Each lobule is composed of one to four highly convoluted SEMINIFEROUS TUBULES. These constitute the exocrine portion of the testis, which is in essence a cytogenous gland whose holocrine secretory product is whole cells, the spermatozoa. The tubules are highly convoluted loops, but they may also branch or end blindly. The seminiferous tubules are enclosed by one or more layers of adventitial cells derived from primitive connective tissue elements of the interstitium. In common laboratory rodents, there is single layer of flattened polygonal cells that meet edge to edge to form a continuous epitheloid sheet surrounding the tubule. These cells are referred to as MYELOID CELLS or PERITUBULAR CONTRACTILE CELLS (Bloom and Fawcett, 1986).

Each adult seminiferous tubule contains supporting cells and numerous microscopically recognizable stages of spermatogenesis, the sequence of events by which spermatogonia are transformed into spermatozoa.

The Sertoli cells are the supporting, nonproliferating, columnar epithelium resting upon the basal lamina of the seminiferous tubule and extending upward through the full thickness of the

epithelium to its free surface. Sertoli cells have a rather large pale-staining nucleus that is commonly located in the basal region of the cell; they are elongated to ovoid, with irregular indentations. The Sertoli cell has homogenous nucleoplasm, except for a large and highly characteristic nucleolus (Ham and Cormack, 1987). The cells comprising the different stages in spermatogenesis are the spermatogonia, spermatocytes, spermatids and spermatozoa.

The spermatogonia are the large, spherical germ cells basally situated adjacent to the surrounding basement membrane. It is the diploid predecessor from which spermatocytes arise. The spermatogonium is of two types : Type A spermatogonium has a spherical or ellipsoid nucleus with very fine chromatin granules and one or two irregularly-shaped nucleoli attached to the inner aspect of the nuclear envelope. The cytoplasm is homogeneous and pale-staining. In some, the nucleoplasm is dark and a large pale-staining nuclear vacuole is present. While Type B spermatogonium has a spherical nucleus, containing chromatin granules of varying size, many of which are distributed along the nuclear envelope. The single nucleolus is centrally located and often has granules of chromatin associated with it. The Type A spermatogonium undergoes a series of divisions that give rise to other Type A

spermatogonia. Of these progenies, certain ones serve as stem cells for future cycles of spermatogonial renewal and spermatogenesis. Others proceed to differentiate through recognizable intermediates into Type B spermatogonia. The division of Type B spermatogonia then gives rise to primary spermatocytes (Bloom and Fawcett, 1986).

The primary spermatocytes at first resemble in size and cytological characteristics the spermatogonia from which they arise, but as they move away from the basal lamina of the germinal epithelium, they accumulate more cytoplasm and become distinctly larger. The primary spermatocyes will undergo the first part of meiosis to become secondary spermatocytes. Secondary spermatocytes are smaller and are situated in the middle layers and the more superficial layers of the seminiferous epithelium. These cells miss the S phase of the cell cycle and almost immediately undergo the second maturation division. The daughter cells of the secondary spermatocytes are the spermatids. At first, spermatids are rather small round cells with a spherical nucleus. They then elongate and the nucleus takes up a peripheral position relative to the lumen of the tubule. Each spermatid undergoes spermiogenesis to change into a spermatozoon (Ham and Cormack, 1987).

Each spermatozoon is made up of a head, a

midpiece that represents the proximal part of the tail and the main part of the tail. The somewhat flattened ellipsoidal head contains the nucleus, which is relatively rigid and is packed with highly condensed chromatin. The anterior end of the nucleus is invested by the acrosomal head cap. The midpiece and the remainder of the tail comprise the flagellum. The midpiece of the spematozoon also incorporates the mitochondrial sheath and a small quantity of cytoplasmic matrix. The rest of the tail consists of a principal piece and an end piece (Ham and Cormack, 1987).

It has been the traditional view that each spermatogonium divided and the daughter cells each developed into a primary spermatocyte; this ultimately divided into two secondaries, and these in turn divided to form four individual spermatids. This interpretation is now known to be incorrect. In all but the earliest spermatogonial division, cytokinesis is incomplete, resulting in groups of conjoined spermatogonia and larger syncytial clusters of primary spermatocytes. These produce double the number of interconnected secondary spematocytes. Their division in turn produces very large numbers of conjoined spermatids. The progeny of a single spermatogonium thus form a cluster of germ cells that remain in protoplasmic continuity

throughout their differentiation. This arrangement is probably responsible for the synchrony of development of large numbers of germ cells in any one area of the seminiferous tubule. Individual sperms are separated from the syncytia at the moment of their release from the epithelium (Bloom and Fawcett, 1986).

The ovaries are slightly flattened ovoid paired organs which are covered with a cuboidal epithelium. The ovary has a thick peripheral zone or CORTEX surrounding the MEDULLA. Embedded in the connective tissue of the cortex are follicles containing the female sex cells, the oocytes. The follicles are present in a wide range of sizes representing various stages of their development. When a follicle reaches maturity, it ruptures at the surface of the ovary to release the ovum, which then enters the open end of the neighboring oviduct. The boundary between the ovarian cortex and medulla is poorly defined. The medulla consists mainly of loose connective tissue and a mass of contorted blood vessels that are large in proportion to the size of the ovaryv (Bloom and Fawcett, 1986).

The ovary is covered by a continuous sheet of squamous or cuboidal epithelium, named the GERMINAL EPITHELIUM. Beneath this is layer of dense connective tissue, the TUNICA ALBUGINEA , the white appearance that its name suggests is due to the

great content of intercellular substance and lack of vascularity. Its fiber bundles and cells are arranged more or less parallel to the surface. Embedded in the stroma of the cortex deep to the tunica albuginea are the follicles. The vast majority of the follicles are the PRIMORDIAL or UNILAMINAR FOLLICLES. Each consists of a large round primary oocyte that is in the resting (dictyotent) stage of the meiosis I. Surrounding the primary oocyte is a single layer of squamous follicular epithelial cells, which are alternatively known as GRANULOSA cells, enclosed within their basement membrane. The oocyte has a large, eccentrically placed vesicular nucleus with a conspicous nucleolus (Bloom and Fawcett, 1986).

As the oocyte enlarges, the follicular cells become cuboidal or low columnar and by mitotic proliferation give rise to a stratified epithelium of granulosa cells, thus transforming the primordial follicle to a multilaminar primary follicle. As the follicular epithelial cells proliferate, the walls of the follicles come to contain more than a single layer of these cells. In addition, an amorphous material accumulates in a space between the oocyte and the granulosa cells to form the ZONA PELLUCIDA which is a highly refractile glycoprotein layer. Also the stromal cells surrounding the follicle

differentiate into a capsule-like theca (Bloom and Fawcett, 1986). However, only one of the follicles that is beginning to mature in each cycle actually completes the process; the remainder undergoes a degenerative process called FOLLICULAR ATRESIA. In atretic follicles, there is the absence of blood clot because bleeding occurs only if the follicle reaches maturity and ruptures, disorganization of the normal arrangement of the follicular cells and pyknosis of their nuclei or shrinkage or other kinds of distortion of the oocyte or its nucleus (Ham and Cormack, 1987).

In the secondary follicle stage, the enlarging follicle becomes oval in shape and the oocyte eccentric in position. Irregular spaces filled with clear fluid appear among the granulosa cells. This fluid, called the LIQUOR FOLLICULI increases in amount as the follicle enlarges and the irregular spaces among the granulosa cells become confluent to form a single crescentic cavity, the ANTRUM. Thenceforth,this secondary follicle is described as antral follicle.The typical small antrum follicle is lined with a stratified epithelium of granulosa cells, which displays a local thickening on one side called the CUMULUS OOPHORUS. This thicker region protruding into the fluid-filled cavity has the oocyte in its center. The oocyte is surrounded by a single layer of cuboidal follicular cells whose

apical processes are firmly anchored in the zona pellucida. This cellular investment is referred to as the CORONA RADIATA (Bloom and Fawcett, 1986).

In the mature follicles (GRAAFIAN FOLLICLES), mitotic figures gradually decrease in number among the granulosa cells. Intercellular spaces among the inner layers of the epithelium become more prominet. THe connection of the ovum and the associated granulosa cells of the cumulus oophorus with the rest of the epithelium is gradually loosened by the development of new, liquid-filled intercellular spaces. In the loosening up of the cumulus, the ovum, together with its zona pellucida and corona radiata are freed of their attachment in preparation for ovulation (Bloom and Fawcett, 1986).

Following ovulation and discharge of the liquor folliculi, the wall of the folliculi collapses, and its granulosa cell lining is thrown into folds. The basal lamina that formerly separated the granulosa and the theca interna is depolymerized. There may be some associated blood from the capillaries, resulting in the formation of a central clot. The cells of the plicated granulosa cells and those of the theca interna then undergo cytological alterations. They enlarge, accumulate lipid and are transformed into plump, pale staining polygonal cells - the lutein cells. After these postovulatory

changes have taken place, the follicle is called the CORPUS LUTEUM (Bloom and Fawcett, 1986).

C. GAMMA RADIATION

George Pahl gave a clear and complete discussion of the nature and properties, and mechanism of interaction of gamma radiation.

Gamma rays are electromagnetic radiations. Electromagnetic radiations are characterized by their wave property. Each has its own wavelength - distance between successive crests, and frequency - number of cycles per unit time. The entire spectrum of electromagnetic radiations is present in our environment in the form of radiowaves, infrared, visible, ultraviolet, x-ray and of course, gamma rays. Their speed is independent of their wavelength, frequency and energy and is the same as that of light (about 3×10^{10} cm/sec). Their wavelength, frequency and energy are correlated by the following equations :

$$c = wf \quad \text{and} \quad E = hf$$

where c is the speed of light, w is the wavelength, f is the frequency, E is the energy and h is Planck's constant, with a numerical value of 4.15×10^{-15} eV-sec.

Gamma-enegy values for the most commonly used gamma emitters vary roughly between 0.2 and 1.5 MeV.

A gamma photon originates from an unstable nucleus through a radioactive decay process. It is either monoenergetic or consists of few discrete energies.

The interaction of gamma radiation with matter may take the form of any of the following :

1. Photoelectric Effect

Upon striking the electron, the gamma photon disappears completely, and its energy is transferred to the electron (usually an electron in the K shell of the atom), which is ejected from the atom, with the consequent formation of an ion pair. The ejected electron is usually called a photoelectron. Photoelectrons have enough kinetic energy to become sources of ionizations, such that along their paths they will strip orbital electrons from other atoms of the absorber, thus producing secondary ion pairs (Figure 2, Appendix B).

2. Compton Scattering

Unlike the photoelectric effect, Compton Scattering is a type of interaction that does not occur with electromagnetic radiation of lower energy than X-rays and gamma rays. The incident gamma photon collides with an orbital electron without disappearing; it is simply deflected (scattered) from its path at an angle, and after the collision it has a longer wavelength and therefore lower frequency and energy than the incident photon. The

energy lost by the photon is imparted to the electron, which is usually one of the outermost orbits; the electron (Compton electron) is ejected from the atom with a certain amount of kinetic energy and thus with the ability of producing ion pairs (Figure 3, Appendix B).

In Compton Scattering, the gamma photon seems to behave like a particle. The interaction between the incident photon and the electrons results in the same type of effects as the collision between two particles. The discovery and explanation of the Compton effect led for the first time to the concept that electromagnetic waves present some properties of particles .

3. Pair Production

This is a type of interaction that cannot take place unless the photon energy is at least 1.02 MeV, and its probability increases with the atomic number of the absorber. Pair production is a rather startling phenomenon because it involves the conversion of energy into matter, as predicted by the general theory of relativity. When a high energy gamma photon passes very close to a nucleus of high atomic number it interacts with the strong nuclear electrostatic field and disappears. Its energy is entirely converted into two material particles, a negative electron (negatron) and a positive electron

(positron), hence pair production. This creation of particles from energy is sometimes called "materialization of energy". For the formation of one negatron and one positron, at least 1.02 MeV of energy is required. Quite frequently, the two electrons possess enough kinetic energy to produce secondary ions along their paths. When the negatron has dissipated all of its energy, it will come to rest and continue to exist either as a free electron or as an orbital electron of an atom. When the positron comes to rest, it combines with a negatron and both together disappear (annihilation of matter). Their matter is converted to energy, and two gamma photons (annihilation of radiation) originate from masses of two annihilated particles,,each having an energy of 0.511 MeV and traveling in opposite directions (Figure 4, Appendix B).

D. RADIATION STUDIES

P. Desaive determined the part played by restoration and by variation of radiosensitivity, in the final effect on the follicular system of the ovary using x-rays given in two types : either as a single dose or as the same total dose but equally fractionated. Three methods were used : a single dose of 2,500 rads (which sterilises the ovary and completely destroys the follicular system) in rabbits,

a single dose of 1,050 rads (which does not destroy or sterilise but is enough to cause much damage, partially restorable , to the follicular system) and the same dose of 1,050 rads, but given as five doses of 210 rads, at intervals of 48 hours. The results of counting primordial follicles in the 146 ovaries he studied showed that after a single sterilising dose (2,500 rads), disappearance of evoluting follicles is rapid and definite, while that of primordial follicles follows a simple exponential law specifically influenced by the factor of radiological destruction. After a single non-sterilising dose (1,050 rads), the curve of primordial follicles decreases first, in a similar manner to that of the 2500 rads, then from the 2nd day, this proportion grows considerably smaller owing to restoration. After a fractionated non-sterilising dose, the ovaries, when examined ten days later, are found to be less damaged than after the same lapse of time and when the same dose had been given in a single irradiation; but the damage is more extensive than on the second day following the single dose irradiation. Afterwards the number of follicles in this experiment decrease so that on about the 80th day the the effect is greater than was obtained in the 2nd experiment .

Cole, Habermeyer and Stolan studied the effects of different levels of single-dose exposure and

fractionated irradiation of X-rays on the fertility of mice. In the single-dose exposure, it was found immediately that there were no offspring after exposure to 810 rads plus single intravenous injection of 5 x 10^6 nucleated cells from the femoral bone marrow of normal, isologous donors. 150 rads, 100 rads, 75 rads and 50 rads were also used. At the 150 and 100 rads , the mice were completely sterile; no offspring were produced over a period of 6 months mating, whether or not bone marrow cells had been administered. In the first experiment with 75 rads, one of the 10 irradiated mice produced offspring (2 in the litter) at the first mating and none at the second mating or thereafter. In the corresponding group receiving 75 rads plus bone marrow, 2 out of 10 produced litters, but no offspring at the second mating or later. Following a single exposure of 50 rads, a definite decrease in productivity was detected after the second and third matings, and not necessarily at the first mating whether the mice received injection of bone marrow or not.

A fractionated irradiation using a total of 50 rads given in 5 consecutive days was done. It was found that fertility loss was less pronounced in the mice receiving 50 r in the fractionated schedule than in those exposed to 50 rads at a single sitting. Also drastic reduction in fertility was evident 12 months post-irradiation as compared with non-irradiated

controls of the same age.

Langendroff also studied the effect of repeated small doses on the fertility of the white mouse. He concluded that there was a cooperation of constitutional factors which add to the decreased fertility.

T.H.S. Hsu and J.I. Fabrikant studied the cell, population kinetics and radiation response of continuously irradiated mouse testes . The changes in spermatogonial cell population sizes and cell kinetic parameters have provided information on the degree of radiosensitivity within the seminiferous epithelium at sublethal continuous irradiation doses and the manner in which the tissue responds. At 1.8 rad/day, spermatogonial cell renewal was maintained at near-normal levels through compensatory cell proliferation and recruitment from the spermatogonia A stem cell pool. However, at 45 rad/day , depopulation of the testes took place through radiation depletion and maturation-differentiation of surviving elements. At this higher dose rate, the five type-A subpopulations were maintained at near-steady states of growth. The seminiferous epithelium displayed a remarkable capacity to recover. After two weeks of continuous irradiation at 45 rad/day, recovery commenced with proliferation from the type A spermatogonial stem cells, leading to full restoration of the seminiferous epithelium.

Grayevsky, Shapiro, Konstantinova and Barakina studied the role of cellular damage in the mammalian radiation syndrome. According to them, one of the most important consequences of irradiation was the early mass destruction of cells in radiosensitive systems. It was shown that : (1) early mass destruction takes place only in directly irradiated tissues; (2) radiosensitivity of bone marrow cells *in vivo* and *in vitro* does not differ significantly; (3) the early destruction is unlikely to be related to the process of division or to some radiation-induced chromosome damage; (4) early death occurs only in those cases where an affected substrate is put into operation. The specific function which makes radiation damage apparent may be assumed to be the process of differentiation.

Erickson and Martin studied the effects of continuous prenatal gamma radiation on the pig and rat. Pigs were irradiated continuously for the first 108 days of their 112-day gestation period at rates of 20, 9, 3 and 1.5 R per 22-hour day. Six pregnant gilts and six controls were employed at each dose rate. Fetal doses were 7, 3, 1 and 0.5 rad/day . Neither the health of the gilt nor the number of live births was affected by any exposure. Postnatal viability was also unaffected. Radiation effects on growth and organ development were assayed at birth, 70 and 150 days of age. Body weight and growth were unaffected by dose

rates of 3 rad/day or less; and other than the gonad, only the weight of the brain was affected by 3 rad/day. At 1 rad/day or less, only the gonadal weight was reduced. At doses of 7 and 3 rad/day, sterility was observed in both sexes. Following 1 rad/day, germ cell number was reduced to 5% and 2% of control in female and male, reaspectively. At 0.5 rad/day, germ cells were reduced to 43% of control in the female and 11% of control in the male. In contrast to the pig, 7 rad/day reduced the germ-cell population of male and female rats to only 49% and 35% of control, respectively, and 1 rad/day produced no apparent effect on either sex. It appears, therefore, that interspecific differences in the response to continuous gamma radiation are large and that the germ cell is the most labile cell type.

E.W. Hupp studied the effects of low-dose rate, continuous lifetime irradiation on Spanish goats. Groups of 11 or 12 goats of each sex were exposed to daily doses of 0, 2.6 , 7.2 , 15 , 29.6 and 40.8 R per 20-hour day from Cobalt-60 gamma radiation. The median lethal exposure at 7.2 , 15, 30 and 40 R/day were 2650, 2415, 2485 and 2004 R, respectively in females, and 8330, 3600, 2840 and 2286 R, respectively in males. Only 25% of the animals exposed to 2.6R/day died from radiation effects during the 48 1/2-month period. Performance of males in an obstacle course was not affected until after clinical signs of radiation sickness were apparent. While

profound decreases in sperm number and semen quality were observed, the ability to survive in the radiation environment was the only limiting factor affecting reproductive performance. Egon Lorenz and Walter Heston studied the effects of continuously irradiating male and female mice of the C_3H strain with 4.4, 1.1, 0.11 and 0.044 rads per 24-hour day. Histologically, only the gonads showed irradiation damage, mainly in animals exposed to 4.4 rads/day. THe damage in the testes consisted of diminished spermatogonia and reduction in the number of mature spermatozoa in the epididymis. This damage is reversible. Testes returned to normal after removal from exposure field. Irradiation damage to the ovaries, observed principally in the mice, receiving continuous doses of 4.4 rads/24-hour day and perhaps also those receiving 1.1 rad/24-hour day, is irreversible and progressive and results in some cases in tubular downgrowths of the germinal epithelium that progresses to early tumor formation. Breeding experiments indicate that C_3H female mice are permanently sterilized with doses of 4645 rads applied at the rate of 4.4 r/ 24-hour day.

In another study, these same authors together with Margaret Deringer cited the results obtained by Russ and Scott, who studied the effect of gamma radiation on young rats exposed continuously for 4 months on three levels such that they received total doses of 1410, 228

and 49.2 rad. Immediately after irradiation both males and females were mated to other irradiated animals or to controls. The males on the highest level never produced any young, the females did, but the young died in utero, at birth,or shortly thereafter. The animals on two lower levels produced normal offspring. Three months later, the males and females were remated. Two of the males and all but one of the females on the highest level produced young. However these young were no more vigorous than those of the preceeding mating. They concluded that the effects of gamma rays were largely cumulative.

Deringer, Heston and Lorenz further cited a work made by Snell who demonstrated that male mice irradiated with total doses of 600 to 800 r exhibited a fertile period of approximately 2 weeks when mated to normal females. This fertile period was followed by a sterile period of three months. The litters produced during the initial fertile period were reduced in size, which indicated that irradiation was responsible for changes in the mature sperm. When these irradiated males were mated to normal females, Snell found the following types of embryo : (1) embryos which were dead and beginning to degenerate, (2) embryos which were alive but with abnormal brains because of the failure of the neural groove to close, and (3) normal embryos.

Metcalf, Blandau and Barnett exposed albino

rats to a single dose of 550 rads of whole-body x-ray radiation and sacrificed them at intervals of around 15 min to 42 days after exposure.

In the testes, degeneration of spermatogonia occured within the first few hours, and shortly thereafter there occured a depletion of spermatogonia and later a disappearance of the remainder of the spermatogenic elements as the process of maturation continued. Spermatogonia began to reappear between the twentieth and fourtieth days.

Spermatozoa from the vasa deferentia exhibited no change in motility prior to the twenty-first day, but after this time motility was reduced or even absent in most cases.

Degenerative changes were observed in the granulosa cells of the ovarian follicles as early as 1 hour after exposure.

Although 25 per cent of the rats died after being exposed to 550 rads, this did not represent an accurate mortality figure for this dosage level since many of the animals were sacrificed prior to the period of highest death rate. Most of the deaths were attributed to septicemia.

Effects of radiations from internal sources on the testis were studied by Heller. She found out that only a few of the internally administered radioactive isotopes are capable of producing permanent sterility. In the doses used in her experiment, radium proved to

be extremely destructive and plutonium only slightly so. Strontium 89, yttrium 91, phosphorus 32, and barium 140-lanthanum 40 gave no evidence for permanent sterility. As a matter of fact, immature rats treated with strontium 89 at a dose about 85 per cent lethal, continued to develop spermia just as the untreated animals did. The entire testis was slightly smaller but it was proportional to the smaller size of the whole animal.

The effects on the ovary were studied by William Bloom. He showed that of the internally administered materials, radium and plutonium caused the most damage to the ovary in the doses administered. In terms of radioactivity rather than weight, 1 unit of intravenously injected plutonium is as effective as 10 units of intraperitoneally injected radium.

In the thesis made by Howell Ho, he exposed tilapia to 0, 0.5, 2 and 5 krads of whole body gamma radiation for a few minutes. He found out that the testes were normal in the 0.5 krad samples but exhibited deleterious effects in the 2 krad and 5 krad sections. The 5 krd samples were the most adversely affected with an almost total wipeout of germinal cells . The ovary was normal in the 0.5 krad sample but reduction in cell density was observed in both the 2 krad and 5 krad sections. At 5 krads, atretic follicles were seen in abundance.

IV. METHODOLOGY

(1) Forty white mice which were around three months old were used : twenty males and twenty females. They were divided into five groups, each group containing two males and two females. The males and the females were distinguished by comparing the ano-genital distance (distance between the anal aperture and the penis or clitoris). The distance was much greater in the male than in the female (Figure 1, Appendix B).

(2) Each group was given whole-body exposure of different levels of cobalt-60 gamma radiation (Figure 6, Appendix B). The different amounts of radiation were manifested in the differences in the amount of exposure time. Since the dose rate used was 52.3 kilorads per hour or 14.28 rads per second, the exposure time for each level was determined by the formula :

dose rate = dose / time

14.28 r/sec = dose / time

time = dose / 14.28 r/sec

where the dose had the values 0, 150, 300, 600 and 750 rads. The exposure time were 0, 10.3, 20.7, 41.3 and 51.7 seconds , respectively (Table 11, Appendix A).

(3) After irradiation, two males and two females from each group were sacrificed. The testes and ovary were located (Figure 5, Appendix B).
The organs were freed from the surrounding tissues and were fixed in formaloacetic acid containing 40 milliliter formalin, 40 ml. acetic acid and 360 ml ethyl alcohol.

(4) The rest of the mice were fed every afternoon with around 8 grams of food pellet and 30 ml of water for one week, after which they were also sacrificed. The testes and ovaries were removed and again fixed.

(5) The organs were cut using the microtome, mounted and stained with hematoxylin-eosin dye. Their histologies were observed under the microscope at different magnifications. The effects of the different levels of gamma radiation for both 24 hours and one week after exposure were studied; and for each level, the tissues for the 24 hours after exposure were compared to those of the one week after exposure.

(6) Photomicrographs of the sections were taken.

V. RESULTS AND OBSERVATIONS :

The gametogenetic cells of the testis were compared at

different levels : 0, 150, 300, 600 and 750 rads. Afterwards, each of the testis in the "after-24-hours" group was compared to its counterpart in the "after-one-week" group. The same procedure was done for the ovaries.

For the testis, the low power objective showed that the control, or the unirradiated one had very minimal spaces in between the seminiferous tubules. And the lumen of each seminiferous tubules was small or of the same thickness as the germinal epithelium (composed of the gametogenic cells and Sertoli cells) (Plate 1, Appendix C). For the irradiated testes, although the lumen of the seminiferous tubules of the ones exposed to 150, 300 and 600 rads were more or less the same as that of the control, the testis exposed to 750 rad showed a large increase in the thickness of the lumen and a sharp decrease in that of the germinal epithelium (Plate 2, Appendix C).

For the control, all the stages of gametogenesis, the spermatogonia, the spermatocyte, spermatid and spermatozoa were present (Plate 3, Appendix C). In the 150 rad, some reduction in the number of spermatogonia was seen. But the other stages seemed to be unaffected (Plate 4, Appendix C). In the 300 rad, the spermatogonia agained decreased, and a few spermatocytes had disappeared, although the spermatids remained the same (Plate 5, Appendix C). In the 600 rad (Plate 6, Appendix C), although a few spermatogonia remained in some of the seminiferous tubules, the other tubules contained almost no spermatogonia. The spermatocytes took up

the positions of these spermatogonia near the basement membrane, but the spermatids and spermatozoa were still numerous. In the 750 rads, the spermatogonia seemed to have been totally wiped out. The spermatocytes were still present, but they have decreased in number. The spermatids formed the majority of the cells in each seminiferous tubules, but these too decreased a lot. The number of spermatozoa was still large (Plate 7, Appendix C).

For both the 150 and 300 rad, the spermatogonia further decreased after one week (Plates 8 and 9 as compared to Plates 4 and 5 respectively). The spermatocytes seemed to be the same. The 600 rad testis after one week showed considerable variation from that of the 24-hour testis. The different stages of gametogenesis had almost disappeared. Only some spermatids developing into spermatozoa were more visible. Instead, one type of cell stood out in number. Each of these cells had a very large nucleus, occupying almost two-third of the whole cell. The nucleus seemed to be very compact and solid, and no trace of chromosome strands was visible. The nucleus stained very dark, the shape of the cell seemed to be a little bit irregular, and some of them were very large. Aside from this, the whole seminiferous tubule seemed to become "loosen" (Plate 10, Appendix C). In the 750 rad, all types of cells in the spermatogenic line decreased in number. (Plate 11, Appendix C).

The various parts of the ovary were observed using the scanner, low power and high power objectives. They were

compared based on the number of follicles and presence to tumors. The control or the unirradiated one showed all the types of follicles : primordial, primary, secondary and mature or Graafian follicles (Plate 12, Appendix C). The 150 rad (24 hours) seemed to be more or less the same as the control except for a very little decrease in the number of primary follicles (Plate 13, Appendix C). Likewise, the ovary of the mouse exposed to the 300 rad still had numerous secondary follicles. But there was the remarkable presence of a CYST lined with tall, columnar epithelium. The columnar cells seemed to have projections towards the lumen, and the nuclei were basally located (Plates 14, 15 and 16, Appendix C). For the 600 rad, Graafian follicles and secondary follicles were still very much present, but primary follicles decreased a lot (Plate 17,Appendix C). In the 750 rad, only a few primordial and primary follicles were present. In fact the whole ovary seemed to have become smaller. A follicle with theca seemed to have been sectioned near its surface. A regressing corpus luteum and an atretic follicle in a later stage were seen. Another follicle supposed to be in its secondary follicular stage was undergoing the early stage of atresia. Although the granulosa cells were still intact, they were beginning to slough off into the antrum which still contained liquor folliculi. A clump of follicle cells was also present. The different parts of the ovary seemed to have been loosened (Plate 18, Appendix C).

In comparing the ovaries exposed to 150 rads and sacrificed after 24 hours and one week (Plates 13 and 19, Appendix C), there seemed to be very little difference in the number of follicles, except that there seemed to be more spaces in-between the follicles and stroma in the one sacrificed after one week. In the 300 rad (after one week), there were fewer secondary follicles. Instead, there were atretic follicles, and obvious degeneration of foolicle cells, as manifested in the more empty spaces inside the ovary. There were also numerous epithelial tubes (Plate 20, Appendix C). In the 600 rad, the ovary seemed to have been "pulled apart", showing again numerous spaces. tho primordial and primary foolicles were nearly nonexistent. While there were numerous secondary follicles, there were lots of granulosa cells having no oocyte inside, like "rings" (Plate 21, Appendix C). In the 750 rads (after one week), numerous spaces were again seen, showing degeneration of follicle cells. The oocytes were not intact anymore within the follicle. two whole clumps of cells, possibly granulosa-cell tumors were present. These cells had nuclei which were dark staining anf cytoplasm which stained with eosin (Plate 22, Appendix C).

VI. DISCUSSION AND ANALYSIS

The above observation showed that spermatogonia are more radiosensitive, followed by spermatocytes. The spermatids and spermatozoa seemed to be radioresistant at

the doses used. The sensitivity of the spermatogonia may be attributed to the inhibition of mitosis. This result seems to agree with the findings of Bergonie and Tribondeau (Pahl, 1980), that is, cells with greater reproductive activity are more sensitive than those which do not undergo cell division. These spermatogonia, having to undergo mitosis, are more senstive than the spermatids, which need to undergo differentiation only, and the spermatozoa. it is possible however that even though these cells do not undergo mitosis, they are still capable of metabolizing and synthesizing cell materials like deoxyribonucleic acid (DNA) and proteins. thus, this might result to cells with numerous DNA and large nuclei. This phenomenon might have happened in the cells found in the testis exposed to 600 rads (after one week). From the results, it can be seen that the mice which were sacrificed after one week had lesser spermatogonia and spermatocytes. It can be seen that repair or regeneration of these types of cells have not yet taken place within the time of experimentation. Although the spermatids decreased a little, these spermatids and the spermatozoa were still numerous. In fact in some tubules, they were the only types of spermatogenic cells which were present.

A period of fertility may ensue (Pahl, 1980). This may be due to the fact that many of the spermatozoa and spermatids which were present before the irradiation were not damaged. These are capable of fertilizing the ova. furthermor, some spermatocytes and spermatogonia which might

not have been damaged may develop and grow into the more differentiated spermatids and spermatozoa.

From the observation of the ovaries, it can be seen that the primary follicles like the spermatogonia were the most radiosensitive, while the mature ones were the most resistant. Even the granulosa or follicle cells were sensitive. It seems that even after one week, the mice are still capable of being fertilized when the doses are 150, 300 and 600 rads. But in the 750 rad, the damage seems to be very widespread. This fertile condition of the mice afterone week may be due to the secondary and mature follicles which were radioresistant, and had not been damaged. Also, the sections showed numerous tumors, like granulosa-cell tumors and other cyts. However, some of these, like the cysts found in the 300 rads (after 24 hours) could not have been caused by the radiation due to the very short time interval. Also, because some cysts seem to be parts of a normal ovary, it can not actually be determined whether these were caused by the radiation or not.

VII. CONCLUSION

From the results, it can be concluded that among the gametogenic cells in the testis of mice, the youngest cells, the spematogonia are the most radiosensitive while the spematozoa are the most radioresistant. However, the Sertoli cells and interstitial cells are even more radioresistant. In the ovary, the primary follicles and primordial follicles

are also very sensitive while tha Graafian follicles are resistant. It is also concluded from the experiment that the cells are not capable of regeneration within onw week after exposure. However, this one week is still a part of a fertile period because spermatids, spermatozoa, and mature, graafian follicles are present in abundance. If no regeneration of "stem" cells occur, it may be hypothesized that a "sterile" period will occur.

VIII. RECOMMENDATION

This experiment indeed has its limitations, so for those who would wish to make further studies, it is recommended that :

(1) the effects of the radiation after one week be followed up to determine whether regeneration would occur or permanent sterility would set in;

(2) a statistic1 method be used to count the cells to have a more accurate result;

(3) effects of fractional doses be studied and compared to the effects of equal dose of radiation applied as a single dose ;

(4) more samples be used to determine whether some structures , e.g. cysts in the ovaries, are really normal occurences or are the results of radiation.

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Table I. Normative Data About the Mice (Jacoby and Fox,1984)

Adult weight	
Male	20-40 gm
Female	18-35 gm
Life span	
Usual	1-3 yr
Maximum reported	4 yr
Surface area	0.03-.06 cm^2
Chromosome number (diploid)	40
Water consumption	6.7 ml/8 wk age
Food consumption	5.0 gms/8 wk age
Body temperature	98.8 ^{o}-99.3 ^{o}F
Puberty	
Male	28-49 days
Female	29-49 days
Breeding season	None
Gestation	19-21 days
Litter size	4-12 pups
Birth weight	1.0-1.5 gm
Eyes open	12-13 days
Weaning	2i days
Heart rate	310-840 beats/min
Blood pressure	
Systolic	133/160 mm Hg
Diastolic	102-110 mm Hg
Blood volume	
Plasma	3.15 ml/100 gm
Whole blood	5.85 ml/100 gm
Respiration frequency	163/min
Tidal volume	0.18(0.09-0.38)ml
Minute volume	24(11-36)ml/min
Stroke volume	1.3-2.0 ml/beat
Plasma	
pH	7.2-7.4
CO_2	21.9 moles mM
CO_2 pressure	40 + 5.4 mm Hg
Leukocyte count	
Total	8.4(5.1-11.6)x10^3/ l
Neutrophils	17.9 (6.7-37.2) %
Lymphocytes	69 (63-75)%
Monocytes	1.2(0.7-2.6) %
Eosinophils	2.1(0.9-3/8) %
Basophils	0.5(0 -1.5) %
Platelets	600(100-1000)x10^3/ l
Packed cell volume	44(42-44)%
Red blood cells	8.7-10.5x10^8/mm^3
Hemoglobin	13.4(12.2-16.2)gm/dl
Maximum volume of single breeding	5 ml/kg
Clotting time	2-10 min

Table II. Amount of Radiation and Exposure Time for Each Group.

	Amount of Radiation (in rads)	Exposure Time (in sec)
Group 1	150	10.3
Group 2	300	20.7
Group 3	600	41.3
Group 4	750	51.7
Control	0	0.0

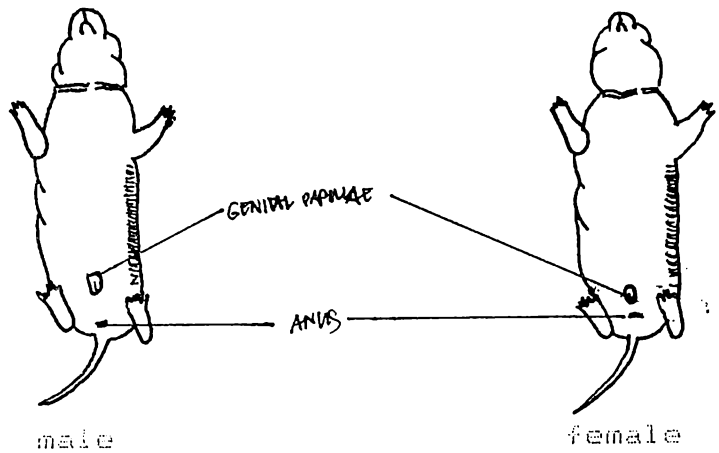


Figure 1 Ano-genital Distance of Male and Female (Greenman and Duhring, 1931)

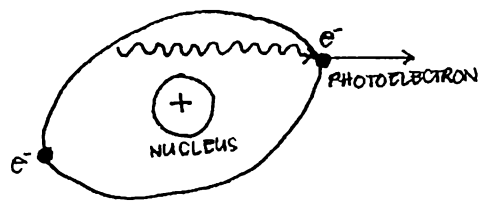


Figure 2. Photoelectric Effect (Pahl, 1980).

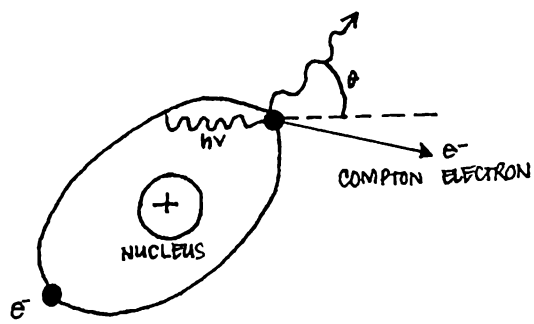


Figure 3. Compton Scattering (Pahl, 1980)

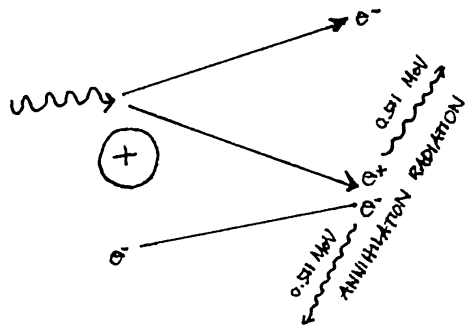


Figure 4. Pair Production (Pahl, 1980).

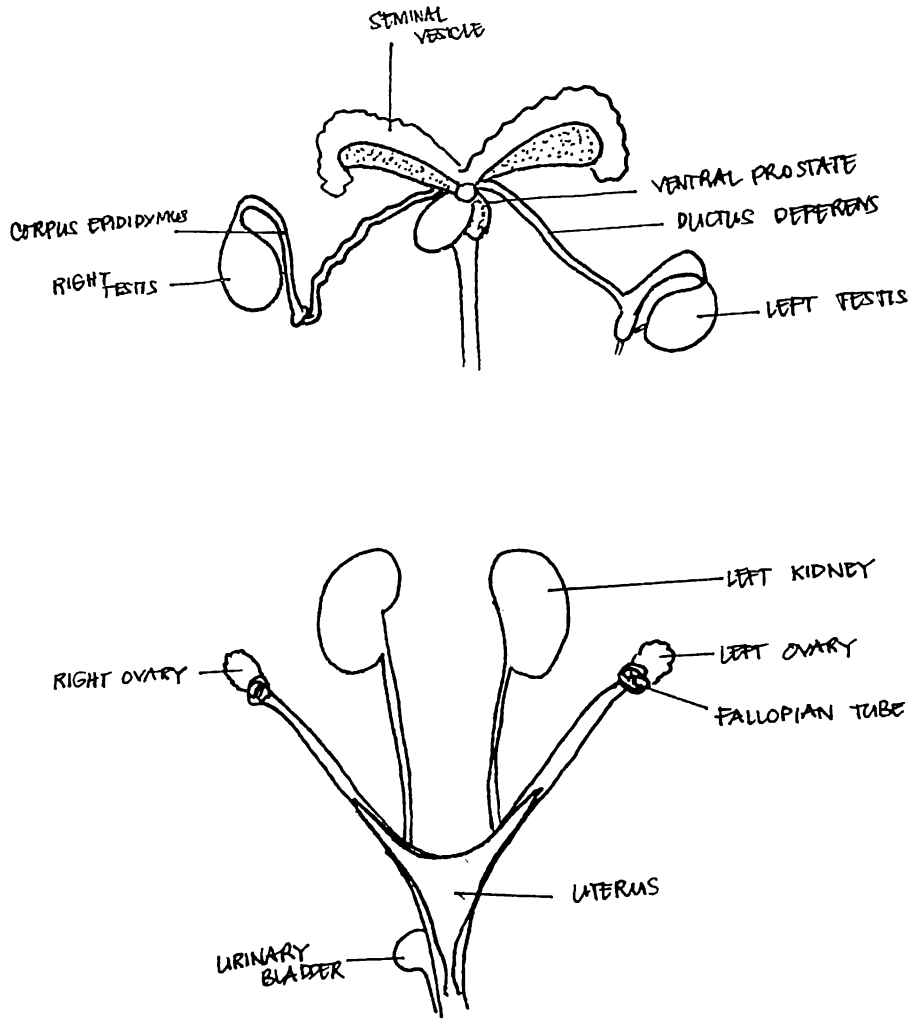
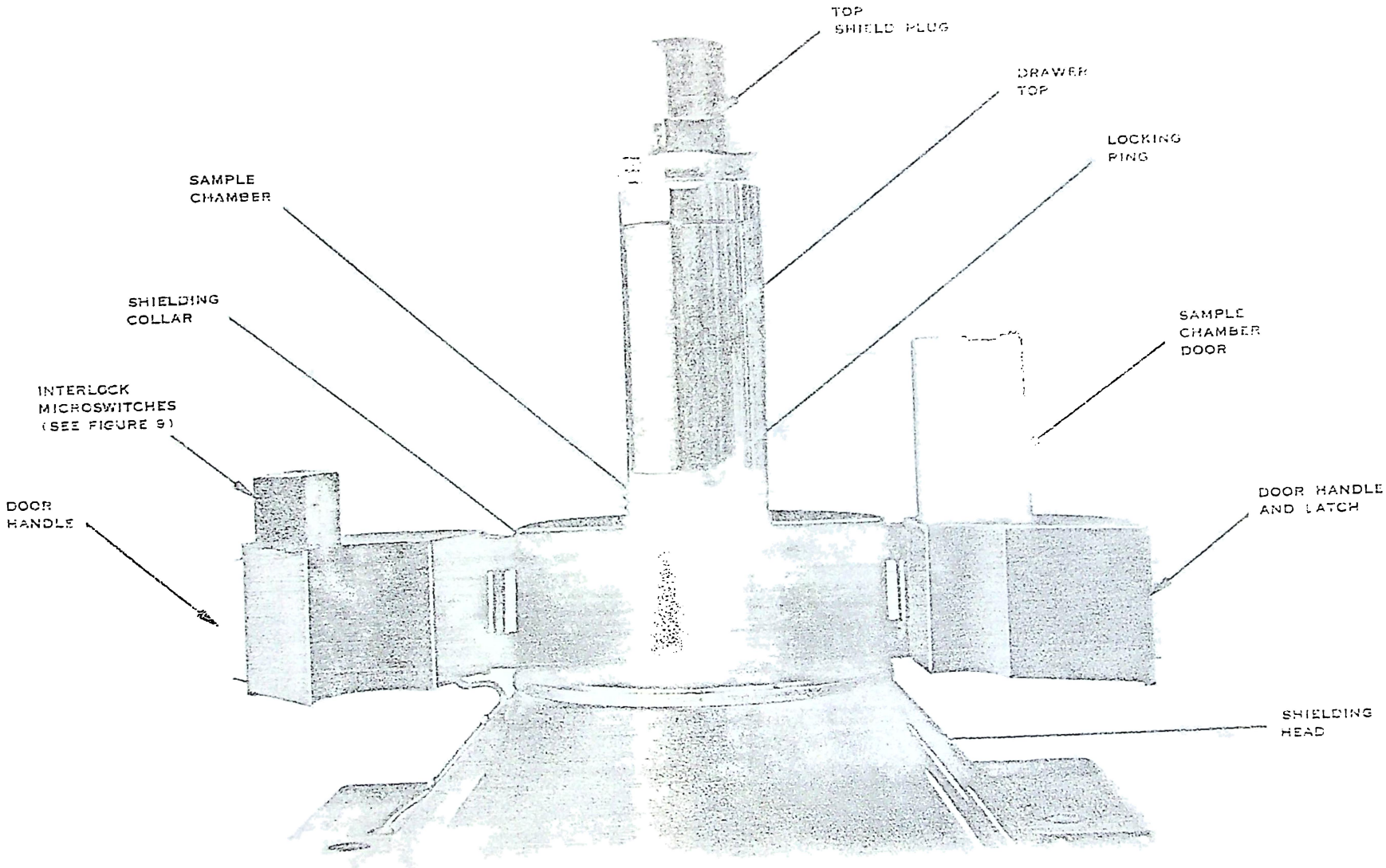


Figure 5. Testis and Ovary of Mice (Jacoby and Fox, 1984).



GAMMACELL 220

COLLAR AND SAMPLE CHAMBER

FIGURE 6

APPENDIX C

Legend (for Plates 1 and 2):

A - Seminiferous tubule
B - Germinal epithelium
C - Lumen of the Seminiferous Tubule

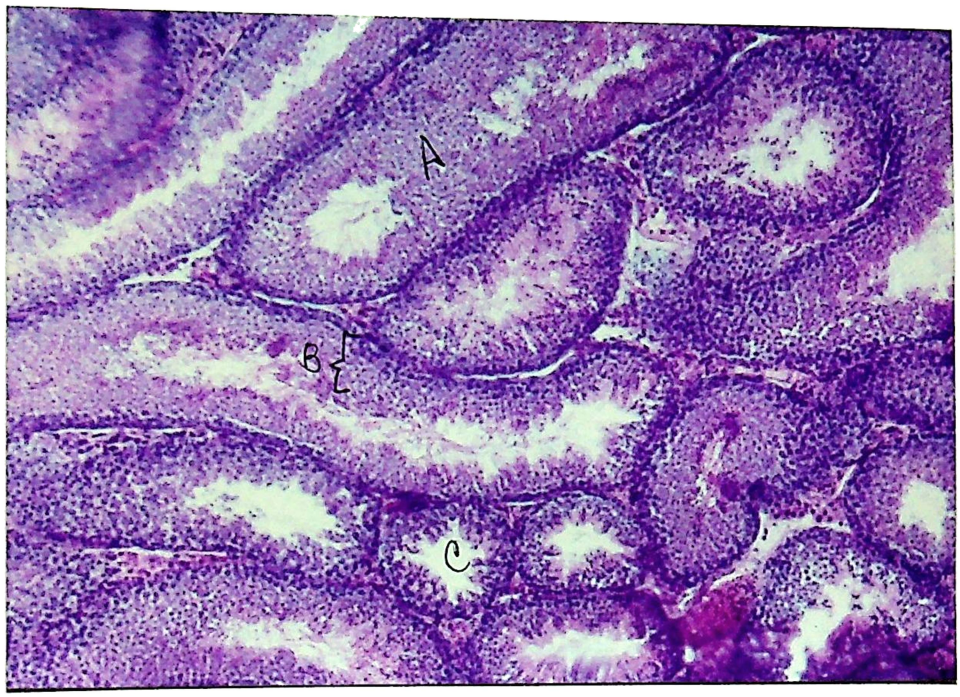


Plate 1. Normal Testis (100x).

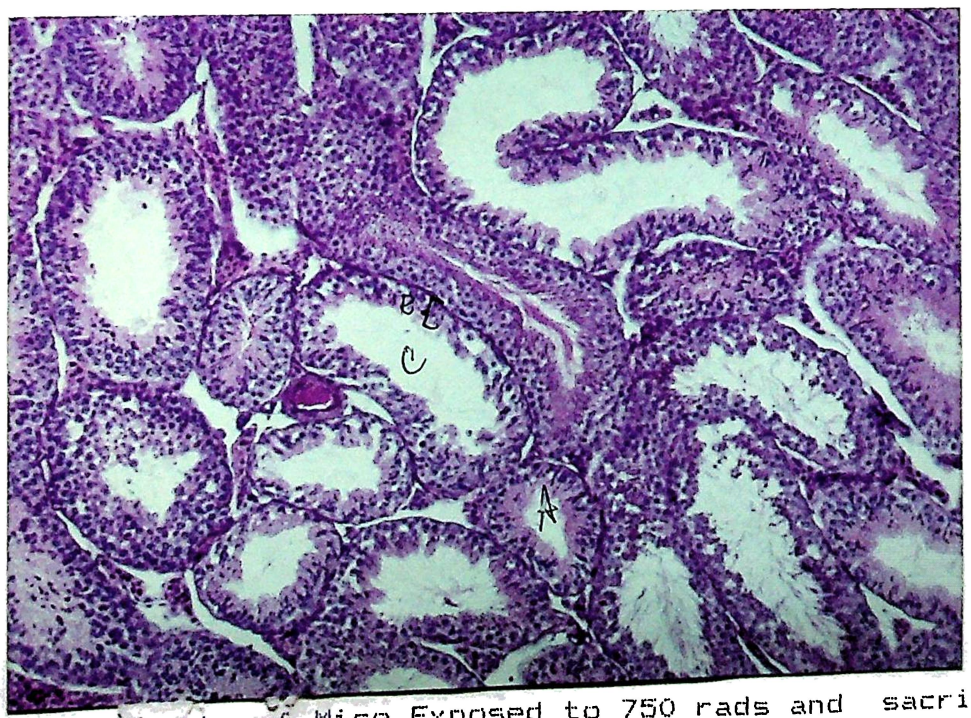


Plate 2. Testis of Mice Exposed to 750 rads and sacrificed after 24 hours (100x).

Legends (for Plates 3 to 11) :

A - Spermatogonia
B - Spermatocyte
C - Spermatid
D - Spermatozoa

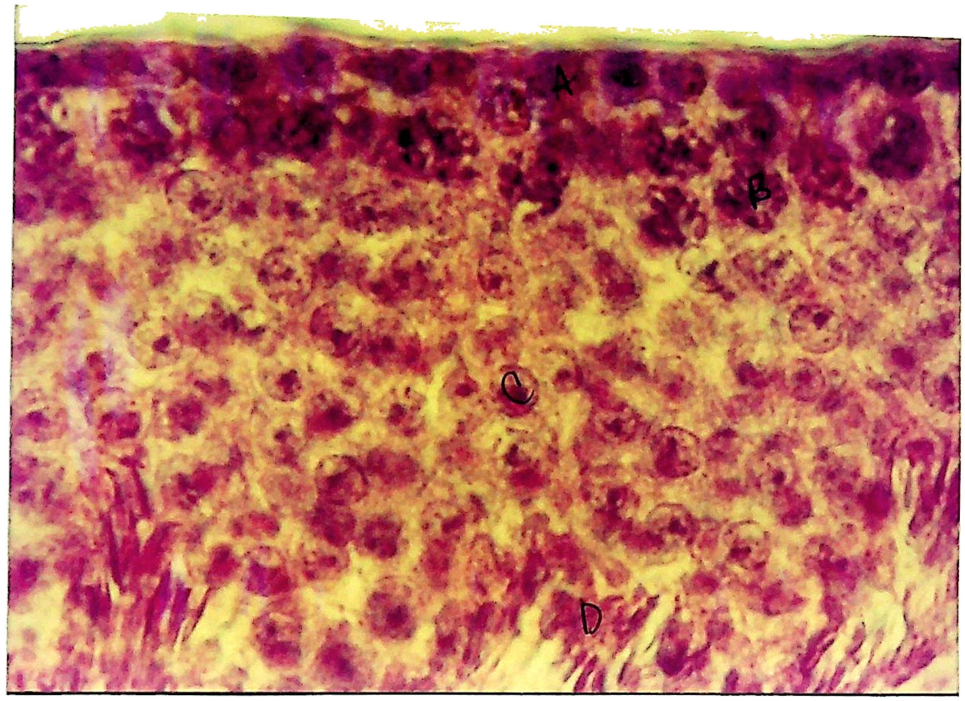


Plate 3. Normal Testis (1000x).

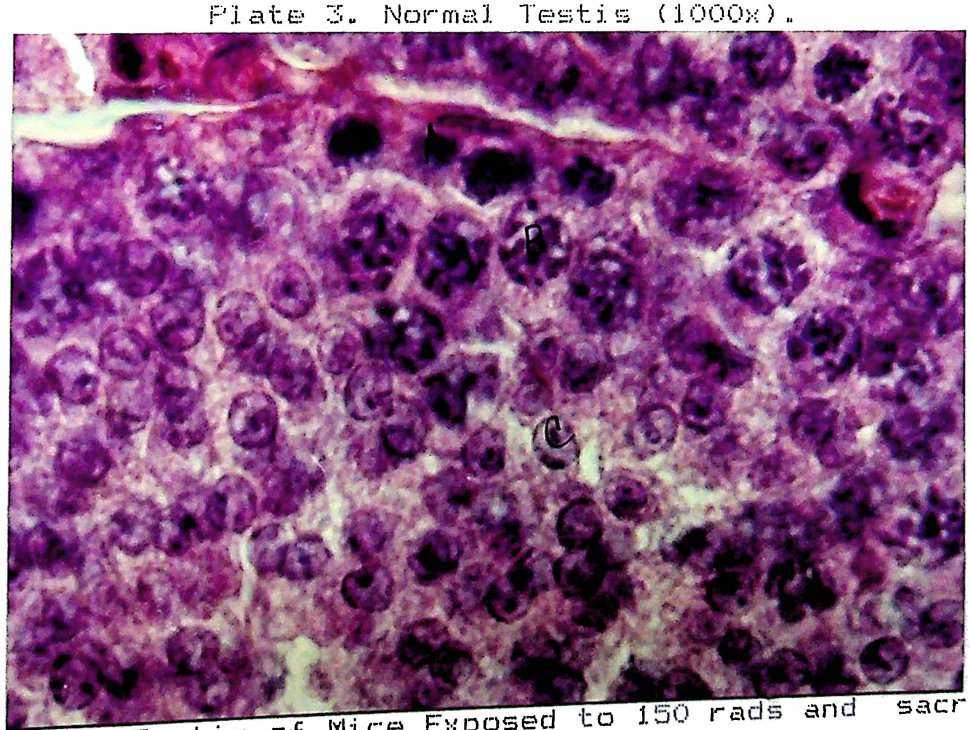


Plate 4. Testis of Mice Exposed to 150 rads and sacrificed after 24 hours (1000x).

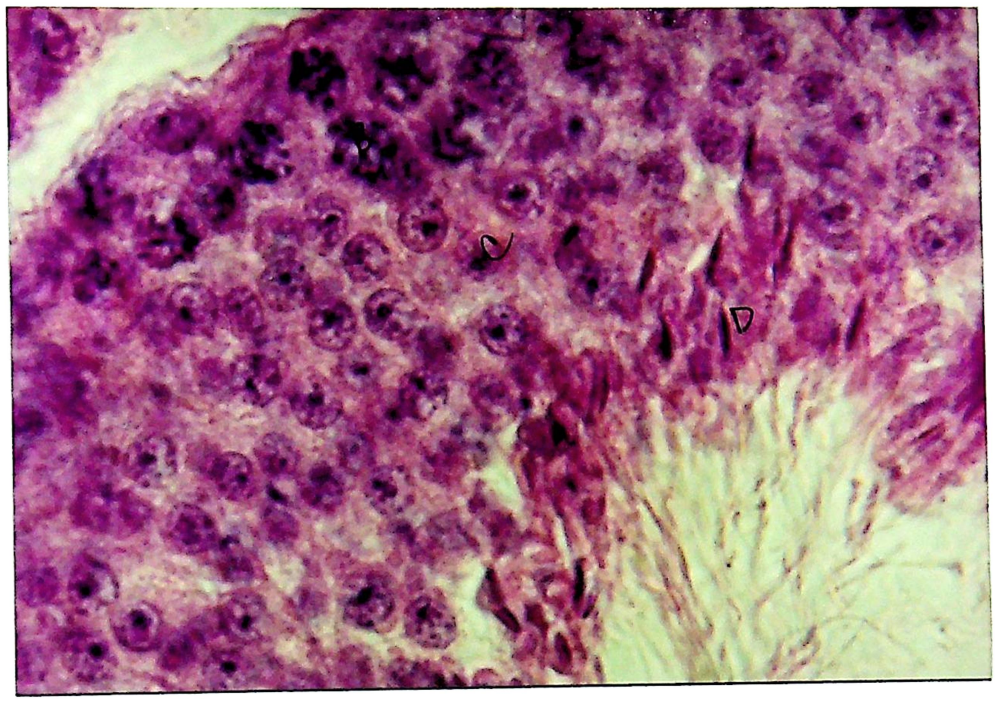


Plate 5. Testis of Mice Exposed to 300 rads and Sacrificed after 24 Hours (1000x).

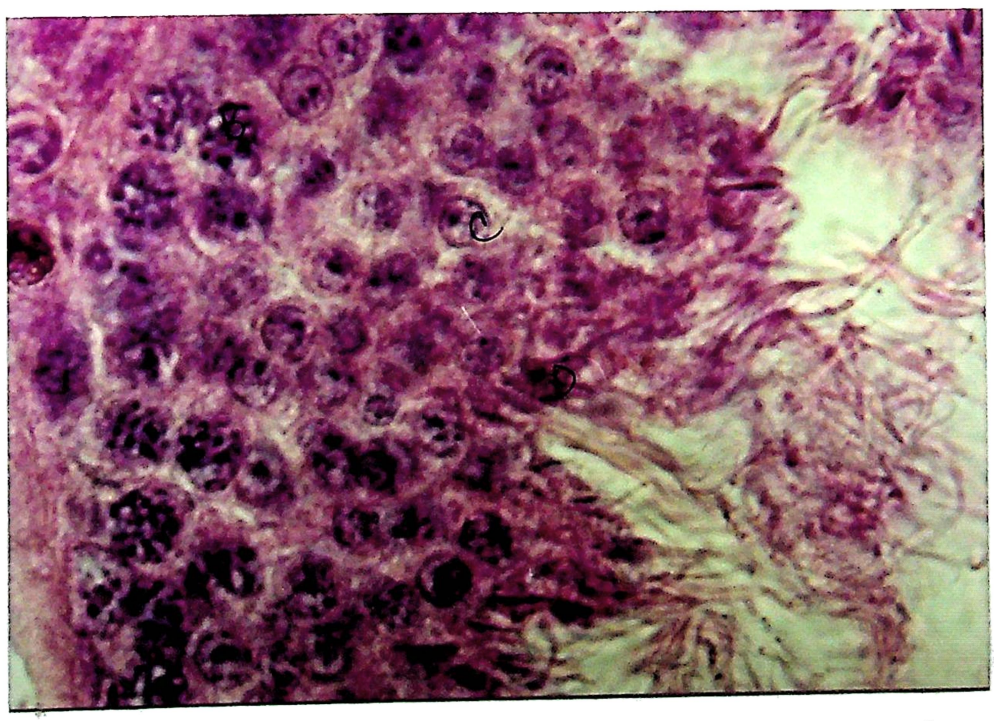


Plate 6. Testis of Mice Exposed to 600 rads and Sacrificed after 600 rads (1000x).

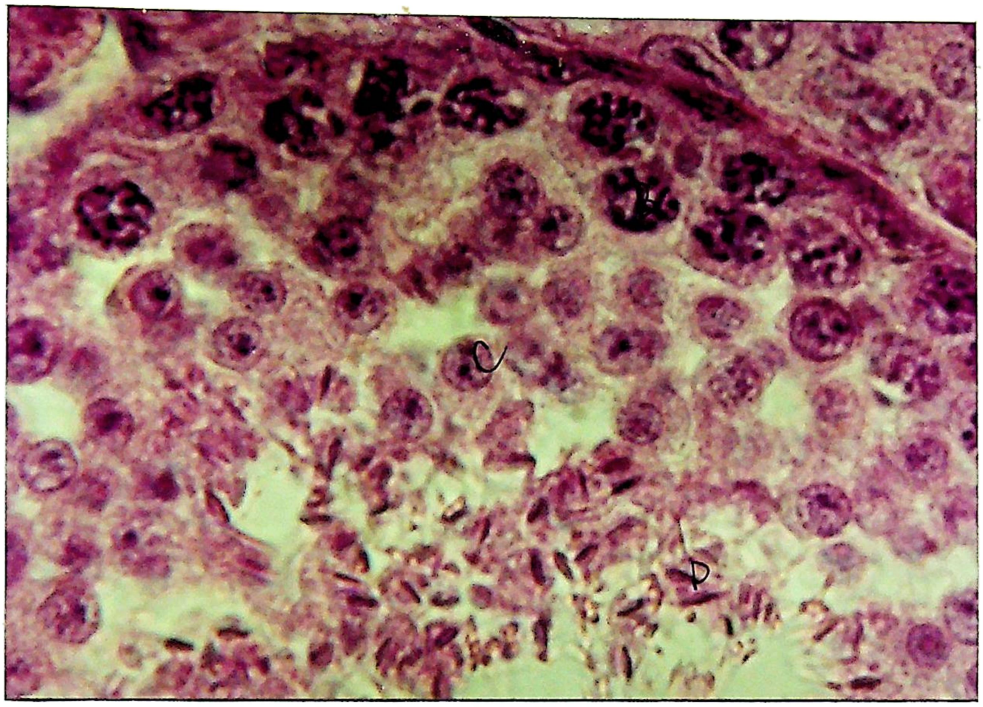


Plate 7. Testis of Mice Exposed to 750 rads and Sacrificed after 24 Hours (1000x).

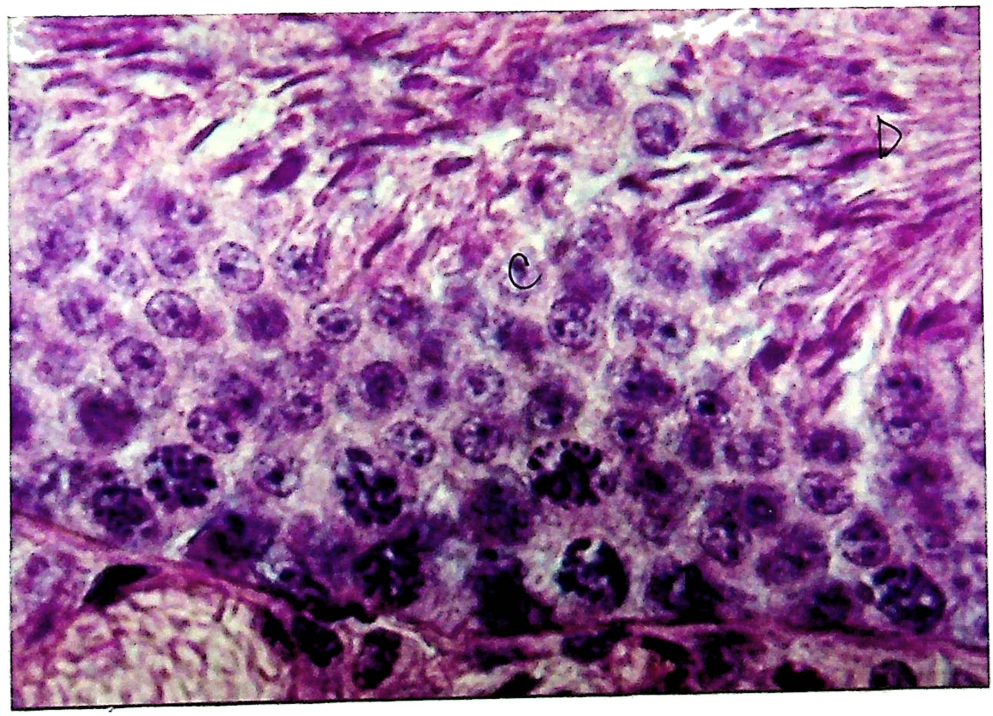


Plate 8. Testis of Mice Exposed to 150 rads and Sacrificed after One Week (1000x).

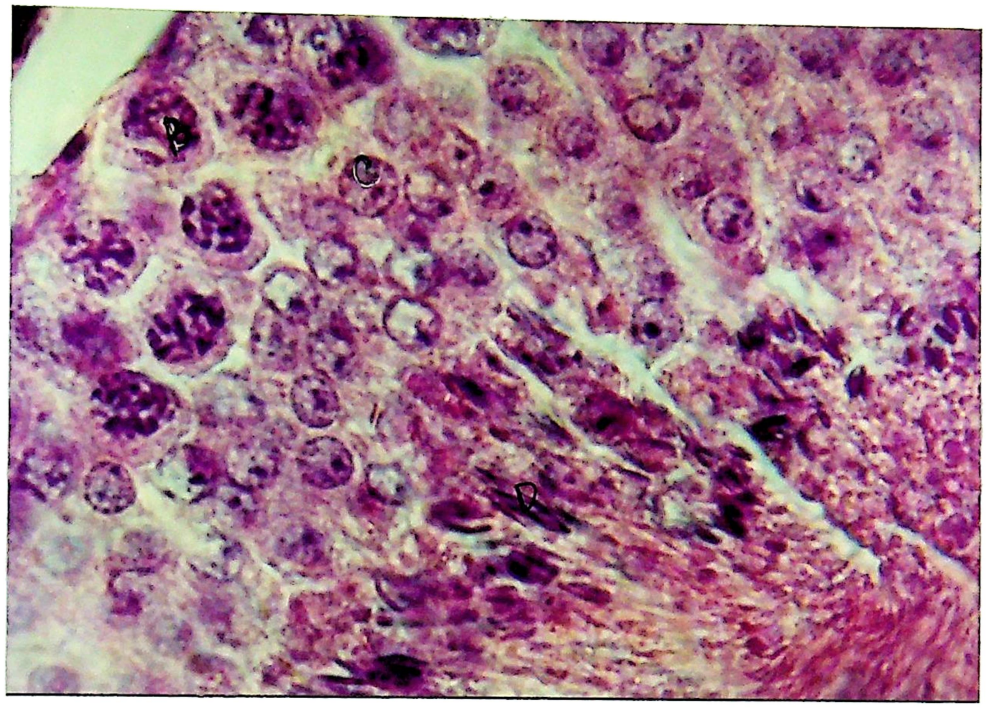


Plate 9. Testis of Mice Exposed to 300 rads and Sacrificed after One Week (1000x).

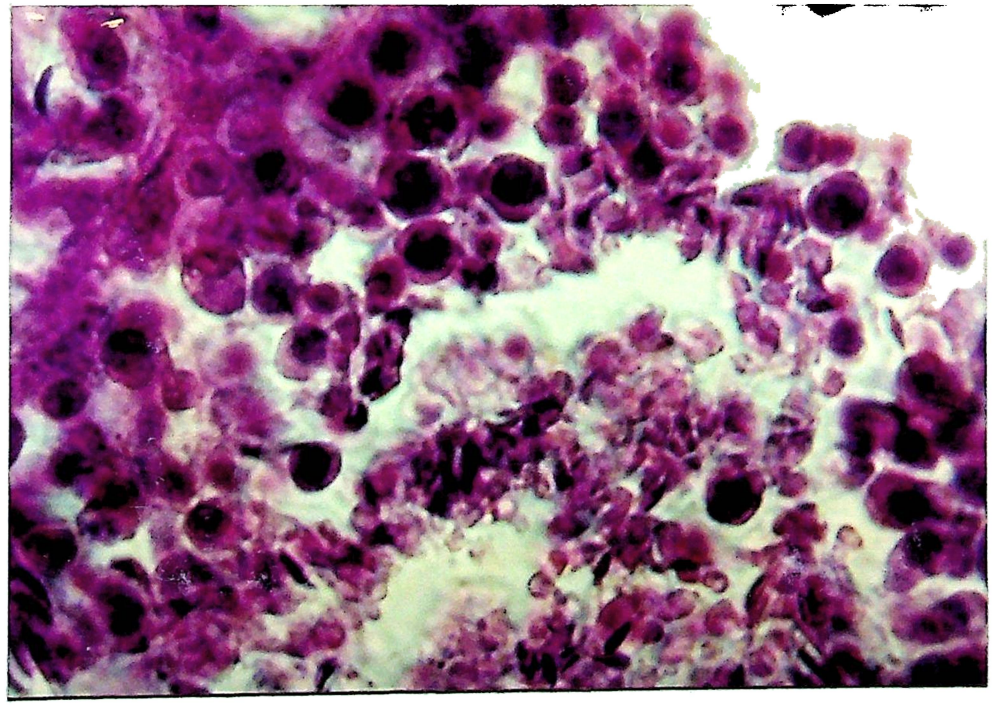


Plate 10. Testis of Mice Exposed to 600 rads and Sacrificed after One Week (1000x).

x.

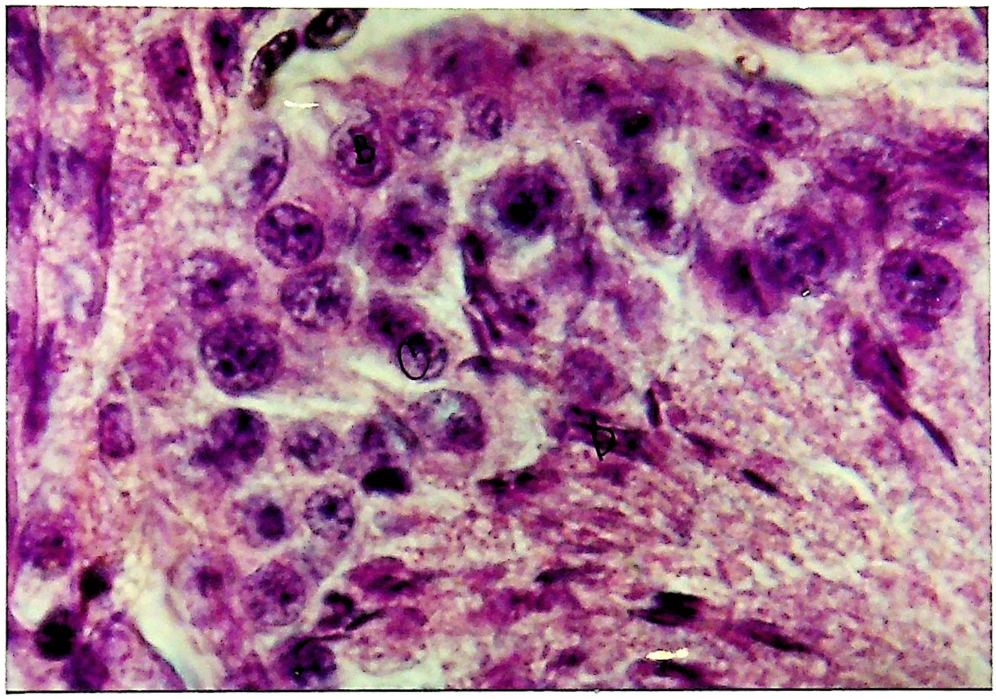


Plate 11. Testis of Mice Exposed to 750 rads and Sacrificed after One Week (1000x).

Legend (for Plates 12 to 22) :

A. Graafian Follicle
B. Secondary Follicle
C. Primary Follicle
D. Primordial Follicle
E. Cyst or Tumor

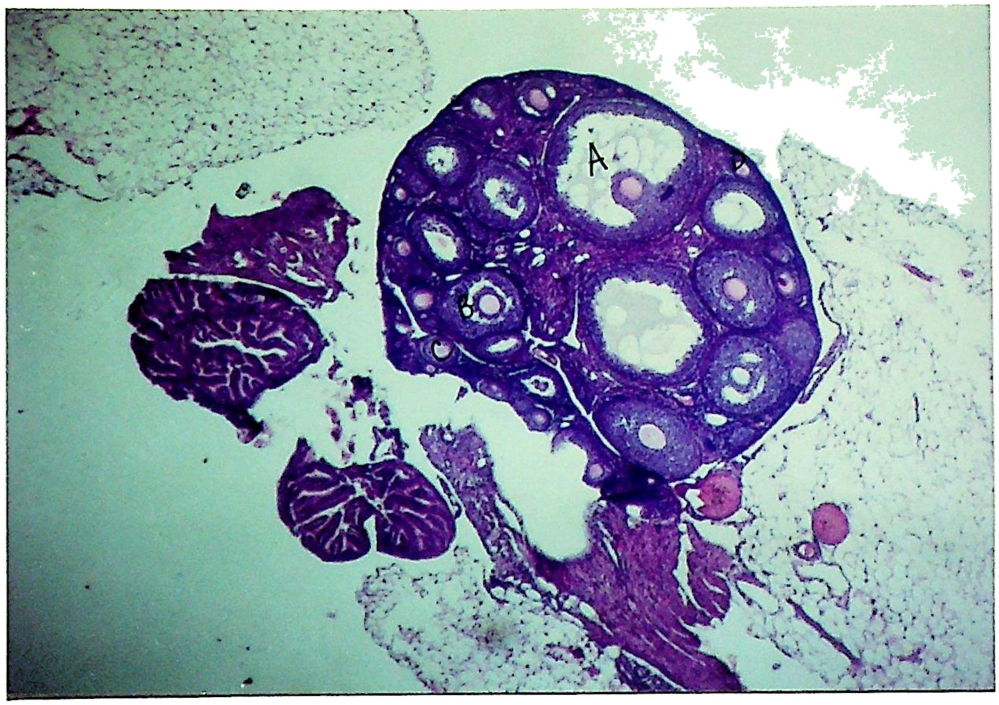


Plate 12. Ovary of Normal Mice (40x).

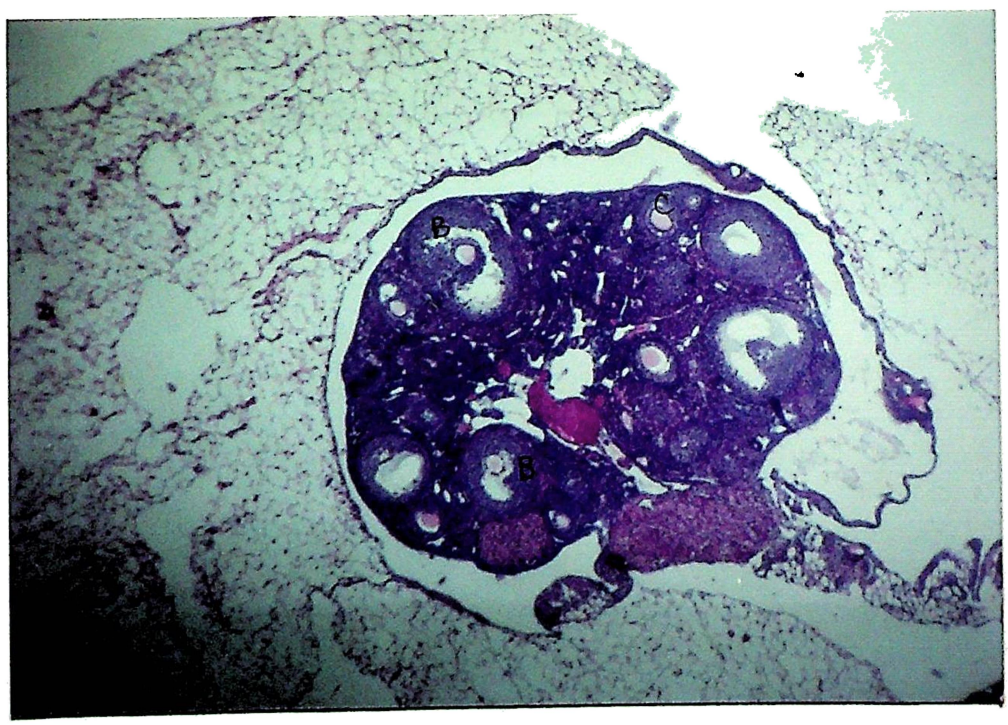


Plate 13. Ovary of Mice Exposed to 150 rads and sacrificed after 24 Hours. (40x)

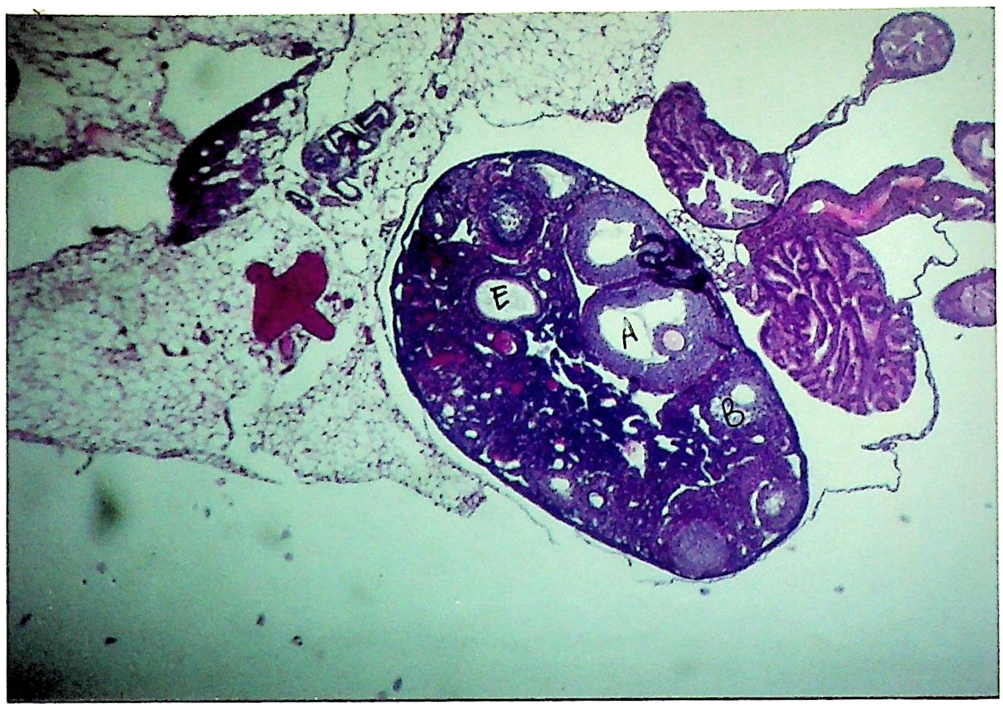


Plate 14. Ovary of Mice Exposed to 300 rads and sacrificed after 24 Hours (40x).

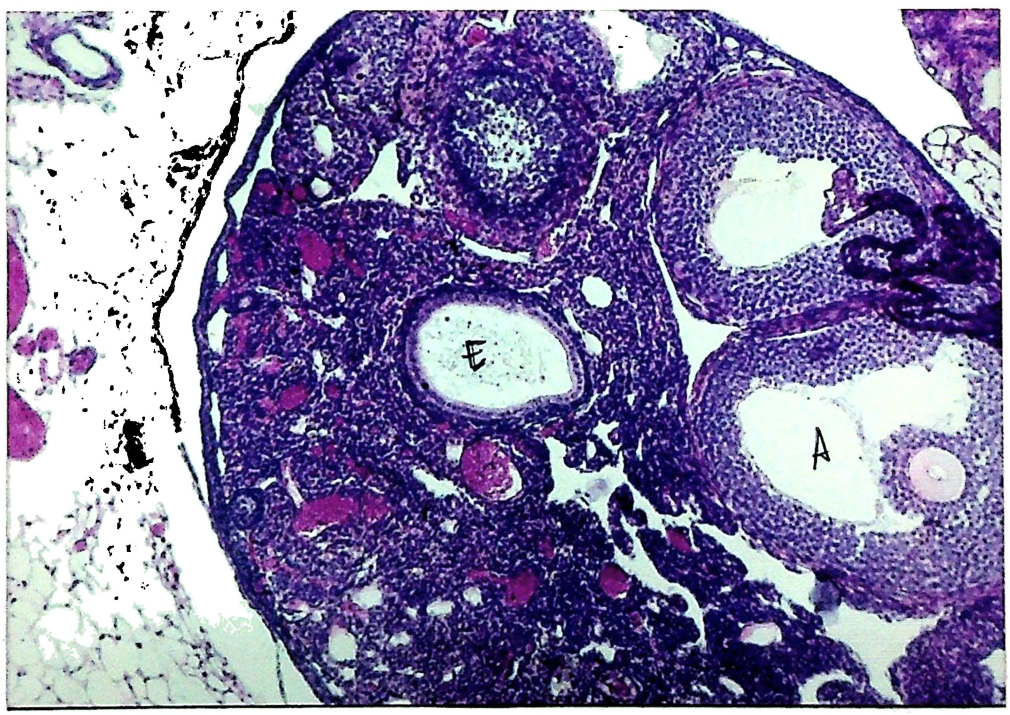


Plate 15. Ovary of Mice Exposed to 300 rads and sacrificed after 24 Hours (100x).

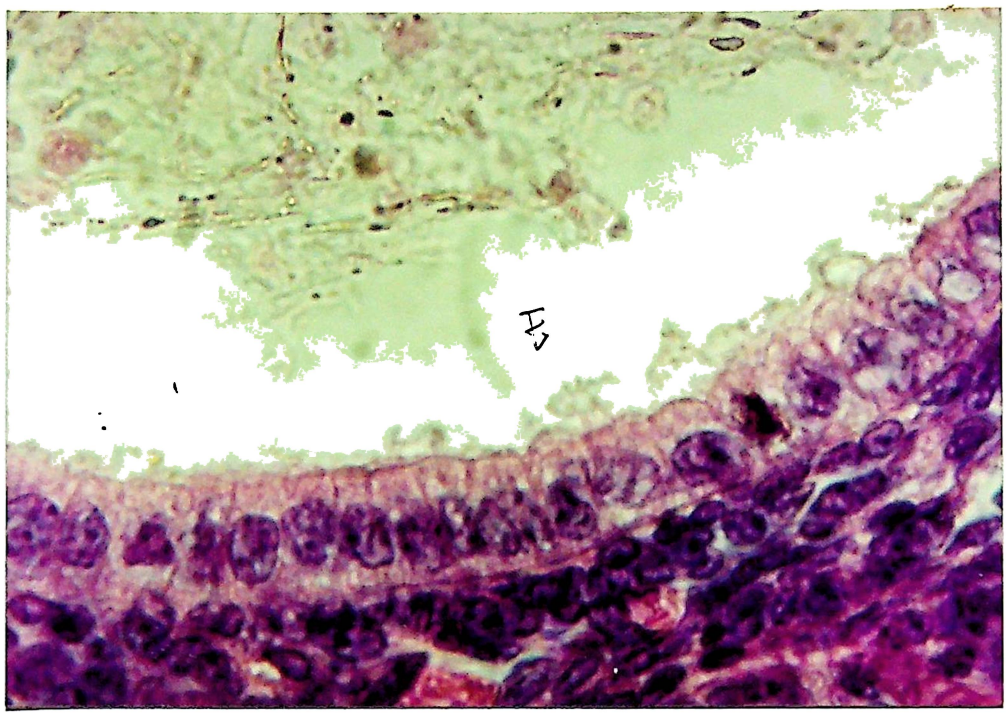


Plate 16. Columnar Cells of Cyst in the Ovary of Mice Exposed to 300 rads and Sacrificed after 24 Hours (1000x).

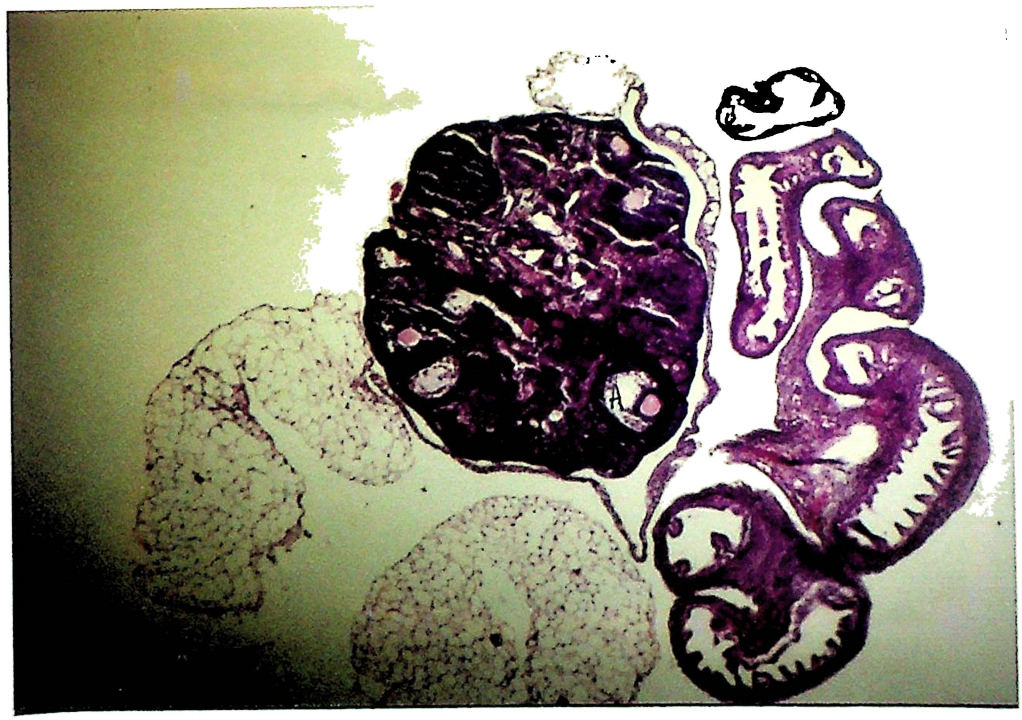


Plate 17. Ovary of Mice Exposed to 600 rads and Sacrificed after 24 Hours (40x).

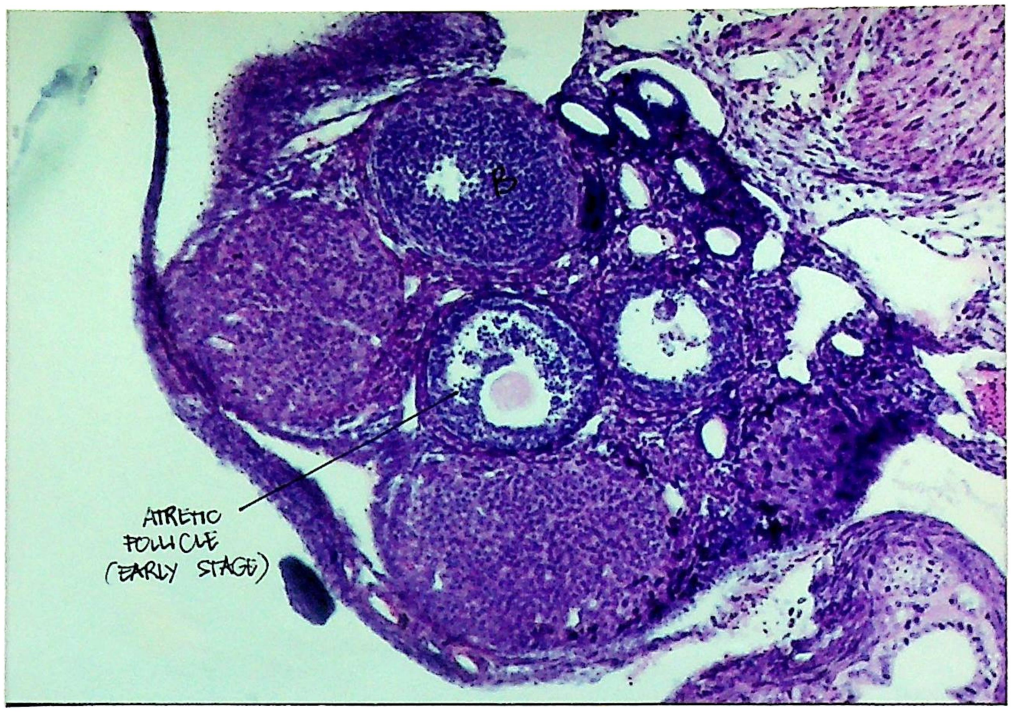


Plate 18. Ovary of Mice exposed to 750 rads and sacrificed after 24 Hours (100x).

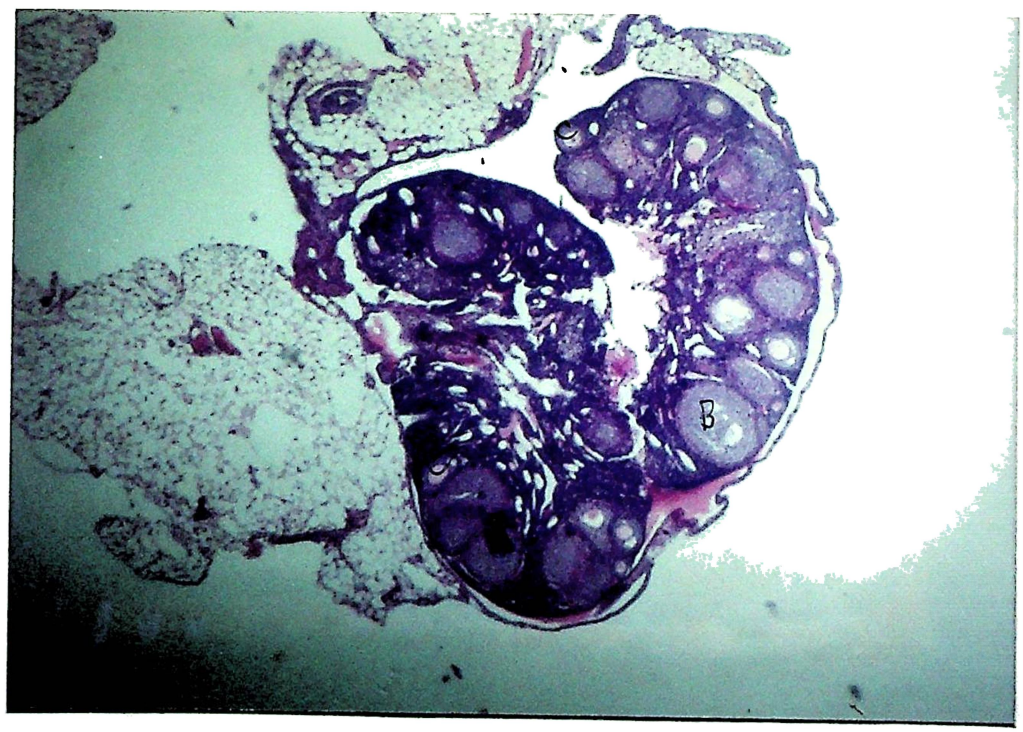


Plate 19. Ovary of Mice Exposed to 150 rads and Sacrificed after One Week (40x).

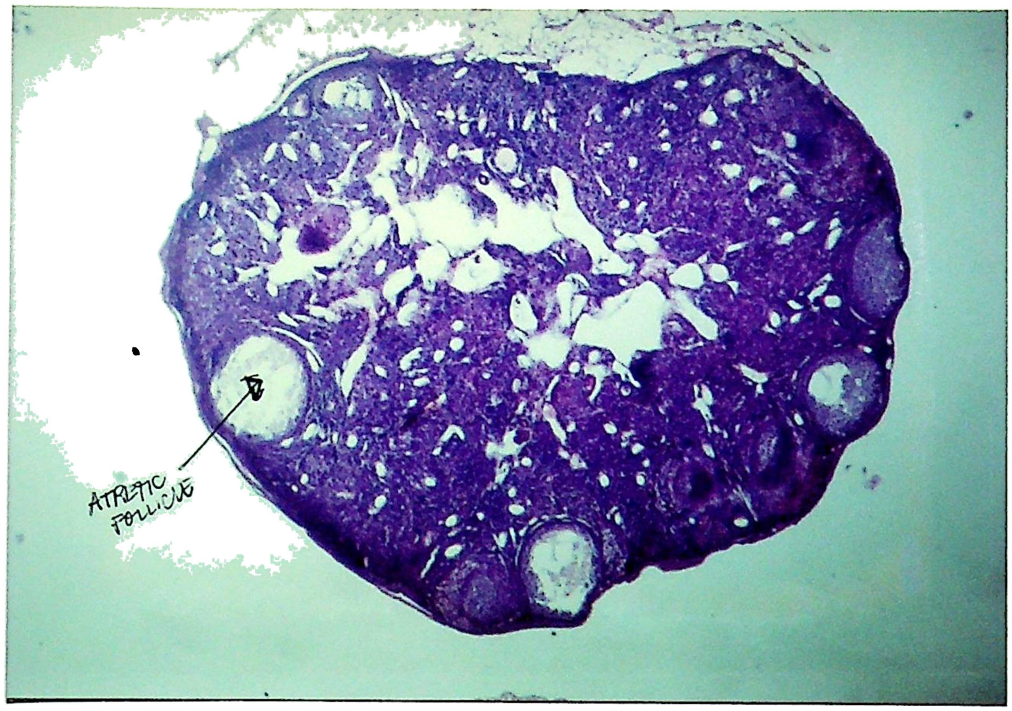


Plate 20. Ovary of Mice Exposed to 300 rads and Sacrificed after One Week (40x).

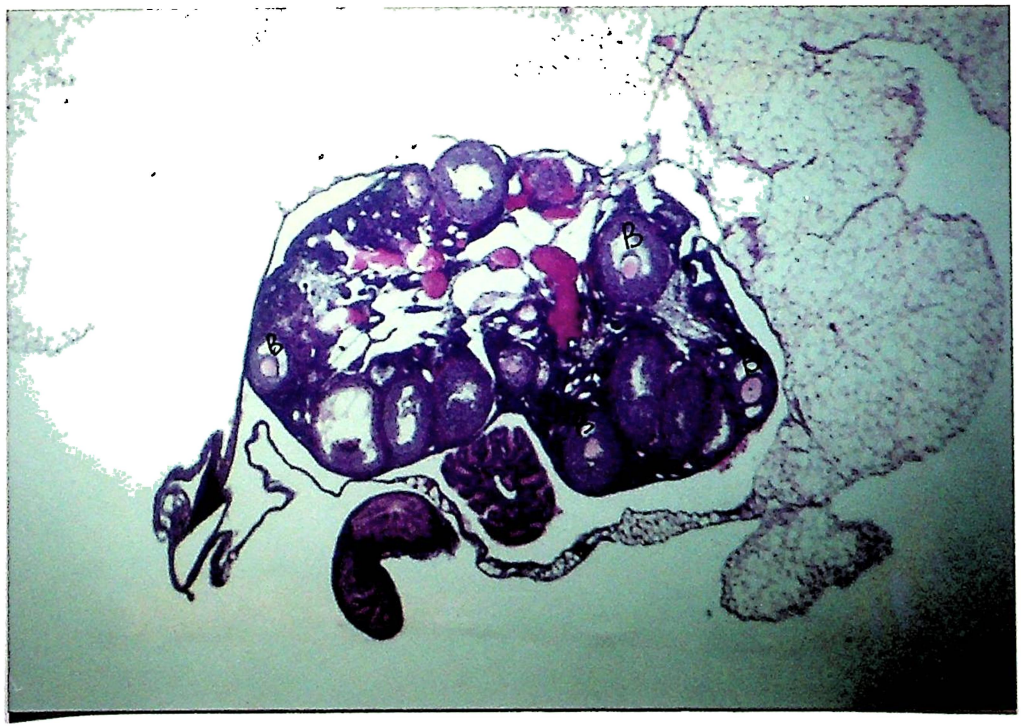


Plate 21. Ovary of Mice Exposed to 600 rads and Sacrificed after One Week (40x).

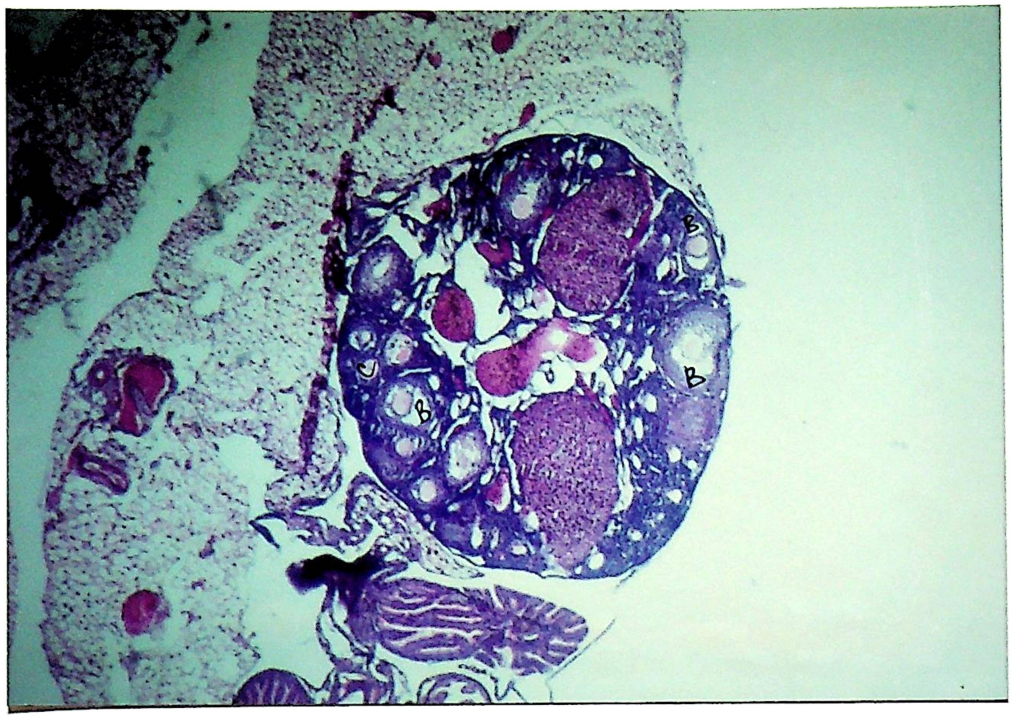


Plate 22. Ovary of Mice Exposed to 750 rads and Sacrificed after One Week (40x).