LOCOMOTION, PHYSICAL DEVELOPMENT, AND BRAIN MYELINATION IN RATS TREATED WITH IONIZING RADIATION IN UTERO.

A DISSERTATION

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I am submitting herewith a dissertation written by Md Sarwar Zaman entitled "Locomotion, Physical Development, and Brain Myelination in Rats Treated with Ionizing Radiation in Utero". I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Radiation Biology.

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DEDICATION

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ABSTRACT

LOCOMOTION, PHYSICAL DEVELOPMENT, AND BRAIN MYELINATION IN RATS TREATED WITH IONIZING RADIATION IN UTERO

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Effects of ionizing radiation on the emergence of lomomotion skill and some physical development parameters were studied in laboratory rats (Fisher F-344 inbred strain). Rats were treated with 3 different doses of radiation (150 R, 15 R, and 6.8 R) delivered on the 20th day of the prenatal life. Results indicated that relatively moderate (15 R) to high (150 R) doses of radiation have effects on certain locomotion and physical development parameters.

Exposure to 150 R affected pivoting, cliff-avoidance, upper jaw tooth eruption, body weight, and organs, such as brain, cerebral cortex, ovary, kidney, heart and spleen weights. Ohter parameters, such as negative geotaxis, eye opening, and lower jaw tooth eruption appeared to be affected in the 150 R treated animals. Exposure to 15 R affected pivoting and cliff-avoidance parameters. The cerebral cortex weight of the 15 R treated animals was found to be reduced at the age of day 30. Exposure to 6.8 R had no adverse effects on these parameters.

Prenatal exposure to 150 R of radiation reduced the cerebral cortex weight by 22.07 percent at 30 days of age, and 20.15 percent at 52 days of age which caused a reduction in cerebral cortex myelin content by 20.16, and 22.89 percent at the ages of day 30 and day 52 respectively. Exposure to 150 R did not affect the myelin content of the cerebellum or the brain stem; or the myelin concentration (mg myelin/g brain tissue weight) of the cerebral cortex, cerebellum, and the brain stem. Exposure to 15 R, and 6.8 R did not affect either the myelin content or the myelin concentration of these brain areas.

TABLE OF CONTENTS

Table of contents continued

Table of contents continued

Table of contents continued

Page

LIST OF TABLES

List of table continued

LIST OF FIGURES

List of figure continued

list of figure continued

CHAPTER I

INTRODUCTION

MYELIN

Myelin is an outer membrane characteristic of vertebrate nerve fibers. Myelin wraps the fibers as a sheath and functions as an insulator increasing the velocity of nerve impulses.

Myelin is composed of a lipid bimolecular leaflet, coated by a layer of protein on each side. In higher vertebrates, compact myelin is formed by two different types of cells. In the central nervous system (CNS) myelin is produced by the oligodendrocyte cells, while in the peripheral nervous system (PNS) myelin is produced by Schwann cells. The myelin sheath is absent during the early developmental stage of the vertebrate nervous system, but its synthesis starts when the population of neurons is established.

RADIATION

Radiation has existed in nature ever since matter was formed. Living organisms are exposed to continuous radiation coming from outer space and also from natural and man-made

radiation sources, all of which are hazards in our environment. Since non-man-made radiation accounts for more than half of the exposure received by living organisms (Rodos, 1979), roughly half of the total radiation humans are exposed to is the by-product of this civilization. The atomic age presents major environmental problems to our civilization, especially in the areas of nuclear war, nuclear reactors, and disposal of medical and industrial radioactive waste.

Gamma rays are produced during the decay of various radioactive materials such as ^{60}Co, ^{90}Sr, ^{190}Hg, ^{200}Bi, and are considered as ionizing radiations. Ionizing radiation is a major environmental hazard since it is responsible for deleterious effects on biological systems.

STATEMENT OF THE PROBLEM

Various drugs and environmental agents cause hypomyelination in the developing brain (Lancaster et al. 1984; Bohn and Friedrich, 1982; Patsalos and Wiggins, 1982; Toews et al. 1980; Druse and Hofteig, 1979; Clarren et al. 1978; Konat and Clausen, 1976; Krigman et al. 1974). Although very few reports are available regarding radiation induced demyelination of the adult nervous system (Lampert, 1959; Love et al. 1986), effects of radiation on developing

brain myelination are not documented. Therefore, studies of effects of various doses of radiation on brain myelination are appropriate areas of investigation.

OBJECTIVES OF THE PROJECT

Specific objectives of this project were:

1. To determine whether gamma radiation would cause hypomyelination in the developing rat brain.
2. To determine whether weights of brain tissues were affected by radiation.
3. To determine whether radiation affects different developmental processes such as pivoting, righting, crawling, negative geotaxis, cliff-avoidance, hindlimb support, eye opening, tooth eruption and body weight in the developing rat offspring.
4. To determine whether any of these effects found were related to the various doses of radiation.

HYPOTHESES

The hypotheses tested in this study were:

1. Gamma radiation decreases myelination in the developing rat brain.

2. Radiation decreases brain weights of the developing rats.
3. Gamma radiation affects different developmental processes such as pivoting, righting crawling, negative geotaxis, cliff-avoidance, hindlimb support, eye opening, tooth eruption and body weight in the developing rat offspring.
4. These effects are related to the various doses of radiation.

CHAPTER II

LITERATURE REVIEW

Myelin

History

Myelin has been known as a structural entity since the mid-nineteenth century. Virchow in 1854 (Raine 1977) reported the presence of sheaths around nerve fibers, and introduced the term 'myelin'. Gothlin in 1913 (Raine 1977) extensively studied the birefringent properties of nerve fibers and reported that two patterns were present, a lipid dependent and a protein dependent birefringence.

Results from polarization microscope studies by Schmidt in 1936 (Peters and Vaughn 1970), and x-ray defraction work by Schmitt et al.(1941) suggested that peripheral myelin had a radial concentric lamellar structure with a periodicity of about 17 to 18 nm. Finean (1953) confirmed that the lamellar nature of myelin consisted of two bimolecular lipid layers, each about 5.5 nm thick, which alternated with a 3 nm thick protein layer. An entire myelin lamella therefore, measured about 17 nm across. Worthington and Blaurock (1986) reported that CNS myelin lamella ranges

from 15.3 to 15.9 nm depending on the species, while peripheral myelin varies between 17.1 to 18.2 nm.

Gasser in 1952 (Peters and Vaughn 1970) showed that 'C' fibers (unmyelinated fibers, having the lowest conduction rate of about 0.5 m/sec) invested by cytoplasm of Schwann cells were connected to the surface of the cell by a membranous channel which was named the 'mesaxon'. Geren (1954) speculated that myelin formation was related to the elongation and spiral wrapping of the mesaxon around the axon to produce a tightly packed myelin sheath. Robertson (1955) tested Geren's interpretation, and the 'jelly roll' theory of myelination became accepted. Mathurana (1960) and Peters (1960) demonstrated that CNS myelin had a spiral configuration analogous to that of the PNS sheath.

Luse (1956) proposed that CNS myelin was the product of a number of plicated cell processes elaborated around the axon by several oligodendroglial cells. Luse (1956) also proposed that these processes become flattened and fused to form lamallae. In contrast, DeRobertis et al. (1958) postulated that CNS myelin was the product of a series of vesicles, formed within an investing oligodendroglial cell, which fused to form lamallae. Conversely, Hild in 1957

(Peters and Vaughn 1970) contended that CNS myelin was the product of the axon itself. Bunge et al. (1961, 1962) finally resolved the issue, by proposing that processes from oligodendroglia invested the axons. Peters (1964), Bunge and Glass (1965), and Hirano (1968) documented the actual connections between oligodendroglia and the myelin sheath.

Development

In the PNS, myelin is produced by the Schwann cells. Geren (1954) and Robertson (1955) stated that Schwann cell myelin consists of compact spiral layers of a modified plasma membrane ensheathing peripheral nerve axons. In the CNS, myelin is produced by oligodendroglial cells. Bunge et al. (1962), and Peter (1964) stated that in the CNS, myelin forming cells can not spiral around the axon, as the oligodendroglial cells are able to ensheath more than one axon.

While the total myelin accumulation is most rapid postnatally, different tracts in human brain myelinate at different times and rates (Gilles et al. 1983). In humans, rapid myelination occurs in the pons, medulla and mesencephalon during weeks 20 to 30 of gestation.

Myelination in the forebrain and corpus callosum starts later, within 30 to 40 weeks, or even postnatally (Wiggins 1986).

In rats, rapid brain myelination begins during the second postnatal week and is maximum at about day 20 (Norton and Poduslo 1973), whereas, sciatic nerve myelination begins on day 3 and is largely completed by day 10 (Wiggins and Morell 1980). The end of the most rapid phase of myelination is at 25 to 30 days in rats (Norton and Poduslo 1973) and at 2 years in humans (Dobbing and Smart 1973).

Effects of different insults on myelination

Inhibiting mitosis during the period of oligodendroglia cell proliferation could have effects on myelination, however, cell proliferation is not necessarily a critical stage of myelination (Wiggins 1986). Bohn and Friedrich (1982) reported that proliferation of oligodendroglia can be inhibited with cortisol, causing hypomyelination, however, myelin recovery was observed after withdrawal of cortisol treatment. Patsalos and Wiggins (1982) reported that phenobarbital, phenytoin, sodium valporate, and the combination of valporate and phenytoin depressed the rate of myelin membrane synthesis relative to

synthesis of the other subcellular membranes.

Clarren et al. (1978) noted that prenatal exposure to alcohol caused an abnormal glial maturation in laboratory animals. Druse and Hofteig (1977) reported that gestational exposure to alcohol caused retardation in postnatal myelination in rats. Lancaster et al. (1984) reported that treating lactating dams with alcohol produced a marked reduction in brain myelin concentration among the suckling offspring.

Studies showed that food deprivation during the first two weeks (postnatally) caused a lasting hypomyelination when compared to deprivation during the actual period of myelin accumulation (2 to 4 weeks) (Wiggins 1986). Wiggins (1982) reported that hypomyelination in the developing brain of undernourished rats was due to a deficit of myelinated fibers. It was observed that myelinated fibers were practically normal, although there was some 'thinning' of myelin sheaths (Wiggins et al. 1984, 1985).

Hexachlorophene, a broad spectrum bactericide and fungicide, has been extensively used in agriculture, antiseptic solutions, cosmetics, and soaps (Kimbrough 1971). Towfighi et al. (1973) observed that hexachlorophene produced status spongiosus in the brain and peripheral nerves and

vacuolation of myelin sheaths in rats. Towfighi et al. (1975) stated that administration of hexachlorophene to newborn rats did not prevent myelination, however, vacuoles rapidly appeared in the myelin when the layers began to accumulate. Shuman et al. (1975) identified the period of 6 to 22 days in rats as especially vulnerable to effect on myelination. Rats younger than day 6 have no myelin, and rats older than day 22 may be protected (in part) by maturation of the liver and subsequent detoxification of hexachlorophene.

Radiation

Cell sensitivity

Different kinds of cells in the body display different types of radiosensitivity (Upton 1969, Arena 1971). Based on decreasing radiation sensitivity, Arena (1971) ranged the body cells in the following order: lymphocytes, erythroblasts, myeloblasts, megakaryocytes, spermatogonia, ova, jejunal and iliac crypts, germinative stratum, sebaceous glands, hair matrix, sweat glands, eye lens cartilage, osteoblasts, blood vessel epithelium, granular epithelium, liver, glia, neuron, muscle, connective tissue, and osteocytes.

Effects on nervous system

Casarett (1968) described nervous tissue as the most radioresistant tissue in the human or animal body. Doses of 10,000 rads or greater destroy virtually all glial cells, the endothelial cells of the capillaries and a number of neurons in the path of the beam of radiation (Casarett 1968).

Casarett (1980) stated that white matter of the CNS appears to be much more suceptible to radionecrosis than the gray matter. Necrosis and loss of CNS cells may be followed by proliferation of glial cells in a process (gliosis) analogous to replacement fibrosis in other organs.

Hicks et al. (1956) reported acute oligodendromyelin necrosis in the forebrain of mice exposed to 10,000 R. Knapp et al. (1986) investigated the neuroglial cell death in white matter tracts of jimpy and normal mice. It was determined that glial death during development (days 4, 7, 11, 12, 16, 20, and 22 postnatally) ranged from 0.5 to 2.7 percent in normal mice, while up to 10.0 percent of the glial population were pyknotic in jimpy mice. An ultrastructural study of dying glial cells presented evidence that the glial cells were oligodendrocytes. The authors concluded that the myelin deficits observed were due to premature death of the oligodendrocytes.

Wolsky (1982) reported that irradiation on the 9th and 10th days prenatally affects the anterior part of the prosencephalon, optic tract and hindbrain. Irradiation on the 11th day mostly affects the telencephalon and optic tract. Irradiation on the 8th or 9th days could produce organotypic malformation such as formation of a single ventricle brain. Casarett (1980) described myelin as relatively radioresistant, but the process of myelin synthesis becomes radiosensitive by the time myelination of nerve fibers begins (Wolsky 1982).

Lampert (1959) reported a case of demyelination in a 32-year old housewife who was exposed to several thousand rads of therapeutic radiation after a cancerous growth was removed from her left external auditory canal. Histological observation of her brain tissue after death revealed considerable demyelination in the cerebellum, mid pons, cerebellar hemispheres (mostly left), and middle cerebellar peduncles, but the mid brain showed no gross changes. The oligodendroglial cells were absent in the demyelinated areas. Love et al. (1986) observed demyelination in mouse peripheral nerve by administering lysophosphatidyl choline and 20 Gy of x-rays in a single dose.

Effects on cellular and molecular levels

Radiation may produce several visible criteria of degenerative processes in cells. These include pyknosis (the nucleus is contracted and the chromatin material is condensed), karyorrhexis (the nuclear membrane is ruptured and the nuclear material is abnormally fragmented and scattered in the cytoplasm), karyolysis (the nucleus and its chromatin disappear), protoplasmic coagulation (irreversible gelation in the cytoplasm or in the nucleus or both), and cytolysis (bursting of the cell)(Casarett 1980).

Cellular changes due to radiation may occur with damage of nucleic acids, proteins, enzymes, and various polysaccharides (glycogen, starch, or cellulose). Molecular changes may lead first to cellular then to organismal changes (Morgan 1981).

All periods of the cell-cycle can be affected by radiation (Casarett 1980), but the most sensitive phases are M and G1/S transition, and the most resistant is late S phase (Grosch and Hopwood 1979). Radiation effects on the cell cycle may cause a temporary mitotic block or mitosis-linked cell necrosis. The G2 phase of the cell cycle has been found to be more sensitive to radiation induced chromosomal breakage. Chromosomal structural

changes could be associated with a high probablity of eventual reproductive failure of the cells (Casarett 1980).

Most probably, DNA represents the primary target in the killing of the cell by ionizing radiation (Dalrymple and Baker 1973). Radiation affects both the structure of DNA and its synthesis. Cells that actively synthesize nucleic acids and associated materials are especially radiosensitive. Relatively larger doses (200 rads) of radiation may depress DNA synthesis to three-quarters of the unirradiated control values (Holmes 1947).

Lett et al. (1967) reported a decrease in size of the DNA molecule following radiation treatment. In mammalian cells, this decrease could be seen after exposure to 1,000 rads or less. Evidence from continuous measurements of DNA content and DNA replication obtained by density gradient studies has indicated a small decrease in DNA synthesis after irradiation (Grosch and Hopwood 1979).

Singh (1974) suggested that the genome and cell membrane should be considered as two components of a "co-operative target", which interact in the killing of cells by radiation. Dalrymple and Baker (1973) postulated that radiation produces sufficient damage to the DNA molecule so that it is unable to serve effectively as a template for

synthesis of DNA as well as RNA. All DNA bases are susceptible to chemical alterations induced by radiation (Wheeler and Lett 1972).

Prenatal exposure

One hundred and fifty rem (150,000 millirems) of radiation on the first day of gestation would produce 60-70 percent embryonic death in humans and in rats (Brent 1980). Data from the study indicated that exposure to 100 rads during 8 to 11 days of gestation in rats or 2 to 4 weeks of gestation in humans would produce maximum malformation in the embryo. At that level 41 percent CNS malformation and 90 percent eye malformation could be observed.

Of all diagnostic x-ray examinations done purposefully during pregnancy, 90 to 95 percent are either abdominal flat plate or x-ray pelvimetry films (Schussman and Lutz 1982). The dose of radiation delivered to the fetus per pelvimetry study ranges from approximately 1 to 4 rad (Stewart and Kneale 1970). Shapiro (1981) stated that one CT (Computed Tomograph) scanning in high-accuracy (lower noise) mode may deliver doses as high as 56 rad.

Radiation exposure of a woman from radiation therapy for cancer of her abdomen, or women within 1,500 meters of

the atomic explosion in Nagasaki-Hirosima would be 50 to 250 rad (Brent 1980). The author also reported that in humans, the first 14 days of gestation showed a very interesting phenomenon called "all or none response", when the embryo could not be malformed regardless of the exposure dose, but it was very sensitive to the killing effects of radiation. During this period 100 rad could eliminate 65 to 70 percent of the mammalian embryos, but the ones that would survive would be completely normal.

Brent (1980) reported that 20 pregnant women who were exposed to therapeutic radiation during their pregnancies to treat them for cancer of the reproductive organs received thousands of rad of radiation. Exposure during the first trimester produced microcephaly, mental retardation and eye malformation in the offspring. Surprisingly, no abnormalities were observed among the offspring of those irradiated during the second and third trimesters.

Therefore, diagnostic or therapeutic doses or radiation (during pregnancy) ranging from 500 mrad (from one x-ray plate) to 100 rad or more, and its effects on the developing offspring are important areas of investigations.

CHAPTER III

MATERIALS AND METHODS

Animal and housing

Adult, Fisher F-344 inbred strain pregnant rats were used in this study. The rats were housed individually in plastic cages with stainless steel tops. Lighting was regulated to 12 h light and 12 h dark. A constant room temperature of 22 ^{o}C was maintained throughout the study period. The regular food was commercially prepared laboratory diet (Purina Lab Chow) and distilled water.

Treatment procedure

Radiation treatments: The radiation treated rats received total body gamma irradiation from the U.S. Nuclear Corporation G-R-9 gamma irradiator. The radiation treated animals were divided into three groups. Group A (n=14), group B (n=14), and group C (n=14), and the treatment groups received 6.8 rad, 15 rad, and 150 rad total body gamma irradiations respectively. The radiation was delivered in a single dose on the 20th day of gestation.

Control treatment: Group D (n=14) served as controls for groups A, B, and C. These animals were

received sham irradiation on day 20 of gestation. Food, water, and caloric consumption of the different treatment groups was measured on a daily basis. The body weights of all groups were measured every three 3 days.

At day 3 postnatally, litter size was adjusted to eight pups. Litter sizes of all treatment groups and several developmental parameters of pups of each treatment group, such as eye opening, pivoting, hind limb support, and tail flick latency were also observed.

Offspring of each different treatment group were sacrificed on days 30 and 52. At both ages, 15 female animals from each treatment group were sacrificed by decapitation and brain and organ (spleen, heart, liver, kidney, adrenal, and ovary) wet weights were recorded and organ/body ratio was compared.

The brains of all sacrificed pups were dissected into three different areas, cerebellum, brain stem, and cerebral cortex and were stored at -85 ^{o}C. The myelin concentration of these brain areas were quantitatively measured.

Developmental parameters: Starting on day 3 and continuing up to day 21, the following developmental parameters were observed postnatally among the pups of the

control and radiation treated groups.

Pivoting: In pivoting forelimbs act as paddles. The hindlimbs do not support movement of the forelimbs in a coordinated fashion, and because the pelvis remains supported by the surface, the forelimb movement produces a circular motion (pivoting) (Altman and Sudarshan 1975). In this study the pups were left on a smooth surface of plywood and were observed for one minute to record if they performed pivoting within this time period.

Righting on a surface: The pups were placed on the back on a wooden platform. The animals tended to right themselves. The righting was scored as 0 (for those that could not right within 15 seconds), 1 (these could right within 15 seconds), 2 (they could right within 10 seconds), and 3 (these could right within 5 seconds).

Crawling: In rats crawling occurs by paddling movenents of the paws. Sometimes sluggish hindlimbs fail to keep up with the forelimbs and are dragged in an extended position with soles of the feet facing upward (Altman and Sudarshan 1975).

To observe the development of crawling movements, each pup was left on a smooth surface of plywood for a period of one minute. The surface was marked by clear lines that indicated 3 cm, 6 cm, and 9 cm distances. Crawling was scored as 0 (crawling less than 3 cm), 1 (crawling atleast 3 cm but less than 6 cm), 2 (crawling at least 6 cm but less than 9 cm), and 3 (crawling 9 cm or more).

Negative geotaxis: When a rat is placed on an incline, with the head pointing downward, it turns to face upward. Crozier and Pincus (1926) called this reaction negative geotropism.

In this study, the pups were placed on a plywood surface with a 20 ^{o} incline. Each animal was given one minute for a trial, to record if it could rotated its body axis 180 ^{o}.

Cliff-avoidance: Each pup was placed on the edge of a thick wooden platform with its nose and forefeet over the edge. The animal tended to move away from the 'cliff' by backing up or by turning sideways. The responses were scored as 0 (no response), 1 (response within 15 seconds), 2 (response within 10 seconds), and 3 (response within 5

seconds) (Altman and Sudarshan 1975). The elapsed time was measured with a stop watch.

Hindlimb support when suspended: Altman et al. (1971) observed that suspended rats tended to grasp a horizontally extended string of wire when their forepaws were brought in contact with it. In this study a 2 mm thick and 45 cm long wire was extended horizontally between two 30 cm high poles. Each pup was held by the nape of the neck and the forepaws were allowed to touch the wire. Grasping occured almost immediately as the pup attempted to pull-up its body with the forelimbs and to support itself with the hindlimbs. Data were collected as successful or unsuccessful attempts.

Eye opening (left and right eyes), and tooth eruption (upper and lower jaws): The pups were monitored on a daily basis to observe and record eye opening, and tooth eruption.

Body weight: The body weights of the pups were recorded on alternate days from day 3 to day 21.

Measurement of Myelin:

The brains were dissected into three different areas, cerebellum, brain stem and cerebral cortex (Glowinski and Iverson 1966). The myelin concentrations of these three different areas were measured quantitatively, using the methods of Norton and Poduslo (1973).

All steps of myelin isolation was carried out at 4 ^{o}C or at the temperature of ice. The reagents used in the isolation procedure were 0.32 M and 0.85 M-sucrose.

The brain tissue was homogenized in 15 ml of 0.32 M-sucrose using a Downs homogenizer (6 strokes with pestal B, and 5 strokes with pestal A). The homogenate was layered over 20 ml of 0.85 M-sucrose in 37 ml transparent polyallamere tubes and was centrifuged at 25' K for 30 min using a swinging bucket type rotor (BECKMAN SW 27.1) and an ultracentrifuge (BECKMAN L7-55).

Myelin was collected from the interface between sucrose layers in a 37 ml polyallamere tube, resuspended with distilled water and centrifuged at 25' K for 15 min. The resulting pellet was resuspended in water and was transferred into a 30 ml corax tube and water stocked over ice for 30 min, and then centrifuged at 10.5' K for 15 min.

The supernatant over the pellet was aspirated and the pellet was resuspended in 15 ml of 0.32 M sucrose. The resuspended pellet was layered over 20 ml of 0.85 M sucrose in a 37 ml polyallamere tube and was centrifuged at 25' K for 30 min.

The final myelin sample was collected from the interface and washed free of sucrose by three cycles of centrifugation from distilled water at 12' K for 15 min. The resulting myelin pellet was resuspended in 1.5 ml of distilled water and transfered into a weighed screw top tube and left at -20 ^{o}C in slanting position for 24 h.

The sample was leyphiolized for 72 h at -50 ^{o}C. The freeze dried sample was weighed and the myelin concentration was calculated as mg myelin/g brain tissue weight.

Statistical analysis

The data obtained were subjected to appropriate statistical analyses including 2-way ANOVA, repeated measure ANOVA, Chi-square, and hierarchical loglinear analysis assisting in the interpretation of the experimental results.

CHAPTER IV

RESULTS

Water consumption during gestation: No differences in daily water consumption were observed between different treatment groups during the gestational period (Fig. 1), but results of ANOVA with repeated measures showed a significant within subject effects of days ($p \leq 0.001$) (Table 1). Mean water consumptions for the entire gestational period were 25.9 ± 1.1 ml, 24.6 ± 1.3 ml, 26.3 ± 1.2 ml, and 25.7 ± 0.9 ml for the control, 150 R, 15 R, and 6.8 R treated animals respectively (Fig. 2). No difference in mean water consumption between different treatment groups was observed during gestation.

Food consumption during gestation: Significant differences in daily food consumption between groups of rats were observed only on day 8 of the gestational period ($P \leq 0.01$) (Fig. 3), when 15 R and 6.8 R treated animals consumed more food than the control and the 150 R treatment groups; but no differences were observed between the 15 R and 6.8 R treated animals, or control and 150 R treated animals. Mean

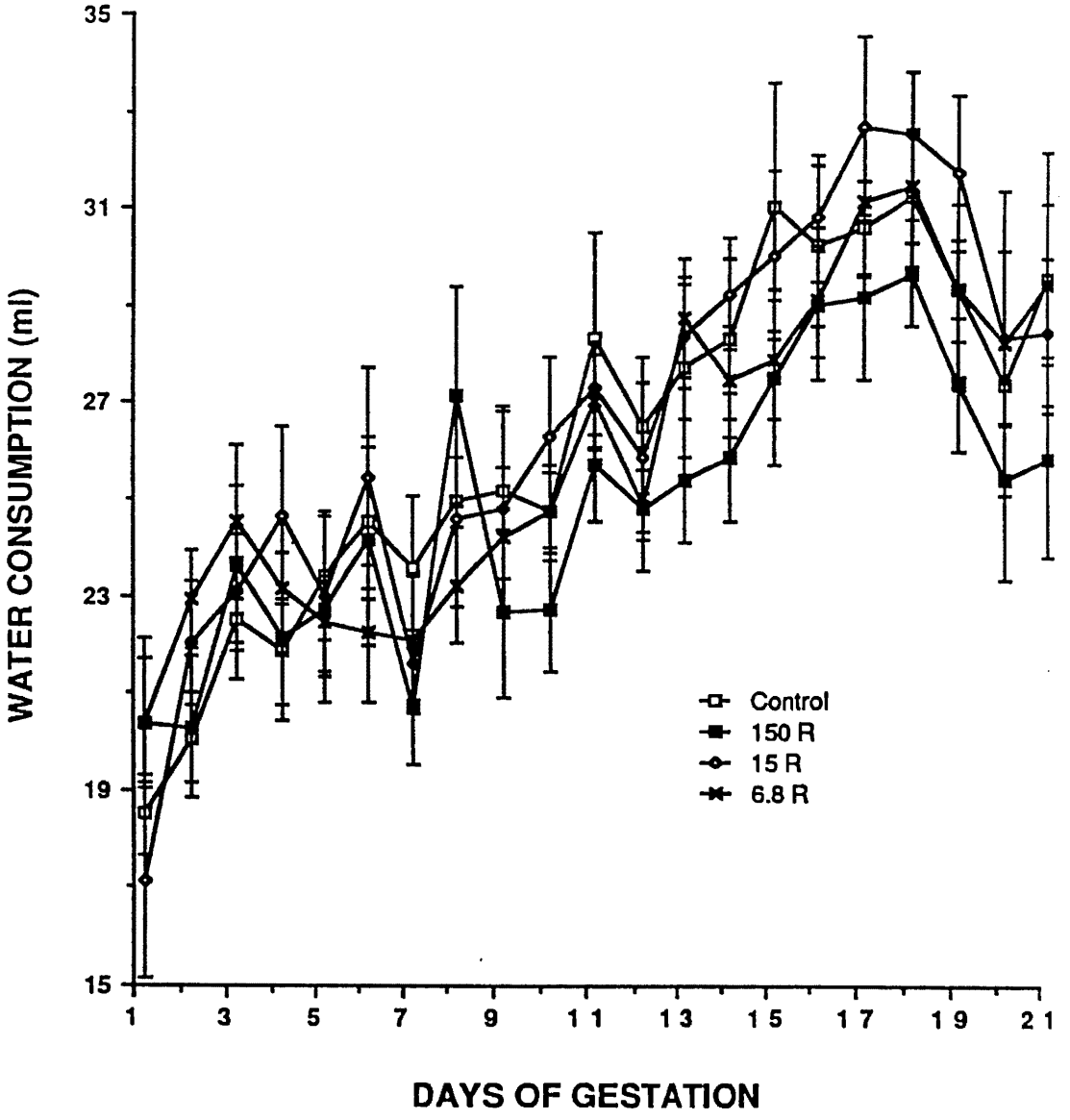

Figure 1. Comparison of daily water consumption during gestation (mean ± SEM) by control and radiation treated dams.

TABLE 1

SUMMARY OF ANOVA WITH REPEATED MEASURES:
WATER CONSUMPTION DURING GESTATION

Source	DF	SS	MS	F	p
Between					
Subjects	55				
Treat	3	461.5	153.8	0.38	0.762
Error between	52	20640.3	396.9		
Within					
Subjects	1120				
Days	20	12359.2	617.9	39.79	0.001
Treat X Days	60	1109.1	18.4	1.19	0.157
Error within	1040	16149.9	15.5		

DF = Degrees of Freedom
SS = Sum of Square
MS = Mean Square
F = F distribution (sampling distribution)
P = Probability

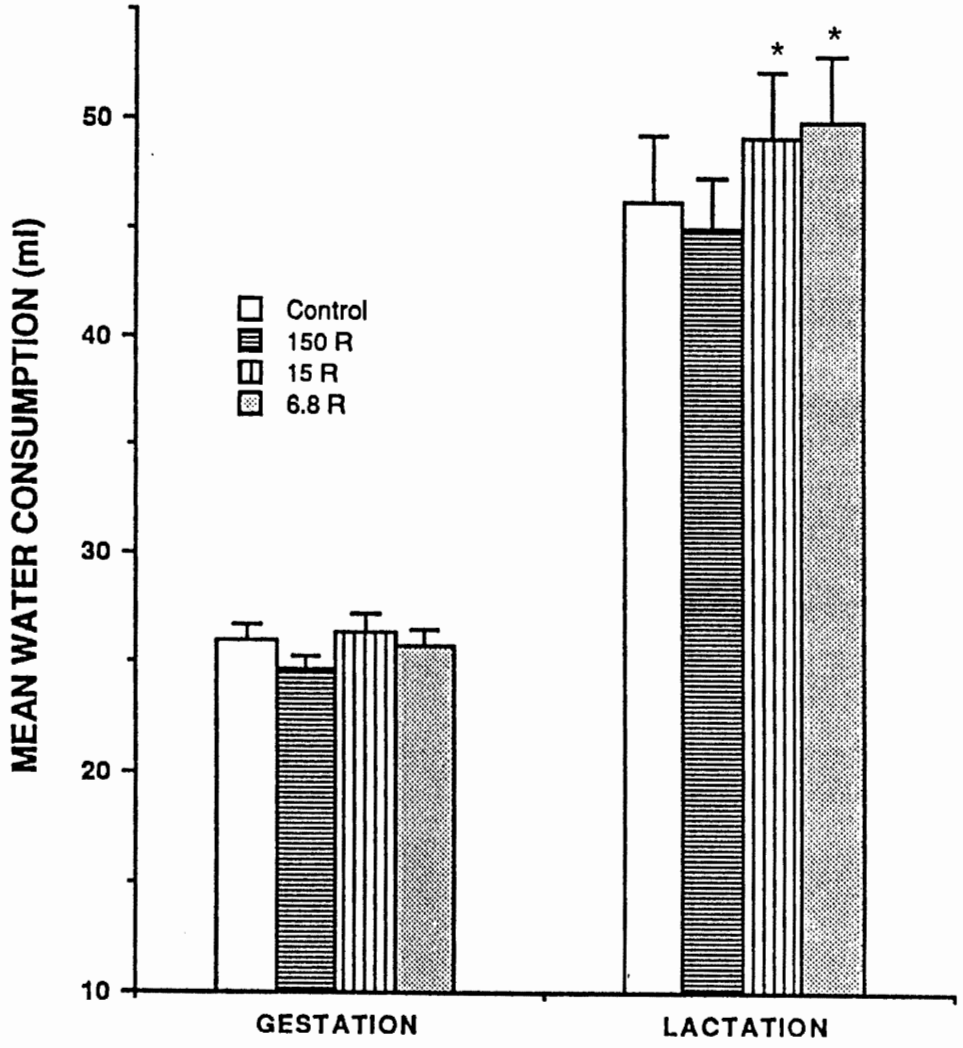

Figure 2. Comparison of mean water consumption during gestation and lactation (mean ± SEM) by control and radiation treated dams.

* Significantly different than control and 150 R at the 0.05 level: Tukey's test.

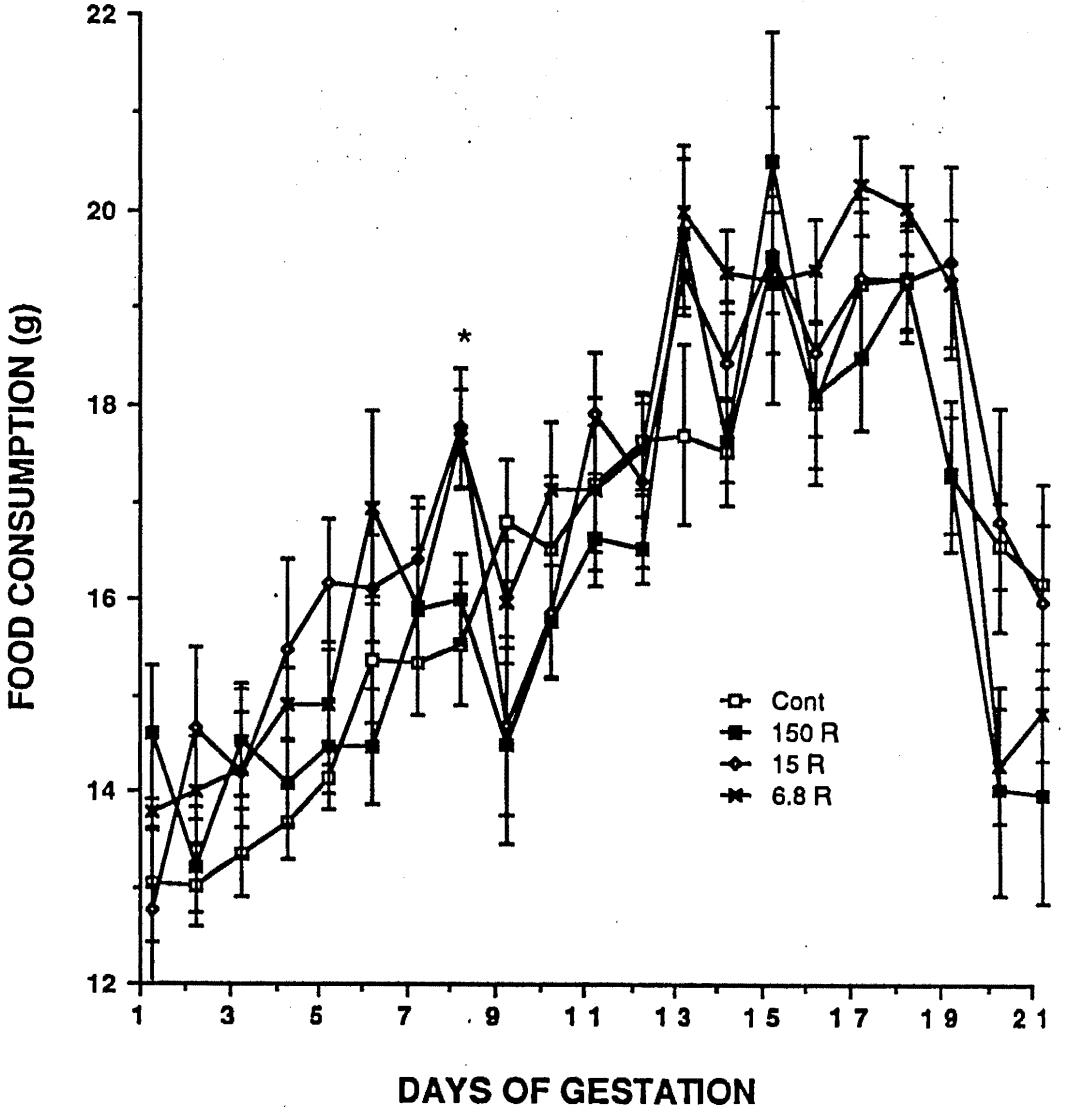

Figure 3. Comparison of daily food consumption during gestation (mean ± SEM) by control and radiation treated dams.

* Significant at the 0.05 level: Tukey's test.

food consumption for the whole gestational period was 16.1 ± 0.3 g, 16.0 ± 0.3, 16.8 ± 0.4, and 16.9 ± 0.2 g for the control, 150 R, 15 R and 6.8 R treatment groups respectively (Fig. 4). No difference in mean food consumption was observed between different treatment groups. Results of ANOVA with repeated measures showed a significant within subject effect of days ($p \leq 0.001$) in gestational food consumption (Table 2).

Body weight during gestation: Body weights of the pregnant rats were observed on days 1, 5, 9, 13, 17, and 21 of the gestational period. The body weights were significantly different ($p \leq 0.0002$) (Fig. 5) only on day 21, when the 15 R and 6.8 R treatment groups weighed more than the control or the 150 R treatment groups; but no significant differences in body weights were observed between the control and the 150 R treated animals, or between the 15 R and the 6.8 R treated animals. Mean body weights for the entire gestational period were 201.00 ± 3.32, 201.99 ± 3.34, 212.15 ± 2.68, and 210.86 ± 1.42 grams for the control, 150 R, 15 R, and 6.8 R treated dams (Fig. 6). Mean body weights of the control and 150 R treated rats were significantly different from the 15 R and the 6.8 R treated rats ($p \leq 0.01$); but no significant differences in mean body weights were observed between the control and the 150 R treated animals, or between

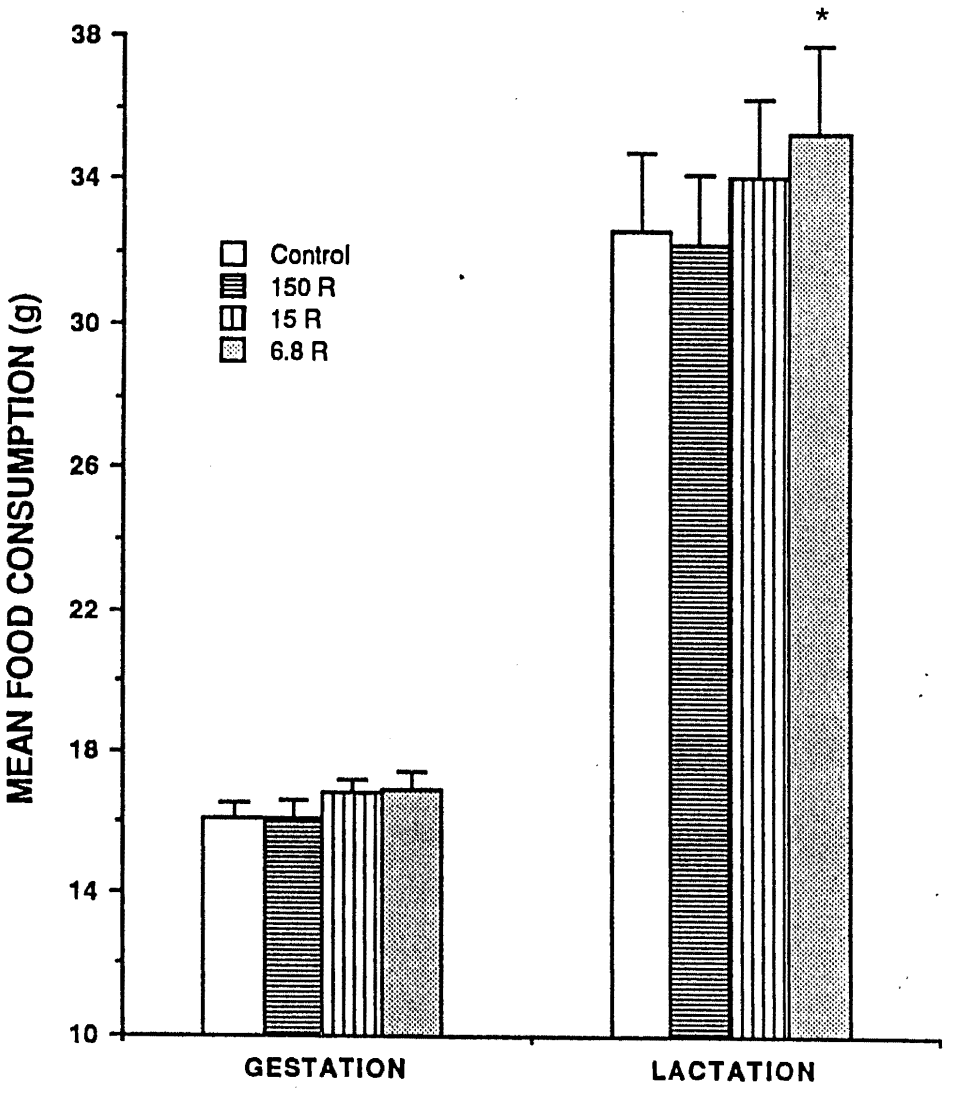

Figure 4. Comparison of mean food consumption during gestation and lactation (mean ± SEM) by control and radiation treated dams.

* Significantly different than control and 150 R at the 0.05 level: Tukey's test.

TABLE 2

SUMMARY OF ANOVA WITH REPEATED MEASURES:
FOOD CONSUMPTION DURING GESTATION

Source	DF	SS	MS	F	p
Between					
Subjects	55				
Treat	3	165.9	55.3	1.40	0.252
Error between	52	2047.4	39.3		
Within					
Subjects	1120				
Days	20	4380.1	219.0	39.58	0.001
Treat X Days	60	467.8	7.7	1.40	0.224
Error within	1040	5753.3	5.5		

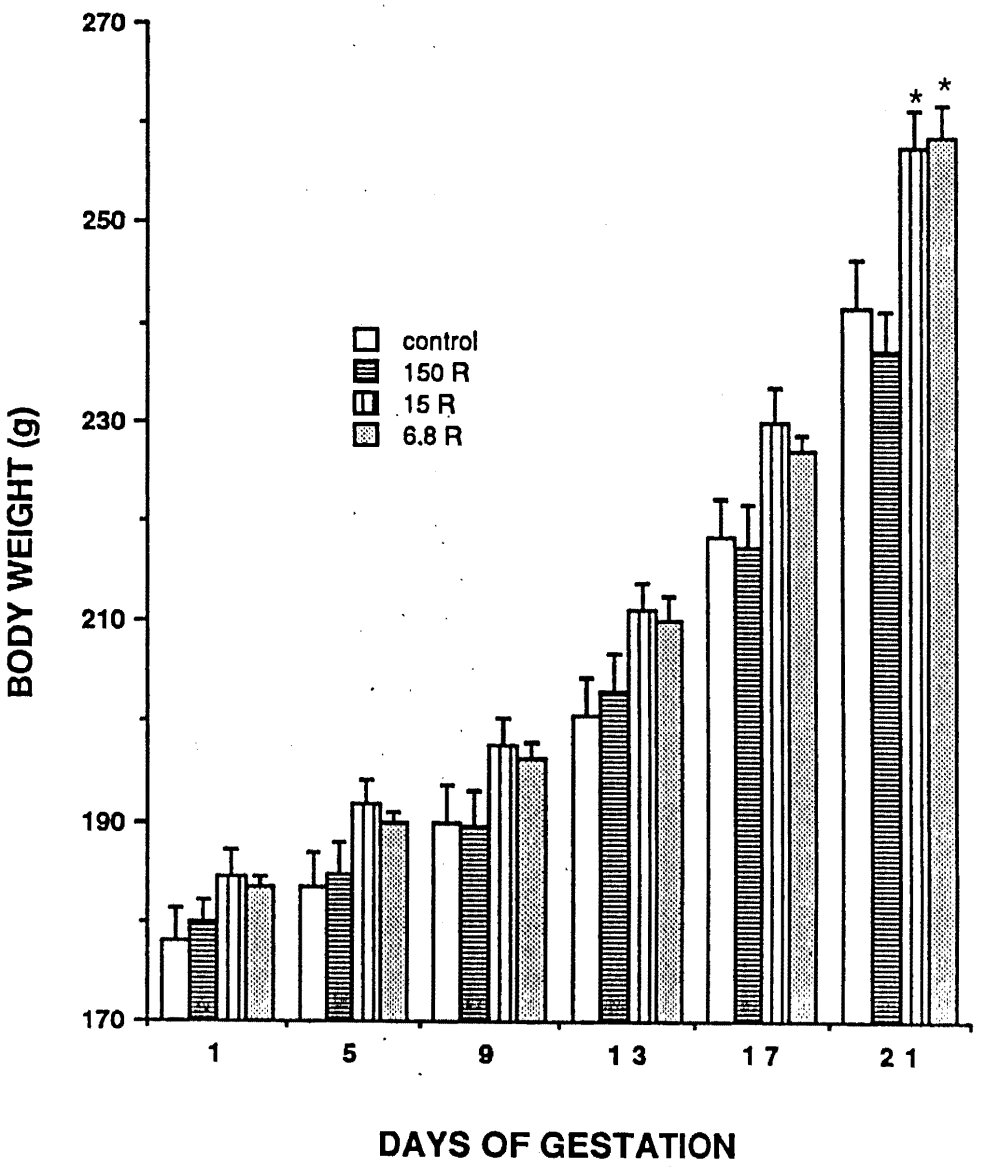

Figure 5. Comparison of body weight during gestation (mean ± SEM) of control and radiation treated dams.

* Significantly different than control and 150 R at the 0.05 level: Tukey's test.

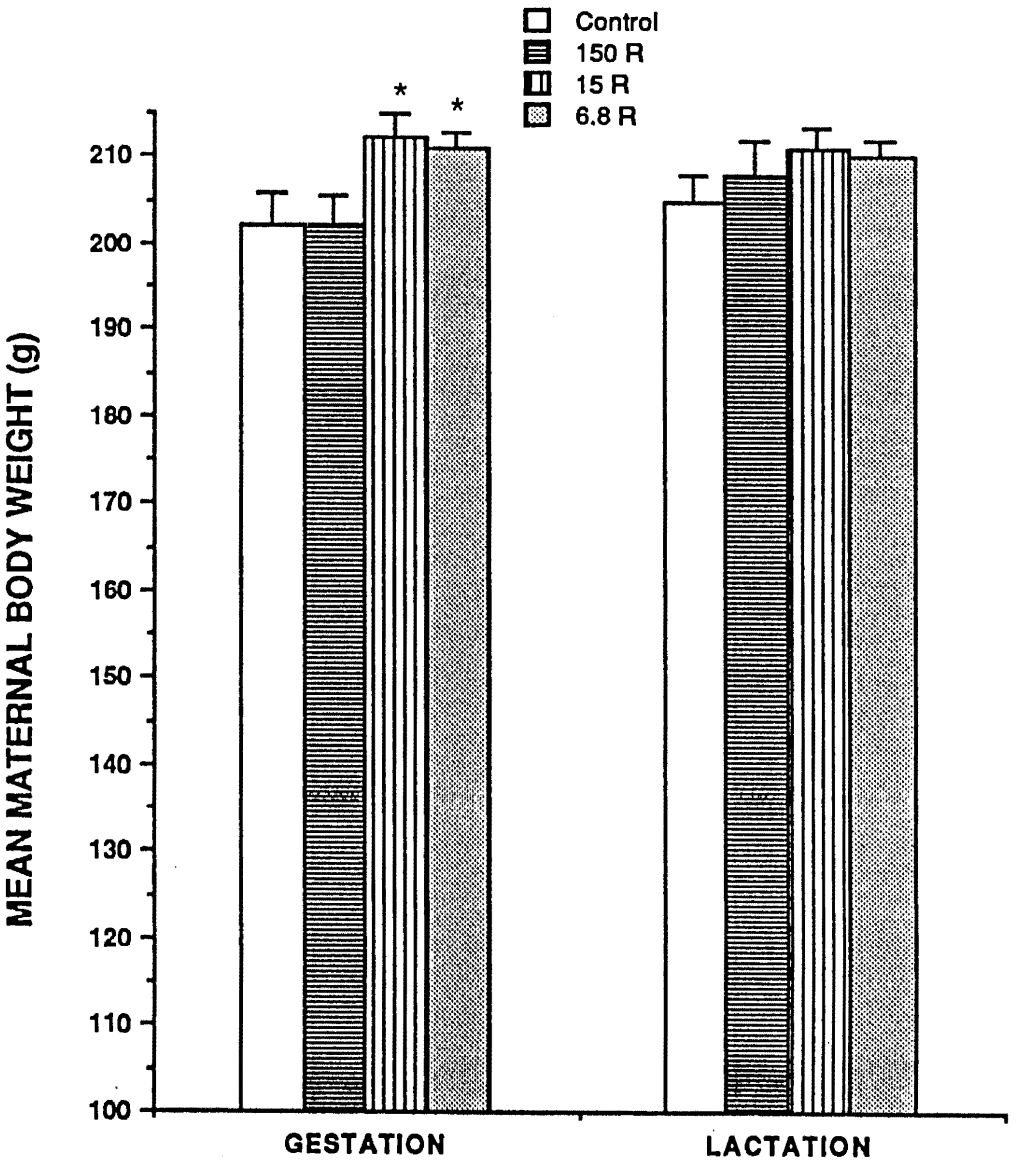

Figure 6. Comparison of mean body weight during gestation and lactation (mean ± SEM) of control and radiation treated dams.

* Significantly different than control and 150 R at the 0.05 level: Tukey's test.

the 15 R and the 6.8 R treated animals. Results of ANOVA with repeated measures showed a significant between subjects effect of treatment ($p \leq 0.01$), and within subjects effect of days ($p \leq 0.001$) (Table 3).

Water consumption during lactation: During lactation significant differences in daily water consumption between groups were observed on days 4, 5, 6, and 18 (Fig. 7). On days 4, 5,and 6 water consumption by the 6.8 R treatment group was significantly higher than for the other treatment groups ($p \leq 0.01$, 0.01, and 0.008 respectively). On day 18 water consumption by 15 R and 6.8 R treated animals were significantly higher than for the control and 150 R treated animals ($p \leq 0.01$) (Fig. 7). Mean water consumptions for the entire lactational period were 46.0 ± 1.3 ml, 45.4 ± 1.5 ml, 50.2 ± 0.8 ml, and 49.5 ± 1.0 ml for the control, 150 R, 15 R, and 6.8 R treatment groups respectively (Fig. 2). Mean water consumption of the control and the 150 R treated rats was significantly different from the 15 R and the 6.8 R treated rats ($p \leq 0.01$); but no significant differences in mean water consumption were observed between the control and the 150 R treated animals, or between the 15 R and the 6.8 R treated animals. Results of ANOVA with repeated measures showed a significant between-subjects effects of treatment

TABLE 3

SUMMARY OF ANOVA WITH REPEATED MEASURES:
BODY WEIGHT DURING GESTATION

Source	DF	SS	MS	F	p
Between					
Subjects	55				
Treat	3	7690.6	2563.5	3.88	0.014
Error between	52	34329.0	660.1		
Within					
Subjects	280				
Days	5	180953.5	36190.7	1046.16	0.001
Treat X Days	15	2002.0	133.4	3.85	0.001
Error within	260	8994.3	34.5		

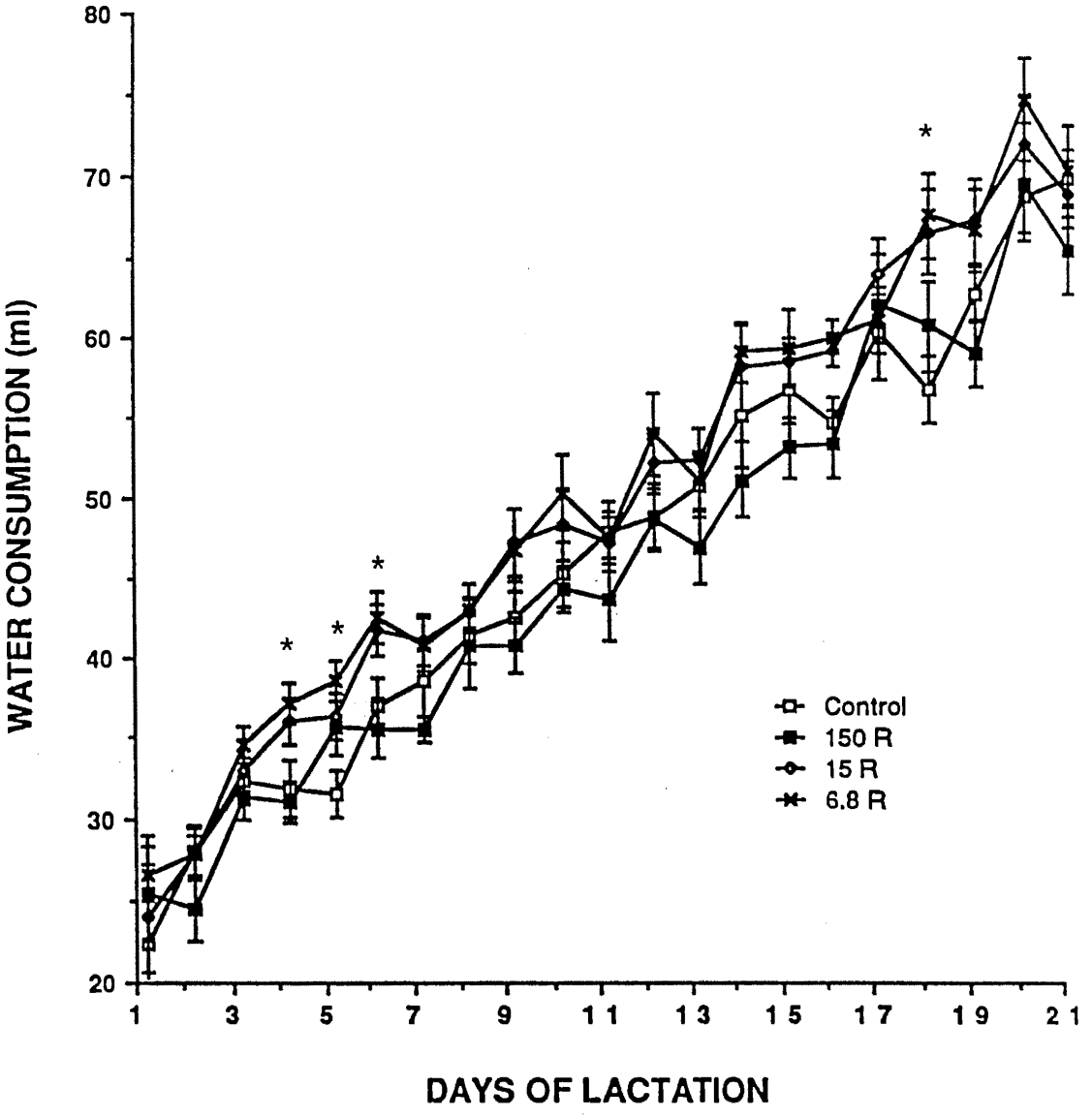

Figure 7. Comparison of daily water consumption during lactation (mean ± SEM) by control and radiation treated dams.

* Significant at the 0.05 level: Tukey's test.

($p \leq 0.01$), and within subjects effects of days ($p \leq 0.001$) in lactational water consumption (Table 4).

Food consumption during lactation: Significant differences in daily food consumption between the groups were observed only on days 12 and 16 of the lactational period ($p \leq 0.004$, and 0.001 respectively) (Fig. 8). On day 12, the 15 R and 6.8 R groups consumed more food than the control and the 150 R groups; but no significant differences in food consumptions were observed between the control and the 150 R groups, or between the 15 R and 6.8 R irradiated groups. On day 16, the 15 R treated animals consumed more food than the control and 150 R groups; and the 6.8 R treatment group consumed more food than the 150 R group; but no significant differences in food consumption were observed between the control and 150 R groups, or between the 15 R and 6.8 R groups (Fig. 8). Mean food consumption for the entire lactational period was 32.7 ± 0.8 g, 32.5 ± 0.6 g, 34.5 ± 0.7 g, and 35.1 ± 0.4 g for the control, 150 R, 15 R, and 6.8 R treatment groups respectively (Fig. 4). Results of ANOVA with repeated measures showed a significant between subjects effect of treatment ($p \leq 0.02$), and within subjects effect of days ($p \leq 0.001$) (Table 5). Mean food consumption of 6.8 R treated dams was significantly higher when compared to the

TABLE 4

SUMMARY OF ANOVA WITH REPEATED MEASURES:
WATER CONSUMPTION DURING LACTATION

Source	DF	SS	MS	F	p
Between					
Subjects	47				
Treat	3	4308.1	1433.0	3.68	0.010
Error between	44	17131.3	389.3		
Within					
Subjects	960				
Days	20	16327.0	8161.8	178.08	0.001
Treat X Days	60	2480.7	41.3	0.90	0.685
Error within	880	40330.4	45.8		

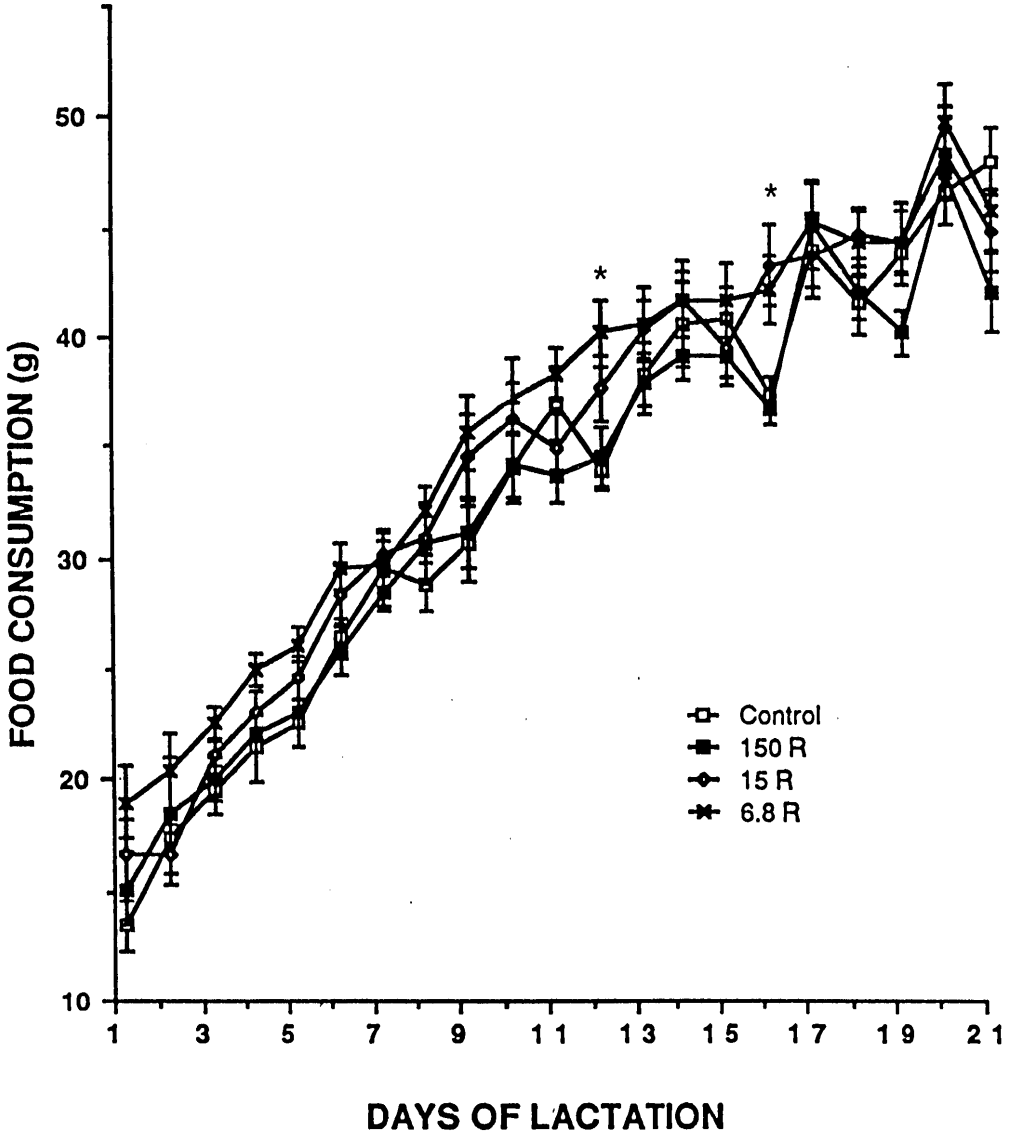

Figure 8. Comparison of daily food consumption during lactation (mean ± SEM) by control and radiation treated dams.

* Significant at the 0.05 level: Tukey's test.

TABLE 5

SUMMARY OF ANOVA WITH REPEATED MEASURES:
FOOD CONSUMPTION DURING LACTATION

Source	DF	SS	MS	F	p
Between					
Subjects	47				
Treat	3	1310.6	436.8	3.51	0.02
Error between	44	5461.3	124.1		
Within					
Subjects	960				
Days	20	80451.6	4022.5	157.05	0.001
Treat X Days	60	1551.8	25.8	1.00	0.457
Error within	880	22539.2	25.6		

150 R treated dams ($p \leq 0.001$), but no differences were observed between control, 15 R, and 6.8 R treated groups, or between control, 150 R, and 15 R treated groups.

Body weight during lactation: The body weights of the lactating rats were observed on days 1, 5, 9, 13, 17, and 21 of the lactational period (Fig. 9). No significant differences in daily mean body weights between the different experimental groups were observed during this period. Mean body weights for the entire lactational period were 204.90 ± 2.80, 206.35 ± 3.03, 210.85 ± 2.24, and 210.85 ± 1.17 grams for the control, 150 R, 15 R, and 6.8 R treated dams respectively (fig. 6). No significant difference in mean body weight was observed between different treatment groups, but results of ANOVA with repeated measures showed a significant within subjects effects of days ($p \leq 0.001$) since the body weights of different treatment groups were gradually increased with days of lactation (Table 6).

DEVELOPMENTAL PARAMETERS

Pivoting: A Chi-square analysis of data obtained on pivoting behavior revealed that the pups irradiated with 150 R on day 20 of prenatal life had significantly lower performance than any other groups on days 15 and 16 of the

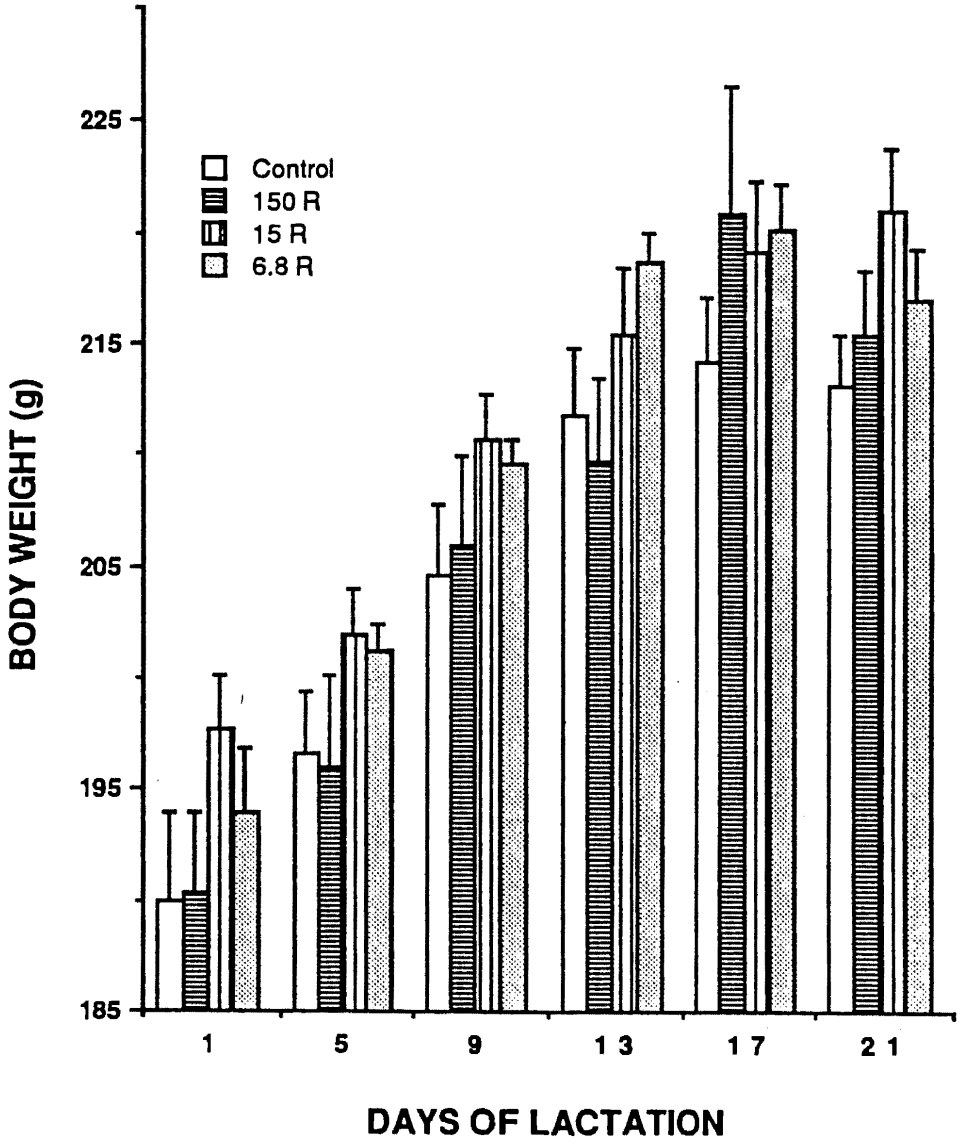

Figure 9. Comparison of body weight during lactation (mean ± SEM) of control and radiation treated dams.

TABLE 6

SUMMARY OF ANOVA WITH REPEATED MEASURES:
BODY WEIGHT DURING LACTATION

Source	DF	SS	MS	F	p
Between					
Subjects	52				
Treat	3	1892.7	630.9	1.32	0.278
Error between	49	23412.8	477.8		
Within					
Subjects	265				
Days	5	28379.8	5675.9	99.68	0.001
Treat X Days	15	706.2	47.0	0.82	0.647
Error within	245	13949.5	56.9		

observation period (Fig. 10). A hierarchical loglinear analysis of the data showed no overall difference between different treatment groups.

Righting on a surface: All treatment groups had similar scores in righting performance. Almost all the animals could right within 5 seconds throughout the observation period. Therefore, analysis of the data showed no differences between different treatment groups.

Crawling: No crawling was observed until day 6 of the postnatal life, and no crawling was observed after day 18, as this movement was eventually replaced by walking. The crawling performance was evaluated on a score basis. All the groups tended to score high on days 11 to 15 (Fig. 11). No significant differences in crawling between the control and radiation treated groups were observed during the observation period. Mean scores for the entire observation period were 1.26 ± 0.10, 0.98 ± 0.16, 1.14 ± 0.19, and 1.01 ± 0.16 for the offspring of the control, 150 R, 15 R, and 6.8 R radiation treated animals (Fig. 12) No significant differences in mean scores were observed between different treatment groups. Results of ANOVA with repeated measures showed significant within subjects effects of days ($p \leq .001$)

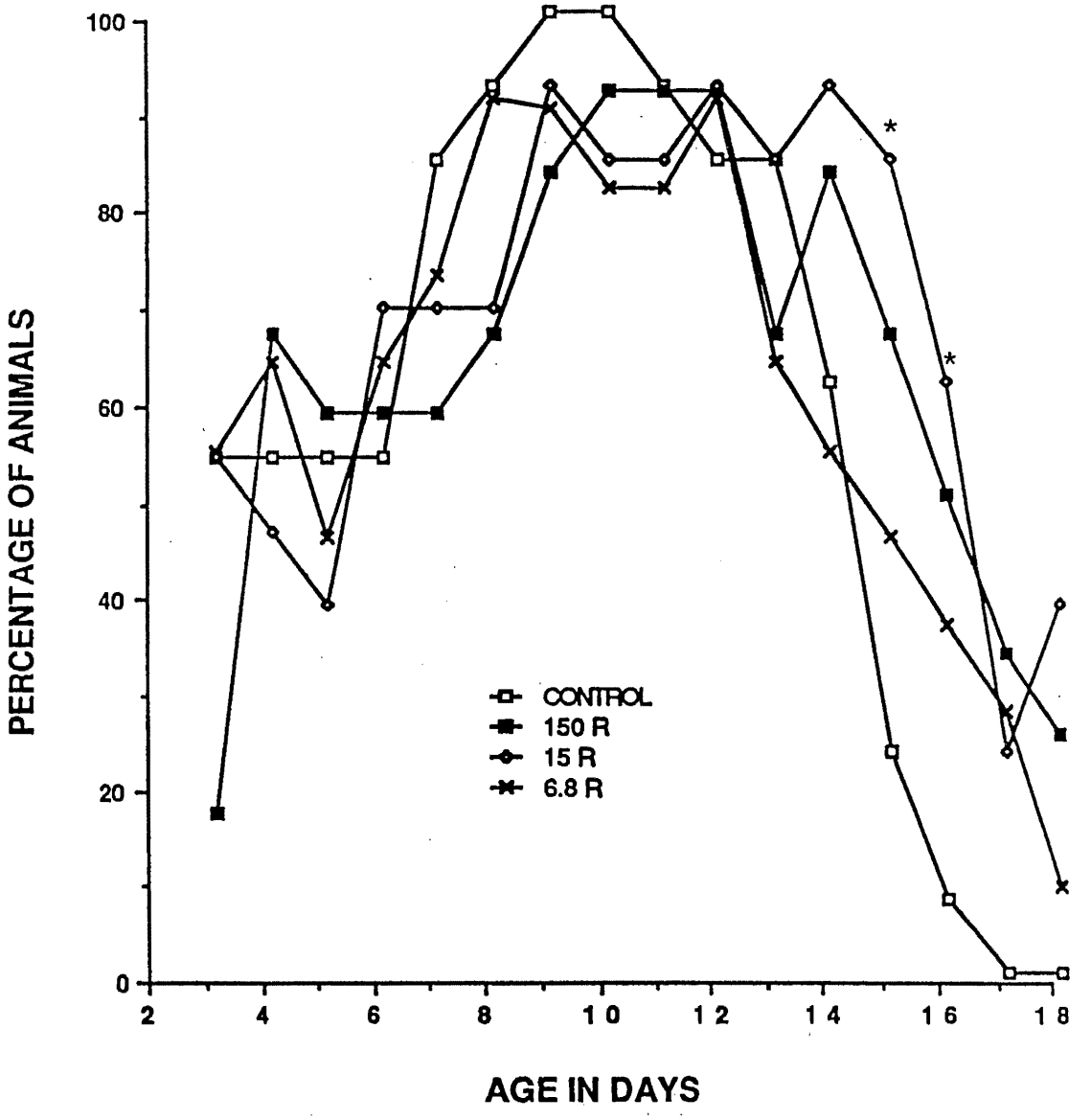

Figure 10. Comparison of pivoting of pups born to control and radiation treated dams.

* Significant at the 0.05 level: Chi-square test.

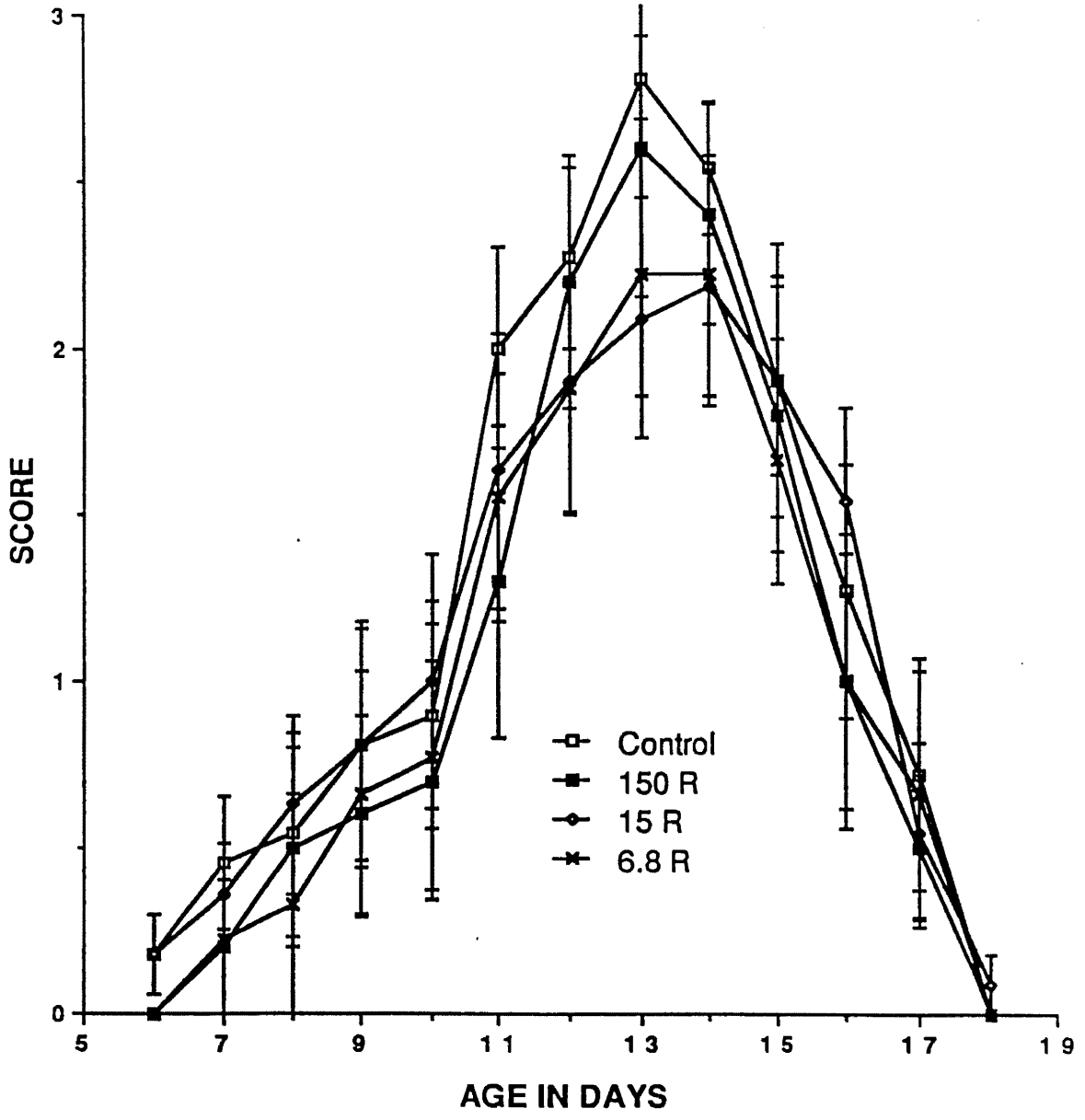

Figure 11. Comparison of crawling (mean ± SEM) of pups born to control and radiation treated dams.

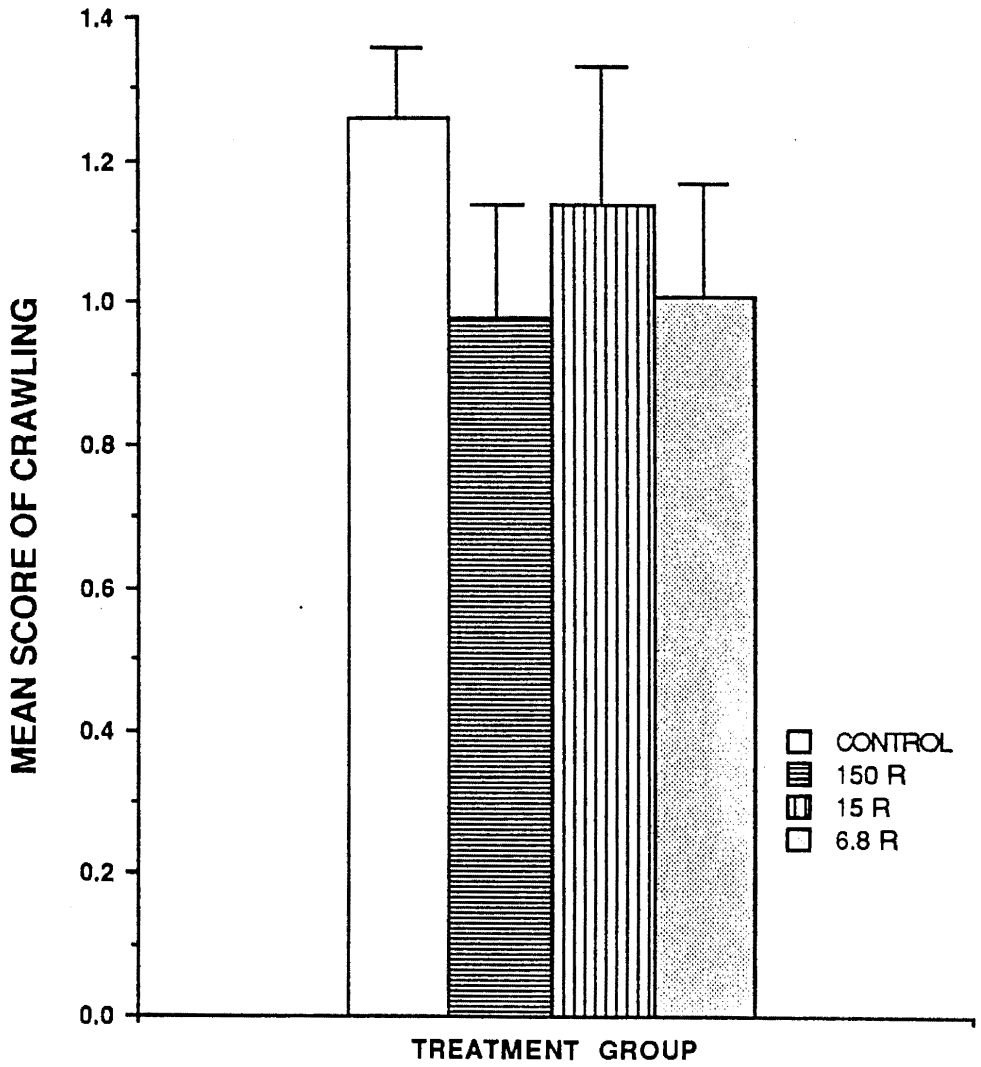

Figure 12. Comparison of mean scores of crawling (mean ± SEM) of pups born to control and radiation treated dams.

(Table 7) since the crawling performance gradually appeared and eventually disappeared with age (days).

Negative geotaxix: Negative geotaxis was observed from day 3 to day 10 of postnatal life. A Chi-square analysis of the data revealed no significant differences between different treatment groups (Fig. 13). Although the pups receiving 150 R appeared to exhibit considerably less geotaxis response than the other groups, the differences were not significant at the 5 percent level of probability.

Cliff-avoidance: Cliff-avoidance was recorded from day 3 to day 10 of postnatal life. This performance was evaluated on a score system. Although the pups of the 150 R treated group appeared to score considerably lower throughout the study period, statistically significant differences in cliff-avoidance scores were observed only on days 8 and 9 (Fig. 14). On day 8, control and 6.8 R treated groups received significantly higher scores than the 15 R and 150 R treatment groups ($p \leq 0.01$), but no significant differences were observed between control and 6.8 R treatment groups, or between 15 R and 150 R treatment groups. On day 9, the 150 R treatment group received a significantly lower score than the other three treatment groups ($p \leq 0.006$), but no significant

TABLE 7

SUMMARY OF ANOVA WITH REPEATED MEASURES: CRAWLING

Source	DF	SS	MS	F	p
Between					
Subjects	40				
Treat	3	6.7	2.2	0.66	0.577
Error between	37	123.9	3.3		
Within					
Subjects	492				
Days	12	331.0	27.5	34.24	0.001
Treat X Days	36	11.0	0.3	0.38	1.000
Error within	444	357.7	0.8		

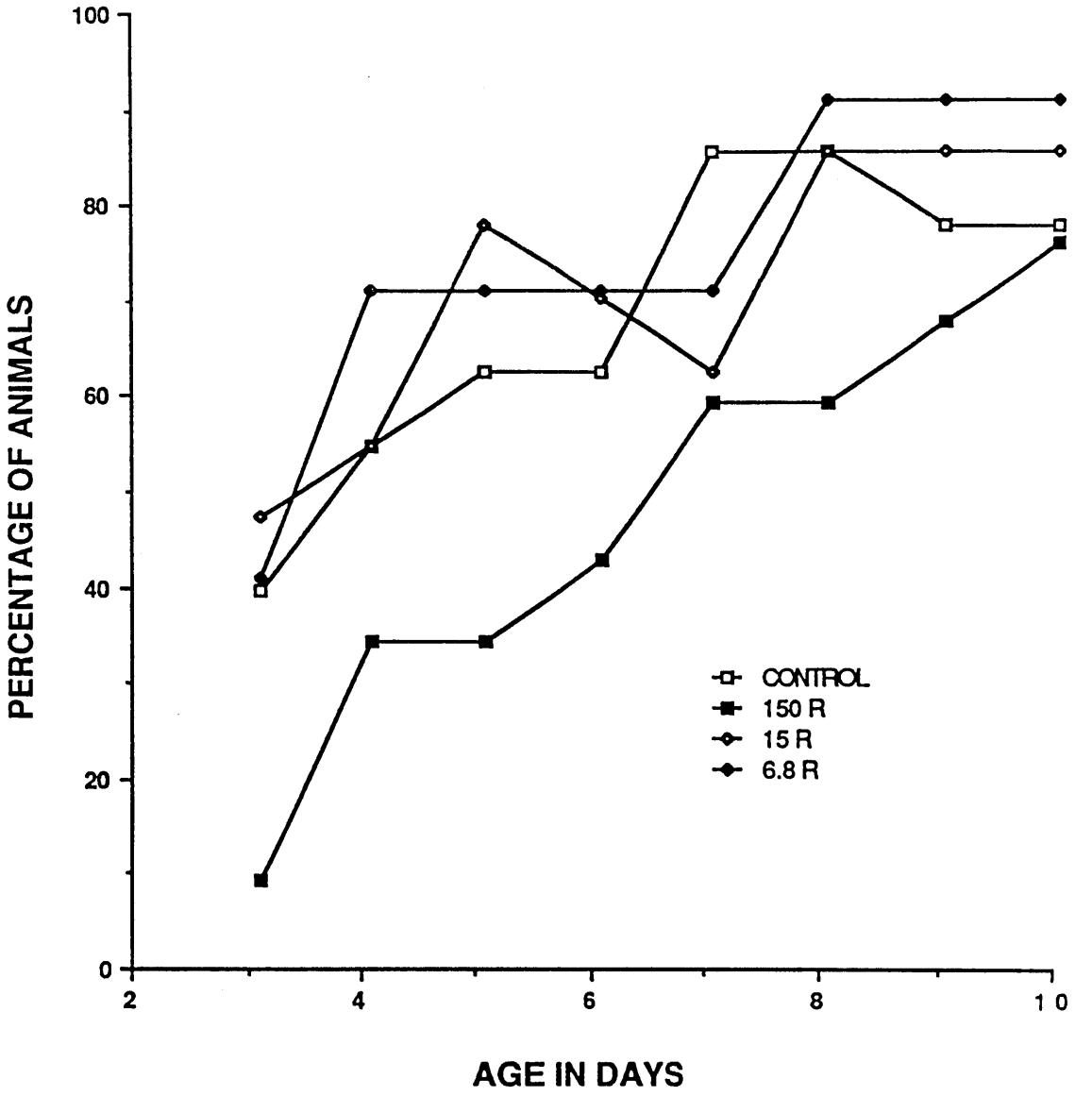

Figure 13. Comparison of negative geotaxis of pups born to control and radiation treated dams.

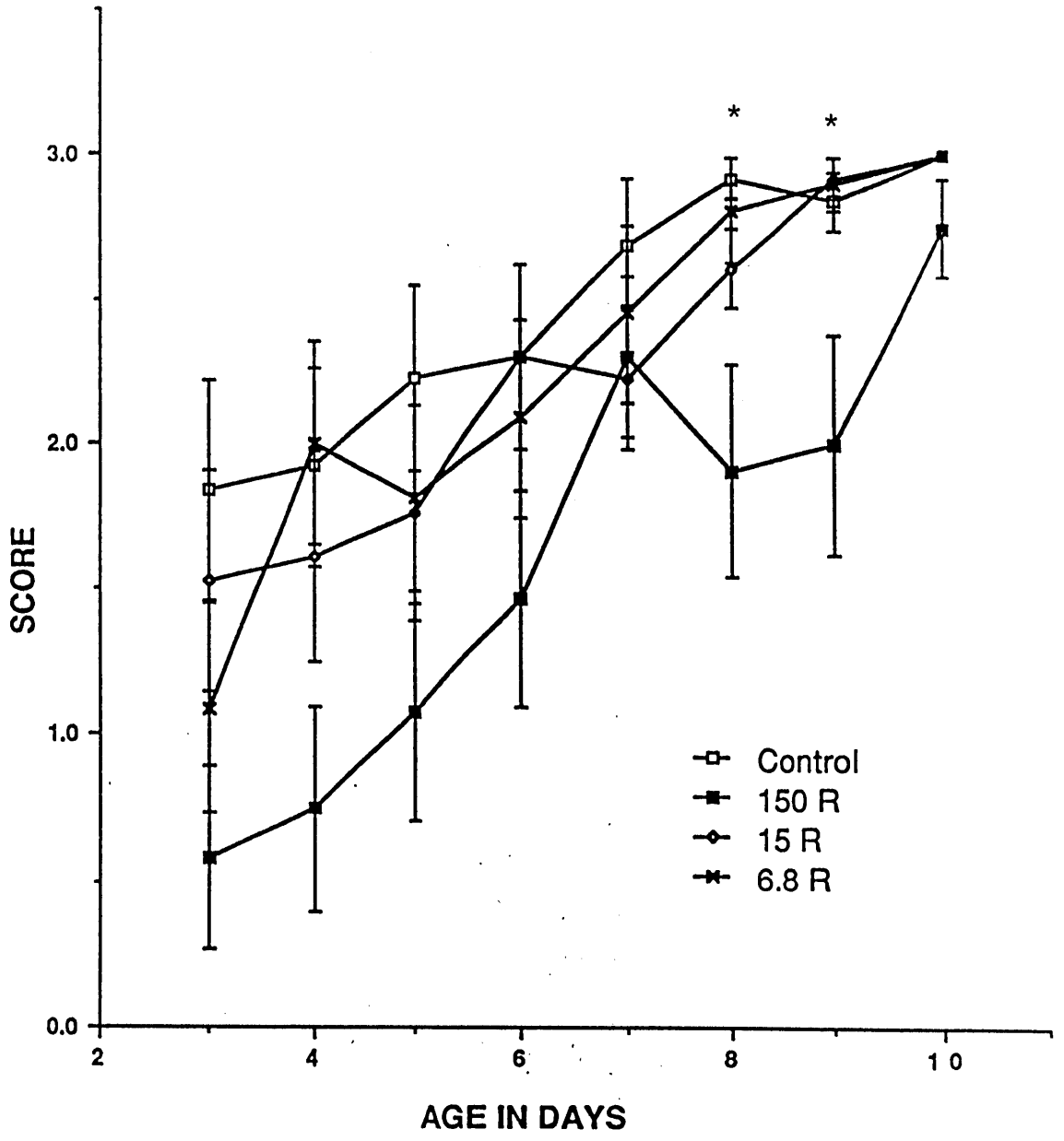

Figure 14. Comparison of cliff-avoidance (mean ± SEM) of pups born to control and radiation treated dams.

* Significant at the 0.05 level: Tukey's test.

differences were observed between control, 15 R, and 6.8 R treatment groups. Mean scores for the entire observation period were 2.47 ± 0.14, 1.62 ± 0.24, 2.25 ± 0.16, and 2.27 ± 0.16 for the offspring of the control, 150 R, 15 R, and 6.8 R treated animals (Fig. 15). Mean score of the control pups was significantly higher when compared to that of the 150 R treated pups ($p \leq 0.001$). No any other significant differences were observed between different treatment groups. Results of ANOVA with repeated measures showed a significant between subjects effect of treatment ($p \leq 0.01$), and within subjects effect of days ($p \leq 0.001$) (Table 8).

Hindlimb support when suspended: No hindlimb support was observed until day 13 of the postnatal life. Chi-square analysis of the data showed no significant differences between different treatment groups (Fig. 16)

Eye opening (Left eye): The mean left eye opening time periods were 17.69 ± 0.75, 18.33 ± 1.07, 17.92 ± 0.64, and 17.90 ± 0.83 days for the control, 150 R, 15 R, and 6.8 R radiation treated groups respectively (Fig. 17). Although the left eye opening appeared to be delayed in the pups of the 150 R treated rats, no significant differences in left eye opening times were observed between different treatment

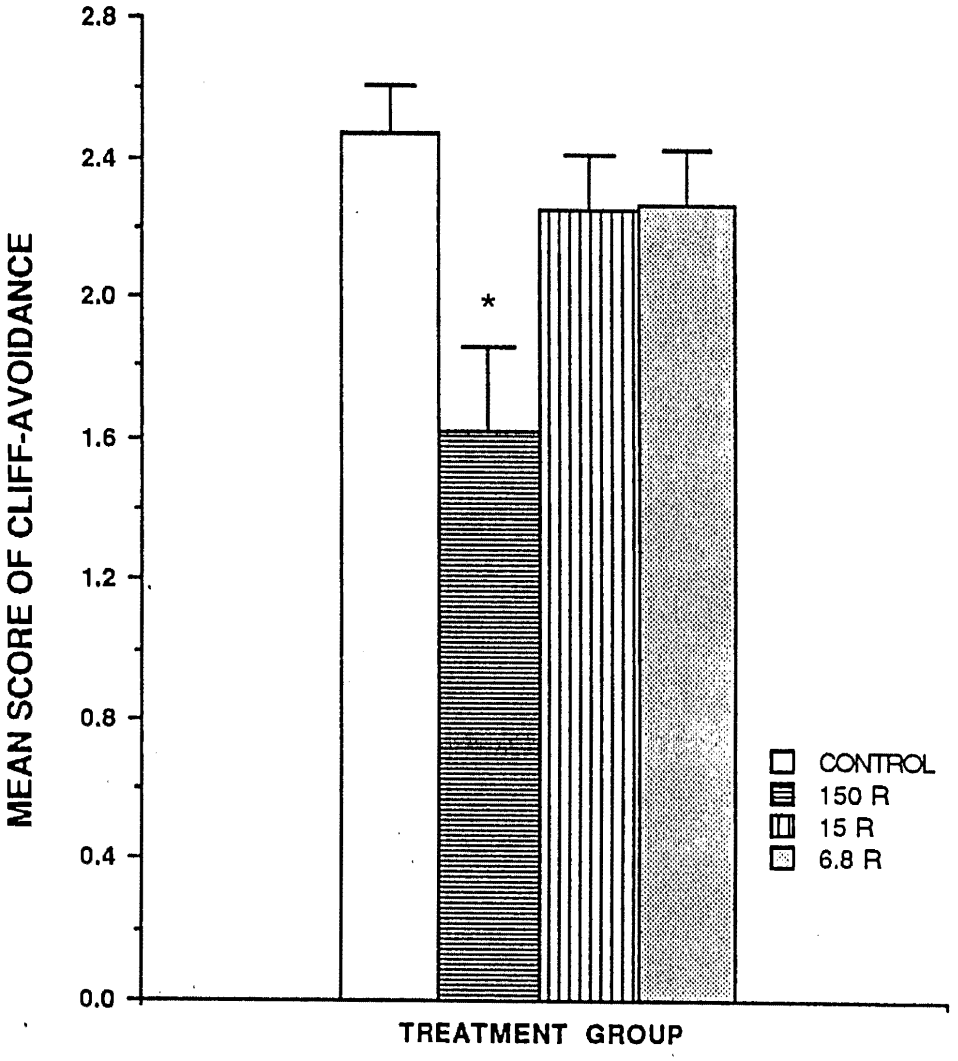

Figure 15. Comparison of mean scores of cliff-avoidance (mean ± SEM) of pups born to control and radiation treated dams.

* Significantly different than control, 15 R and 6.8 R at the 0.05 level: Tukey's test.

TABLE 8

SUMMARY OF ANOVA WITH REPEATED MEASURES: CLIFF-AVOIDANCE

Source	DF	SS	MS	F	p
Between					
Subjects	48				
Treat	3	39.5	13.1	4.12	0.011
Error between	45	143.7	3.1		
Within					
Subjects	343				
Days	7	119.6	17.0	23.30	0.001
Treat X Days	21	15.0	0.7	0.98	0.487
Error within	315	230.9	0.7		

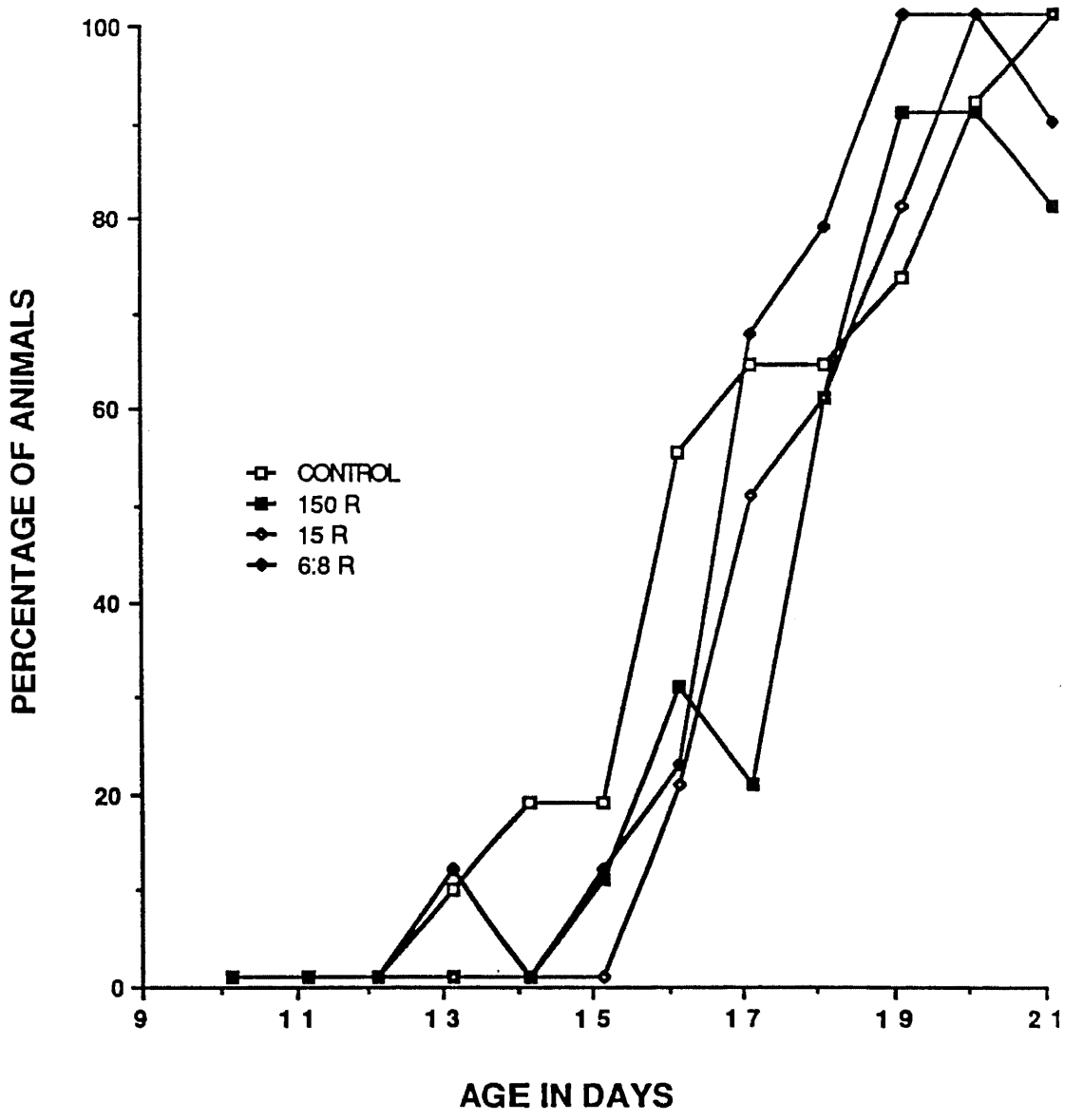

Figure 16. Comparison of hindlimb support of pups born to control and radiation treated dams.

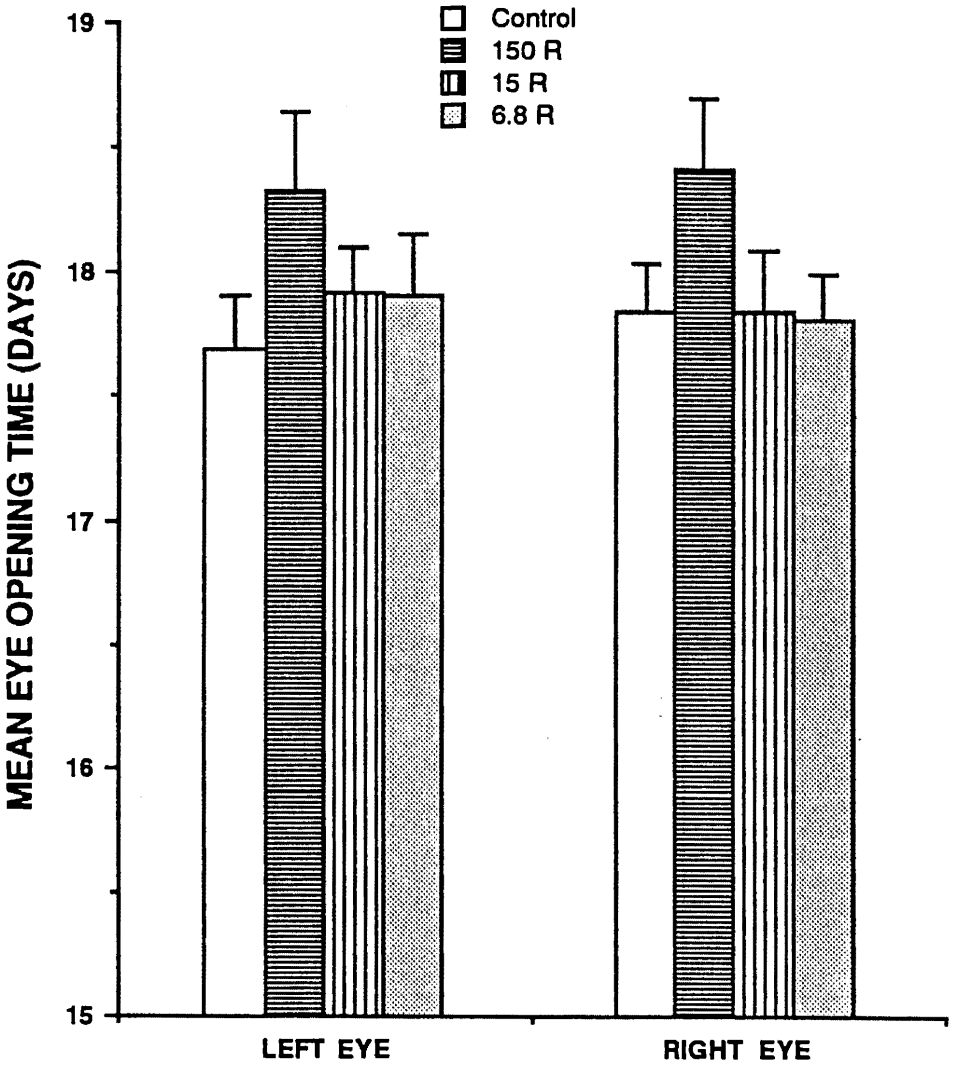

Figure 17. Comparison of mean left and right eye opening time (mean ± SEM) of pups born to control and radiation treated dams.

groups.

Eye opening (Right eye): The mean right eye opening time periods of different experimental groups were very similar to the left eye opening time periods (Fig. 17). No significant differences in right eye opening times were observed between different treatment groups, although it also appeared to be delayed in the pups of the 150 R treatment group.

Tooth eruption (Upper jaw): The mean time periods for upper jaw tooth eruptions were 9.15 ± 0.19, 10.33 ± 0.37, 10.00 ± 0.27, and 9.54 ± 0.20 days for the control, 150 R, 15 R, and 6.8 R radiation treated groups respectively (Fig. 18). There was a dose related delay in upper jaw tooth eruption for all three irradiated groups, but the only significant difference ($p \leq 0.003$) was observed between the control and 150 R treatment groups.

Tooth eruption (Lower jaw): The mean time periods for lower jaw tooth eruptions were 11.38 ± 0.28, 12.50 ± 0.41, 11.92 ± 0.41, and 12.16 ± 0.42 days for the control, 150 R, 15 R, and 6.8 R treatment groups respectively (Fig. 18). While no significant differences in lower jaw tooth eruption were observed between different treatment groups,

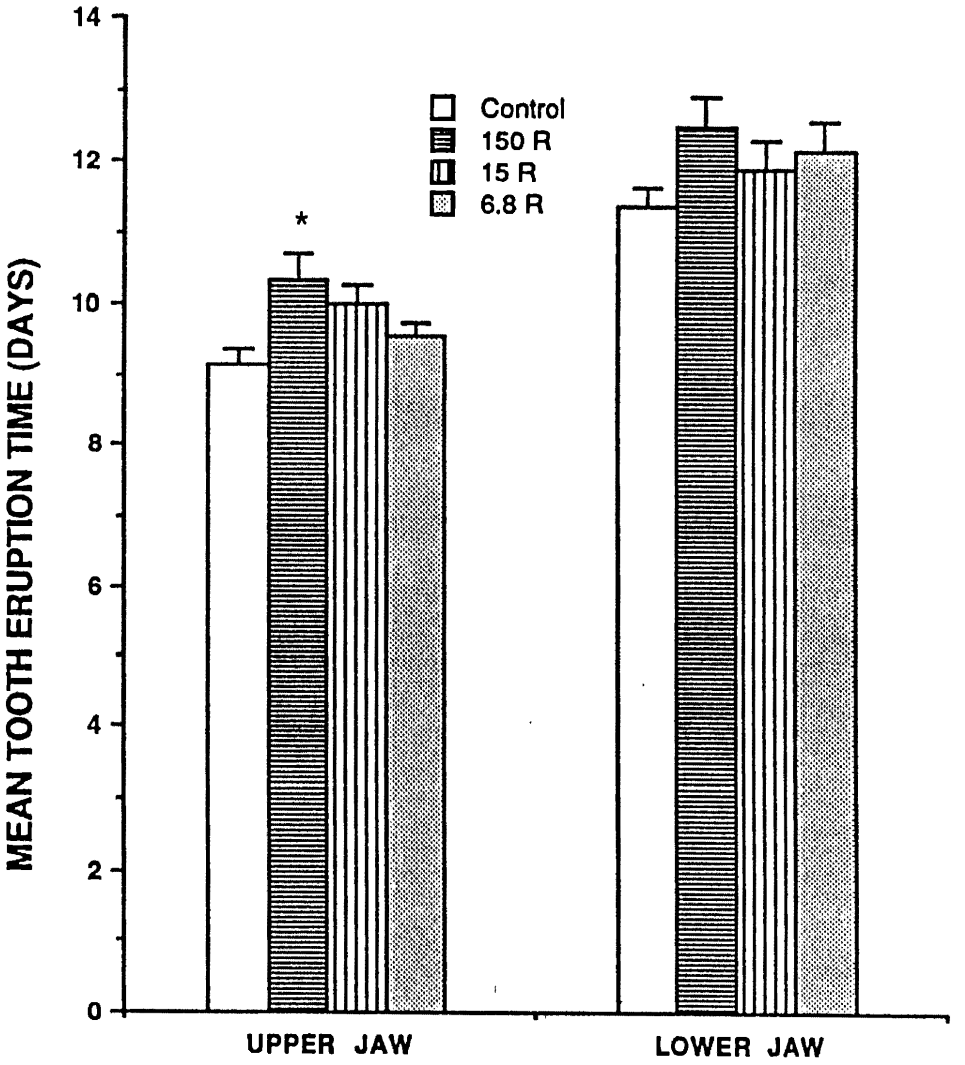

Figure 18. Comparison of mean upper and lower jaw tooth eruption time (mean ± SEM) of pups born to control and radiation treated dams.

* Significantly different than control at the 0.05 level: Tukey's test.

eruption was delayed in all treatment groups, with the greatest effect being observed in the 150 R group.

Body weight: Pups were weighed on every alternate day from day 3 to day 21 of postnatal life, then on day 30,and day 52. Body weights of some treatment groups were significantly different on all days except days 3,5 and 9 (Fig. 19). On days 7, 11, 15, 21, the 6.8 R treatment group had significantly higher body weights than 150 R treatment group ($p \leq 0.02$, 0.03, 0.01, and 0.01 respectively), but no significant differences were observed between control, 15 R, and 6.8 R treatment groups. On days 13, and 19 control and 6.8 R treatment groups had significantly higher weights than the 150 R treatment group ($P \leq 0.008$, and 0.005 respectively) (Fig. 19). No differences were observed between control, 6.8 R, and 15 R treatment groups or between 15 R and 150 R treatment groups. On days 17, 30, and 52, control, 6.8 R, and 15 R treatment groups had significantly higher body weight than 150 R treatment group ($p \leq 0.002$, 0.004, and 0.005 respectively), but no significant differences were observed between the control, 6.8 R, and 150 R treatment groups (Fig. 19). Mean body weights from day 3 to day 52 were 29.59 ± 0.55, 25.36 ± 1.59, 29.22 ± 0.54, and 30.54 ± 0.80 grams for the offspring of the control, 150 R, 15 R, and

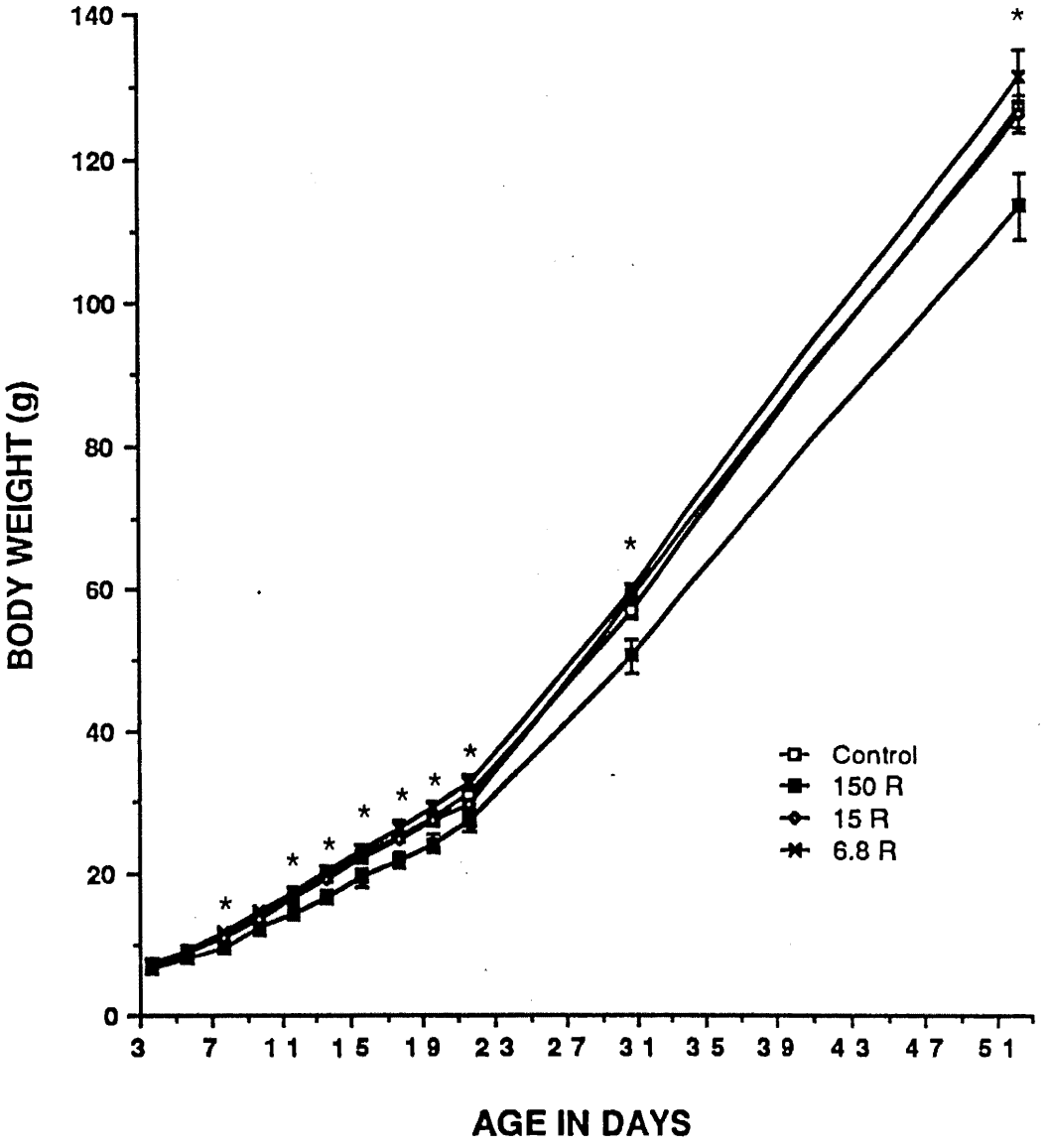

Figure 19. Comparison of body weight (mean ± SEM) of pups born to control and radiation treated dams.

* Significant at the 0.05 level: Tukey's test.

6.8 R treated animals (Fig. 20). Mean body weight of the 150 R treated offspring was significantly lower when compared to that of any of the other treatment groups ($p \leq 0.02$). Results of ANOVA with repeated mesures showed a significant between subject effect of treatment ($p \leq 0.02$), and within subjects effects of days ($p \leq 0.001$) (Table 9).

Organ weights, and the ratio of organs to body weight (day 30) : In the pups sacrificed on day 30 of postnatal life, brain, ovary, kidney, heart, and spleen weights of 150 R treatment group were significantly lower ($p \leq 0.0001$) than that of any other treatment groups (Table 10 and 11). Liver weight of the 150 R treatment group was significantly lower ($p \leq 0.008$) than that of the 15 R and 6.8 R treatment groups, but no significant differences were observed between the control and the 150 R treatment groups, or between 15 R and the 6.8 R treatment groups. Adrenal and lung weights were comparable between all treatment groups.

A comparison of the organs to body weight ratio (brain, ovary, adrenal, kidney, liver, heart, spleen, and lung) showed no significant differences between different treatment groups (Table 12 and 13).

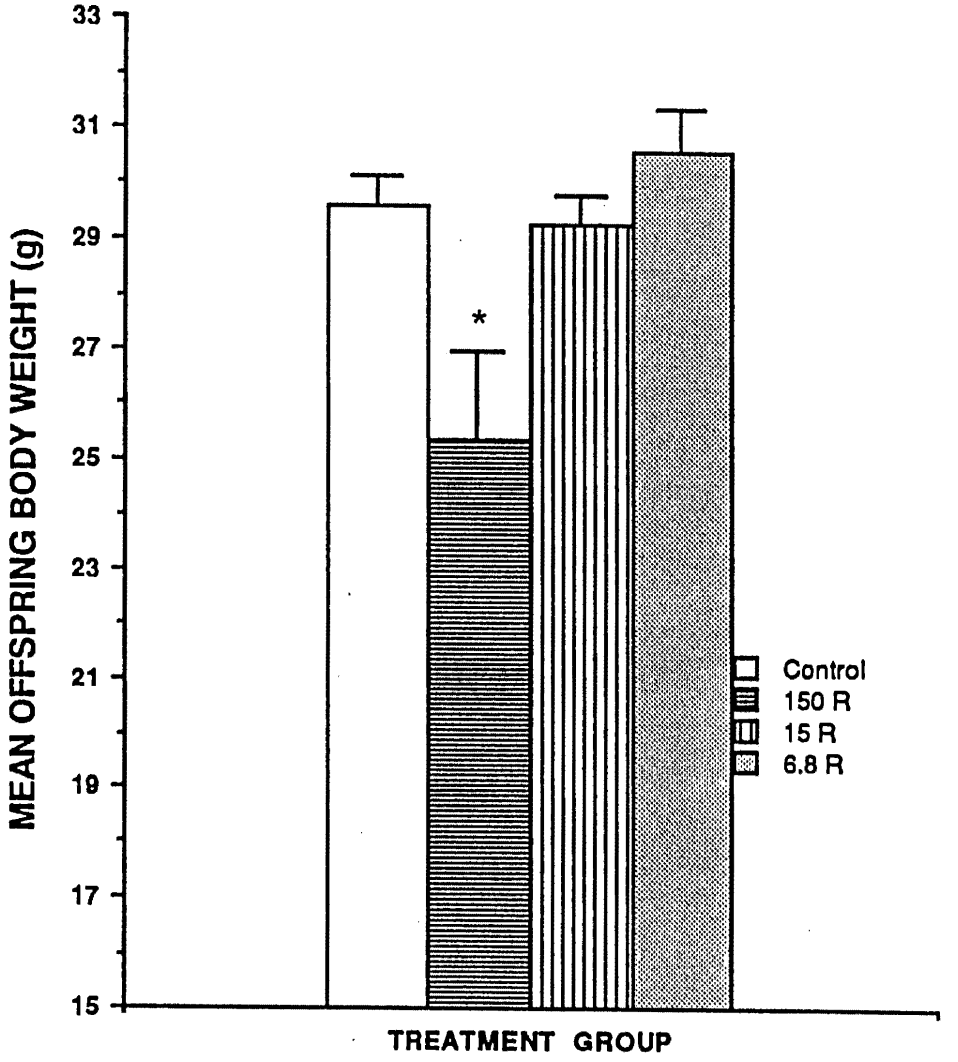

Figure 20. Comparison of mean body weight (mean ± SEM) of pups born to control and radiation treated dams.

* Significantly different than control, 15 R and 6.8 R at the 0.05 level: Tukey's test.

TABLE 9

SUMMARY OF ANOVA WITH REPEATED MEASURES: OFFSPRING BODY WEIGHT

Source	DF	SS	MS	F	p
Between					
Subjects	41				
Treat	3	446.4	148.8	3.59	0.022
Error between	38	1572.6	41.4		
Within					
Subjects	378				
Days	9	23189.3	2576.6	1875.90	0.001
Treat X Days	27	114.7	4.3	3.09	0.001
Error within	342	469.7	1.4		

TABLE 10

A comparison of body, brain, and brain area weights of offspring of control, and radiation treated rats. Dams were exposed to gamma radiation on the 20th day of gestation.

Age (days)	Groups (n = 14)	Body (g ± SEM)	Brain (g ± SEM)	Cerebral cortex (g ± SEM)	Cerebellum (g ± SEM)	Brain stem (g ± SEM)
30	CONTROL	55.81 ± 1.29	1.42 ± .01	0.797 ± .01	0.184 ± .003	0.182 ± .007
	150 R	49.26 ± 2.40•	1.19 ± .03•	0.621 ± .02•	0.176 ± .004	0.170 ± .008
	15 R	57.68 ± 1.16	1.42 ± .01	0.799 ± .01	0.180 ± .004	0.178 ± .004
	6.8 R	58.55 ± 1.00	1.41 ± .01	0.798 ± .01	0.180 ± .003	0.173 ± .005
52	CONTROL	126.02 ± 3.06	1.64 ± .01	0.883 ± .01	0.225 ± .004*	0.228 ± .005
	150 R	111.99 ± 4.59•	1.40 ± .04•	0.705 ± .03•	0.207 ± .005Δ•	0.242 ± .004
	15 R	124.89 ± 2.53	1.62 ± .02	0.872 ± .01	0.222 ± .004*•	0.235 ± .012
	6.8 R	130.01 ± 3.62	1.64 ± .02	0.864 ± .01	0.223 ± .004*•	0.226 ± .005

•, Δ, *, Groups with different characters are significantly different from other groups at the $p \leq 0.05$ level.

TABLE 11

A comparison of organ weights of offspring of control and radiation treated rats. Dams were exposed to gamma radiation on the 20th day of gestation.

Age (days)	Groups (n=14)	Ovary (g ± SEM)	Adrenal (g ± SEM)	Kidney (g ± SEM)	Liver (g ± SEM)	Heart (g ± SEM)	Spleen (g ± SEM)	Lung (g ± SEM)
30	CONTROL	0.026 ± .001	0.026 ± .011	0.61 ± .01	2.14 ± .03Δ	0.21 ± .01	0.19 ± .01	0.37 ± .01
	150 R	0.022 ± .001•	0.014 ± .001	0.55 ± .02•	1.93 ± .10Δ	0.19 ± .01•	0.16 ± .01•	0.34 ± .01
	15 R	0.027 ± .001	0.015 ± .001	0.63 ± .01	2.19 ± .05•	0.22 ± .01	0.20 ± .01	0.37 ± .01
	6.8 R	0.026 ± .001	0.015 ± .001	0.64 ± .01	2.19 ± .04•	0.22 ± .01	0.20 ± .01	0.38 ± .01
52	CONTROL	0.055 ± .002	0.033 ± .001	1.17 ± .02	4.93 ± .14	0.40 ± .01	0.33 ± .01	0.61 ± .02
	150 R	0.053 ± .002	0.033 ± .002	1.09 ± .04	4.62 ± .18	0.37 ± .01	0.31 ± .01	0.59 ± .03
	15 R	0.057 ± .001	0.036 ± .001	1.17 ± .02	4.92 ± .11	0.40 ± .01	0.34 ± .01	0.47 ± .02•
	6.8 R	0.060 ± .001	0.035 ± .002	1.17 ± .02	4.91 ± .10	0.40 ± .01	0.34 ± .01	0.62 ± .02

•, Δ, Groups with different characters are significantly different from other groups at the $p \leq 0.05$ level.

TABLE 12

A comparison of body, brain and brain area weight ratio of offspring of control and gamma radiation treated rats. Dams were exposed to radiation on the 20th day of gestation.

Age (days)	Groups (n = 14)	Body (g ± SEM)	Brain (% b. wt. ± SEM)	Cerebral cortex (% b. wt. ± SEM)	Cerebellum (% b. wt. ± SEM)	Brain stem (% b. wt. ± SEM)
30	CONTROL	55.81 ± 1.29	2.55 ± .06	1.44 ± .03•	0.331 ± .007	0.328 ± .017
	150 R	49.26 ± 2.40•	2.45 ± .08	1.28 ± .04Δ*	0.343 ± .013	0.334 ± .010
	15 R	57.68 ± 1.16	2.46 ± .04	1.31 ± .04Δ*	0.313 ± .006	0.314 ± .008
	6.8 R	58.55 ± 1.00	2.40 ± .03	1.37 ± .02•*	0.313 ± .006	0.312 ± .007
52	CONTROL	126.02 ± 3.06	1.31 ± .03	0.70 ± .02	0.178 ± .002	0.182 ± .007
	150 R	111.99 ± 4.59•	1.23 ± .02	0.62 ± .01•	0.182 ± .002	0.200 ± .004
	15 R	124.89 ± 2.53	1.28 ± .01	0.69 ± .01	0.178 ± .003	0.176 ± .005
	6.8 R	130.01 ± 3.62	1.27 ± .02	0.68 ± .02	0.172 ± .003	0.176 ± .006

•, Δ, *, Groups with different characters are significantly different from other groups at the $p < 0.05$ level.

TABLE 13

A comparison of body and organ weights ratio of offspring of control and radiation treated rats. Dams were exposed to gamma radiation on the 20th day of gestation.

Age (days)	Groups (n=14)	Ovary (% b. wt. ± SEM)	Adrenal (% b. wt. ± SEM)	Kidney (% b. wt. ± SEM)	Liver (% b. wt. ± SEM)	Heart (% b. wt. ± SEM)	Spleen (% b. wt. ± SEM)	Lung (% b. wt. ± SEM)
30	CONTROL	0.047 ± .002	0.027 ± .000	1.09 ± .01	3.77 ± .06	0.38 ± .01	0.35 ± .01	0.67 ± .01
	150 R	0.045 ± .001	0.028 ± .001	1.12 ± .03	3.91 ± .05	0.39 ± .01	0.33 ± .01	0.69 ± .01
	15 R	0.046 ± .001	0.027 ± .001	1.08 ± .01	3.79 ± .03	0.38 ± .01	0.35 ± .01	0.64 ± .02
	6.8 R	0.044 ± .002	0.026 ± .001	1.08 ± .01	3.74 ± .05	0.37 ± .01	0.34 ± .01	0.65 ± .02
52	CONTROL	0.044 ± .001	0.026 ± .001	0.94 ± .01	3.95 ± .07	0.32 ± .01	0.26 ± .01	0.49 ± .01
	150 R	0.048 ± .001	0.030 ± .000	0.97 ± .01	4.14 ± .07	0.33 ± .01	0.28 ± .01	0.53 ± .02
	15 R	0.046 ± .001	0.028 ± .000	0.93 ± .01	3.95 ± .08	0.33 ± .01	0.27 ± .01	0.47 ± .02
	6.8 R	0.048 ± .001	0.028 ± .001	0.93 ± .01	3.90 ± .08	0.32 ± .01	0.27 ± .01	0.48 ± .01

• No significant differences were observed between groups at the $p \leq 0.05$ level.

Organ weights, and the ratio of organs to body weight (day 52): Brain weight of the 150 R treatment group was significantly lower ($p \leq 0.0001$) than in all other treatment groups (Fig. 21). No significant differences in brain weights were observed between control, 15 R, and 6.8 R treatment groups (Fig. 21). Lung weight of the 15 R treatment group was significantly higher ($p \leq 0.0001$) than in any other treatment group (Table 11), but lung weights were comparable among other treatment groups (Table 11). All other organ weights were comparable between different treatment groups (Table 11). A comparison of the ratio of all organs to body weight revealed no significant differences between different treatment groups (Table 12 and 13).

Brain areas (day 30): A comparison of weights of different brain parts showed that the cortex weight of 150 R treatment group was significantly lower ($p \leq 0.0001$) than in any other treatment groups (Table 10), but cerebellum and brain stem weights were comparable among all treatment groups (Table 10).

When the ratio of brain parts to body weight were compared, it was observed that the control treatment animals had significantly higher ($p \leq 0.006$) cortex weight than 150 R, and 15 R treatment groups, but no differences were

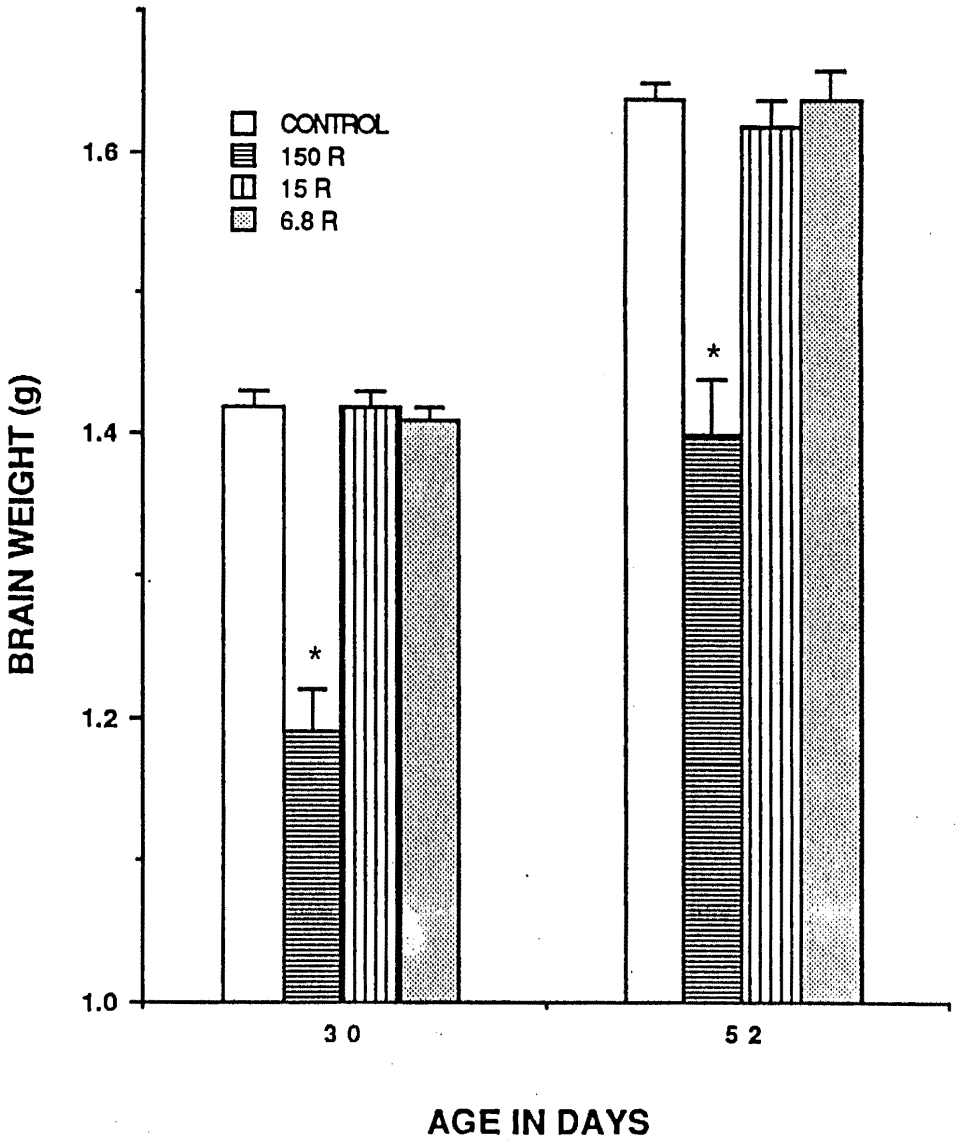

Figure 21. Comparison of brain weight (mean ± SEM) of pups born to control and radiation treated dams.

* Significantly different than control, 15 R and 6.8 R at the 0.05 level: Tukey's test.

observed between the control and 6.8 R, or between 15 R and the 150 R treatment groups (Table 12). Comparison of other brain areas (cerebellum, and brain stem) did not show any significant differences between different treatment groups (Table 12).

Brain areas (day 52): On day 52, cortex weight of the 150 R treatment group was significantly lower ($p \leq 0.0001$) than in any other treatment groups, and was comparable among control, 15 R, and 6.8 R treatment groups (Table 10). Cerebellum weight of the control treatment group was significantly higher ($p \leq 0.03$) than of any other treatment group, and was comparable among 150 R, 15 R, and 6.8 R treatment groups (Table 10). Weight of brain stem was comparable among all treatment groups (Table 10).

The ratio of the cortex to the body weight of 150 R treatment group was significantly lower ($p \leq 0.001$) than of all other treatment groups (Table 12). Cerebellum to the body weight, and brain stem to the body weight ratios were comparable between different treatment groups (Table 12).

Cerebral cortex myelin content (day 30): At day 30, the mean cerebral cortex myelin contents were 7.43 ± 0.84, 5.93 ± 0.68, 7.50 ± 1.07, and 7.8500 ± 0.87 mg for the

offspring of the control, 150 R, 15 R, and 6.8 R radiation treated rats (Fig. 22). The mean myelin content of the 150 R treatment group was considerably less than that of other treatment groups but it was not significantly different.

The cerebral cortex myelin concentrations in mg/g of wet tissue were 9.38 ± 1.04, 9.18 ± 1.14, 9.25 ± 1.29, and 9.64 ± 1.07 mg/g for control, 150 R, 15 R, and 6.8 R radiation treated groups respectively (Fig. 23). None of these values were significantly different from each other.

Cerebral cortex myelin content (day 52): At day 52, offspring of the 150 R radiation treated dams had lower myelin content than the offspring of control, 15 R, and 6.8 R treated dams, but the differences were not statistically significant. The mean myelin contents were 8.08 ± 0.49, 6.23 ± 1.81, 8.34 ± 0.70, and 8.15 ± 0.66 g for the offspring of the control, 150 R, 15 R, and 6.8 R radiation treated rats respectively (Fig. 22). Although the myelin content of 150 R treatment group was lower than that of any other treatment groups, the differences were not statistically significant.

At day 52, the cerebral cortex myelin concentration mg/g of weight tissue averaged 9.02 ± 0.58 mg/g for the control group, 9.01 ± 0.88 mg/g for the 150 R group, 9.47 ± 0.89

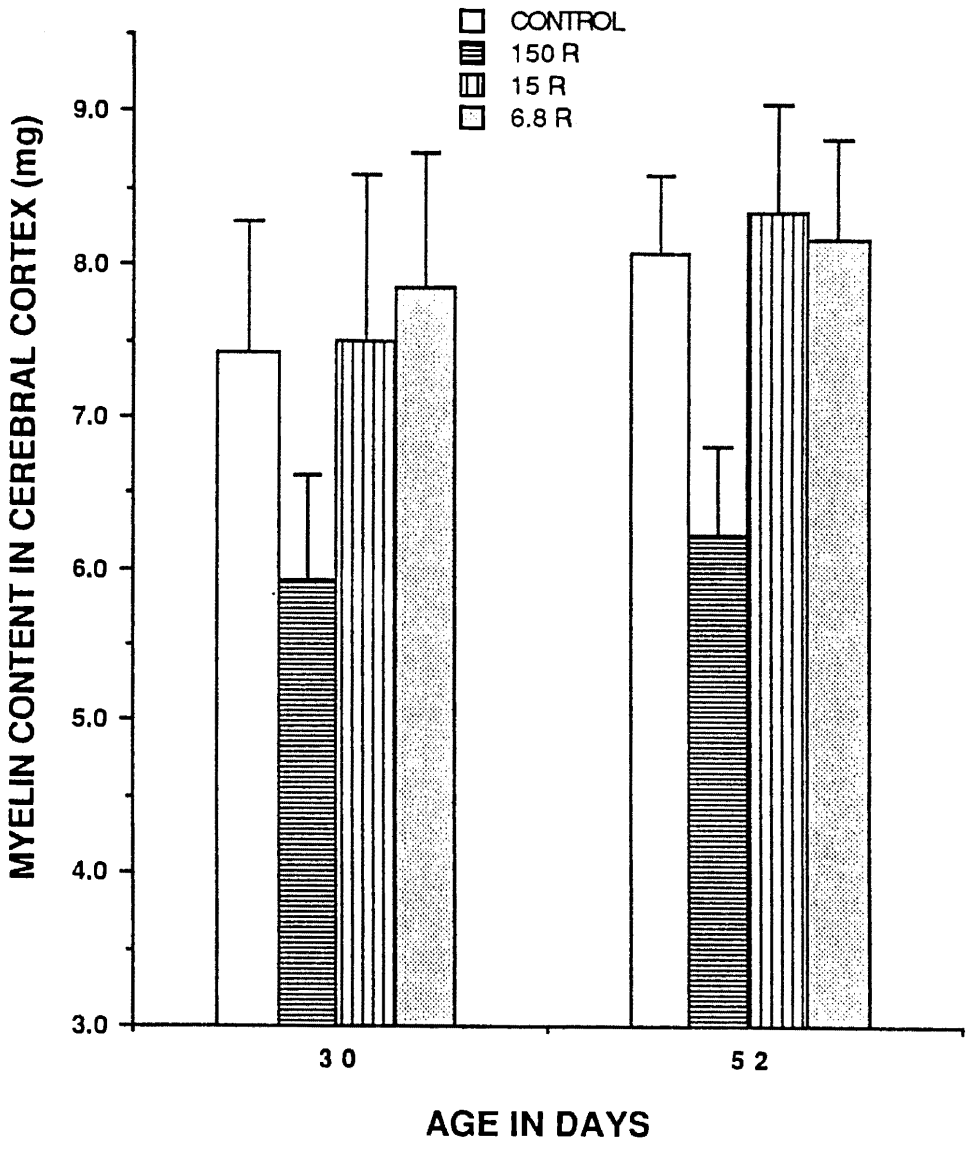

Figure 22. Comparison of myelin content (mean ± SEM) of cerebral cortex of pups born to control and radiation treated dams.

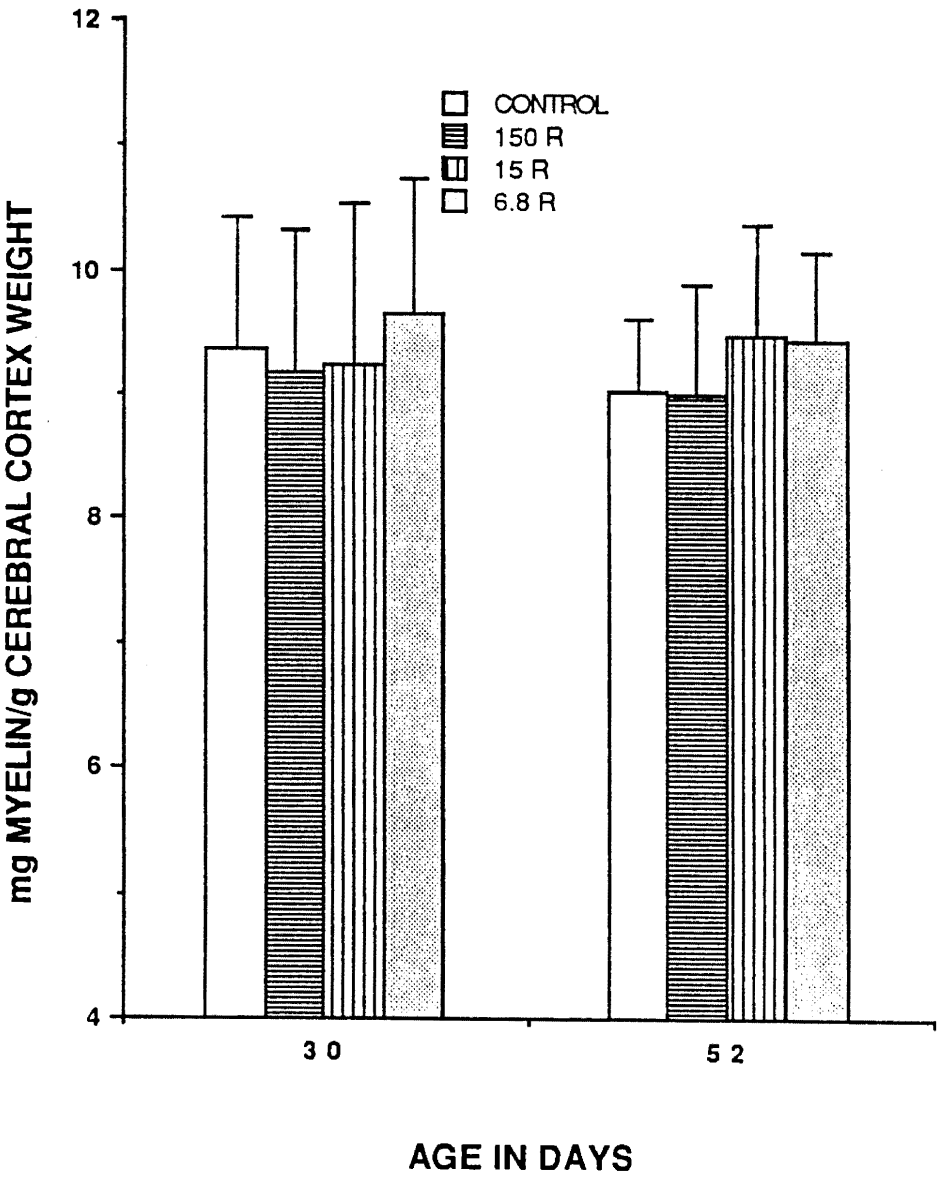

Figure 23. Comparison of myelin concentration (mean ± SEM) of cerebral cortex of pups born to control and radiation treated dams.

mg/g for the 15 R group, and 9.43 ± 0.70 mg/g for the 6.8 R group (Fig. 23). There was no significant difference in myelin concentration in mg/g of tissue between different treatment groups.

Cerebellum myelin content (day 30): At day 30, the mean cerebellum myelin contents were 2.17 ± 0.16, 1.97 ± 0.10, 1.99 ± 0.23, and 2.11 ± 0.21 mg for the offspring of the control, 150 R, 15 R, and 6.8 R radiation treated rats respectively (Fig. 24). No differences in cerebellum myelin content were observed between different treatment groups.

The cerebellum myelin concentrations in mg/g of wet tissue were 11.71 ± 0.91, 11.08 ± 0.67, 11.02 ± 1.42, and 11.96 ± 1.29 mg/g for control, 150 R, 15 R, and 6.8 R radiation treated groups respectively (Fig. 25). None of these values were significantly different from each other.

Cerebellum myelin content (day 52): At day 52, no difference in cerebellum myelin content was observed between different treatment groups. The mean cerebellum myelin weights were 3.25 ± 0.16, 3.65 ± 0.64, 3.65 ± 0.14, and 3.51 ± 0.18 g for the offspring of the control, 150 R, 15 R, and 6.8 R radiation treated rats respectively (Fig. 24).

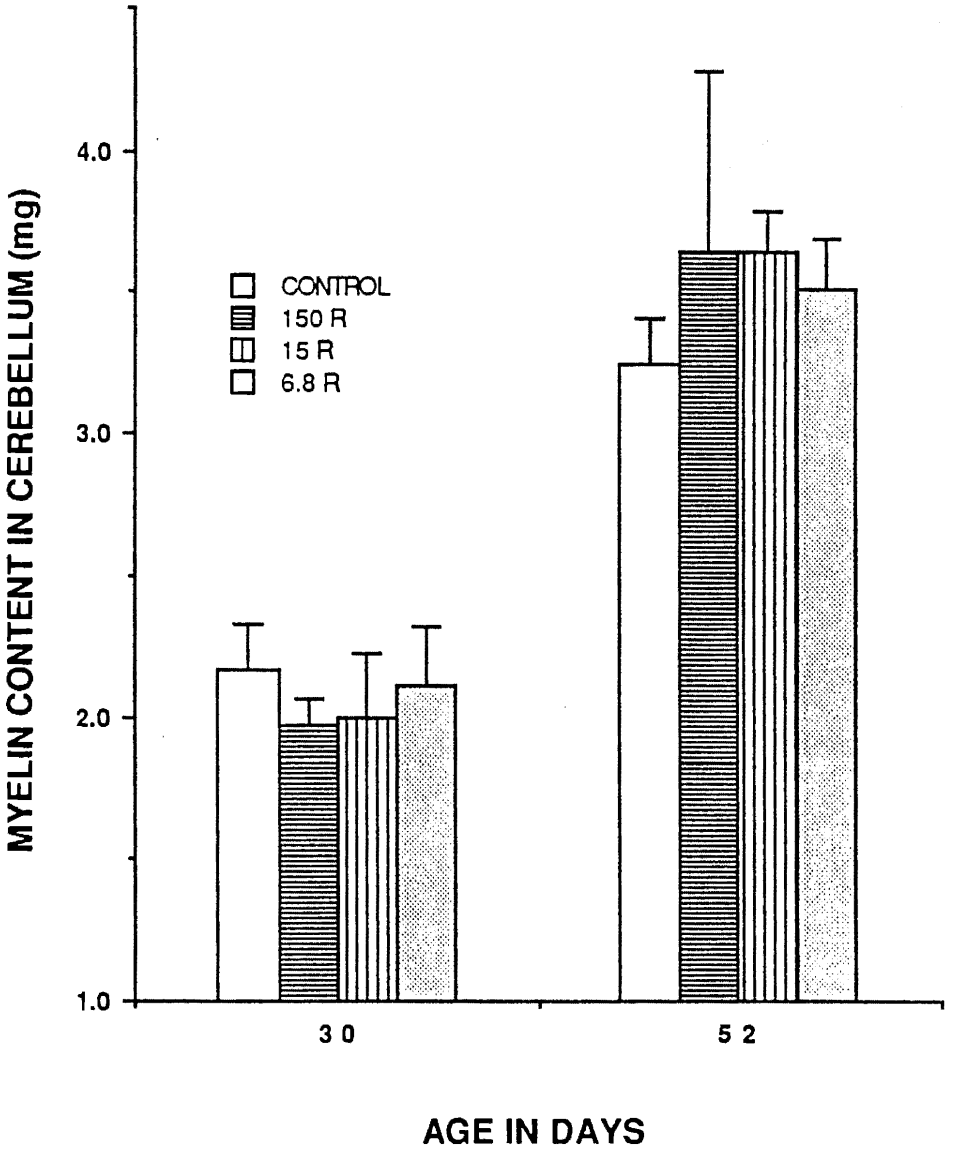

Figure 24. Comparison of myelin content (mean ± SEM) of cerebellum of pups born to control and radiation treated dams.

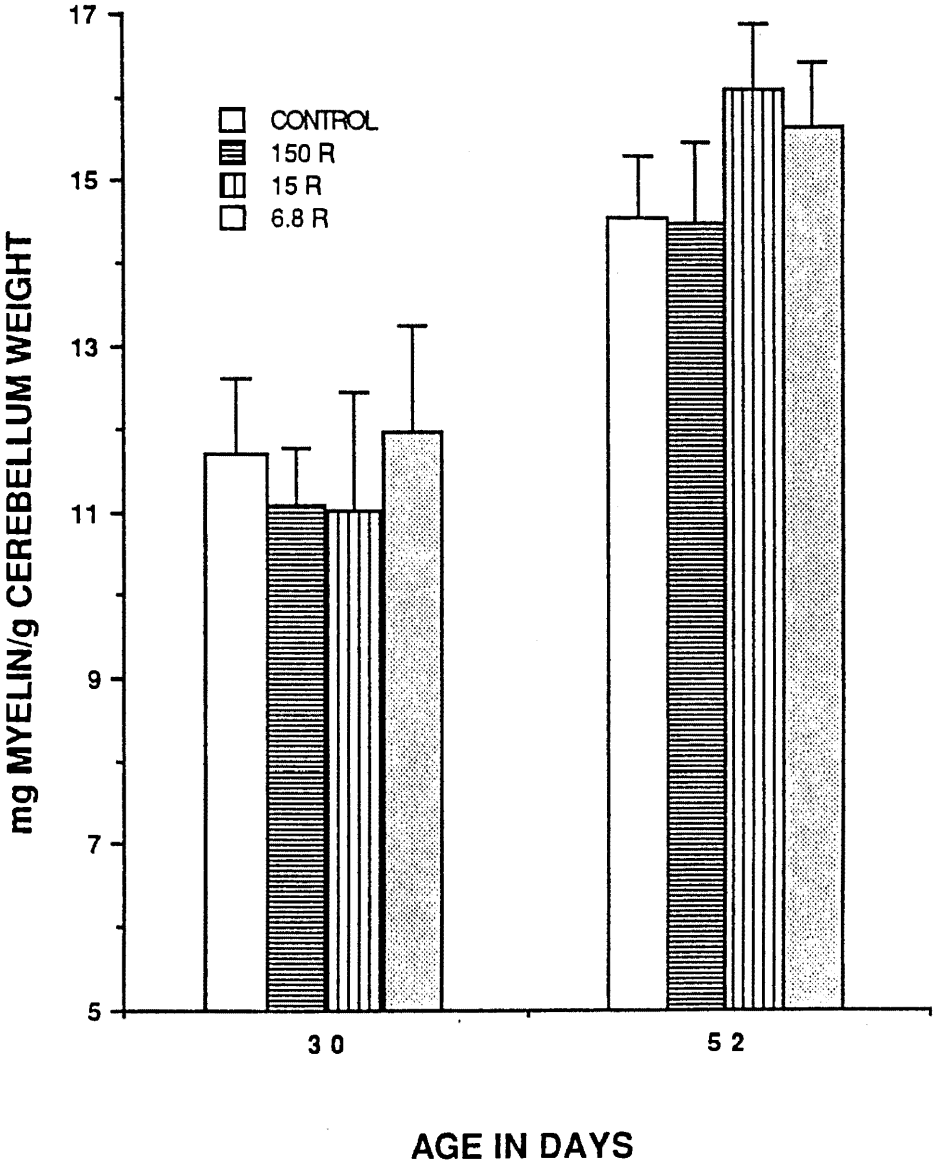

Figure 25. Comparison of myelin concentration (mean ± SEM) of cerebellum of pups born to control and radiation treated dams.

At day 52, the cerebellum myelin concentration mg/g of weight tissue averaged 14.55 ± 0.76 mg/g for the control group, 14.51 ± 0.95 mg/g for the 150 R group, 16.11 ± 0.80 mg/g for the 15 R group, and 15.67 ± 0.78 mg/g for the 6.8 R group (Fig. 25). There was no significant difference in myelin concentration in mg/g of tissue between different treatment groups.

Brain stem myelin content (day 30): At day 30, the mean brain stem myelin contents were 4.96 ± 0.45, 4.86 ± 0.33, 4.68 ± 0.37, and 4.37 ± 0.24 mg for the offspring of the control, 150 R, 15 R, and 6.8 R radiation treated rats respectively (Fig. 26). None of these values were significantly different from each other.

The brain stem myelin concentrations in mg per g of wet tissue were 29.74 ± 2.35, 25.72 ± 1.50, 26.94 ± 2.30, and 25.75 ± 1.57 mg/g for control, 150 R, 15 R, and 6.8 R radiation treated groups respectively (Fig. 27). These values were not significantly different from each other.

Brain stem myelin content (day 52): At day 52, no difference in brain stem myelin content was observed between different treatment groups. The mean cerebellum myelin contents were 12.29 ± 0.56, 11.01 ± 0.98, 12.01 ± 0.59, and

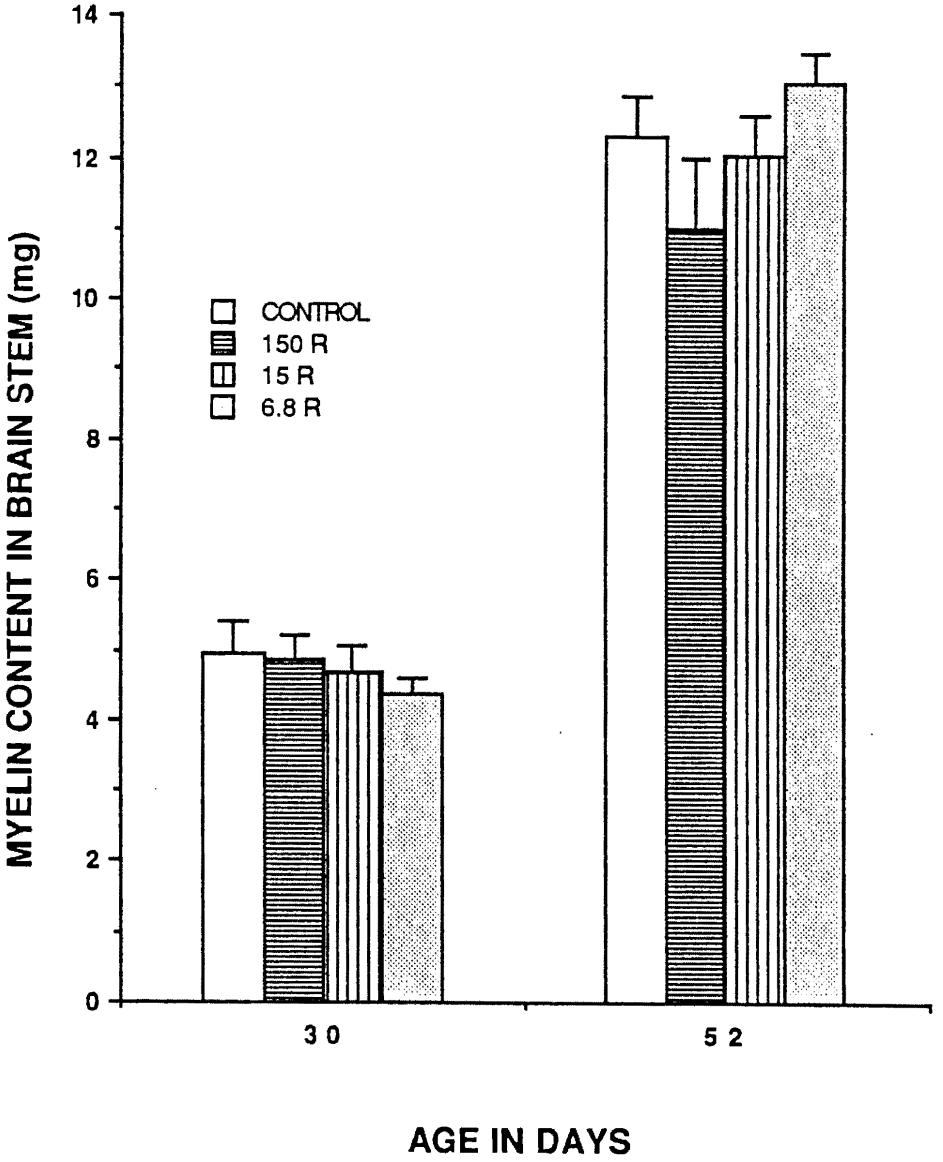

Figure 26. Comparison of myelin content (mean ± SEM) of brain stem of pups born to control and radiation treated dams.

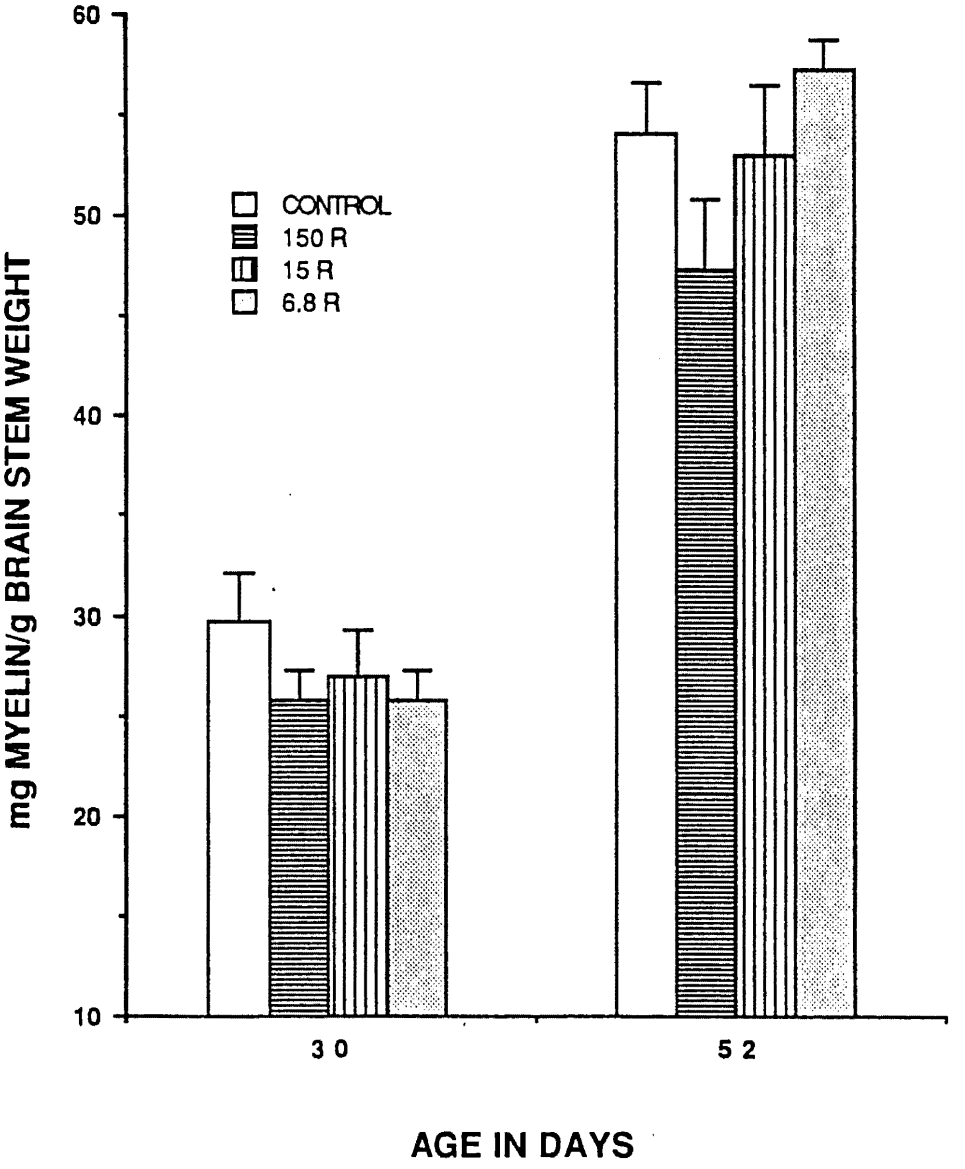

Figure 27. Comparison of myelin concentration (mean ± SEM) of brain stem of pups born to control and radiation treated dams.

13.05 ± 0.41 g for the offspring of the control, 150 R, 15 R, and 6.8 R radiation treated rats respectively (Fig. 26).

At day 52, the brain stem myelin concentration mg/g of weight tissue averaged 54.11 ± 2.48 mg/g for the control group, 47.27 ± 3.57 mg/g for the 150 R group, 53.04 ± 3.40 mg/g for the 15 R group, and 57.35 ± 1.39 mg/g for the 6.8 R group (Fig. 27). There was no significant difference in myelin concentration in mg/g of tissue between different treatment groups.

CHAPTER V

DISCUSSION AND CONCLUSIONS

In this study, daily mean gestational food intake values of all the treatment groups ranged between 16.0 ± 0.3 to 16.9 ± 0.2 g. Although the food intakes of 15 R and 6.8 R treated dams were higher than the 150 R and control dams, all the treatment groups had similar nutritional status since none of the daily mean food intake values were significantly different from each other.

During gestation no differences in the daily water consumptions were observed between the different treatment groups. No differences in caloric and water intakes during gestation were expected because the radiation was delivered on the 20th day of gestation (one day before the termination of pregnancy) and until then there were no treatment differences between different groups of rats.

In this study the daily mean lactational food intake values of all treatment groups ranged between 32.5 ± 0.6 to 35.1 ± 0.4 g. For the entire lactational period the mean food intakes of the 15 R, and 6.8 R treated dams were slightly higher than the 150 R and control dams, but the

values were not significantly different from each other, therefore, all the treatment groups had a very similar nutritional status. The mean water consumptions for the entire lactational period were not significantly different between the treatment groups, which suggested that radiation did not produce any effects on food and water intakes during lactation.

During gestation and lactation, the mean body weights of the 6.8 R and 15 R treated dams were slightly higher than the control and 150 R treated animals which occured due to increased caloric intake, but the differences in body weights between groups were not significantly different form each other. Since the caloric and water intake values of all the treatment groups were very similar during the gestational and lactational periods, that resulted in a comparable mean body weights of the animals of different treatment groups.

Wallace and Altman (1970) reported that neonatal irradiation of cerebellum with 1 to 4x200 R with x-ray affected the weight pulling, and irradiation with 200 R affected rope climbing parameters but none of the parameters were significantly different when compared to the unirradiated controls. Altman et al. (1971) reported that focal irradiation of the cerebellum (0-1 days of age) with

2x150 R (low dose) affected the general locomotion (crawling and walking) but did not affect the effective locomotion (number of squares travelled). In the same study the authors also observed that pivoting in low radiation (2x150 R) treated animals was not different from that observed in the control animals. Although pivoting appeared to be affected in the intermediate irradiation (6x150 R) group, the differences with respect to the controls were not significant.

In our study we observed that exposure to 6.8 R (low irradiation), 15 R (intermediate irradiation), or 150 R (high irradiation) did not affect the crawling parameter. Although pivoting was affected in 15 R, and 150 R treated pups on postnatal days 15 and 16, no overall differences between different treatment groups were observed. These findings agreed with the findings of Altman et al. (1971).

Certain other locomotion development parameters were affected by radiation. The results indicated that 150 R and 15 R of radiation affected the cliff-avoidance parameters in the developing rat offspring. In cliff-avoidance although the statistically significant differences in performances were observed on days 8 and 9 of the postnatal life, the pups of the 150 R treated group appeared to score

considerably lower throughout the study period, except on day 7.

Crawling, righting, and hindlimb support performances were not affected by radiation. Although negative geotaxis data revealed no significant differences between different treatment groups, this parameter appeared to be affected in the pups of the 150 R treated animals. Probably a larger sample size would have detected statistically significant differences.

Left and right eye openings were delayed in the 150 R exposed pups, although the time differences were not statistically significant when compared to other groups. Eye opening was observed with 24 h intervals and possibly that introduced a larger variable in the study. Probably observation with shorter intervals (6 to 12 hours) would minimize the variability and thus would produce a significant difference.

Upper jaw tooth eruption was delayed in the pups of 150 R treated group, and the data also suggested that the upper jaw tooth eruption time was related to the different doses of radiation. Lower jaw tooth eruption appeared to be delayed in the same group of animals, but the time difference was not statistically significant.

Body weight was significantly reduced in the 150 R radiation exposed pups. This finding supported several previous studies where radiation caused a reduction in body weights (Grosch and Hopwood 1979). Mean body weights of 6.8 R treated pups were slightly higher (but not significantly different) than the unirradiated controls. Probably it was due to increased caloric intakes by 6.8 R treated dams during gestation and lactation.

Ovary, kidney, heart, and spleen weights were significantly reduced in the offspring of the 150 R radiation treated rats at day 30, but no differences were observed at day 52, which probably indicated a recovery.

Brain and cerebral cortex weights were significantly reduced in the 150 R treated group at days 30 and 52. This indicated that although some of the organs (ovary, kidney, heart, and spleen) showed some recovery at day 52, the high radiation dose produced an apparently permanent damage to the brain tissue, as indicated by no recovery. At day 52, all radiation exposed pups had significantly lower cerebellum weights when compared to the control pups.

Norton and Poduslo (1973) reported that in 30-day old rats when the average brain weight was 1.44 g, the actual myelin yield per whole brain was 23.6 mg. Therefore, the

estimated average myelin concentration would be 16.39 mg/g weight of brain tissue.

In our study the brain was dissected into cerebral cortex, cerebellum, and brain stem (pons and medulla) to study the total myelin content and myelin concentration in those tissues. The other brain areas such as corpus callosum, and the mid brain (thalamus, subthalamus, and hypothalamus) were discarded. In 30-day old untreated control rats the pooled myelin content of the three brain areas was 14.56 ± 1.39 mg, and the mean myelin concentration of the three brain areas was 16.94 ± 1.43 mg/g when the mean brain weight was 1.42 ± 0.01 g. The pooled myelin concentration value agrees very well with the value reported by Norton and Poduslo, 1973.

Norton and Poduslo (1973) also reported that in 60-day old rats with an average brain weight of 1.67 g, the average myelin yield per whole brain was 39.0 mg; therefore, the estimated myelin concentration would be about 23.35 mg/g. In our study with 52-day old rats the mean myelin concentration value for the three different brain areas of the untreated control rats was 25.89 ± 1.27 mg/g which agrees with the previously estimated value (Norton and Poduslo 1973).

The lipid protein ratio of the myelin is constant with age, and in rats the lipid comprises about 72.3 percent of

the total dry myelin weight (Norton and Poduslo 1973); therefore, the estimated protein should be about 27.7 percent of the total dry myelin weight. Wiggins and Fuller (1978) reported that in 60-day old rats the myelin protein concentrations for cerebral cortex, cerebellum and medulla were 3.97 mg/g, 2.54 mg/g, and 14.4 mg/g respectively. Since the estimated protein content in myelin was about 27.7 percent of the total dry weight of the myelin, the estimated myelin concentrations would be 14.33 mg/g, 9.16 mg/g, and 51.98 mg/g for cerebral cortex, cerebellum, and brain stem respectively; and the mean myelin concentration for all three brain areas would be 25.15 mg/g. In our study with 52-day old rats, the pooled myelin concentration of three different brain areas was 25.89 ± 1.27 mg/g which perfectly agreed with the value estimated from the study of Wiggins and Fuller, 1978. The individual myelin concentration value of each brain area of our study also agreed with the estimated values obtained from the previous study (Wiggins and Fuller 1978).

A careful review of the literature failed to reveal any study of brain myelinaltion of different brain areas with 30-day, or approximately 30-day old rats. Therefore, the data we obtained on myelination of each specific brain area

with 30-day old rats can not be compared with any other study.

The results of the present study indicate that exposure to 150 R of radiation during the 20th day of prenatal life reduced the total myelin content of the cerebral cortex, although the reductions were not statistically significant. Exposure to 150 R reduced the brain weight by 15.79 and 14.79 percent at the ages day-30, and day-52 respectively. This exposure also reduced the cerebral cortex weight by 22.07 percent at age day-30, and 20.15 percent at age day-52; which caused a reduction in cerebral cortex myelin content by 20.16, and 22.89 percent in 30-day, and 52-day old animals respectively. Although the myelin contents of cerebral cortex of 150 R treated rats were reduced, the myelin concentrations (mg myelin/g brain tissue) were unaffected. The reduction of total myelin content in cerebral cortex was due to reduced brain tissue weights in the 150 R treated rats. Exposure to 15 R and 6.8 R did not affect either the brain tissue weight or the myelin content or the myelin concentration.

We were concerned whether exposure to low levels of clinical radiation during pregnancy could affect the myelination in the offspring. Data from our study suggested that radiation doses ranging between 6.8 R to 15 R would

neither affect the total myelin content nor the myelin concentration of the brain tissue.

The present findings do not rule out the possibility that radiation may affect the CNS myelination in developing brain, because in this study radiation was delivered on the 20th day day of prenatal life in a single dose; exposure to radiation in a single or fractionated doses at different times of pernatal life other than day 20 or during early postnatal life may produce teratogenic effects on the CNS myelination. Therefore, studies of effects of single or fractionated doses of radiation during different periods of development on CNS myelination would be the appropriate areas of future investigation.

Locomotion development in the rat offspring is related to the motor development, and motor development is related to the development of the nervous system. Data from this study suggest that radiation produces damage to the brain tissue which may affect several locomotion parameters.

These locomotion parameters are voluntory movements, and the areas of the cerebral cortex that are involved in these processes are somatosensory and sensory cortex (areas 1,2, and 3), primary cortex (area 4) and the premotor cortex (area 6). Nuclei of the brain stem, pons, and cerebellum

also could be involved in these processes. Therefore, by observing the data we may speculate that these are the probable brain areas which could be affected by radiation.

Cerebral cortex weight was significantly reduced and the total myelin content of the cerebral cortex was reduced by a similar amount. Although the myelin content of cerebellum and brain stem, and myelin concentrations of whole cerebral cortex, cerebellum, and brain stem remained unaffected by radiation, myelination of the previously mentioned specific sites of these brain areas could be affected since we know that the delayed reflex is related to hypomyelination. Therefore, we propose that in future studies several aspects could be investigated such as (1) glial morphology and neuron positioning, since neuron movement is assisted by glial cells; and (2) myelination of these specific sites of the brain areas. These investigations would involve cytohistology and electron microscopy work.

In this study several hypotheses have been tested. The hypothesis "gamma radiation affects different developmental processes in developing rat offspring" is partly rejected because although radiation significantly affected several developmental parameters, several other parameters remained unaffected. The cerebral cortex myelin

content was found to be reduced by 20 to 23 percent in 150 R radiation treated pups, therefore, the hypothesis "gamma radiation decreases myelination in the developing rat brain" could be accepted.

Data from this study indicate that the brain weight was reduced at ages day-30 and day-52, therefore, the hypothesis "radiation decreases brain weight of the developing rats" may be accepted. The effects of radiation that we observe in this study is dose related as we find that 150 R affected several parameters, exposure to 15 R affected only few parameters, and the 6.8 R treated group did not differ from the control treated group; Therefore, the hypothesis "these effects are related to the various doses of radiation can be accepted.

The overall analysis of data revealed that exposure to 150 R of radiation on day 20 of the prenatal life affected several locomotion and developmental parameters in the developing rat offspring, such as pivoting, cliff-avoidance, upper jaw tooth eruption, body weight, and organs, such as brain, brain cortex, ovary, kidney, heart and spleen weights. Other parameters, such as negative geotaxis, eye opening, lower jaw tooth eruption and myelin content of the cerebral cortex appeared to be affected in the 150 R treated animals, although the effects were not statistically significant.

Exposure to 15 R affected pivoting and cliff-avoidance parameters. The cerebral cortex weight of the 15 R treated group was found to be reduced at the age of day 30. Exposure to 6.8 R had no adverse effects on the parameters studied. Since all the treatment groups had identical nutritional status during gestation and lactation, and none of the groups was under malnutrition we may conclude that these effects were produced by the radiation alone.

CHAPTER VI

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