CADMIUM DISTRIBUTION AND SELECTED TISSUE HISTOLOGY IN RATS FOLLOWING ADMINISTRATION OF CADMIUM CHLORIDE AND/OR GAMMA RADIATION

A DISSERTATION

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We hereby recommend that the Dissertation prepared under our supervision by Yashdev R. Kundomal entitled "Cadmium Distribution and Selected Tissue Histology in Rats Following Administration of Cadmium Chloride and/or Gamma Radiation."

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DEDICATION

This dissertation is dedicated to my mother and father, whose encouragement, love, support, and understanding have made its completion possible.

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CHAPTER I

INTRODUCTION

There is evidence from studies on animals and man that radiation is both beneficial and harmful. The current and future need for electricity has caused a number of nations to develop nuclear power reactors. As a result there is great concern about the possible long-term deleterious effects, on biological systems, which may arise from the radioactive by-products. There is also concern about the disposal of radioactive waste products, and the possibility of a nuclear war. Besides artificially-made nuclear fission products, all organisms are exposed to environmental irradiation from cosmic rays as well as from external and internal natural radioactive materials from the earth's crust. On the other hand, radiation has been shown to be beneficial in agriculture, industry, dentistry and medicine (Arena, 1971).

The number of heavy metals to which man and animals are exposed has increased tremendously due to modern technology. One of these metals is cadmium and there is great concern about the possible health effect that this metal might have on biological systems. Cadmium is found in silver and pot polishes, shoe whiteners, and some lead-free paints. It is widely used in plating certain cooking and

baking utensils as well as in the manufacture of batteries and electronic components (Berman, 1967; Flatau and Aubert, 1979). A considerable number of studies have been done involving the effects of cadmium on biological systems (Boisset et al., 1978). Research has shown that cadmium is deposited and accumulated in various body tissues, and is also found in varying concentrations throughout all environmental compartments such as air, food, soil, and water (Flick et al., 1971). The interactions between cadmium and radiation have also been investigated. Cadmium has been shown to result in a linear decrease in $LD_{50(30)}$ values when it is used as a co-insult with X-irradiation (Lappenbush and Gile, 1975).

The purposes of this research were:

1. To determine the distribution of cadmium in tissues of vital organs of laboratory rats following single treatments of cadmium or co-insult of cadmium and gamma (γ) radiation, and
2. To determine whether cadmium and γ-irradiation singly or as co-insults have any significant histological effect on certain tissues of these animals.

Two major statistical hypotheses were formulated for this investigation:

Hypothesis 1. There is no significant difference in distribution of cadmium chloride ($CdCl_2$) among the

tissues of the control, the cadmium-injected groups, or cadmium-injected and γ-irradiated groups.

Hypothesis 2. There is no significant histological effect of $CdCl_2$ and/or γ-irradiation upon the selected tissues of treated animals as compared to that of the controls.

CHAPTER II

REVIEW OF LITERATURE

Radiation

The accumulated radiation burden to man is increasing as a result of the medical, occupational, and military uses of X-rays, and other radiation sources as well as nuclear energy. Ionizing radiation has pronounced effects on a number of organs and organ systems; these effects are reviewed below. Following exposure to radiation, the pathologic events in biological systems are determined by the radiation sensitivity of the organ vasculature and stroma. Different kinds of cells of an organism display different types of radiosensitivities (Arena, 1971). The following cells are classified based on an increasing radiation sensitivity: osteocytes, connective tissue, muscle, nerve, glial, liver, glandular epithelium, blood vessel epithelium, osteoblasts, eye lens cartilage, sweat glands, hair matrix, sebaceous glands, germinative stratum, jejunal and iliac crypts, ova, spermatogonia, megakaryocytes, myeloblasts, erythroblasts, and lymphocytes. On the other hand, nerve tissue has been reported to be the most radiation resistant in human as well as in experimental animals (Casarett, 1968); however, the damage which appears is slow to develop

in relation to other radioresistant tissues. The kidneys have been shown to be radiosensitive, with damage primarily to the arterioles and capillaries, resulting in benign and malignant hypertension (Schroeder et al., 1966).

Doses of ionizing radiation greater than 3,500 rads have been reported to damage the bone marrow to an extent where repopulation of the hemopoietic stem cells is ineffective (DeGowin et al., 1974). Zherbin et al. (1978) studied the inactivation of different cell series of human bone marrow, cultured in organ cultures during the first four days following γ-irradiation, as a function of the radiation dose. Mature granulocytes and macrophages in the bone marrow were found to be in significantly greater numbers than those in the control culture group.

Bone marrow, liver and plasma lipid fractions of 32 albino rabbits, 10 to 12 weeks of age, were determined 24 hours following 1,000 R of whole-body X-irradiation (Elko and Di Luzio, 1959). In the plasma, cholesterol, phospholipid, and total lipid fractions in the X-rayed groups were markedly elevated above the control values. Although no alteration was observed in liver cholesterol concentration, liver phosphatide and neutral fat fractions were significantly increased in the X-irradiated group. The cholesterol, phospholipid, and neutral fat fractions of bone marrow, obtained either from the femur or from other

bone marrow sites, showed no significant alteration in the X-irradiated group. These findings indicated that bone marrow lipids did not contribute to the post-irradiation hyperlipemia and that they were not markedly altered one day following lethal whole-body X-irradiation.

The cause of an increase in fat content of rat liver following irradiation has been studied (Agostini et al., 1964). Albino rats of both sexes were treated with small doses of X-rays. Microscopic examination showed that the liver cells were infiltrated with small droplets of fat uniformly distributed throughout the lobules. After a single dose of 1,150 R only a few droplets of fat were detectable. No modification was induced in glucose-6-phosphatase activity of hepatic microsomes; but, coenzyme A content of the liver was decreased. Based on these results it was postulated that coenzyme A deficiency and the increase in free fatty acid release from peripheral adipose tissue probably have an important part to play in the pathogenesis of fatty liver produced by X-rays.

Radiation carcinogenesis has been extensively studied because of the increasing concern due to new radiation sources. Diamond et al. (1973) reported that children who were exposed in utero to diagnostic X-rays had an increased risk of leukemia. Leukemia was found among many survivors of the Hiroshima and Nagasaki atomic bomb attacks (Ishimaru

et al., 1971). The effects of γ-irradiation on skin have also been studied (Upton, 1975). A dose of 1,000 R or greater produced skin carcinomas and if the exposure was prolonged to less than 4 R/day, neoplasia resulted. At doses below 2,000-4,000 R the effect was less due to the survival of only a few follicular cells which were capable of proliferation.

Ionizing radiation has pronounced effects at the cellular and molecular levels as well. Deoxyribonucleic acid (DNA) is most probably the primary target for the killing of the cell by ionizing radiation (Dalrymple and Baker, 1973). However, the cell membrane has also been reported as another probable site for the radiation-induced modification of the cellular response (Shenoy et al., 1974). 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. The possible mechanisms of the rejoining of single-strand breaks have been studied by Wheeler and Lett (1972), with the conclusion that all DNA bases are susceptible to chemical alteration induced by radiation.

Cadmium

In the past decade many studies have been conducted dealing with possible effects of metals on biological

systems. One of the metals studied was cadmium, which was reported to be a hazardous trace element in man (Voors and Shuman, 1977) as well as in animals (Aoki and Hoffer, 1978). Cadmium is accessible to the biological systems via air, food, and water (Webb, 1975). Menden et al. (1972) detected the metal in dairy products, grains, meats, vegetables, and also in cigarette smoke. Experimental findings revealed that cadmium may be an etiological factor for various pathological processes including testicular tumors, kidney malfunction, hypertension, arteriosclerosis, growth inhibition, chronic diseases of old age, and cancer (Flick et al., 1971).

The quantity of cadmium which is toxic to animals and man depends on various factors, including the species (Ammerman et al., 1973), length of exposure (Friberg et al., 1971), and amount given (Ellis et al., 1979). Some studies have revealed accumulation of cadmium in the body as a cause of hypertension. Female rats on a cadmium-free diet exhibited fluctuating systolic hypertension when given cadmium in drinking water at subtoxic levels (5 ppm) from the time of weaning to 180-240 days. Cadmium accumulated in small amounts in the kidney and liver, and hypertension was the only sign of toxicity (Schroeder and Vinton, 1962). The average concentration of cadmium in the air of 20 cities in the U.S. showed a marked correlation with death rates from

cardiovascular disease (Carroll, 1966). Cadmium and heart disease tended to increase with city size and degree of industrialization. Fischer and Thind (1971) determined the concentrations of cadmium in different-sized blood vessels, heart, kidney and liver of cadmium hypertensive and normal male rabbits. Normal kidney had a significantly higher cadmium level than any other normal tissue, while heart had the lowest concentration. The level of cadmium in all tissues of hypertensive animals was found to be significantly higher than the corresponding control tissues. Within the hypertensive group kidney and liver had the largest cadmium deposition.

Intraperitoneal injection of cadmium in rats induced prompt hypertension which persisted for at least one hour (Perry and Erlanger, 1971). Cadmium was avidly bound by the liver and the amount concentrated there increased steadily for two hours until that organ had as much as 40 percent of the total amount of the metal injected. Rats given 1 to 5 ppm of cadmium over a period of one year showed significantly greater increases in systolic pressure than did the control animals (Perry and Erlanger, 1974). However, rats given 10 and 25 ppm showed smaller increases in pressure; whereas, rats given 50 ppm of cadmium were sick and had significant decreases in blood pressure. Perry et al. (1976) suggested that these acute hypertensions induced by

small doses of cadmium probably resulted from a direct effect on vascular smooth muscle.

The mechanism of cadmium-induced hypertension was studied by measuring noradrenaline metabolism. Revis (1978) showed that cadmium *in vitro* inhibited both the enzymes which inactivated the neurotransmitters noradrenaline and adrenaline; however, *in vivo* the two enzymes were inhibited significantly only in the aorta. Thus, the effects of noradrenaline on vascular smooth muscle was increased and prolonged.

Early changes in the microvascular bed of the testis resulting from treatment with cadmium were studied using trypan blue, electron dense tracers and by vascular injections (Aoki and Hoffer, 1978). Leakage of fluids and electrolytes from the testicular blood vessels to the interstitium was demonstrated at the light and electron microscope levels as early as 1 to 2 hours after parenteral injection of cadmium. Discontinuities in the endothelial lining were detected by the presence of carbon particles in the walls of the testicular capillaries and venules. Three hours after injection, the extravasation of carbon had increased, such that particles were seen labeling large veins. Within the vessels, a local increase in the concentration of erythrocytes was noted and numerous platelets adhering to exposed subendothelial structures were seen

plugging the endothelial gaps. At 4 hours, a striking decrease in labeling of veins was noted due to the fact that delivery of the tracers towards the venous system was almost completely obstructed as a result of the blockage and progressive deterioration of the smaller vessels; ischemia of the testis ensued from the obstruction of the microvascular circulation. The results showed that the degeneration of the seminiferous epithelium and all biochemical and physiological changes known to occur in the testis at later time intervals following cadmium treatment are secondary to ischemia rather than due to a direct effect of the cadmium.

A study by Clegg and Carr (1967) has shown that cadmium acts principally on the blood vessels of the testis and epididymis of rats, making them more permeable, resulting in slower bloodflow. One group of animals was given a subcutaneous or intraperitoneal injection of 0.5 mg of $CdCl_2$/100 g of body weight. The other group (control) received an equivalent volume of Ringer's solution. Before sacrificing, each animal was injected with a solution of Evan's blue in half strength of Ringer's solution at a dose of 6 mg dye/100 g of body weight, together with a colloidal suspension of saccharated iron oxide at a dose of 20 mg of iron/100 g of body weight.

Animals were sacrificed at 20 minutes, 30 minutes, 1, 1.5, 2, 3 and 4 hours, 6 or 12 hours, 24, 48 hours, and 7 days after injection of $CdCl_2$ or Ringer's solution. Capillaries and venules begin to leak within 10 minutes of the injection and after two hours arterioles and arteries had become affected. Permeability was increased for at least 48 hours, but by seven days it appeared to be normal. Administration of BoL 148, an antiserotonin, had no effect on the development of the vascular lesions. Study of ultrastructures showed that leakage occurred mainly within testicular epididymis and differential blood vessels.

Male lambs were fed ad libitum diets containing various concentrations of cadmium for six months (Doyle et al., 1974). Dietary cadmium at concentrations of 30 and 60 ppm produced a significant reduction in growth rate and feed-intake but no effects on feed efficiency. A negligible quantity of cadmium was noted in excreted urine. Little cadmium accumulated in blood, fat or muscle; but, large amounts accumulated in kidney and liver. The blood hematocrit was significantly higher at the higher level of cadmium when compared with the control groups.

The effects of acute cadmium administration on the liver and kidney of rats have been determined (Hoffmann et al., 1975). Rats received a single intravenous injection of cadmium acetate and 16 hours later liver damage was

observed but no changes were noted in the kidney. Study of ultrastructure of the liver revealed more profound changes in parenchymal cells than in Kupffer cells. These changes were single parenchymal cell necrosis, deterioration of rough endoplasmic reticulum, proliferation of smooth endoplasmic reticulum, autophagocytosis, and mitochondrial degeneration. Lesions of Kupffer cells were not prominent.

Male Wistar rats were given concentrations of 0, 0.25, 0.50, and 0.75 mg of $CdCl_2$/kg of body weight three times weekly for eight weeks (Faeder et al., 1977). Increases in activities of aspartate aminotransferase (AAT) and γ-glutamyl transpeptidase (GT) were observed after six weeks of treatment. Small but significant changes in red blood cell carbonic anhydrase activity also occurred. Electron micrographs of liver tissue indicated dilation of the rough endoplasmic reticulum and proliferation of prominent connective tissue fiber bundle at six weeks. These results indicated a correlation between ultrastructural liver changes and elevations in plasma enzyme activities, which were considered caused by chronic liver damage. No further elevation of enzymes was noted after eight weeks of treatment.

Rats given an acute dose of lead acetate and cadmium acetate intravenously were more susceptible to an intravenous challenge with Escherichia coli by approximately

1000-fold (Cook et al., 1975). Since equivalent vulnerability of lead- or cadmium-treated rats to killed E. coli was observed, toxicity was, therefore, due to the endotoxin content of the bacteria. This is further supported by the observation that equal doses of viable cultures of the Gram-negative bacteria Staphylococcus epidermidis failed to elicit lethality in the acute lead-intoxicated rats.

Wilson et al. (1941) showed that cadmium administered in the food of rats resulted in decreased rates of growth, the greater effects being obtained with higher concentrations of the metal. It was also noted that the incisor teeth of these animals were more easily bleached following treatment with cadmium. Powell et al. (1964) showed that growth rate, food consumption, water intake, and testicular development decreased progressively as the concentration of cadmium in the diet increased. The level of blood hemoglobin decreased slightly when 40 to 160 ppm of cadmium in the diet were consumed; however, with higher levels of cadmium (640 and 2500 ppm) an increase in the blood hemoglobin was observed. Stowe et al. (1972) showed that rabbits injected with an average of 14.9 mg $CdCl_2$/kg of body weight/day for 200 days had retarded growth, anemia, neutrophilia, lymphopenia, hypoalbuminemia, and renal enlargement as well as interstitial renal and interlobular hepatic fibrosis.

The effect of chronic low-level cadmium intoxication on the haversian system has been determined (Anderson and Danylchuk, 1979). Mature male beagles were exposed to 25 ppm, 10 ppm and less than 1 ppb in a sequential manner for six months. Biopsies were performed from the mid-shaft of a rib before the exposure to cadmium and at the end of each exposure period. Biochemical and hematologic results of all dogs monitored monthly showed no significant changes during the experimental periods, nor did they differ significantly from untreated control beagles. Tissue levels of cadmium in the treated groups were significantly different than those of control in liver (5.75 vs. 0.03 μg/g), kidney (32.8 vs. 0.15 μg/g), and bone (1.20 vs. 1.96 μg/g). Statistically significant differences in haversian bone remodeling measurements were observed between experimental and control animals in activation frequency, radial closure rates, number of osteoid seams/mm^2, and bone formation rates when the experimental animals were subjected to 25 ppm $CdCl_2$. These differences tended to disappear at the end of the 10 ppm exposure period and completely disappeared when the test organisms were given ordinary tap water. The results suggested that the effect of cadmium on haversian bone remodeling is not related to mechanisms mediated through interference with parathyroid hormone activity or the metabolism of vitamin D, that the effect may

represent a toxic effect at the cell level in bone, and that this effect is reversible.

Workers from a handmade silver jewelry factory were evaluated medically to determine cadmium intoxication (Baker et al., 1979). Blood cadmium levels in workers exposed to cadmium were higher (0.93 mg/100 ml) than in unexposed (0.38 mg/100 ml) workers. A dose-relationship was observed between blood cadmium level and symptom prevalence in four systems, namely dyspnea, chest pain, dysuria, and dizziness. Segmental hair analysis revealed highest cadmium concentrations (up to 19 mg/g) in segments formed prior to cadmium exposure, suggesting that extrinsic contamination was the primary source of cadmium in the hair. β_2-microglobulin levels were within normal limits. No significant renal or pulmonary dysfunctions were noted. Symptoms ceased after a cadmium-containing brazing alloy used in jewelry production was replaced, yet urine cadmium levels remained persistantly high in some workers. Blood cadmium determinations were found to be useful in evaluating symptoms potentially related to cadmium intoxication.

Cadmium fumes have been found to cause permanent damage to the lungs (Boisset et al., 1978). Fifty-five young male specific pathogen free, Sprague-Dawley derived rats weighing 130-140 g were randomly divided into 11 groups. Seven groups of five animals each were exposed for

30 min/day for five consecutive days to cadmium oxide (CdO) particles, the other groups were used as controls. Treated groups were sacrificed immediately after exposure and after 7, 14, 21, 28, 42, 56, and 84 days. Control groups were killed at 0, 14, 42, and 84 days. Time-course changes of lung, liver and kidney weight as well as cadmium content of these organs were studied by polynomial regression analysis. Growth of lungs was disturbed in rats exposed to cadmium fumes and some degree of permanent damage to pulmonary lobes was evidenced. Liver growth was affected to a less extent and no effect on the growth of the kidneys was noted. Results indicated that 12 percent of the inhaled cadmium was deposited in lungs. Clearance of pulmonary cadmium was low, exponential and monophasic (biological half-life 56 days). A slight, but statistically significant, accumulation of cadmium in liver and kidney was observed. The results suggested that 60 percent of the lung-deposited cadmium was absorbed.

The biochemical accumulation and clinical pathological conditions induced by intraperitoneal injection of cadmium were studied in rats (Colucci et al., 1975). Cadmium was injected as $CdCl_2$ in 0.85 percent sterile saline solutions. In the experiment 42 male Sprague-Dawley rats, weighing 350 g, were divided into seven injection groups of 0, 0.5, 1.0, 2.0, 2.5, 3.0 or 4.0 mg $CdCl_2$/kg of body

weight and injections were given for six consecutive days. In the second experiment, 16 male Sprague-Dawley rats, weighing 200 g, were divided into four groups. Each group received a dose of 0, 0.5, 1.0, or 2.0 mg $CdCl_2$/kg of body weight. Animals were injected five times and were sacrificed after six days. Tissues were fixed in formalin for light microscopy and livers for each surviving groups were homogenized and centrifuged with 0.25 M sucrose. The supernatant fractions were concentrated by lyophilization and chromatographed on Sephadex G-75 equilibrated and eluted with 0.05 M potassium phosphate. The elution fractions were assayed for metals by atomic absorption spectroscopy. It was observed that cadmium caused induction of metallothionein. At low doses of cadmium (0.5 to 1.0 mg/kg of body weight) the metal was predominantly complexed to metallothionein and no clinical or pathological signs or symptoms of toxicity were observed. The critical determining factor of toxicity did not appear to be the injection dose, but rather the tissue concentration. Animals in the 0.5 and 1.0 mg $CdCl_2$/kg of body weight groups revealed several degenerating hepatocytes. There were no differences in the apparent numbers of degenerating cells in these latter three groups. When compared with the normal hepatocytes, the degenerating cells were smaller, the cytoplasm was

darker and less granular, and the nuclei were darker staining and smaller in size.

The effects of cadmium on adrenal and thyroid functions in rats have been studied (Der et al., 1977). Seventy sexually-matured male Sprague-Dawley rats weighing 300 g were divided into seven equal groups. Groups 1 and 2 served as controls and were injected daily with 328 µg sodium acetate and 269 µg sodium chloride respectively. Groups 3 and 4 were injected with 50 and 250 µg of lead respectively; groups 5 and 6 were given 50 and 250 µg of cadmium respectively; and group 7 was administered 25 µg of both lead and cadmium. Thyroxine secretion rate (TSR) was determined at 10 days before treatment and 30 and 60 days after treatment. After 70 days of injections, the rats were sacrificed. Body weight and organ weights were obtained and histology of the adrenals was made. Blood was analyzed for triiodothyronine (T_3), thyroxine (T_4), and corticosterone. Thyroxine secretion rate results showed that 250 µg cadmium had a stimulating effect but 1/10 of the dose of cadmium plus 25 µg lead had the opposite effect. Plasma thyroid hormone levels indicated that 50 µg lead had a stimulating effect on T_4 but cadmium and a mixture of lead and cadmium had a suppressing effect on both T_4 and T_3. Plasma corticosterone and adrenal cortical

histology indicated an increase in adrenal function in animals treated with 50 µg lead, 50 µg and 250 µg cadmium and a mixture of lead and cadmium. The data showed that cadmium had more deleterious effects on thyroid and adrenal function than lead had.

The interactions between dietary cadmium, iron, and zinc in the growing rat were studied with respect to body weight gain, blood hemoglobin concentration and tissue levels of trace elements (Banis et al., 1973). Weanling rats each weighing about 46 g were fed diets identical in all respects, within each experiment, except for the levels of cadmium, iron, and zinc. One hundred and seventy rats were divided into four groups to determine the effect of zinc and iron added to the diet alone and in combination in preventing cadmium toxicity. Cadmium added to the diet at a level of 100 ppm depressed weight gain and blood hemoglobin in all four experiments. Supplemental iron at 300 ppm in the diet offset the effect of cadmium on weight gain and hemoglobin. The amount of copper in the liver was reduced and that of zinc was increased by cadmium. The amount of iron in the liver was reduced by cadmium at 100 ppm dietary iron but not at 400 ppm. Depression of weight gain by cadmium in one experiment was prevented by 68 ppm of iron plus 200 ppm of zinc added to a low iron-low zinc diet, but

not when either was added singly. Weight gain and hemoglobin of rats fed cadmium were increased when rats were kept in galvanized cages as compared to stainless steel cages in 1 of 2 experiments.

Co-Insult (Cadmium and Radiation)

Co-insult studies involving metals (e.g. cadmium) and ionizing radiation in mammalian systems are scarce. Complete histopathological studies following co-insult treatment with metal and ionizing radiation do not appear in the published literature.

The effect of co-insults of cadmium and X-irradiation was determined in rats (Lappenbusch and Gile, 1975). Rats were injected twice weekly for 30 days with 0, 12.5, 62.5, 125 or 250 μg of $CdCl_2$/kg of body weight and then X-irradiated with 0, 450, 525, 600 or 750 rads at 26 rads/min. The results showed a linear decrease in $LD_{50(30)}$ values. Co-insults also decreased the numbers of red and white blood cells, and the neutrophile and lymphocyte ratios were lowered significantly over a period of time. High concentrations of cadmium were found in the liver and kidney followed by lesser concentrations in the spleen, heart, intestine, lung, stomach, muscle, blood and brain.

Cadmium chloride has also been shown to cause degradation of DNA in human- and rat-embryo cells (Zasukhina et al., 1977). Incubation of the cells in a medium free of cadmium did not change the profile of DNA sedimentation. Marked inhibition of the virus-induced synthesis of interferon was also noted. The introduction of $CdCl_2$ into diploid cells infected by the leucosis virus caused a 3-4 fold increase in the yield of virus-induced transformation loci. Experiments with γ-irradiation were performed to show the activity of the repair system in these cells. Cells irradiated post-incubation in a complete medium resulted in a complete repair of the initial DNA structure; however, post-incubation experiments in the presence of $CdCl_2$ yielded negative results. Possible interpretations of this phenomenon might be either repression of the repair system by $CdCl_2$ or induction of non-repairable DNA changes by $CdCl_2$.

Synergistic effects, using methylmercury chloride and X-rays, have been observed (Johnson and Cember, 1977). Methylmercury chloride was administered to rats in drinking water at doses of 0, 10, and 50 ppm. Three groups, one at each mercury level, were sham-irradiated. Irradiation of the experimental organisms was done three weeks following initiation of drinking mercurated water. The treated

animals received a single exposure of 500, 1,000 or 5,000 R of X-rays. Mercurated water was fed to the experimental animals until either death occurred or study ended. Ten days following exposure to radiation, measurements of work performance via a treadmill began. The results indicated that at high mercury level (50 ppm) and high irradiation dose (1,000 R) the work output of the treated animals deteriorated immediately following treatments and continued until death two months later. At the low level of mercury (10 ppm) and radiation (500 R), rats began to exhibit impaired work performance about one month after X-ray treatment.

Hupp et al. (1977) observed an interaction between methylmercuric chloride and ionizing radiation on nervous systems of hamsters, rats, and squirrel monkeys. A number of parameters were evaluated: blood-brain barrier, brain electrical activity, brain histology, behavior measurements, concentration of mercury in various brain locations, and lethality. The results indicated that in a number of cases the effects of the co-insults were less than, or the same as one of the two insults applied alone. Nine kR of X-rays were more effective than the combined effects of 8 mg methylmercury/kg of body weight and 9 kR of radiation in inducing behavioral decrement in rats 2-4 hours

post-irradiation. In monkeys, the behavior was not affected as much with co-insults of 300 R of irradiation and 6 mg methlymercury/kg of body weight as with methylmercury alone. With doses of radiation in the $LD_{50(30)}$ range, the two agents were partially additive.

Additional effects of cadmium and radiation have been studied on mouse fetuses (Ueda and Yoshizawa, 1973). Virgin female mice were mated with mature males, and the females were checked for the existence of vaginal plugs on the following morning. The day, when vaginal plugs were found, was considered to be day zero in gestation age. The pregnant mice were then exposed to γ-ray of Cesium-137 at a dose rate of 3.5 R/min until a total exposure of 200 R was reached in a single exposure. Cadmium chloride was injected intraperitoneally in sterile isotonic sodium chloride at dose level of 1, 19, 50 or 100 µg prior to irradiation. On day 18 of gestation, the pregnant mice were sacrificed to observe their fetuses. A total of 2312 fetuses were examined. The cadmium and radiation showed additional effects in regard to three kinds of biological effects, namely, the reduction in body weight of fetus, the digital malformation and the delay of ossification in the caudal vertebrae. These results suggested that a synergistic relation existed between radiation and cadmium.

Morgan (1981) studied the interacting effect of cadmium and γ-irradiation on the hematopoietic system in the albino rat. Significant decreases were observed in hemoglobin, hematocrit, and red blood cell with 543 R and 2.5 mg $CdCl_2$/kg of body weight. Decreases were also seen in animals treated with high dose of $CdCl_2$ (2.5 mg/kg of body weight) alone. In general, $CdCl_2$ and radiation had contrasting actions on total white blood cell counts, radiation producing a leukopenia and cadmium a leukocytosis. Dose effectiveness of radiation on the total white blood cell count was greatest on days 1 and 7 following radiation. Intergroup differences were also observed between high dose co-insult (543 R, 2.5 mg $CdCl_2$/kg of body weight) and low dose co-insult (362 R, 1.0 mg $CdCl_2$/kg of body weight) groups. Statistically significant decreases of lymphocytes were seen in all treated groups when compared with the control for the same day post-irradiation. Serum glutamic oxaloacetate transaminase enzyme levels were increased in high cadmium groups at days 1 and 7, whereas lactate dehydrogenase enzyme and alkaline phosphatase enzyme levels were increased in the high radiation groups at days 1 and 7. Serum iron elevations were observed in co-insult and radiation groups at post-irradiation day 1. Significant

increases were also noted at days 7 and 21 in low dose cadmium-high dose radiation and high radiation groups.

CHAPTER III

MATERIALS AND METHODS

Test Organism

The organism used in the experiment was the Sprague-Dawley derived rat, Rattus norvegicus. This species was selected because it exhibits many advantages which make it valuable in biological research. The attractiveness of the Sprague-Dawley rat as a research animal in this aspect is enhanced by its availability at any time of the year, and its relatively short life span. The species has the ability to thrive well on minimal care and space, and is relatively free from diseases. The white rat is large enough from which to collect tissues, yet small enough to maintain in large numbers. Many studies have been done in evaluating the effects of various metals in Rattus norvegicus. Radiation-induced lethality studies have also been done using this species.

Procedures

All rats were about 80 days old and weighed 180-250 g. They were randomly selected from the rat colony maintained at Texas Woman's University for this investigation. All test organisms were ear-notched for

identification purposes. Six to eight rats were assigned at random per cage (67 x 25 x 175 cm), and given Purina Laboratory Chow and water ad libitum. The temperature of the animal facility was maintained at 24° ± 1° Celsius (C) and the relative humidity control was set to provide a minimum relative humidity of approximately 50 percent. The fluorescent lights were automatically turned on and off every 12 hours so as to create a diurnal environment. Animals were transferred to clean cages every week.

Preliminary Studies

Study 1: acute cadmium chloride lethality

One hundred and twelve male and 96 female white rats were used. Each group of rats received a single intraperitoneal injection of $CdCl_2$ based on body weight of the test organism. The experimental protocol is given in Table 1. Mortality was recorded daily for 30 days in order to determine the lethal dose that killed 50 percent of the population within 30 days ($LD_{50(30)}$).

Study 2: chronic cadmium chloride lethality

Ninety females and 90 males were assigned to 10 different groups. Each rat was injected intraperitoneally with $CdCl_2$ twice weekly for 29 days for a total of nine injections with one of various concentrations of $CdCl_2$.

TABLE 1

EXPERIMENTAL TREATMENT FOR DETERMINING ACUTE CADMIUM LETHALITY IN RATS

Group No.	No. of Animals		Total Amount of $CdCl_2$ Injected (mg/kg of body weight)
	Male	Female	
1	14	12	0
2	14	12	2.0
3	14	12	4.0
4	14	12	5.0
5	14	12	6.0
6	14	12	8.0
7	14	12	16.0
8	14	12	24.0

The experimental design is given in Table 2. Mortality was recorded during the injection period and for an additional 30 days.

Study 3: acute radiation lethality

Sixty male and 60 female rats were each exposed to a single whole-body dose of ^{60}Co γ-radiation in the U.S. Nuclear Corporation model GR-9 irradiator according to the scheme of the experimental design (Table 3). Those receiving no irradiation were sham-irradiated in the same room. The irradiation of the rats was conducted at room temperature. Mortality was recorded during a 30-day period following irradiation to determine the $LD_{50(30)}$.

Final Co-insult Study

Two hundred and sixteen male rats were assigned at random to various $CdCl_2$ and/or radiation groups to determine the interacting effect of cadmium and radiation in these animals (Table 4).

Cadmium chloride injection

The white rats were intraperitoneally injected twice a week for 29 days for a total of nine injections. The animals received 0, 1.0 or 2.5 mg $CdCl_2$/kg of body weight in distilled water at each injection, based on the current body weight of the animals. All cadmium treated animals

TABLE 2

EXPERIMENTAL TREATMENT FOR DETERMINING CHRONIC CADMIUM LETHALITY IN RATS

Group No.	No. of Animals		Amount of $CdCl_2$ Injected*
	Male	Female	(mg/kg of body weight)
1	8	8	0
2	10	10	1.0
3	8	8	2.0
4	10	10	3.0
5	8	8	4.0
6	10	10	5.0
7	10	10	6.0
8	10	10	7.0
9	8	8	8.0
10	8	8	16.0

*Animals received these amounts with each injection.

TABLE 3

EXPERIMENTAL TREATMENT FOR DETERMINING ACUTE RADIATION LETHALITY IN RATS

Group No.	No. of Animals		Radiation Dose (R)*
	Male	Female	
1	10	10	0
2	10	10	675
3	10	10	700
4	10	10	725
5	10	10	750
6	10	10	775

*At a dose rate of 304 R/min.

TABLE 4

EXPERIMENTAL TREATMENTS IN THE CO-INSULT STUDY OF CADMIUM AND GAMMA RADIATION

Group No.	No. of Animals	Injections of $CdCl_2$* (mg/kg of body wt.)	Radiation Dose** (R)	Number of Animals Sacrificed Days After Radiation		
				1	7	21
1	24	0	0	8	8	8
2	24	2.5	543	8	8	8
3	24	1.0	543	8	8	8
4	24	0	543	8	8	8
5	24	2.5	362	8	8	8
6	24	1.0	362	8	8	8
7	24	0	362	8	8	8
8	24	2.5	0	8	8	8
9	24	1.0	0	8	8	8

*Animals were injected with these amounts twice weekly for 29 days for a total of nine injections.

**Twenty-four hours after the last treatment with cadmium chloride, animals were irradiated at a dose rate of 300 R/min.

were injected with a standard solution of 4.0 mg of $CdCl_2$/ml of distilled water. The control groups received distilled water only. Justification for the use of high and low doses of $CdCl_2$ was based on results of the preliminary studies. A high dose $CdCl_2$ value of 2.5 mg/kg of body weight was chosen, based on the probit analysis. The low dose of 1.0 mg/kg of body weight was selected since no deaths occurred at this dose.

Radiation treatment

Preliminary radiation studies (Study 3) using ^{60}Co gamma source produced an $LD_{50(30)}$ of 725 R, as determined by probit analysis. A high dose of 543 R was selected because this was approximately 75 percent of the $LD_{50(30)}$. The low dose of 362 R was chosen because no deaths occurred at that dose. Thus, a maximum radiation dose was obtained with less lethal effects.

On day 30 the white rats were subjected to γ-irradiation according to the scheme of the experimental design. Those receiving no irradiation were sham-irradiated in the same room.

Collection of tissues

All rats were fasted for at least 10 hours before sacrificing. The animals were sacrificed using

pentobarbital sodium anesthesia (4.0 mg/100 g of body weight) on day 1, 7, or 21 post-irradiation. The following tissues were collected: blood, brain, heart, intestine, kidney, liver, lung, muscle, spleen, stomach, and testis.

Histological analysis

Immediately after collection, the tissues were weighed and half of each tissue was placed in a modified Tellyseniczky's formol-alcohol fixative for 24 hours then stored in 70 percent ethyl alcohol (Humason, 1972). The other half of the tissues were used for cadmium analysis. A Fisher Tissuematon was used for dehydrating, and infiltrating the tissues with paraffin. After blocking in paraffin, the tissues were sectioned 7 μm thick using an American Optical "820" Spencer microtome and mounted on glass slides. The sections were deparaffinized in xylene which was removed by washing with absolute alcohol. The slides were stained with hematoxylin and eosin. The glass cover slips were mounted using Permount.

Tissues were studied with a Reichert light microscope at 43X and 100X powers. Standard histological observations of the experimental tissues were compared with those of the controls.

Analyses of tissues for cadmium

The tissues were stored in a freezer at -20° C until digestion in nitric acid (HNO_3), sulfuric acid (H_2SO_4), and perchloric acid ($HClO_4$) in the ratio of 3:3:1 by volume, using 1.0 ml/g of wet material (Bauer et al., 1979). The digestion was done in Kjeldahl digestion flasks at a temperature not exceeding 195° C. The digested material was allowed to cool and the pH adjusted to five using ammonium hydroxide (NH_4OH).

The concentrations of cadmium in the tissues were measured by a Perkin-Elmer model 370 atomic absorption spectrophotometer using the standard instrumental parameters recommended by the manufacturer.

Statistical methods

Lethality studies. The LD_{50} for the lethality studies was determined by an analysis of probit against the log (dose) as described by Finney (1952, 1971). The SAS statistical package (SAS, 1979) as implemented by the North Texas State University Computer Center was used for this computation. This procedure calls for the calculation of the maximum likelihood estimates of the intercept, the slope, and the natural (threshold) response rate for lethality data.

Tissue studies. The basic statistical model employed in this investigation was the three by three by three factorial arrangement with eight replicates. Replicates were completely random.

The main effects were levels of $CdCl_2$ (0, 1.0, and 2.5 mg/kg of body weight), levels of radiation (0, 362, and 543 R), and post-irradiation intervals to sacrifice (1, 7, and 21 days). Since the effects were fixed, the analysis of variance (ANOVA) was used to test main and interactions against error mean square. The Student-Newman-Keuls test was used where appropriate (Anderson and McLean, 1974). The BMDP statistical package (BMDP, 1977) as implemented by the Texas Woman's University DEC System-20 computer was used to do the analysis of variance procedure.

CHAPTER IV

RESULTS AND DISCUSSION

Lethality Studies

Studies were performed to investigate acute and chronic lethality of $CdCl_2$ and acute lethality of ^{60}Co γ-irradiation. Effects of cadmium and radiation singly or as co-insults on one particular organ or vascular tissue or the interaction of effects in several organs produced characteristic syndromes as observed in this study. Some of these syndromes were accompanied by tissue damage. The time of appearance of these syndromes, their duration, and the survival of the organism depend on various factors, including the species (Ammerman et al., 1973), length of exposure (Friberg et al., 1971), and the dose (Arena, 1971). The data accumulated in this investigation provide a better understanding of the mechanisms whereby deleterious effects resulted in lethality by cadmium and radiation singly and as co-insults. The results and discussions of these studies follow.

Acute cadmium chloride lethality

Following an intraperitoneal injection of one of the various concentrations of $CdCl_2$, deaths of experimental

animals were recorded daily for 30 days to determine the $LD_{50(30)}$ value, that is, the dose that killed 50 percent of those treated within 30 days post-treatment. The numbers of rats dying at each dose were recorded and the percents lethality were calculated (Table 5). The 24 mg $CdCl_2$/kg of body weight dose was 100 percent lethal to male and female rats by 30 days after injection. Fifty percent of the male rats administered an acute dose of 6.0 mg $CdCl_2$/kg of body weight died within 30 days following treatment, whereas 50 percent of the females receiving the acute dose of 5.0 mg $CdCl_2$/kg of body weight succumbed. Most of these deaths for both males and females occurred between days 3 and 5 following $CdCl_2$ treatment (Table 6). Animals receiving an acute injection of 8, 16 or 24 mg $CdCl_2$/kg of body weight succumbed within 4 days post-treatment (Table 6).

The probit $LD_{50(30)}$ value for males was 5.99 mg with a 95 percent fiducial limits of 4.71-7.54 mg $CdCl_2$/kg of body weight (Table 7, Column 2; Figure 1A) and that of females was 7.13 (5.81-8.85) mg $CdCl_2$/kg of body weight (Table 7, Column 3; Figure 1B). The probit $LD_{50(30)}$ for male rats obtained in this investigation compared reasonably well with the 7.0 mg $CdCl_2$/kg of body weight value reported by Krasny and Holbrook (1977). A 20 percent lethality with 250 µg $CdCl_2$/rat was reported (Lappenbusch

TABLE 5

NUMBERS OF MALE AND FEMALE RATS GIVEN AN ACUTE INTRAPERITONEAL INJECTION OF ONE OF VARIOUS CONCENTRATIONS OF $CdCl_2$ AND THE NUMBERS AND PERCENTAGES OF DEATHS 30 DAYS POST-INJECTION

$CdCl_2$ Injection (mg/kg body weight)	No. Rats Injected			No. Rats Dead at 30 Days			% Lethality		
	Males	Females	Combined	Males	Females	Combined	Males	Females	Combined
0	14	12	26	0	0	0	0	0	0
2	14	12	26	0	0	0	0	0	0
4	14	12	26	1	3	4	7	25	15
5	14	12	26	6	6	12	43	50	46
6	14	12	26	7	7	14	50	58	54
8	14	12	26	8	8	16	57	67	62
16	14	12	26	11	10	21	79	83	81
24	14	12	26	14	12	26	100	100	100

TABLE 6

NUMBERS OF RATS INJECTED, NUMBERS OF DEAD RATS AND THE DAYS OF DEATH FOLLOWING AN ACUTE INTRAPERITONEAL INJECTION OF CADMIUM CHLORIDE

$CdCl_2$ Injection (mg/kg of body weight)	Males		Females	
	No. Injected	No./Day of Death	No. Injected	No./Day of Death
0	14	0	12	0
2	14	0	12	0
4	14	1/6	12	3/5
5	14	4/3, 2/4	12	3/3, 3/4
6	14	3/3, 2/4, 2/5	12	2/2, 3/3, 2/4
8	14	6/2, 3/4	12	6/2, 2/3
16	14	11/2	12	10/2
24	14	12/4, 2/2	12	9/1, 3/1

TABLE 7

PROBIT ANALYSES FOR ESTIMATION OF DOSE EFFECTS IN TERMS OF LETHAL DOSE VALUES FOR ACUTE AND CHRONIC $CdCl_2$ LETHALITY AND ACUTE RADIATION LETHALITY

	Acute $CdCl_2$ (mg/kg body weight)		Chronic $CdCl_2$ (mg/kg body weight)				Acute ^{60}Co (R)	
			30 Days		60 Days			
LD	Males	Females	Males	Females	Males	Females	Males	Females
01	1.60	1.58	1.39	2.31	1.08	2.35	641	640
05	1.95	2.46	1.93	2.92	1.51	2.87	668	665
10	2.50	3.13	2.30	3.31	1.80	3.19	682	679
25	3.78	4.61	3.08	4.08	2.43	3.81	706	702
50	5.99	7.13	4.25	5.14	3.38	4.65	733	729
75	9.47	11.04	5.88	6.15	4.71	5.66	761	757
90	14.31	16.36	7.87	8.00	6.35	6.77	787	784
95	18.32	20.70	9.36	9.07	7.59	7.53	804	800
99	29.14	32.17	12.99	11.47	10.61	9.20	835	830

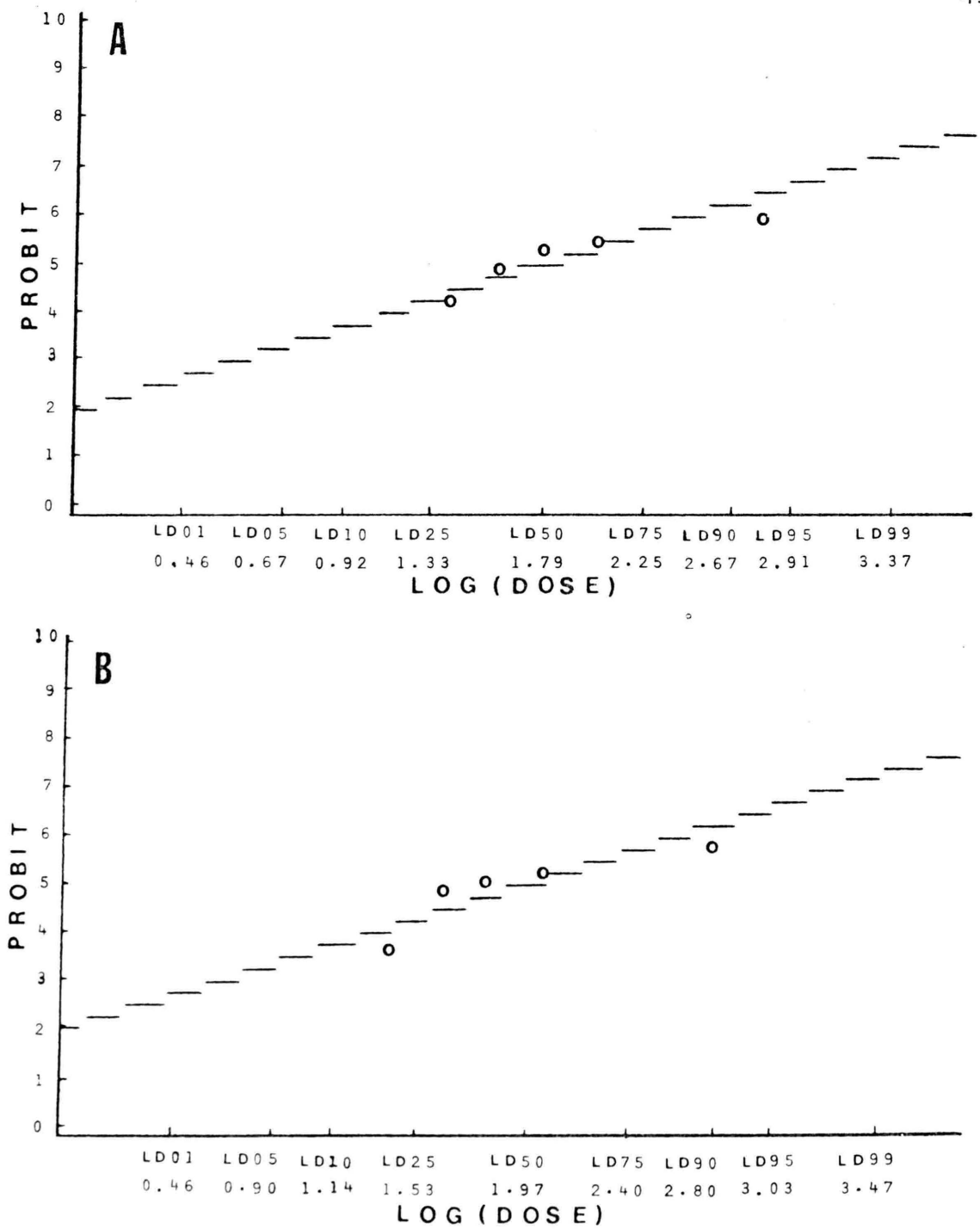


Figure 1. LD_{50} ($_{30}$) probit lines on log (dose) for acute lethality of $CdCl_2$ for (A) male and (B) female rats. "O" denotes data points.

and Gile, 1975); however, this study did not include an $LD_{50(30)}$ determination. Values for female rats were not reported. Hupp et al. (1977) reported that male rats subjected to intraperitoneal injections of methylmercury were more susceptible than females of the same species. Also in this cadmium study the males were more susceptible than the females.

Chronic cadmium chloride lethality

Deaths of rats were scored during the 30 day period of injections and for an additional 30 days post-injections. The numbers of animals which succumbed during that time are presented in Table 8. All of the rats of both sexes receiving a dose of 16 mg $CdCl_2$/kg of body weight died within 30 days. Fifty percent of the male rats administered a dose of 4.0 mg of $CdCl_2$/kg of body weight were dead at 30 days, whereas at 60 days 50 percent of male rats receiving a dose in the concentration of 3.0 mg of $CdCl_2$/kg of body weight were dead. In contrast, at 30 days, only 25 percent of female rats administered a similar dose of 4.0 mg of $CdCl_2$/kg of body weight were dead and with a concentration of 3.0 mg/kg of $CdCl_2$ of body weight only 10 percent of the female rats were dead at 60 days. Based on these data, the probit $LD_{50(30)}$ for male rats at the

TABLE 8

NUMBERS OF MALE AND FEMALE RATS GIVEN CHRONIC INTRAPERITONEAL INJECTIONS OF ONE OF VARIOUS CONCENTRATIONS OF $CdCl_2$ AND THE NUMBERS AND PERCENTAGES OF DEATHS AFTER THE LAST INJECTION AND 30 DAYS POST-INJECTIONS.

$CdCl_2$ Injections (mg/kg body weight)	No. Rats Injected			No. Rats Dead					
				30 Days*			60 Days**		
	Males	Females	Combined	Males	Females	Combined	Males	Females	Combined
0	8	8	16	0	0	0	0	0	0
1	10	10	20	0	0	0	0	0	0
2	8	8	16	1	0	1	1	0	1
3	10	10	20	1	1	2	5	1	6
4	8	8	16	4	2	6	5	2	7
5	10	10	20	7	3	10	7	6	13
6	10	10	20	7	7	14	9	8	17
7	10	10	20	9	9	18	9	9	18
8	8	8	16	7	7	14	8	8	16
16	8	8	16	8	8	16	8	8	16

*Rats injected with $CdCl_2$ 9 times over a 29-day period. **Rats did not receive additional $CdCl_2$ to day 60.

conclusion of injections was 4.25 (3.42-5.05) mg of $CdCl_2$/kg of body weight (Table 7, Column 4; Figure 2A); whereas, the probit $LD_{50}(_{60})$ value (30 days post-injections) was 3.38 (2.61-4.05) mg $CdCl_2$/kg of body weight (Table 7, Column 6; Figure 3A). In contrast, the corresponding probit LD_{50} values for females were 5.14 (4.42-5.92) mg of $CdCl_2$/kg of body weight (Table 7, Column 5; Figure 2B) and 4.65 (4.00-5.25) mg $CdCl_2$/kg of body weight (Table 7, Column 7; Figure 3B), respectively.

When compared with those of the acute $CdCl_2$ lethality study, $LD_{50}(_{30})$ values for the chronic $CdCl_2$ lethality were generally lower, indicating an increase in effectiveness of dose as time of injections was protracted. Forney (1978) reported a similar finding whereby an $LD_{50}(_{7\ day})$ of 2.78 mg $CdCl_2$/kg of body weight in male rats was found; however, over an extended period of time, the $LD_{50}(_{10\ month})$ value of 1.73 mg $CdCl_2$/kg of body weight was observed. The acute $LD_{50}(_{30})$ of methylmercury chloride has been estimated as 10.1 mg/kg in rats, 15.2 mg/kg in hamsters and 4.7-6.4 mg/kg in monkeys (Hoskins and Hupp, 1978). Susceptibility to cumulative doses given daily for five days, in hamsters, was as great as that to a single injection dose. They suggested that detoxification mechanisms, especially

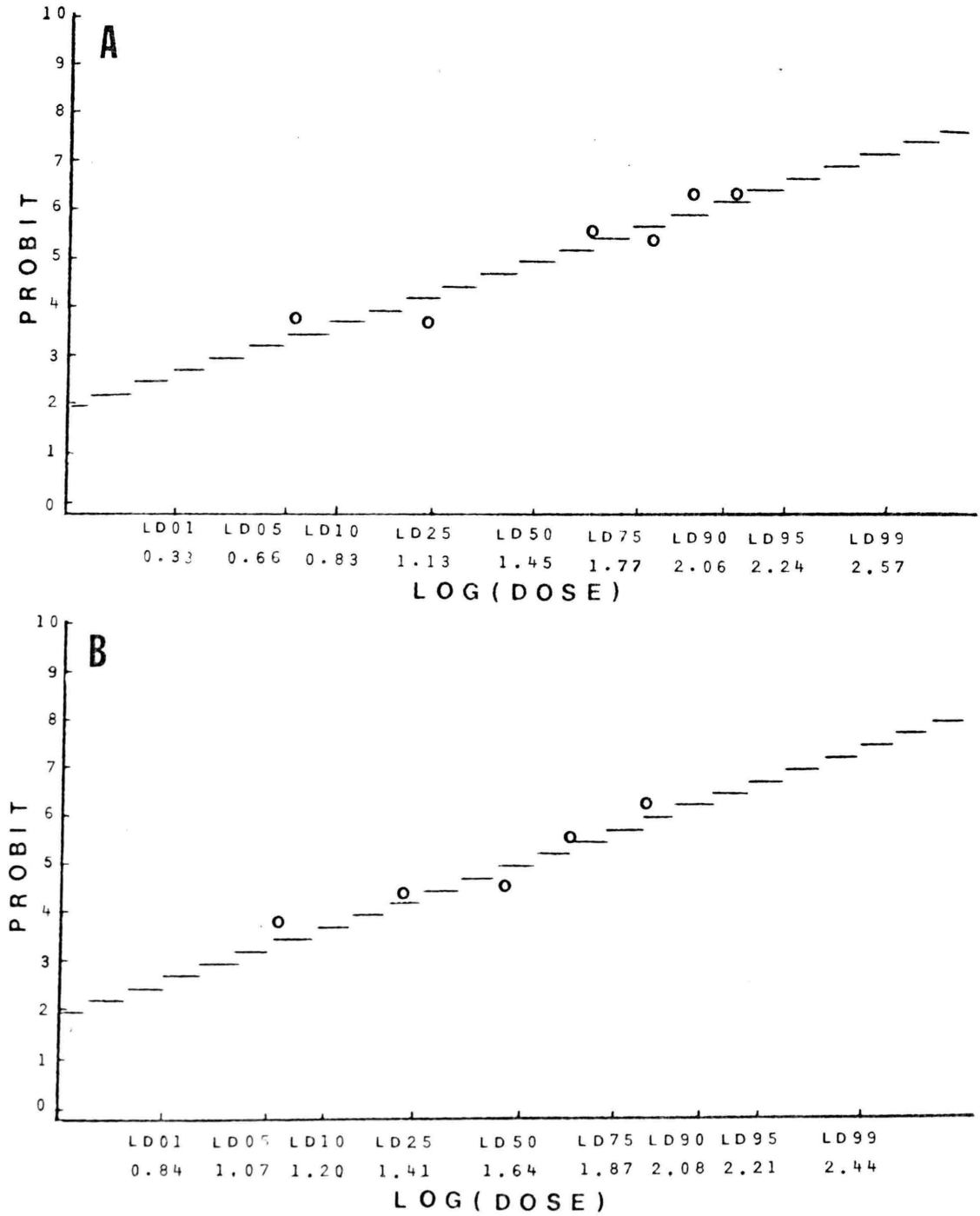


Figure 2. $LD_{50(30)}$ probit lines on log (dose) for chronic lethality of $CdCl_2$ for (A) male and (B) female rats. "0" denotes data points.

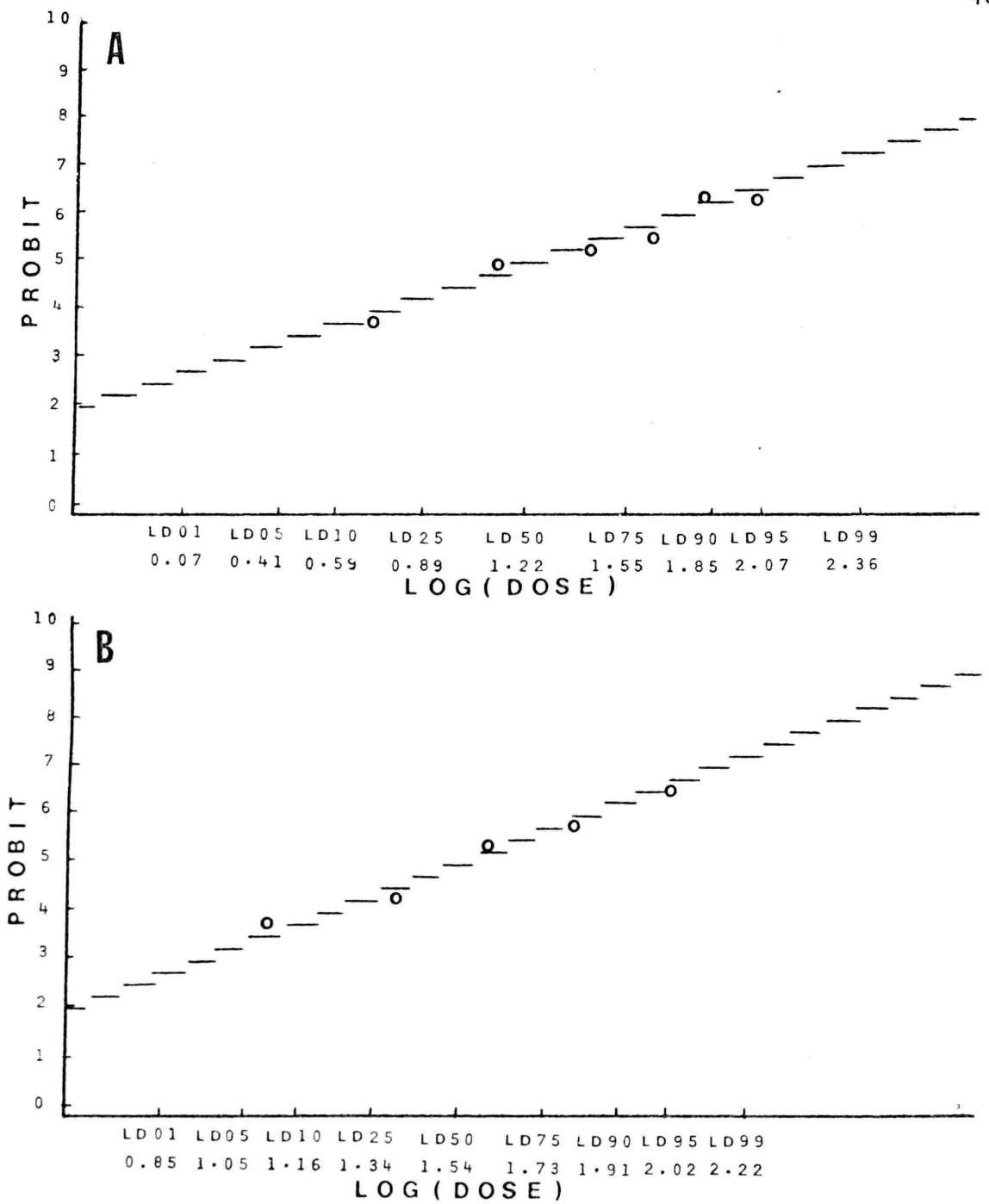


Figure 3. $LD_{50(60)}$ probit lines on log (dose) for chronic lethality of $CdCl_2$ for (A) male and (B) female rats. "0" denotes data points.

via the kidney, were immediately effective following the first injection.

As expected an inverse correlation was observed between dose and time of death in both sexes. At higher doses of $CdCl_2$, the first deaths were observed within 48 hours after the first injection (Table 9).

Although both sexes were administered comparable doses of $CdCl_2$ based on total body weight, there was a tendency for female rats to die sooner. Animals surviving the higher chronic doses of $CdCl_2$ showed lethargy, difficulty in breathing, bloody diarrhea, and excessive weight losses due to decreased food intake. Pronounced edema and nasal hemorrhages were also observed in some animals.

Acute ^{60}Co gamma radiation lethality

Experimental animals exposed to various doses of an acute γ-irradiation were observed for 30 days post-treatment and deaths were recorded daily to obtain the $LD_{50(30)}$ values (Table 10). Sixty percent of the males irradiated with a dose of 750 R succumbed, whereas only 50 percent of the females died after that dose. After an exposure of 775 R, death in both sexes occurred within 30 days post-irradiation (Table 11). The probit $LD_{50(30)}$ value for male rats (Table 7, Column 8; Figure 4A) was

TABLE 9

NUMBERS OF RATS INJECTED, NUMBERS OF DEAD RATS AND THE DAYS OF DEATH FOLLOWING CHRONIC INTRAPERITONEAL INJECTIONS OF CADMIUM CHLORIDE

$CdCl_2$ Injections (mg/kg body weight)	Males		Females	
	No. Injected	No./Day of Death	No. Injected	No./Day of Death
0	8	0	8	0
1	10	0	10	0
2	8	1/26	8	0
3	10	1/34, 3/36, 1/41, 1/43	10	1/26
4	8	3/14, 1/29, 1/37	8	1/2, 1/25
5	10	2/6, 1/9, 1/12, 2/13, 1/20	10	1/17, 1/19, 1/23, 1/34, 1/44, 1/51
6	10	6/6, 1/9, 1/37, 1/49	10	5/2, 1/4, 1/8, 1/38
7	10	7/2, 1/6, 1/27	10	7/2, 2/3
8	8	2/4, 2/6, 1/17, 1/22, 1/26, 1/51	8	5/2, 2/3, 1/47
16	8	6/1, 1/3, 1/10	8	8/1

TABLE 10

NUMBERS OF MALE AND FEMALE RATS EXPOSED TO ONE OF VARIOUS ACUTE DOSES OF ^{60}Co GAMMA RADIATION AND THE NUMBERS OF DEATHS 30 DAYS POST-TREATMENT

Dose (R)	No. Rats Irradiated			No. Rats Dead at 30 Days		
	Males	Females	Combined	Males	Females	Combined
0	10	10	20	0	0	0
675	10	10	20	1	1	2
700	10	10	20	2	3	5
725	10	10	20	4	4	8
750	10	10	20	6	5	11
775	10	10	20	9	10	19

TABLE 11

NUMBERS OF RATS IRRADIATED, NUMBERS OF DEAD RATS AND THE DAYS OF DEATH FOLLOWING AN ACUTE ^{60}Co GAMMA RADIATION EXPOSURE

Dose (R)	Males		Females	
	No. Irradiated	No./Day of Death	No. Irradiated	No./Day of Death
0	10	0	10	0
675	10	1/15	10	1/18
700	10	1/3, 1/16	10	1/20, 2/23
725	10	1/12, 1/15, 1/19, 1/20	10	1/16, 1/18, 2/22
750	10	2/12, 1/14, 2/17, 1/19	10	1/14, 1/17, 2/18, 1/25
775	10	2/15, 1/17, 2/20, 1/22 1/24, 2/28	10	1/9, 4/15, 2/19, 3/24

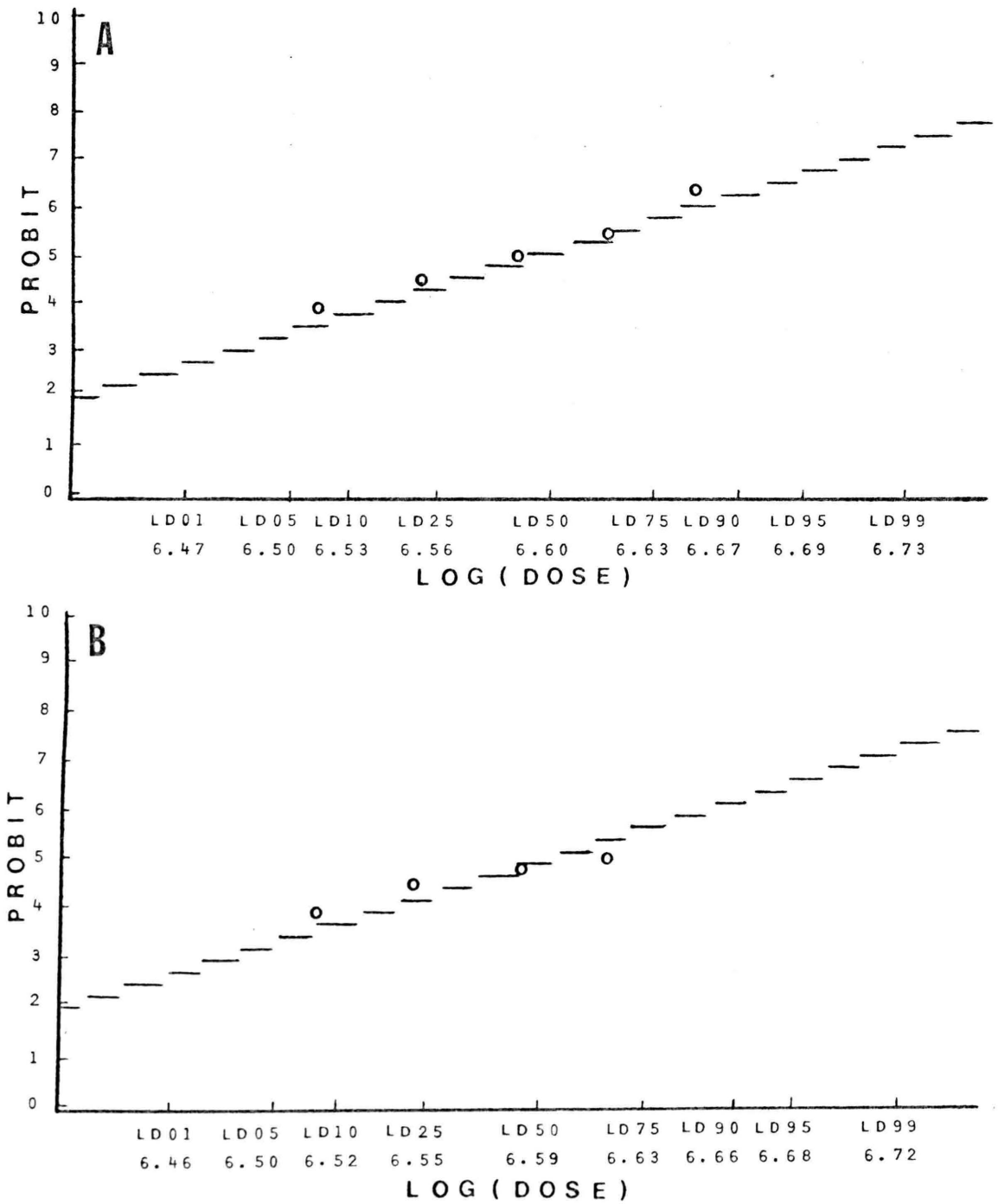


Figure 4. $LD_{50(30)}$ probit lines on log (dose) for acute lethality of gamma-irradiation for (A) male and (B) female rats. "0" denotes data points.

773 (713-745) R, and that of the females (Table 7, Column 9; Figure 4B) was 729 (712-750) R. $LD_{50}(_{30})$ probit values were slightly lower than actual $LD_{50}(_{30})$ values.

Probit $LD_{50}(_{30})$ values of 733 R obtained for male rats and 729 R for females agree with the $LD_{50}(_{30})$ values of 700 rads, 714 rads, and 750 rads as reported by Coggle (1971), Claus (1958), and Casarett (1968), respectively. The present investigation revealed that with higher doses of total-body irradiation survival time decreases. Deaths following whole body exposure to radiation were attributed to injury to the bone-marrow, small intestine, and the central nervous system (Upton, 1969), and blood cells (Morgan, 1981). Deaths occurring during this study of acute radiation lethality were due primarily to injury to these organs or vascular tissue. It was obvious that total-body irradiation also caused damage to the gastro-intestinal system as evident by bloody diarrhea and weight losses by the animals.

Cadmium Concentrations in Tissues

The metal concentrations in all samples are expressed as µg/g (ppm) on a wet weight basis. Mean values ± standard errors (S.E.) for cadmium contents in the organs and vascular tissues of the animals are presented in

Appendix A. The significance of differences between values were compared at the 0.05 and 0.01 levels for the respective degree of freedom. A summary of these values is depicted in Table 12. The highest concentrations of cadmium accumulated were in the liver; succeeded in order of decreasing concentrations by kidney, spleen, intestine, stomach, heart, testis, lung, blood cells, brain and muscle (Appendix B, Table 35). Following are the results and discussions of these findings.

Liver

Regardless of treatments and days on which the animals were sacrificed, the cadmium concentrations were higher in the liver than in any other tissues analyzed. The liver had an average concentration of 47.44 and 21.94 µg/g of wet tissue for the 2.5 and 1.0 mg $CdCl_2$ groups respectively (Appendix B, Table 35).

A significant ($p < 0.05$) amount of cadmium was observed in all groups in which cadmium was used singly or in combination with radiation at the three intervals after irradiation (Figure 5). As expected the groups which received the higher dose of cadmium (2.5 mg/kg of body weight) showed higher concentrations of the metal in the liver, thus indicating a dose dependent accumulation.

TABLE 12

SUMMARY OF CADMIUM CONCENTRATIONS IN TISSUES WHICH DIFFER SIGNIFICANTLY FROM THE CONTROL

Tissue	Days Sacrificed Post-Irradiation																							
	1								7								21							
Group:	2	3	4	5	6	7	8	9	2	3	4	5	6	7	8	9	2	3	4	5	6	7	8	9
Liver	↑↑*	↑**		↑↑	↑		↑↑	↑	↑↑	↑		↑↑	↑↑		↑↑	↑	↑↑	↑		↑↑	↑		↑	↑
Kidney	↑↑	↑		↑↑	↑↑		↑↑	↑↑	↑↑	↑		↑↑	↑		↑↑	↑	↑↑	↑		↑	↑		↑↑	↑
Spleen	↑↑	↑		↑	↑			↑						↓										
Intestine	↑↑			↑↑			↑↑	↑↑	↑			↑			↑↑		↑↑			↑↑			↑↑	
Stomach	↑↑	↑		↑↑	↑↑		↑↑	↑↑	↑					↓				↑					↑↑	
Heart	↑	↑	↓	↑		↓	↑↑	↑							↑↑	↑↑								
Testis								↑																
Lung			↓			↓	↑↑				↑	↑			↑	↑								
Blood Cells	↑↑	↓	↓			↓↓			↑	↓↓	↓↓		↑				↑			↑				↑
Brain								↑↑																
Muscle	↓		↓	↓		↓				↓				↓										

*Significantly increased (↑↑) or decreased (↓↓) at the 0.01 level.

**Significantly increased (↑) or decreased (↓) at the 0.05 level.

Group: 1--control (not shown)
2--2.5 mg $CdCl_2$-543 R
3--1.0 mg $CdCl_2$-543 R
4--0.0 mg $CdCl_2$-543 R
5--2.5 mg $CdCl_2$-362 R
6--1.0 mg $CdCl_2$-362 R
7--0.0 mg $CdCl_2$-362 R
8--2.5 mg $CdCl_2$-0 R
9--1.0 mg $CdCl_2$-0 R

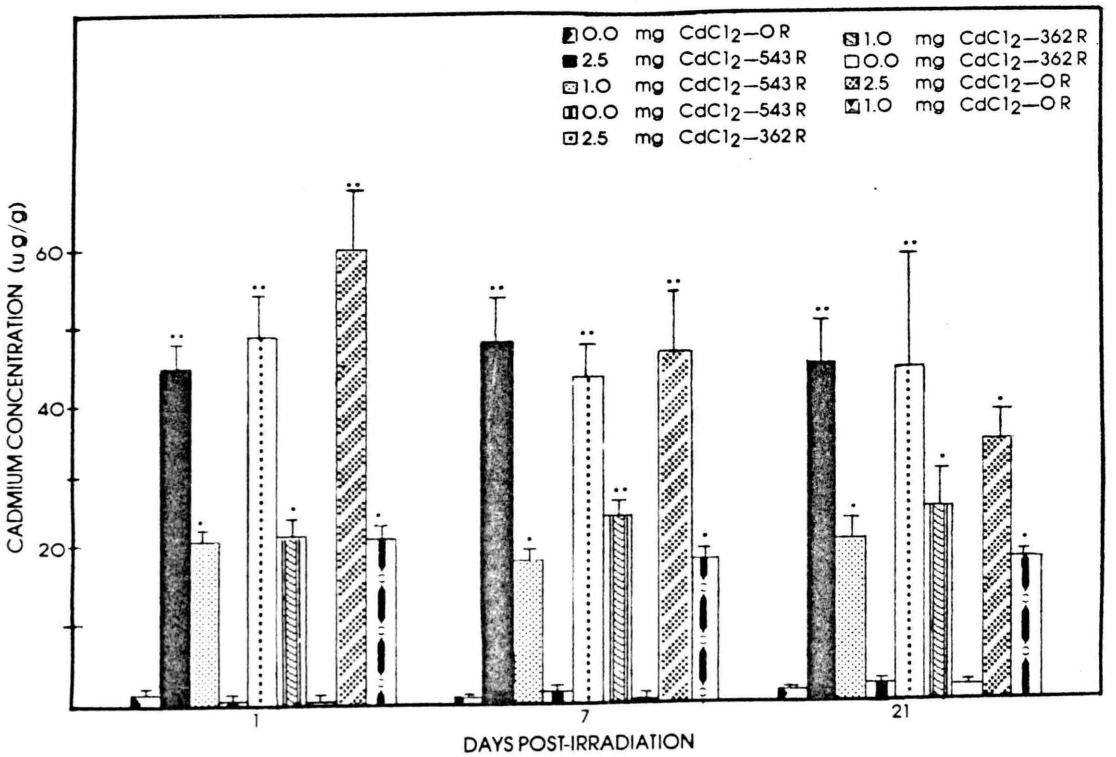


Figure 5. Mean ± S. E. of cadmium concentrations in livers of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

The levels of cadmium in these groups were retained through day 21 post-treatments, showing that although the liver is a detoxifying center it requires a long time to reduce and eliminate the metal.

The high concentration of cadmium found in the liver could be partially explained by the presence of metallothionein, a protein of low molecular weight (6,000-12,000) very rich in cysteine residues and deficient in aromatic amino acids (Kägi and Vallee, 1961). The synthesis of this protein in the liver is induced by cadmium (Winge and Rajagopalan, 1972; Colucci et al., 1975).

The exact mechanism by which cadmium stimulates thionein synthesis is not completely clear; however, Webb (1972) suggested a control mechanism on induction at the translational step which would occur only if the messenger ribonucleic acid (mRNA) for the protein was already present in the cell; therefore cadmium activates the messenger. After binding, the cadmium-thionein complex within the cell may become saturated and the excess cadmium binds to other proteins (Winge and Rajagopalan, 1972). Metallothinein is known to chelate cadmium as well as other heavy metals. Colucci et al. (1975) suggested that this mechanism is responsible for

the accumulation of cadmium in the liver. Studies conducted by Webb (1972) indicated that such induction of metallothionein by toxic cations persisted in the liver for a long period of time. Maximum cadmium concentrations were maintained in the liver of rats for a period of 13 weeks following an injection of 2.2 μmol $CdCl_2$/100 g body weight (Webb, 1972; Colucci et al., 1975). The high concentrations of liver cadmium found in the present study are in agreement with these studies (Webb, 1972; Winge and Rajagopalan, 1972). This investigation seems to suggest that metallothionein does not have a role in the transport of toxic metal, but rather acts as a sequestering agent.

The sublethal dose of radiation did not have any noticeable effect on cadmium accumulation in the liver at days 1, 7, and 21 following irradiation or sham-irradiation (Figure 5). This can be attributed to the fact that since the liver is a radioresistant organ (Arena, 1971) it did not suffer sufficient radiation damage to cause a shift in cadmium concentrations. No significant interactions were noted among cadmium, radiation, and the number of days post-irradiation; however, highly significant ($p < 0.01$) effects were seen in the cadmium levels (Table 13). This

further confirms that the deposition of cadmium in the liver is proportional to the amounts administered.

TABLE 13

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN LIVERS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	39140.10	213.71	0.000
Radiation dose(R)	2	35.52	0.19	0.824
Days(D)	2	243.49	1.33	0.267
CR	4	65.60	0.36	0.838
CD	4	236.30	1.29	0.275
RD	4	209.62	1.15	0.337
CRD	8	123.83	0.68	0.712
Error	189	183.15		

Kidney

The kidney was the organ with the next highest average of cadmium concentration. The means of cadmium in this organ were 28.09 and 12.22 µg/g of wet tissue for the 2.5 and 1.0 mg $CdCl_2$/kg of body weight groups respectively (Appendix B, Table 35). The patterns of accumulation and retention of cadmium in this excretory organ were similar to those of the liver (Potts et al., 1950; Webb, 1972;

Winge and Rajagopalan, 1972; Hoffmann et al., 1975; Uthe and Chou, 1979; Uthe et al., 1979). On day 1 following irradiation or sham-irradiation, highly significant ($p < 0.01$) accumulations of the metal were seen in five of the six groups in which cadmium was used singly or as a co-insult with radiation (Figure 6). The exception was the 1.0 mg $CdCl_2$-543 R group; however, it was significantly ($p < 0.05$) increased. Kidneys from rats given the highest concentration of cadmium (2.5 mg/kg of body weight) showed the highest deposition of this toxic metal regardless if radiation was an insult or not (Figure 6). Further, these kidneys retained the highest concentrations of cadmium through the 21-day observation period. Rats treated with the low dose of cadmium (1.0 mg/kg of body weight) also showed significantly higher concentrations of the metal regardless if the animals were exposed to radiation or not (Figure 6). On days 7 and 21, kidneys from those rats retained similar and significantly ($p < 0.01$) higher concentrations of this heavy metal.

Induction of metallothionein in the kidney by the presence of toxic levels of cadmium has been proposed (Webb, 1972; Chen and Ganther, 1975b; Colucci et al., 1975). They suggested that the presence of this low

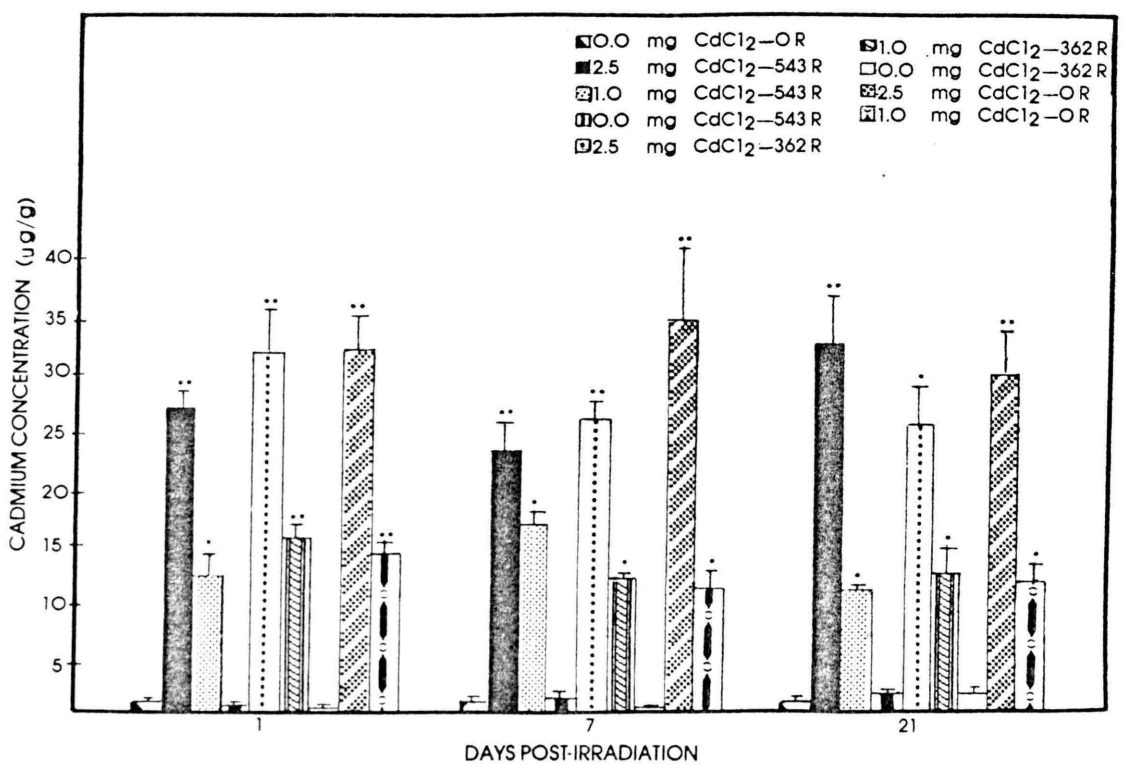


Figure 6. Mean ± S. E. of cadmium concentrations in kidneys of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

molecular weight protein seems to be the factor responsible for the accumulation of this metal in the kidney as it is in the liver. Although the glomerular filtration of this low molecular weight protein could be feasible, other researchers support the concept that the kidney cells are responsible for the de novo synthesis of this protein (Webb, 1972; Chen and Ganther, 1975b; Nordberg et al., 1975). Different electrophoretic mobilities have been identified in the protein components of the liver and the kidney fractions by column chromatography, so it seems unlikely that the metallothionein is being transported from the liver to the kidney as has been suggested by Piscator (1966). Renal synthesis of metallothionein (Shaikh and Lucis, 1970; Webb and Daniel, 1975) is also supported by the absence of this protein in the blood plasma of animals treated with cadmium (Faeder et al., 1977).

Most of the cadmium accumulated in the kidney is confined to the cells of the proximal tubules (Stowe et al., 1972; Friberg et al., 1974). Toxic effects to this portion of the nephrons are associated with chronic accumulation of this metal. The mechanisms involved in the renal impairment are not well understood, but it seems unlikely that cadmium-thionein is directly involved since the complex appears to be biologically inactive (Nordberg et al.,

1975). Tissue damage appears to be related partially to the free cadmium resulting from the complete saturation of metallothionein (Winge and Rajagopalan, 1972; Friberg et al., 1974).

No significant effects were observed for interaction among cadmium levels, radiation levels and post-irradiation days to sacrifice or in the other variables except cadmium levels (Table 14). Like in the liver, the accumulation of this toxic metal in the kidney is proportional to the dose injected.

TABLE 14

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN KIDNEYS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	13538.99	328.84	0.000
Radiation dose(R)	2	33.94	0.82	0.440
Days(D)	2	22.08	0.54	0.586
CR	4	71.71	1.74	0.142
CD	4	24.41	0.59	0.668
RD	4	41.50	1.01	0.405
CRD	8	70.47	1.71	0.098
Error	189	41.17		

Spleen

The average concentrations of cadmium in the spleen of the 2.5 and 1.0 mg $CdCl_2$ groups were 3.46 and 3.56 µg/g of wet tissue respectively (Appendix B, Table 35). These are 7.2 and 16.2 percents of the concentrations found in the 2.5 and 1.0 mg $CdCl_2$ groups of the liver respectively. These percentages agree with those reported by Potts et al. (1950). Spleens of dogs injected with this metal had 9 to 11 percent accumulation of that of the liver deposition.

Of the control tissues in this research, the spleen had the highest mean cadmium concentration (2.04 µg/g of wet tissue). This is fairly high when compared with an average of less than 1.0 µg/g of wet tissue found in the other control tissues. Perhaps this might be explained by the fact that the spleen is a part of the reticuloendothelial system. Those worn-out cells which contained cadmium are phagocytized in the spleen causing an elevation of cadmium in this organ. This may further be explained by the observation that when a high dose of $CdCl_2$ (2.5 mg) was administered, the concentration in the spleen did not significantly change from that of the one given 1.0 mg $CdCl_2$ indicating that the spleen was not taking up any more of the metal. The 1.0 mg $CdCl_2$/kg of body weight group would have been enough to saturate the spleen.

Following treatments with cadmium singly or in combination with radiation, an increase in cadmium content was observed in the spleen at day 1 post-irradiation (Figure 7). In the 2.5 mg $CdCl_2$-543 R group a highly significant ($p < 0.01$) deposition of cadmium was seen on day 1 post-irradiation. In the 2.5 mg $CdCl_2$-362 R group, the 1.0 mg $CdCl_2$-543 R group, the 1.0 mg $CdCl_2$-362 R group and the 1.0 mg $CdCl_2$-0 R group a significant ($p < 0.05$) increase was seen on day 1 following irradiation or sham-irradiation. Differences among treated groups at days 7 and 21 post-irradiation were not significant except in one group (2.5 mg $CdCl_2$-365 R). The accumulation of cadmium in the spleen can be partially explained by the presence of metallothionein found in the cytoplasm of the spleen (Amacher and Ewing, 1975b). This cadmium-binding substance has been identified as metallothionein, a low molecular weight protein. This protein may have bound with cadmium and thus elevated the concentration of this organ. However, by day 7 post-treatment no significant difference was seen when compared with the control groups. This can be accounted for by the rapid cell turnover in this blood forming organ, thus it circulated the cadmium to other parts of the body of the animals.

Both sublethal doses of 362 and 543 R of radiation were ineffective alone or in combination with cadmium to

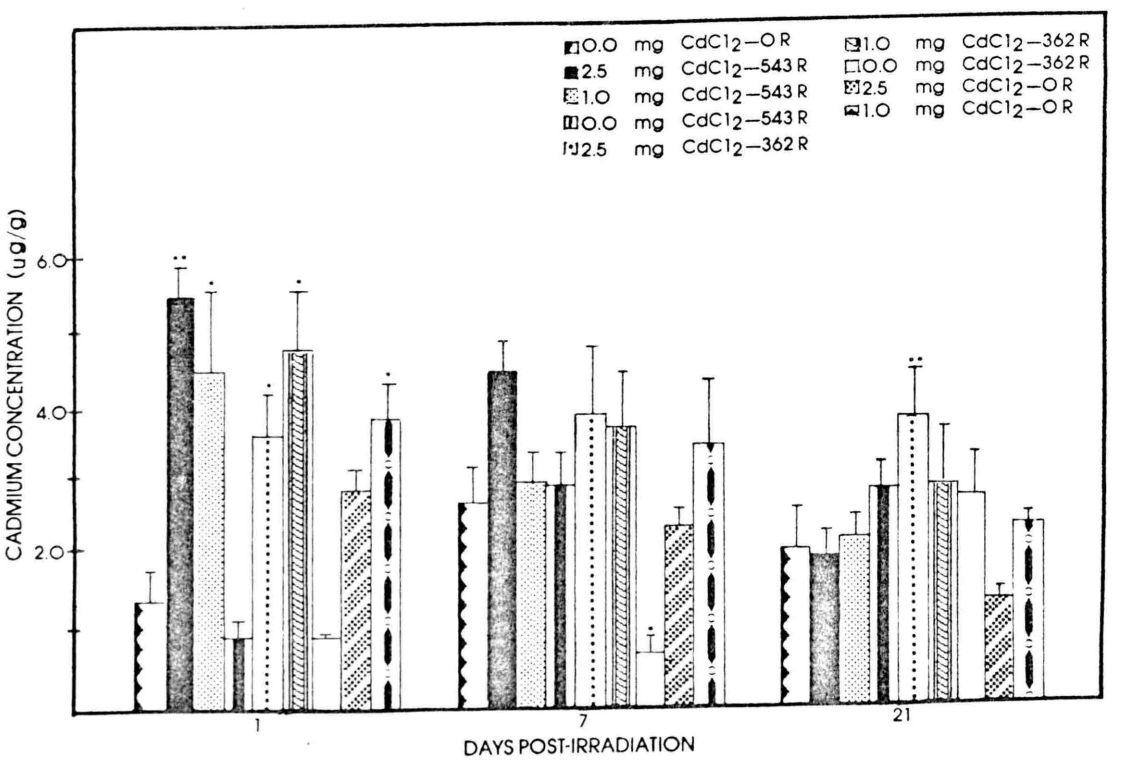


Figure 7. Mean ± S. E. of cadmium concentrations in spleens of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

counteract or intensify the tissue cadmium concentration levels. Highly significant ($p < 0.01$) interacting effects were seen in all variables except between radiation doses and the number of days to sacrifice post-irradiation as evidenced by the analysis of variance in Table 15.

TABLE 15

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN SPLEENS

	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	74.72	30.11	0.000
Radiation dose(R)	2	13.04	5.25	0.006
Days(D)	2	19.75	7.96	0.000
CR	4	22.58	9.10	0.000
CD	4	31.01	12.50	0.000
RD	4	4.33	1.75	0.142
CRD	8	10.48	4.22	0.000
Error	189	2.48		

Intestine

The average cadmium depositions in the intestines were 2.57 and 1.55 µg/g of wet tissue for the 2.5 and 1.0 mg $CdCl_2$ groups respectively (Appendix B, Table 35). These are only 7.0 and 5.4 percents of the amounts observed in the

respective groups in the liver. Regardless of the radiation dose given, the cadmium contentrations in the intestines were significantly higher in the 2.5 mg $CdCl_2$ groups than in the control group of the same day after irradiation (Figure 8). This pattern is consistent with that found in the liver and the kidney. While the cadmium concentrations in the intestines of the 2.5 mg $CdCl_2$ groups at day 21 post-irradiation had dropped to approximately half of their respective day 1 values, they were still highly significantly greater than those of the controls.

The presence of cadmium in the small intestine could have originated from circulatory distribution or biliary secretions (Caujolle et al., 1971; Cikrt and Tichy, 1974), especially when considering that the liver is the largest site for cadmium deposition. This accumulation and retention of cadmium in the intestine of the high dose groups can be attributed to the protein binding metallothionein. This mechanism has been documented in various species of animals (Starcher, 1969; Evans et al., 1970; Sugawara and Sugawara, 1975). The synthesis of this protein in the intestine is induced by heavy metals such as zinc and copper. On the other hand, metallothionein preferentially binds cadmium over copper, indicating an antagonistic effect in the intestine. Starcher (1969) found cadmium to inhibit

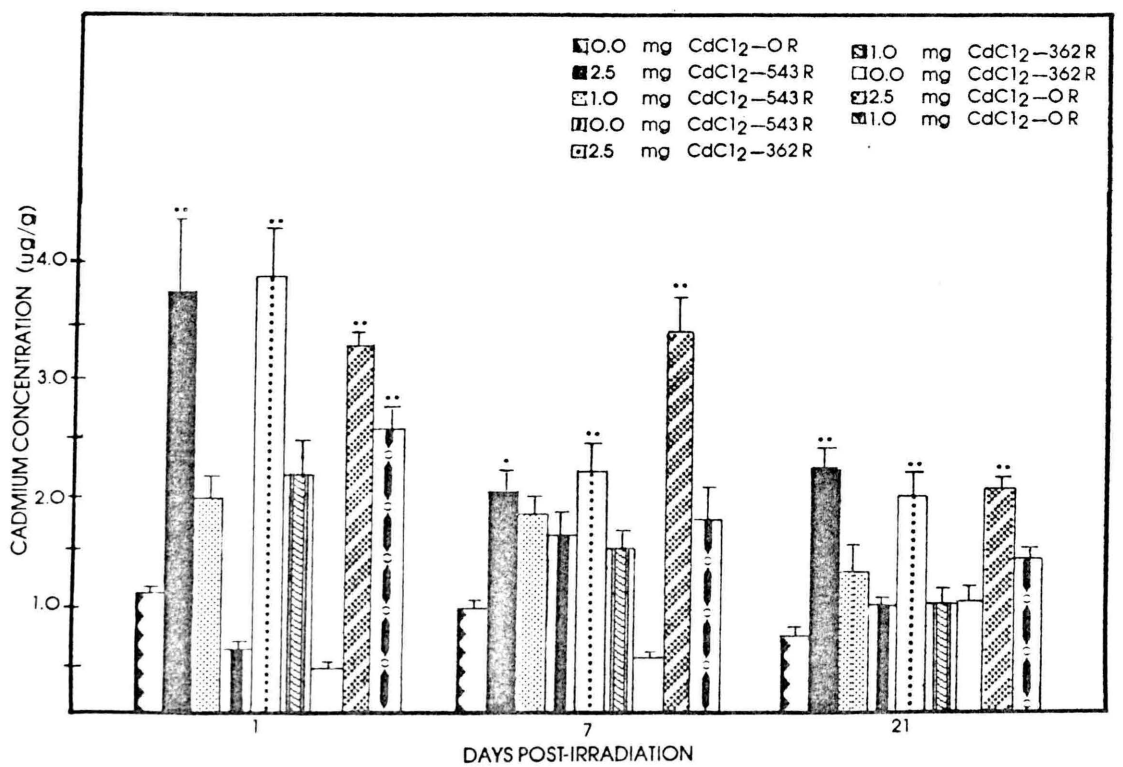


Figure 8. Mean ± S. E. of cadmium concentrations in intestines of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

copper absorption by absorbing to, and competing for, the same active sites essential for copper in chicks. The role of this protein in the absorptive process is still not clear.

In the group where a low dose of cadmium (1.0 mg) was used singly a highly significant ($p < 0.01$) increase in deposition was also noted on day 1 only. In the groups where radiation was an insult, no significant changes in cadmium deposition were seen post-irradiation days to sacrifice. Cadmium accumulations in the intestines of all three groups receiving a low dose of cadmium (1.0 mg) were not different from those of the controls at days 7 and 21 post-irradiation, indicating that cadmium was cleared by day 7. The mechanism by which cadmium is accumulated and retained in the intestine appears to be dose dependent, that is the higher the dose the greater the accumulation and retention capabilities.

Like the spleen, radiation singly or in combination with cadmium did not contribute in any way to cadmium concentration in this organ. No interactions between cadmium levels and radiation doses were observed (Table 16).

TABLE 16

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN INTESTINES

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	66.49	151.66	0.000
Radiation dose(R)	2	2.96	6.75	0.001
Days(D)	2	19.91	45.41	0.000
CR	4	0.86	1.97	0.100
CD	4	7.76	17.70	0.000
RD	4	0.57	1.31	0.000
CRD	8	3.28	7.48	0.000
Error	189	0.44		

Stomach

The mean cadmium accumulations in the stomach were 2.17 and 1.60 µg/g of wet tissue for the 2.5 and 1.0 mg $CdCl_2$ groups respectively (Appendix B, Table 35). These observed values are only 7.0 and 4.5 percents of the amounts seen in the respective groups of the liver. On day 1 post-irradiation highly significant ($p < 0.01$) increases in cadmium content were noted in five of the six treated groups that were treated with cadmium singly or as a co-insult with radiation (Figure 9). In the 1.0 mg

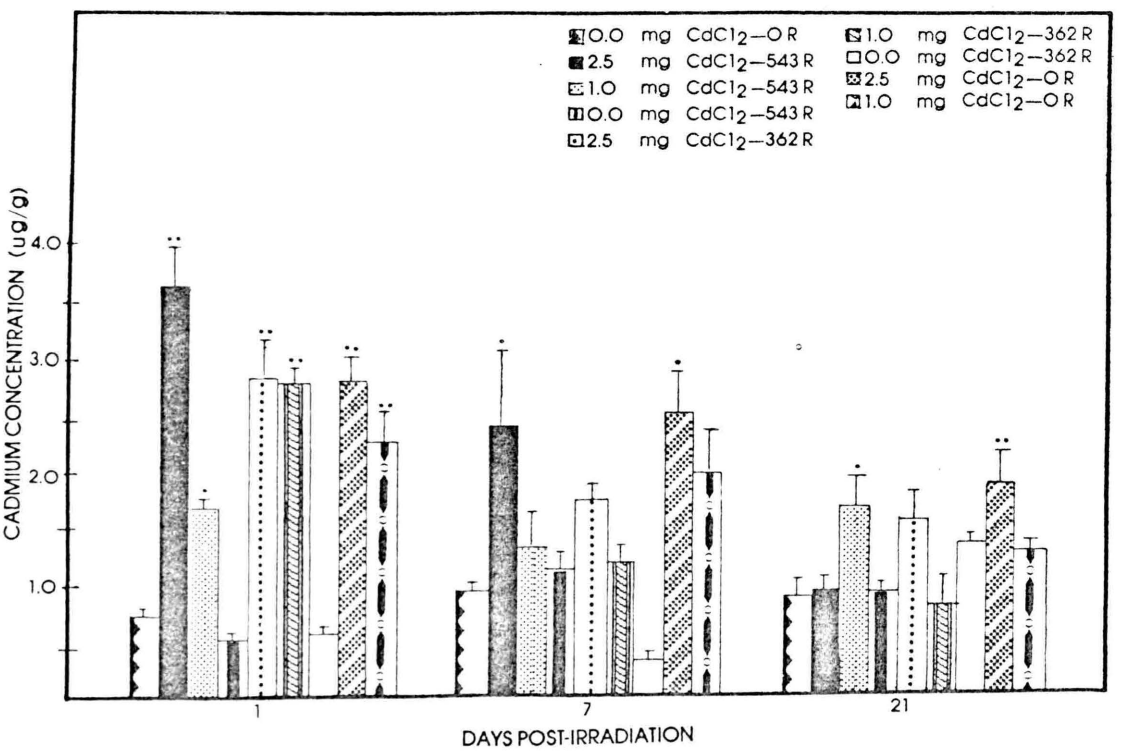


Figure 9. Mean ± S. E. of cadmium concentrations in stomachs of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

$CdCl_2$-543 R group the increase in cadmium level was statistically significant at the 0.05 level. However, at day 7 post-irradiation, only the 2.5 mg $CdCl_2$-543 R group and the 2.5 mg $CdCl_2$-0 R group differed significantly ($p < 0.05$) from the control. Clearance of cadmium from the other groups were seen, indicating no significant differences from control. This reduction in cadmium concentration is possibly due to distribution of the metal to other parts of the gastrointestinal tract, as indicated by the significant retention of cadmium by the intestine on days 7 and 21 post-irradiation described above. On day 21 following irradiation or sham-irradiation two groups namely, the 1.0 mg $CdCl_2$-543 R group and the 2.5 mg $CdCl_2$-0 R group had significantly more cadmium than controls. Although these groups were statistically significantly different from the control, they might not be biologically significant since clearances in the other groups were noted.

No references were found in the literature which deal with the concentrations of cadmium in the stomach. Since the concentrations of cadmium in the stomach were similar to those of the intestine, perhaps the same mechanism operates in the stomach as does in the intestine. Cadmium reached the stomach via blood, whereas in the intestine

cadmium had different ways to get there. Besides via blood circulation, cadmium can get access to the intestine via bile from the liver, where the concentrations of cadmium are very high as seen over the 21-day observation period, and also from the stomach itself in the digestive process (Cikrt and Tichy, 1974; Vostal and Cherian, 1974). Perhaps this further explains why cadmium was retained by the intestine and not by the stomach over the observation period.

No significant interactions were observed between the levels of cadmium and the doses of radiation (Table 17). Significant ($p < 0.05$) interacting effects were seen between radiation levels and the number of days killed post-irradiation, and highly significant ($p < 0.01$) interactions were seen among all other variables.

Heart

Perry and Erlanger (1971) found cadmium to incorporate steadily in the heart muscle of rats treated with intraperitoneal injections of 0.44-4.40 μmol of the metal. In the present study the average concentrations of 1.65 and 1.33 μg/g of heart wet weight were seen in the 2.5 and 1.0 mg $CdCl_2$/kg of body weight groups respectively (Appendix B, Table 35). Significant increases in cadmium

concentrations were observed in the co-insult groups of 2.5 mg $CdCl_2$-543 R, 1.0 mg $CdCl_2$-543 R and 2.5 mg $CdCl_2$-362 R post-irradiation day 1 (Figure 10). A highly significant ($p < 0.01$) increase was noted in the group which received 2.5 mg $CdCl_2$-0 R; and a significant ($p < 0.05$) increase was seen in the 1.0 mg $CdCl_2$-0 R group post-irradiation day 1. Only one cadmium treated group (1.0 mg $CdCl_2$-362 R) did not significantly differ in cadmium concentration from the control at day 1 following treatment. Cadmium levels in the heart were still significantly high in the 2.5 mg and 1.0 mg $CdCl_2$ with 0 R groups at day 7 following sham-irradiation. It appeared that radiation contributed to the clearance of cadmium from the other groups.

This pattern was not seen in any other tissues analyzed. Accumulations of cadmium in the heart have been observed by Amacher and Ewing (1975a) after an acute injection of 1.0 mg $CdCl_2$/kg of body weight using dogs. Although the concentrations found in the heart tissue were slightly greater than in the present study, a similar trend in cadmium clearance subsequent to exposure was observed. Only trace amounts of cadmium-bound protein have been detected from heart of rats treated with small acute concentrations (1.0 µmol/ml) of $CdCl_2$ (Chen and Ganther, 1975b).

TABLE 17

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN STOMACHS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	36.93	91.70	0.000
Radiation dose(R)	2	2.14	5.32	0.006
Days(D)	2	13.32	33.07	0.000
CR	4	0.66	1.63	0.169
CD	4	7.47	18.54	0.000
RD	4	0.99	2.56	0.047
CRD	8	2.26	5.61	0.000
Error	189	0.40		

Significant ($p < 0.05$) interaction effects were seen in days to sacrifice post-irradiation, all other variables showed highly significant ($p < 0.01$) interacting effects (Table 18).

Testis

The mean concentrations of cadmium found in the 2.5 and 1.0 mg $CdCl_2$ groups regardless if radiation was an insult or not were 1.34 and 1.39 µg/g of wet tissue respectively (Appendix B, Table 35). The 1.0 mg $CdCl_2$-0 R group showed a statistically significant increase in the

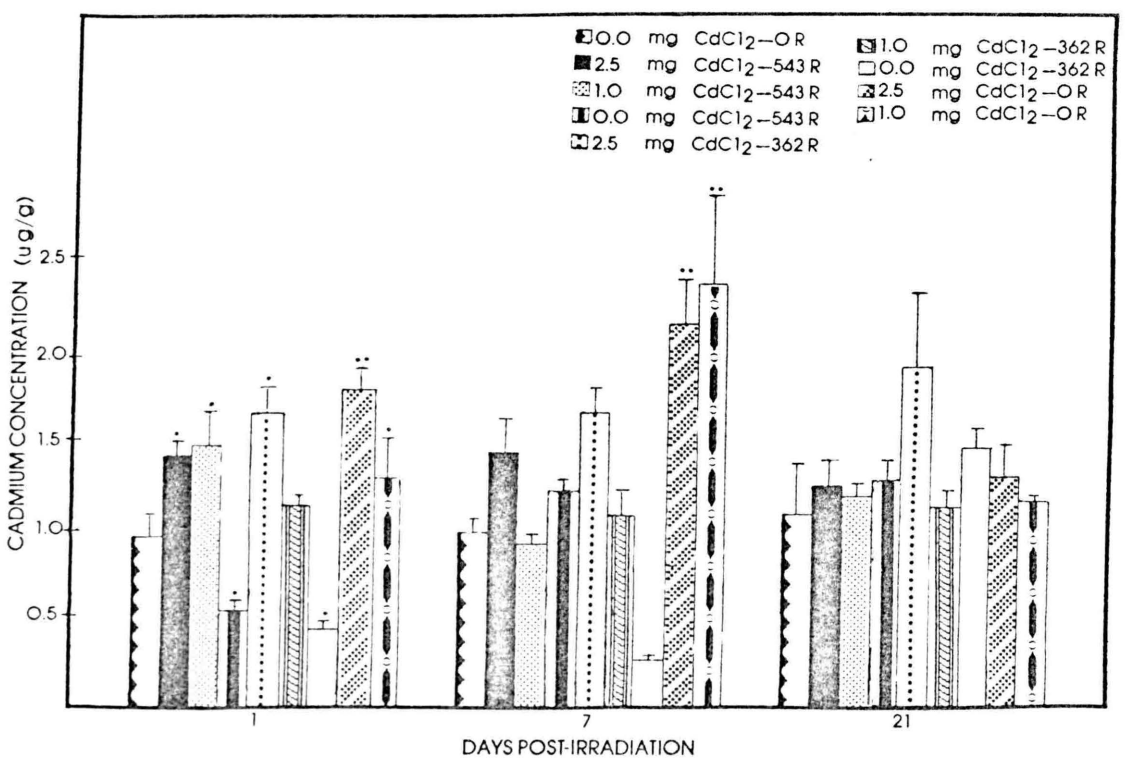


Figure 10. Mean ± S. E. of cadmium concentrations in hearts of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

amount of cadmium when compared to the control of the same day (day 1) following sham-irradiation (Figure 11).

TABLE 18

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN HEARTS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	16.61	57.87	0.000
Radiation dose(R)	2	4.35	15.14	0.000
Days(D)	2	1.27	4.41	0.013
CR	4	6.74	23.49	0.000
CD	4	5.78	20.14	0.000
RD	4	2.91	10.14	0.000
CRD	8	1.85	6.45	0.000
Error	189	0.29		

Testicular atrophy was observed in the high and low cadmium dose groups. The mean weights of the testes $\pm$ standard error are shown in Appendix C, Table 36. Regardless of radiation dose and the days sacrificed post-irradiation, the means of testicular weight for the 2.5 and 1.0 mg $CdCl_2$/kg of body weight were 1.20 and 2.29 g, respectively, while the controls were 3.03 g, indicating a

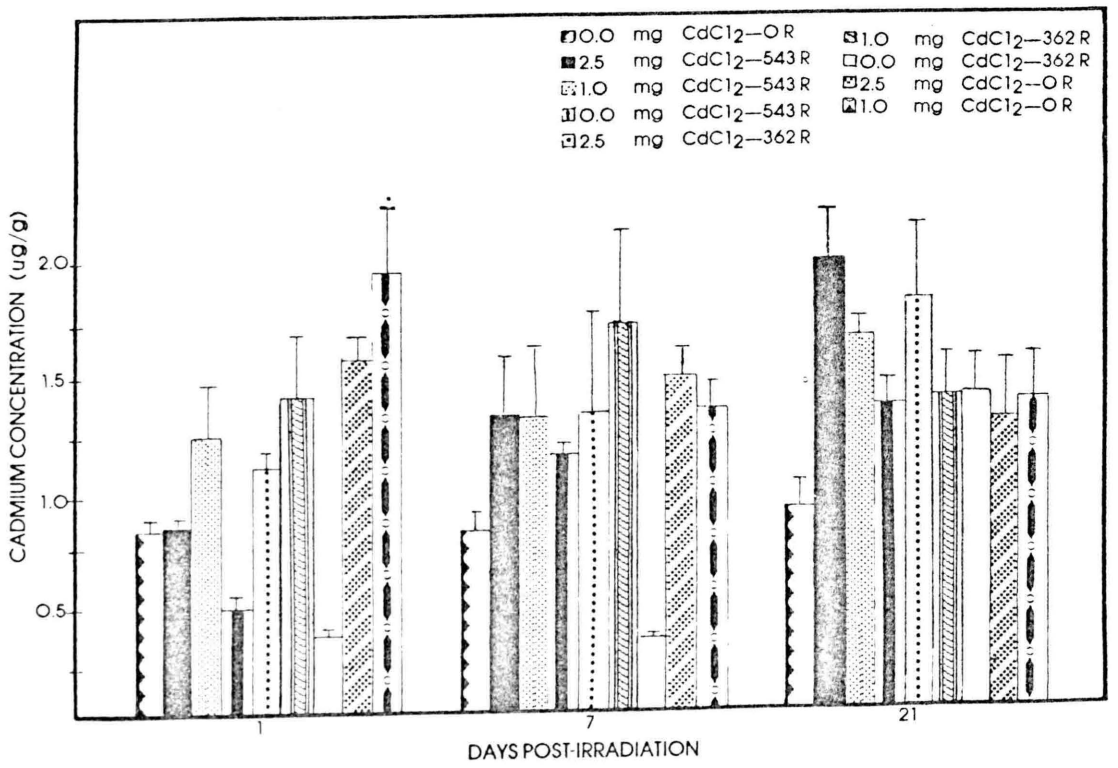


Figure 11. Mean ± S. E. of cadmium concentrations in testes of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

dose dependent relationship. The analysis of variance on testis weight showed significant weight changes (Appendix C, Table 37). All variables were highly significant ($p < 0.01$) except the three-way interactions of cadmium levels, radiation levels and the days to sacrifice post-irradiation. The lowest effective dose that has been reported to cause necrosis of the testis is 0.12 mg Cd/kg of body weight in calves (Pate et al., 1970). These atrophies have been attributed to the consequences of several interdependent factors triggered by injury to the testicular endothelium (Aoki and Hoffer, 1978). Obstruction of the microvascular bed of the testis following treatment with cadmium singly or in combination with radiation may have limited the testicular blood flow thus limiting the accumulation of cadmium in the testis as observed on days 1, 7 and 21 post-irradiation (Figure 11).

Other mechanisms could also have been involved. Chen and Ganther (1975a, 1975b) have identified a unique protein molecule of high molecular weight (30,000) in the testis which has not been detected in other tissues. This protein was found to have a slightly less affinity for cadmium than metallothionein but was associated with cadmium injury and sensitivity of this tissue. Webb (1972) found decreases in the contents of DNA, RNA and in the activities of

certain zinc-containing enzymes in the testis of rats and mice treated with subcutaneous injections of 2.2 μmol $CdCl_2$/100 g of body weight. These alterations were evident despite a very small deposition (0.5-2.0 μg/g wet tissue) of the metal in this tissue. Webb's findings support the fact that these alterations in the testis are independent of cadmium concentration.

Radiation did not have any noticeable effect on cadmium concentration in the testis. This could be attributed to the sublethal doses (543 and 362 R) used in this investigation.

Highly significant ($p < 0.01$) interacting effects were noted in all variables (Table 19). Main effects, first order interactions and second order interactions were observed as presented in the ANOVA table (Table 19).

Lung

The average depositions of cadmium in the lung of the rats, regardless of radiation or days sacrificed post-treatment were 1.39 and 1.11 μg/g of wet tissue in the high dose (2.5 mg $CdCl_2$) and low dose (1.0 mg $CdCl_2$) groups respectively (Appendix B, Table 35). These concentrations are similar or slightly lower than those found in the heart and testis.

TABLE 19

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN TESTES

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	23.27	50.65	0.000
Radiation dose(R)	2	5.00	10.88	0.000
Days(D)	2	3.87	8.43	0.000
CR	4	9.48	20.64	0.000
CD	4	7.07	15.38	0.000
RD	4	7.95	17.29	0.000
CRD	8	4.14	9.01	0.000
Error	189	0.46		

It was noted that the high cadmium dose (2.5 mg/kg of body weight) when used singly increased the cadmium level in the lung significantly ($p < 0.01$) when compared with the control group at post-irradiation day 1 (Figure 12). The lung retained most of the accumulated cadmium in this group through day 7, but was similar to control level by day 21 post-treatment. This suggests a dose dependent deposition and retention mechanism. An inducible cadmium binding protein has been isolated from the lung of rats (Chen and Ganther, 1975b) and hamsters (Benson and Henderson,

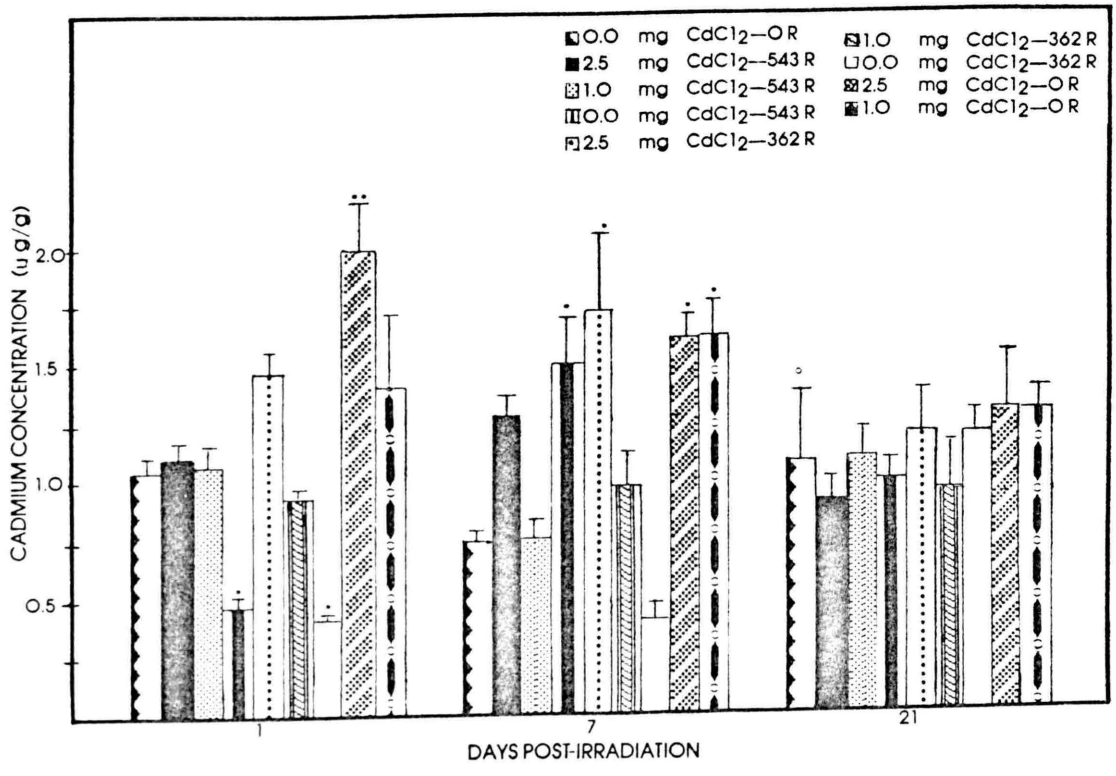


Figure 12. Mean ± S. E. of cadmium concentrations in lungs of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

1979). It is possible that the accumulation and retention of cadmium in the lung is due to the presence of this metallothionein-like protein. Cadmium deposition in the other groups at day 1 were not significantly higher than control. On the other hand, the 0 mg $CdCl_2$-543 R and 0 mg $CdCl_2$-362 R groups had significantly lower amounts of lung cadmium than the controls did. This indicates an effect of radiation on the innate cadmium content of the lung. This is further supported by the observation that when cadmium was given as a co-insult with radiation (2.5 mg $CdCl_2$-543 R, 1.0 mg $CdCl_2$-543 R, 2.5 mg $CdCl_2$-362 R and 1.0 mg $CdCl_2$-362 R), no significant accumulations were observed on day 1 post-irradiation. At day 7 increases in cadmium concentrations in three additional groups besides the 2.5 mg $CdCl_2$-0 R group were seen. The groups of 0 mg $CdCl_2$-543 R, 2.5 mg $CdCl_2$-362 R and 1.0 mg $CdCl_2$-0 R showed significant ($p < 0.05$) increases in the lung cadmium levels. The erratic and rather inconsistent pattern observed at day 7 in groups 0 mg $CdCl_2$-543 R and 2.5 mg $CdCl_2$-362 R may be due to the large deviations of cadmium contents observed (Appendix A, Table 31). The higher lung deposition of cadmium in the 1.0 mg $CdCl_2$-0 R group at day 7 suggested an accumulation of the metal at

a rate lower than that seen in the higher dose group (2.5 mg $CdCl_2$-0 R). Recovery to control values was noted at day 21 post-irradiation in these four groups.

No significant effects were due to days sacrificed post-irradiation (Table 20). However, highly significant ($p < 0.01$) effects were seen in all other sources of variability.

TABLE 20

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN LUNGS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	7.37	35.13	0.000
Radiation dose(R)	2	3.50	16.67	0.000
Days(D)	2	0.56	2.64	0.074
CR	4	2.76	13.14	0.000
CD	4	2.43	11.59	0.000
RD	4	1.73	8.26	0.000
CRD	8	1.16	5.53	0.000
Error	189	0.21		

Blood Cells

The means of cadmium concentrations in blood cells were 1.31 and 1.07 µg/g of wet tissue in the 2.5 and 1.0 mg $CdCl_2$ groups, respectively (Appendix B, Table 35), regardless of days sacrificed post-treatment. Rabbits receiving a daily subcutaneous injection of 0.65 mg cadmium sulphate/kg of body weight for 4 weeks and 10 weeks respectively showed a progressive accumulation of the metal in the red blood cells (Friberg, 1952). The range of cadmium in the group treated for 4 weeks was between 0.2-0.6 µg/ml of whole blood, while those animals treated for 10 weeks ranged between 0.7-1.3 µg/ml of whole blood.

The pattern of deposition and retention of cadmium in the blood cells of the various groups was not consistent. The high cadmium dose (2.5 mg) did not have a significant effect when compared with control, except when used as a co-insult with a high radiation dose (543 R), at all three intervals post-treatments and with a low radiation dose (362 R) on day 21 post-irradiation (Figure 13). Significantly ($p < 0.05$) higher concentrations of cadmium than control levels were observed in these groups. A low cadmium dose (1.0 mg) resulted in a significant ($p < 0.05$) increase in cadmium deposition when used singly at day 21,

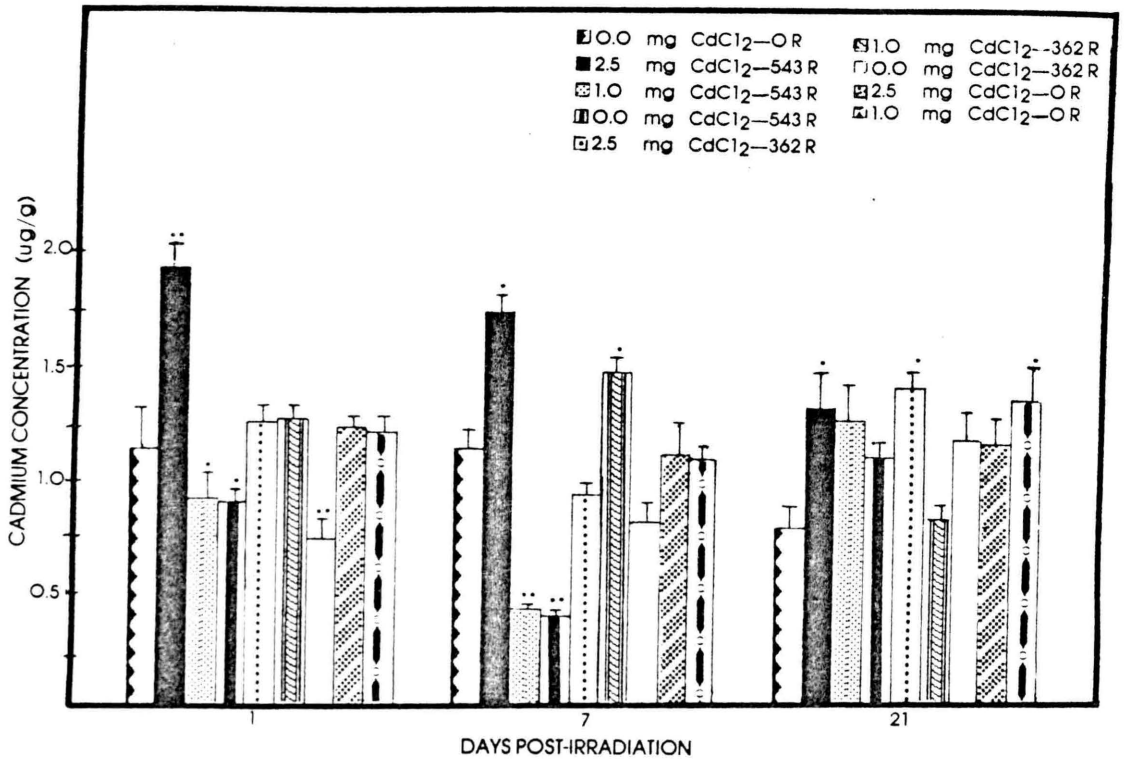


Figure 13. Mean ± S. E. of cadmium concentrations in blood cells of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

and when used in combination with a radiation dose of 362 R on day 7 post-irradiation. On the other hand, a low cadmium-high radiation dose showed a significantly ($p < 0.05$) lower concentration of cadmium at days 1 and 7 post-treatment as compared with control of the same days. Investigations performed in different species of animals using various techniques and different cadmium concentrations indicated a greater accumulation of cadmium in the erythrocyte portion of the blood than in the plasma. Plasma levels are indicative of recent cadmium exposure, while the red blood cells deposition gradually increases as the plasma concentration decreases. Cadmium accumulations in the red blood cells have been found to be 10-20 times higher than that of the plasma in experimental animals (Carlton and Friberg, 1957; Horner and Smith, 1975). The presence of cadmium in the red blood cells has been partially associated with the hemoglobin molecule (Carlton and Friberg, 1957) and partially to a low molecular weight protein probably metallothionein (Nordberg et al., 1971). Hemolysis of erythrocytes observed with chronic cadmium toxicity (Berlin and Friberg, 1960; Piscator, 1963; Nordberg et al., 1971) could result in a leakage of the protein-bound cadmium component, thus resulting in a shift of this

complex to other tissues. This could partially explain why the high dose of cadmium (2.5 mg/kg of body weight) when used singly did not seem to cause a significant increase in cadmium concentration. A similar trend was seen in the group of 1.0 mg $CdCl_2$-0 R on days 1 and 7 following sham-irradiation. A decrease production of erythrocytes resulting from chronic cadmium poisoning could also explain these findings (Swensson, 1957; Berlin and Friberg, 1960). The carrying capacity of the red blood cells could have been limited due to the decreased numbers of erythrocytes. Restoration of blood cells to the normal level could have occurred after day 7 post-treatment in the group receiving the lower dose of cadmium (1.0 mg) as evidenced by the increase in cadmium accumulation on day 21 post-treatment.

When radiation was used as a single insult, significantly lower cadmium levels were evident on days 1 and 7 with a 543 R dose and on day 1 with a 362 R dose post-irradiation. This shift in cadmium concentrations can probably be attributed to a decrease in blood cells. Cornatzer et al. (1953) found that dogs exposed to an acute dose of 500 R of X-irradiation had a pronounced decrease in the numbers of red blood cells.

Highly significant ($p < 0.01$) interacting effects were observed in the cadmium levels, and in the three intervals to sacrifice post-irradiation. No significant effects were seen due to radiation alone. While only significant ($p < 0.05$) interacting effects were noted between cadmium and days post-treatment, highly significant ($p < 0.01$) interactions were observed between cadmium and radiation, between radiation and days, and among the levels of cadmium, the levels of radiation and the numbers of days to sacrifice post-irradiation.

TABLE 21

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN BLOOD CELLS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	2.79	35.37	0.000
Radiation dose(R)	2	0.14	1.73	0.180
Days(D)	2	0.72	9.10	0.000
CR	4	1.49	18.89	0.000
CD	4	0.09	1.18	0.032
RD	4	0.40	5.06	0.001
CRD	8	1.02	12.88	0.000
Error	189	0.08		

Brain

In this study the means of cadmium deposition in the groups receiving 2.5 and 1.0 mg $CdCl_2$/kg of body weight were 0.91 and 1.09 µg/g of wet weight, respectively (Appendix B, Table 35). The findings are comparable to the mean value obtained by Stowe et al. (1972). In their study, they reported that rabbits given 160 ppm of cadmium in drinking water daily for 200 days accumulated 1.18 µg/g of wet brain tissue.

Following treatment with cadmium and/or radiation no significant accumulation of the metal was noted in the brain tissue except in one group (Figure 14). Highly significant ($p < 0.01$) increases in cadmium concentration were seen in the low cadmium and sham-irradiated group post-treatment day 1. However, recovery to control value was observed in this group by day 7 and thereafter. This cadmium accumulation observed can possibly be explained by the observation that the standard deviation in this group was larger than in any other group at day 1 post-irradiation. The mean value of the 1.0 mg $CdCl_2$-0 R group at day 1 post-treatment is comparable with the same treatment group at days 7 and 21 post-irradiation. The latter, however, were not significantly different from the control value or any other group

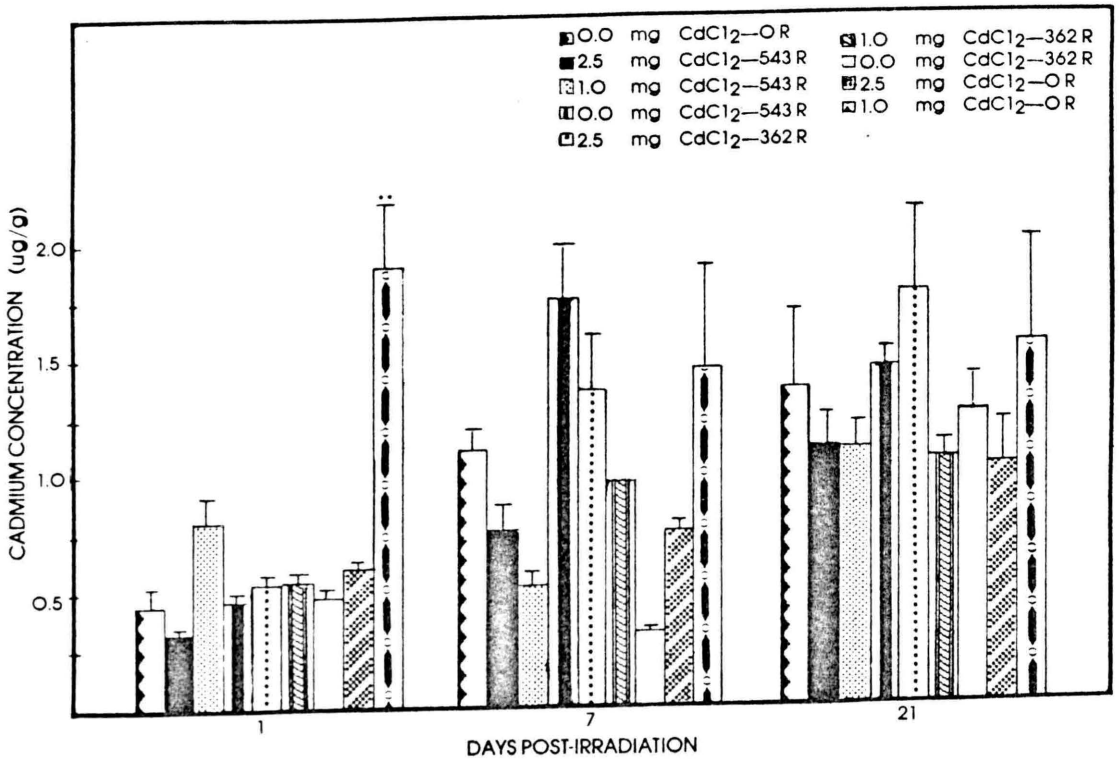


Figure 14. Mean ± S. E. of cadmium concentrations in brains of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

of the same day post-irradiation. Therefore, this indicates no biological significant increase in cadmium deposition in the brain.

In this investigation, the brain appears to have a blood-barrier against the accumulation and retention of cadmium. Wong and Klaassen (1980) observed that the content of cadmium in brain of new born rats was 5-6 times higher than in adults of the same species, indicating that a blood-brain barrier toward cadmium developed with age. This finding is in contrast to the report on mercury. Mercury has been found to accumulate and be retained in the brain 21 days after treatment with methylmercury chloride (Hoskins and Hupp, 1978). Mercury was found to cross the blood-brain barrier of monkeys and hamsters and deposited in the cerebral hemispheres and in the brain stem. Although both cadmium and mercury are divalent heavy metals, the mode of action of these metals seems to differ.

Radiation, when used singly or in combination with cadmium produced no effect which was significantly different from the control value. Highly statistically significant ($p < 0.01$) effects were noted in all variables except one (Table 22). The levels of cadmium were the only source of variability which did not show any significant effect.

TABLE 22

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN BRAINS

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	0.37	1.22	0.296
Radiation dose(R)	2	3.36	11.03	0.000
Days(D)	2	3.19	10.47	0.000
CR	4	10.03	32.89	0.000
CD	4	4.01	13.14	0.000
RD	4	4.31	14.14	0.000
CRD	8	2.48	8.14	0.000
Error	189	0.31		

Muscle

Although the muscle represents a large proportion of the body mass, it showed the least amount of the total body cadmium. The mean concentrations of cadmium in the high and low cadmium dose groups regardless of other treatments were found to be 0.59 and 0.61 µg/g of wet weight respectively (Appendix B, Table 35). These values are similar to the control value of 0.67 µg/g of wet weight observed in this study. The values observed in this investigation are in agreement with the findings of Matsubara-Khan and

Machida (1975). A relative concentration of 0.60 μg/g of muscle tissue was reported following intraperitoneal injections of 6.5 μCi of ^{109}Cd/kg of body weight of mice treated twice weekly for 130 days. Berlin and Ullberg (1963) treated mice with ^{109}Cd and noted by radioautography only traces of the element in the muscle.

One day after radiation exposure the groups of 2.5 mg $CdCl_2$-543 R, 0 mg $CdCl_2$-543 R, 2.5 mg $CdCl_2$-362 R and 0 mg $CdCl_2$-362 R animals exhibited significantly ($p < 0.05$) lower cadmium contents as compared to control of the same day post-irradiation (Figure 15). However, return to values not significantly different than control was seen in three of these groups (2.5 mg $CdCl_2$-543 R, 0 mg $CdCl_2$-543 R and 2.5 mg $CdCl_2$-362 R) at day 7 post-treatment with radiation. The low radiation group maintained a cadmium level below that of the control at day 7. A significantly ($p < 0.05$) low cadmium concentration was noted in the low cadmium-high radiation group on day 7 post-irradiation. Although this shift in cadmium content appears to indicate a role of γ-radiation in cadmium deposition in the muscle, the inconsistent pattern and variability of the mean values do not support these findings. No differences were noted in any group by day 21 post-irradiation (Figure 15).

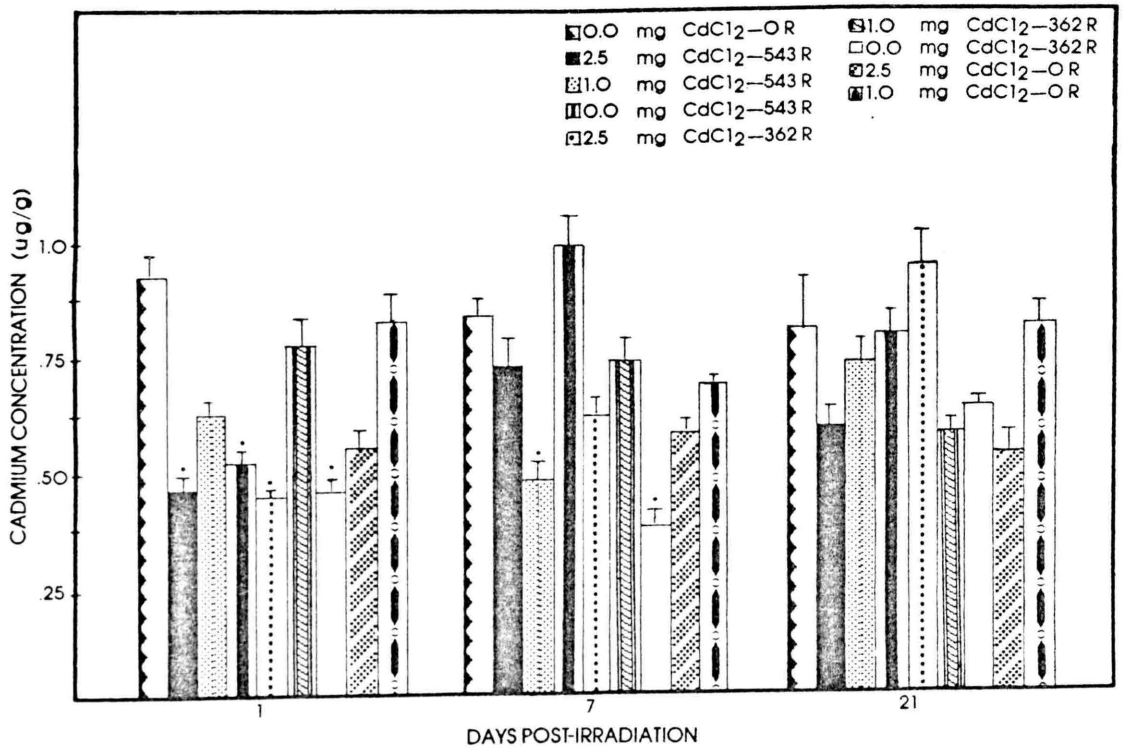


Figure 15. Mean ± S. E. of cadmium concentrations in muscles of rats injected with various concentrations of $CdCl_2$ and/or given different amounts of ^{60}Co gamma radiation. Observations were made on days 1, 7, and 21. Levels of significance were 0.05 (•) and 0.01 (••).

Highly significant ($p < 0.01$) interactions were observed in all variables except one. No significant interactions were due to days sacrificed post-irradiation (Table 23).

TABLE 23

ANALYSIS OF VARIANCE FOR MEAN CONCENTRATIONS OF CADMIUM IN MUSCLES

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	0.31	4.79	0.009
Radiation dose(R)	2	0.78	11.97	0.000
Days(D)	2	0.10	1.51	0.223
CR	4	1.93	29.58	0.000
CD	4	1.64	25.12	0.000
RD	4	1.41	21.67	0.000
CRD	8	0.56	8.63	0.000
Error	189	0.07		

Tissue Histology

The cadmium concentrations observed in the various tissues analyzed showed that the liver and kidney accumulated and retained the highest concentrations of the metal (Appendix B, Table 35). Based on these findings, survey observations were made to determine whether there was any

histopathological alteration in the liver and the kidney of the experimental animals subjected to cadmium and/or γ-radiation according to the scheme of the experimental design (Table 3). Different methodology, age, animal species, routes of administration and sex no doubt contribute to discrepancies in the tissue histology.

Light microscopy of the rat liver

Tissue sections of the liver were examined by light microscopy to identify morphological effects. Histological examination of the liver sections of the control group that was sham-injected and sham-irradiated revealed no abnormalities post-treatment days 1, 7 and 21 (Figure 16A). Tissues from the high cadmium and high radiation dose group days 1, 7 and 21 post-irradiation showed only minor lesions (Figure 16B). Certain nuclei were pyknotic and irregular in shape in some of the slides examined (Figure 16B). This is in agreement with the work of Hoffmann et al. (1975), in which pyknosis of the nuclei was apparent. They also observed that a single intravenous injection of 0.6 mg cadmium acetate/100 g of body weight produced single liver cell necrosis with inflammatory infiltrates in male rats.

The co-insult group, in this research, treated with 1.0 mg $CdCl_2$/kg of body weight and 362 R showed no evidence

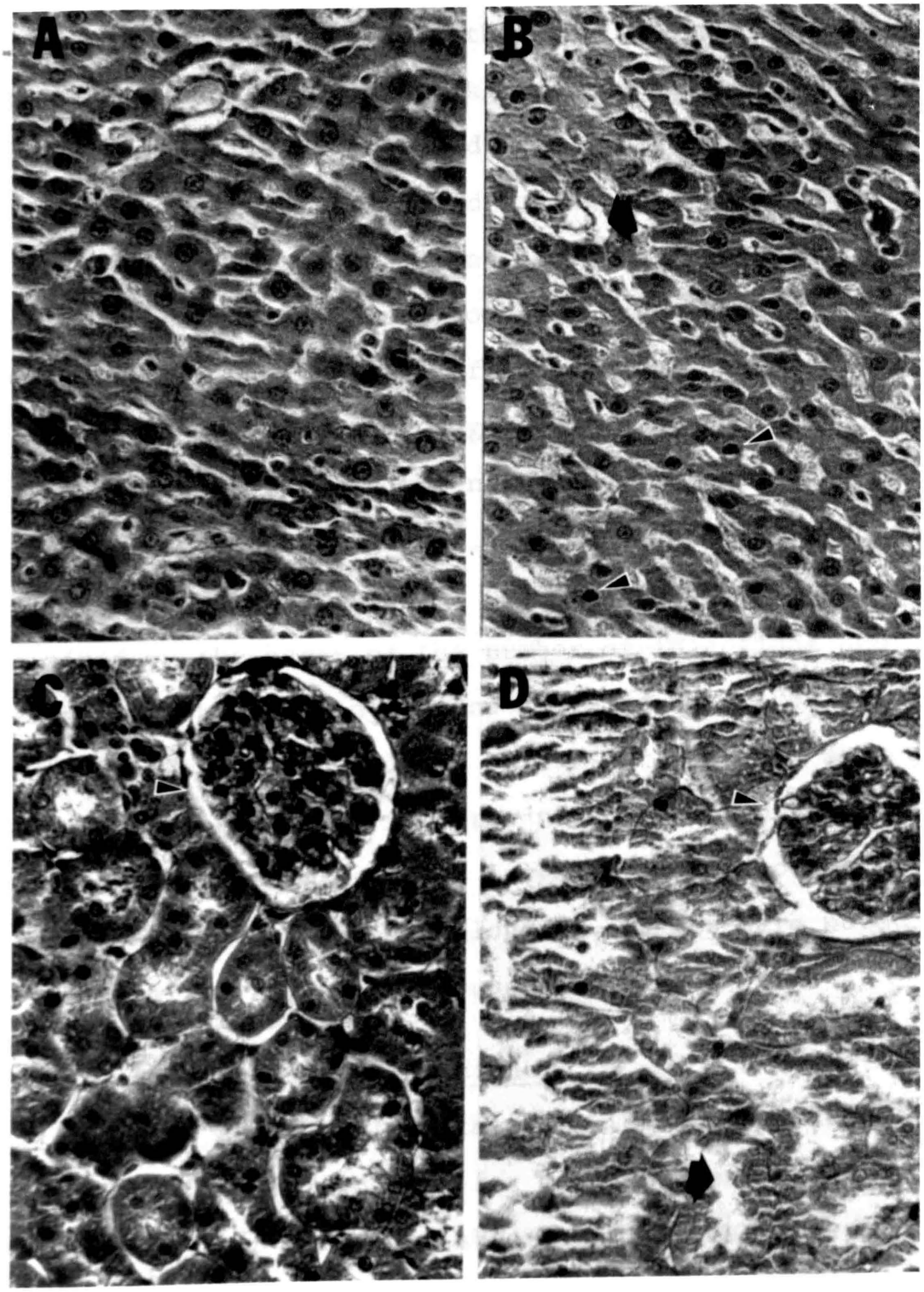
Figure 16. Light photomicrographs of liver (A-B) and kidney (C-D) sections from rats. All slides were stained with hematoxylin and eosin.

A. Liver section from a control rat appears normal with a homogeneous matrix at day 7 following sham-irradiation. X100

B. Day 7 post-irradiation liver section of cadmium and radiation treated rats revealed pyknotic nuclei (arrowhead) and slight lesions of some cells (arrow). X100

C. Kidney section from a control rat appears normal with a homogeneous matrix at day 21 post-sham-irradiation. Bowman's capsule is round (arrowhead). X450

D. Day 21 post-irradiation kidney section of cadmium and radiation treated rats showed cellular disorganization, larger lumens (arrow) and flattened and slightly smaller glomeruli in the Bowman's capsule (arrowhead). X450


A
B
C
D

of histological changes. Further, no abnormalities were observed in the 2.5 mg $CdCl_2$/kg of body weight group. Colucci et al. (1975) injected male Sprague-Dawley rats intraperitoneally with 0.5 and 1.0 mg $CdCl_2$/kg of body weight per day for six days and found no microscopic abnormalities in the liver; however, rats injected with 2.0, 2.5, or 3.0 mg $CdCl_2$/kg of body weight, on microscopic examination, revealed some degenerating hepatocytes. No differences were seen in the numbers of degenerating cells in each of these three groups. Perhaps the differences between this work and that of Colucci and others could be explained on the basis of the number of injections and the interval between injections. The technique in the present investigation allowed sufficient time for some of the metal to be removed from the tissue and thus the amount accumulated was not as large as that reported by Colucci and others. No histological changes were noted in the liver of any other groups examined.

Light microscopy of the rat kidney

Tissue sections from the rat kidneys were also examined by light microscopy for morphological damages. The control group showed no abnormalities post-treatment days 1, 7 and 21 (Figure 16C). Tissues from the high

co-insult group (2.5 mg $CdCl_2$-543 R) on post-irradiation days 1, 7 and 21 showed only slight lesions (Figure 16C). These histological examinations disclosed neither degeneration nor necrosis of the renal epithelial cells of the proximal convoluted and collecting tubules in the kidneys of rats. The glomeruli appeared slightly smaller than normal, and there was cellular disorganization and larger lumens were visible (Figure 16D). These findings are in agreement with those of Hoffmann et al. (1975), who showed that following a single intravenous injection of 0.6 mg cadmium acetate/100 mg of body weight in male rats, the kidney had consistently insignificant changes in the proximal tubules with occasional nuclear pyknosis in the isolated epithelial cells. In this study, no lesions were seen in the glomeruli and blood vessels of these animals at the light microscope level.

In cadmium-treated rabbits, Axelsson et al. (1968) reported that renal lesions were not seen following subcutaneous injections of 0.25 mg $CdCl_2$/kg of body weight five days weekly for 11 and 17 weeks, respectively. Other studies on renal damage in humans following exposure to cadmium showed that the renal changes were predominantly tubular (Piscator, 1966; Baum and Worthen, 1967). In contrast, Bonnell et al. (1960) reported that ischemic

degenerative glomerular changes found in most lesions of rats treated with cadmium were similar to those observed in the kidneys of one human who died from uremia after chronic cadmium exposure. This ischemic degeneration can partially be explained by an increase in filtration fraction and a decrease in inulin clearance (Itokawa et al., 1974; Kawamura et al., 1978).

In the present study, hyalinization was not apparent in the glomerular capillaries. This is in contrast with the work of Anwar et al.(1961), who reported hyalinized glomeruli and atrophic tubules in dogs following ingestion of $CdCl_2$ in drinking water fed to the animals for a 4-year period. No histological alterations were seen in any other groups examined in the present study.

CHAPTER V

SUMMARY AND CONCLUSIONS

The study of interactions between two insults always raises the question of how to best characterize the effects observed when two agents, namely cadmium and γ-radiation are administered. Secondly, it poses the problem of whether the effects seen following administration of the combination deviate significantly from what could have been predicted from the individual action of the two agents. Before attempting to answer these questions, three lethality studies, namely acute $CdCl_2$, chronic $CdCl_2$, and acute γ-radiation were performed to determine sublethal doses for the co-insult study of cadmium and γ-radiation. Based on the co-insult study, cadmium concentrations in various tissues were determined by atomic absorption spectrophotometry, and the effects of cadmium on tissue histology were determined.

Lethality Studies

Acute cadmium chloride lethality

The acute $CdCl_2$ probit $LD_{50}(_{30})$ value of 5.99 mg $CdCl_2$/kg of body weight for male rats was smaller than

the value of 7.13 mg $CdCl_2$/kg of body weight for females of the same species, indicating that the males were more susceptible to cadmium toxicity than females.

Chronic cadmium chloride lethality

The probit $LD_{50(30)}$ value for male rats at the conclusion of $CdCl_2$ injections was 4.25 mg $CdCl_2$/kg of body weight, while the probit $LD_{50(60)}$ value was 3.38 mg $CdCl_2$/kg of body weight. In contrast, the corresponding probit values for females were 5.14 and 4.65 mg $CdCl_2$/kg of body weight, respectively. When compared with those of the acute $CdCl_2$ lethality study, the $LD_{50(30)}$ values for the chronic $CdCl_2$ lethality were generally lower, indicating an increase in effectiveness of doses as time of injections was protracted.

Acute ^{60}Co gamma radiation lethality

Following an acute whole-body exposure to γ-radiation, the probit $LD_{50(30)}$ values for male and female rats were 733 R and 729 R, respectively. Higher doses of total-body irradiation decreased survival time.

Cadmium Concentrations in Tissues

1. On days 1, 7 and 21 following sham-irradiation, all groups treated with cadmium singly showed a high concentration and retention of the metal in the liver and the

kidney. Then following in decreasing order of cadmium accumulation were spleen, intestine, stomach, heart, testis, lung, blood cells, brain and muscle. While the concentration of cadmium in the liver and the kidney persisted over a 21-day observation period, the concentrations in the other tissues analyzed decreased considerably, some to control values.

2. When radiation was used as a single insult, in general, no cadmium accumulations were observed above the background levels post-irradiation days 1, 7 and 21.

3. At post-irradiation days 1, 7 and 21 groups treated with a co-insult of cadmium and radiation showed a high deposition and retention of cadmium in the liver and kidney. Then followed in order of decreasing cadmium accumulations were the spleen, intestine, stomach, heart, testis, lung, blood cells, brain and muscle. While most of the cadmium was retained by the liver and kidney by day 21 post-irradiation, the concentrations of the metal in the other tissues analyzed decreased to control values. Ionizing radiation did not act as an antagonistic or synergistic agent on cadmium concentration or retention in any tissue except, it appeared that radiation contributed to the clearance of cadmium from the heart at days 7 and 21 post-irradiation. However, the heart is not a critical organ

for cadmium deposition and retention as is the liver and kidney.

Tissue Histology

Histologic examination of the liver and kidney sections of the control groups showed no morphological abnormalities. Only the co-insult of cadmium and radiation treated groups revealed slight morphological tissue abnormalities following irradiation. A few lesions were apparent showing enlarged lumens and pyknosis of some nuclei in the liver. In the kidney, disruption of some cells was seen as well as slightly smaller glomeruli in the Bowman's capsule. Changes observed at all three intervals to sacrifice post-irradiation were similar.

General Conclusions

Contamination of the environment by cadmium is a serious hazard because the metal accumulates and is retained in the liver and kidney. This is evident from the data obtained in this study. Cadmium excretion from these critical organs is extremely slow and this in turn is reflected in a long biological half-life. Data from humans and other animals indicated that 200 μg Cd/g of tissue in the renal cortex is the critical level for renal dysfunction. In order to reach 200 μg of cadmium in the

renal cortex after 50 years of exposure with a retention rate of 5 percent, a daily intake of 150-200 μg of cadmium is necessary for humans. Although this is not likely in the general population, occupationally exposed workers could be at risk. The accumulation and retention of cadmium in the body should be studied further in connection with genetic and teratogenic effects.

The present investigation indicates that exposure of rats to sublethal concentrations of cadmium increases the concentration of this metal in several organs and organ systems. Generally, co-insult of cadmium and radiation at the sublethal doses used did not have an overall antagonistic or synergistic effect on cadmium concentrations in these tissues, except in the heart, whereby radiation seems to have contributed in the clearance of cadmium from this tissue. However, the heart is not the critical organ for cadmium accumulation.

The co-insult appeared to be slightly more effective than either agent alone in producing tissue damage. Further studies are necessary to elucidate the mechanisms of action of this heavy metal in the kidney and liver, and perhaps also to serve as a morphological basis for the better understanding of toxic effects of this metal resulting

from single or multiple interaction with radiation or other substances.

APPENDICES

APPENDIX A

TABLES OF MEANS AND STANDARD ERRORS FOR CADMIUM CONCENTRATIONS IN RAT TISSUES OBTAINED FROM THIS STUDY

TABLE 24

CADMIUM CONCENTRATIONS (μg/g) IN LIVERS ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	1.09 ± 0.07[a]**	0.73 ± 0.06[a]	0.74 ± 0.12[a]
2	2.5	543	45.76 ± 3.50[c]	48.88 ± 7.57[c]	45.80 ± 6.42[c]
3	1.0	543	22.64 ± 1.44[b]	19.18 ± 1.52[b]	21.68 ± 2.85[ab]
4	0.0	543	0.42 ± 0.03[a]	1.10 ± 0.13[a]	1.22 ± 0.34[a]
5	2.5	362	50.76 ± 5.55[cd]	45.13 ± 3.56[c]	45.37 ± 14.29[c]
6	1.0	362	23.27 ± 1.78[b]	24.91 ± 1.74[b]	25.98 ± 5.44[bc]
7	0.0	362	0.38 ± 0.10[a]	0.21 ± 0.03[a]	1.09 ± 0.17[a]
8	2.5	0	62.27 ± 10.89[d]	47.41 ± 8.24[c]	35.65 ± 4.15[bc]
9	1.0	0	22.77 ± 1.87[b]	18.06 ± 1.30[b]	18.95 ± 1.32[ab]

*Each figure represents the mean of 8 replicates.

** Those averages with the same superscript in the same column do no differ significantly at the 0.05 level.

TABLE 25

CADMIUM CONCENTRATIONS (μg/g) IN KIDNEYS ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	0.91 ± 0.01^{a}**	0.70 ± 0.09^{a}	0.78 ± 0.11^{a}
2	2.5	543	25.97 ± 1.49^{c}	22.21 ± 2.70^{cd}	31.30 ± 3.74^{c}
3	1.0	543	11.62 ± 1.77^{b}	15.90 ± 0.95^{bc}	10.09 ± 0.82^{b}
4	0.0	543	0.35 ± 0.03^{a}	1.29 ± 0.20^{a}	1.20 ± 0.34^{a}
5	2.5	362	30.70 ± 3.66^{c}	25.00 ± 1.54^{d}	24.18 ± 3.58^{c}
6	1.0	362	14.81 ± 1.16^{b}	11.30 ± 0.81^{b}	11.68 ± 2.21^{b}
7	0.0	362	0.29 ± 0.03^{a}	0.23 ± 0.03^{a}	1.29 ± 0.23^{a}
8	2.5	0	31.17 ± 2.76^{c}	33.46 ± 6.78^{c}	28.79 ± 3.96^{c}
9	1.0	0	13.20 ± 1.53^{b}	10.50 ± 1.51^{b}	10.89 ± 1.15^{b}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 26

CADMIUM CONCENTRATIONS (μg/g) IN SPLEENS ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	1.59 ± 0.43^{a}**	2.83 ± 0.49^{b}	2.13 ± 0.54^{ab}
2	2.5	543	5.64 ± 0.44^{c}	4.63 ± 0.39^{b}	2.05 ± 0.32^{ab}
3	1.0	543	4.63 ± 1.17^{bc}	3.15 ± 0.42^{b}	2.34 ± 0.29^{ab}
4	0.0	543	1.05 ± 0.17^{a}	3.12 ± 0.46^{b}	2.99 ± 0.35^{ab}
5	2.5	362	3.78 ± 0.64^{bc}	4.04 ± 0.94^{b}	3.96 ± 0.71^{b}
6	1.0	362	4.89 ± 0.79^{bc}	3.85 ± 0.73^{b}	3.12 ± 0.78^{ab}
7	0.0	362	1.01 ± 0.05^{a}	0.76 ± 0.22^{a}	2.87 ± 0.60^{ab}
8	2.5	0	3.04 ± 0.33^{ab}	2.48 ± 0.23^{b}	1.53 ± 0.20^{a}
9	1.0	0	3.99 ± 0.43^{bc}	3.59 ± 0.91^{b}	2.55 ± 0.14^{ab}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 27

CADMIUM CONCENTRATIONS (μg/g) IN INTESTINES ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	1.01 ± 0.04^{ab}**	0.84 ± 0.06^{ab}	0.57 ± 0.09^{a}
2	2.5	543	3.59 ± 0.63^{c}	1.84 ± 0.20^{c}	2.02 ± 0.18^{b}
3	1.0	543	1.81 ± 0.19^{b}	1.64 ± 0.14^{bc}	1.13 ± 0.21^{a}
4	0.0	543	0.52 ± 0.07^{a}	1.45 ± 0.21^{bc}	0.84 ± 0.06^{a}
5	2.5	362	3.70 ± 0.43^{c}	2.01 ± 0.26^{c}	1.77 ± 0.21^{b}
6	1.0	362	2.00 ± 0.30^{b}	1.33 ± 0.19^{bc}	0.85 ± 0.13^{a}
7	0.0	362	0.32 ± 0.02^{a}	0.40 ± 0.02^{a}	0.87 ± 0.11^{a}
8	2.5	0	3.11 ± 0.13^{c}	3.22 ± 0.42^{d}	1.84 ± 0.18^{b}
9	1.0	0	2.38 ± 0.21^{b}	1.58 ± 0.29^{bc}	1.23 ± 0.18^{a}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 28

CADMIUM CONCENTRATIONS (μg/g) IN STOMACHS ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	0.71 ± 0.07^{a}**	0.89 ± 0.07^{ab}	0.82 ± 0.17^{a}
2	2.5	543	3.53 ± 0.36^{d}	2.33 ± 0.58^{c}	0.87 ± 0.12^{ab}
3	1.0	543	1.62 ± 0.12^{b}	1.36 ± 0.21^{abc}	1.59 ± 0.26^{bc}
4	0.0	543	0.49 ± 0.04^{a}	1.07 ± 0.12^{ab}	0.87 ± 0.06^{ab}
5	2.5	362	2.75 ± 0.32^{c}	1.67 ± 0.16^{bc}	1.47 ± 0.26^{abc}
6	1.0	362	2.69 ± 0.12^{c}	1.15 ± 0.11^{ab}	0.74 ± 0.12^{a}
7	0.0	362	0.54 ± 0.06^{a}	0.28 ± 0.01^{a}	1.28 ± 0.08^{abc}
8	2.5	0	2.73 ± 0.19^{c}	2.43 ± 0.35^{c}	1.81 ± 0.27^{c}
9	1.0	0	2.17 ± 0.31^{c}	1.89 ± 0.34^{bc}	1.20 ± 0.11^{abc}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 29

CADMIUM CONCENTRATIONS (μg/g) IN HEARTS ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	0.97 ± 0.09^{b}**	0.99 ± 0.08^{ab}	1.10 ± 0.30^{a}
2	2.5	543	1.44 ± 0.07^{cd}	1.46 ± 0.19^{b}	1.27 ± 0.14^{a}
3	1.0	543	1.50 ± 0.21^{cd}	0.93 ± 0.06^{ab}	1.21 ± 0.09^{a}
4	0.0	543	0.54 ± 0.04^{a}	1.24 ± 0.08^{b}	1.30 ± 0.10^{a}
5	2.5	362	1.69 ± 0.13^{d}	1.69 ± 0.16^{b}	1.95 ± 0.42^{b}
6	1.0	362	1.15 ± 0.05^{bc}	1.10 ± 0.15^{b}	1.14 ± 0.10^{a}
7	0.0	362	0.44 ± 0.04^{a}	0.27 ± 0.03^{a}	1.48 ± 0.14^{a}
8	2.5	0	1.82 ± 0.13^{d}	2.20 ± 0.27^{c}	1.32 ± 0.21^{a}
9	1.0	0	1.32 ± 0.20^{cd}	2.43 ± 0.53^{c}	1.17 ± 0.03^{a}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 30

CADMIUM CONCENTRATIONS (μg/g) IN TESTES ± STANDARD ERROR (S. E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	Treatments ^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	0.77 ± 0.05^{abc}**	0.75 ± 0.08^{ab}	0.82 ± 0.13^{a}
2	2.5	543	0.78 ± 0.04^{abc}	1.24 ± 0.25^{ab}	1.99 ± 0.19^{ab}
3	1.0	543	1.14 ± 0.22^{bc}	1.23 ± 0.29^{ab}	1.44 ± 0.08^{a}
4	0.0	543	0.43 ± 0.05^{ab}	1.06 ± 0.05^{ab}	1.27 ± 0.11^{a}
5	2.5	362	1.03 ± 0.07^{abc}	1.24 ± 0.46^{ab}	1.72 ± 0.33^{ab}
6	1.0	362	1.34 ± 0.27^{c}	1.62 ± 0.43^{b}	1.31 ± 0.17^{a}
7	0.0	362	0.31 ± 0.03^{a}	0.27 ± 0.02^{a}	1.32 ± 0.16^{a}
8	2.5	0	1.49 ± 0.11^{c}	1.40 ± 0.11^{b}	1.21 ± 0.24^{a}
9	1.0	0	1.86 ± 0.30^{d}	1.25 ± 0.13^{ab}	1.30 ± 0.18^{a}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 31

CADMIUM CONCENTRATIONS (μg/g) IN LUNGS ± STANDARD ERROR (S. E.)*

Group No.	Treatments		Day Sacrificed Following Irradiation		
	$CdCl_2$ (mg/kg)	^{60}Co R	1	7	21
1	0.0	0	1.03 ± 0.07^{b}**	0.73 ± 0.06^{ab}	1.07 ± 0.29^{a}
2	2.5	543	1.10 ± 0.07^{b}	1.27 ± 0.12^{bc}	0.91 ± 0.10^{a}
3	1.0	543	1.06 ± 0.11^{b}	0.74 ± 0.08^{ab}	1.09 ± 0.12^{a}
4	0.0	543	0.47 ± 0.05^{a}	1.48 ± 0.26^{c}	0.99 ± 0.09^{a}
5	2.5	362	1.45 ± 0.10^{b}	1.72 ± 0.32^{c}	1.19 ± 0.21^{a}
6	1.0	362	0.92 ± 0.04^{b}	0.97 ± 0.16^{abc}	0.94 ± 0.22^{a}
7	0.0	362	0.41 ± 0.03^{a}	0.38 ± 0.08^{a}	1.19 ± 0.10^{a}
8	2.5	0	1.99 ± 0.21^{c}	1.59 ± 0.12^{c}	1.28 ± 0.21^{b}
9	1.0	0	1.38 ± 0.29^{b}	1.61 ± 0.16^{c}	1.27 ± 0.10^{a}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the column do not differ significantly at the 0.05 level.

TABLE 32

CADMIUM CONCENTRATIONS (μg/g) IN BLOOD CELLS ± STANDARD ERROR (S. E.)*

Group No.	Treatments		Day Sacrificed Following Irradiation		
	$CdCl_2$ (mg/kg)	^{60}Co R	1	7	21
1	0.0	0	1.10 ± 0.17^{b}**	1.11 ± 0.09^{b}	0.77 ± 0.10^{a}
2	2.5	543	1.87 ± 0.11^{d}	1.66 ± 0.10^{c}	1.29 ± 0.14^{bc}
3	1.0	543	0.88 ± 0.13^{ab}	0.41 ± 0.01^{a}	1.23 ± 0.16^{abc}
4	0.0	543	0.87 ± 0.05^{ab}	0.37 ± 0.02^{a}	1.08 ± 0.07^{abc}
5	2.5	362	1.22 ± 0.07^{bc}	0.91 ± 0.06^{b}	1.37 ± 0.08^{c}
6	1.0	362	1.23 ± 0.06^{bc}	1.44 ± 0.04^{c}	0.82 ± 0.05^{ab}
7	0.0	362	0.72 ± 0.09^{a}	0.80 ± 0.09^{b}	1.16 ± 0.13^{abc}
8	2.5	0	1.20 ± 0.04^{bc}	1.12 ± 0.17^{b}	1.15 ± 0.11^{abc}
9	1.0	0	1.19 ± 0.06^{bc}	1.06 ± 0.07^{b}	1.33 ± 0.15^{c}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 33

CADMIUM CONCENTRATIONS (μg/g) IN BRAINS ± STANDARD ERROR (S.E.)*

Group No.	Treatments $CdCl_2$ (mg/kg)	^{60}Co R	Day Sacrificed Following Irradiation 1	7	21
1	0.0	0	0.43 ± 0.08^{a}**	1.10 ± 0.09^{abc}	1.36 ± 0.35^{a}
2	2.5	543	0.32 ± 0.02^{a}	0.75 ± 0.11^{ab}	1.10 ± 0.14^{a}
3	1.0	543	0.80 ± 0.11^{a}	0.51 ± 0.06^{a}	1.09 ± 0.12^{a}
4	0.0	543	0.46 ± 0.03^{a}	1.75 ± 0.23^{c}	1.43 ± 0.08^{a}
5	2.5	362	0.53 ± 0.05^{a}	1.35 ± 0.23^{bc}	1.77 ± 0.36^{b}
6	1.0	362	0.54 ± 0.03^{a}	0.96 ± 0.11^{ab}	1.04 ± 0.07^{a}
7	0.0	362	0.47 ± 0.04^{a}	0.31 ± 0.02^{a}	1.23 ± 0.14^{a}
8	2.5	0	0.59 ± 0.02^{a}	0.74 ± 0.04^{ab}	1.02 ± 0.19^{a}
9	1.0	0	1.88 ± 0.27^{b}	1.45 ± 0.41^{bc}	1.55 ± 0.43^{a}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

TABLE 34

CADMIUM CONCENTRATIONS (μg/g) IN MUSCLES ± STANDARD ERROR (S.E.)*

Group No.	Treatments		Day Sacrificed Following Irradiation		
	$CdCl_2$ (mg/kg)	^{60}Co R	1	7	21
1	0.0	0	$0.89 \pm 0.05^{b**}$	0.81 ± 0.05^{cd}	0.77 ± 0.20^{a}
2	2.5	543	0.43 ± 0.04^{a}	0.69 ± 0.09^{bcd}	0.62 ± 0.07^{a}
3	1.0	543	0.60 ± 0.05^{ab}	0.46 ± 0.06^{ab}	0.69 ± 0.10^{a}
4	0.0	543	0.49 ± 0.06^{a}	0.96 ± 0.11^{d}	0.76 ± 0.08^{a}
5	2.5	362	0.42 ± 0.03^{a}	0.63 ± 0.07^{bcd}	0.91 ± 0.14^{b}
6	1.0	362	0.74 ± 0.11^{ab}	0.71 ± 0.10^{bcd}	0.54 ± 0.06^{a}
7	0.0	362	0.43 ± 0.05^{a}	0.35 ± 0.05^{a}	0.59 ± 0.04^{a}
8	2.5	0	0.55 ± 0.08^{ab}	0.55 ± 0.04^{abc}	0.51 ± 0.08^{a}
9	1.0	0	0.79 ± 0.12^{ab}	0.66 ± 0.02^{bc}	0.78 ± 0.08^{a}

*Each figure represents the mean of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the 0.05 level.

APPENDIX B

TABLE OF MEANS FOR CADMIUM CONCENTRATIONS IN RAT TISSUES BASED ON CADMIUM DOSE AND REGARDLESS OF OTHER TREATMENTS

TABLE 35

MEAN CONCENTRATIONS OF CADMIUM (μg/g) IN WET TISSUES OF RATS BASED ON CADMIUM DOSE AND REGARDLESS OF OTHER TREATMENTS

Tissue	Dose (mg/kg of body weight)		
	0	1.0	2.5
Liver	0.78	21.94	47.44
Kidney	0.78	12.22	28.09
Spleen	2.04	3.56	3.46
Intestine	0.76	1.55	2.57
Stomach	0.77	1.60	2.17
Heart	0.93	1.33	1.65
Testis	0.78	1.39	1.34
Lung	0.86	1.11	1.39
Blood cells	0.89	1.07	1.31
Brain	0.95	1.09	0.91
Muscle	0.67	0.66	0.59

APPENDIX C

TABLES OF MEAN WEIGHTS AND ANALYSIS OF VARIANCE FOR MEAN WEIGHTS OF RAT TESTES

TABLE 36

MEAN WEIGHTS OF RAT TESTES ± STANDARD ERROR (S. E.)*

Group No.	Treatments		Days Sacrificed Following Irradiation		
	$CdCl_2$ (mg/kg)	^{60}Co R	1	7	21
1	0.0	0	$3.52 \pm 0.12^{a**}$	3.51 ± 0.13^{a}	3.08 ± 0.29^{a}
2	2.5	543	1.20 ± 0.04^{b}	1.15 ± 0.03^{b}	1.24 ± 0.04^{b}
3	1.0	543	2.47 ± 0.33^{c}	1.81 ± 0.30^{bc}	1.91 ± 0.19^{c}
4	0.0	543	3.32 ± 0.10^{a}	3.14 ± 0.10^{a}	2.30 ± 0.16^{c}
5	2.5	362	1.15 ± 0.05^{b}	1.22 ± 0.05^{b}	1.35 ± 0.08^{b}
6	1.0	362	2.64 ± 0.24^{c}	2.37 ± 0.23^{c}	2.15 ± 0.25^{c}
7	0.0	362	3.29 ± 0.10^{a}	2.96 ± 0.22^{a}	2.12 ± 0.23^{c}
8	2.5	0	1.20 ± 0.04^{b}	1.18 ± 0.04^{b}	1.18 ± 0.04^{b}
9	1.0	0	2.37 ± 0.32^{c}	1.92 ± 0.26^{c}	3.00 ± 0.21^{a}

*Each figure represents the mean in grams of 8 replicates.

**Those averages with the same superscript in the same column do not differ significantly at the .05 level.

TABLE 37

ANALYSIS OF VARIANCE FOR MEAN WEIGHTS OF RAT TESTES

Source	Degrees of Freedom	Mean Square	F	Tail Probability
Cadmium level(C)	2	1.38	5.07	0.007
Radiation dose(R)	2	60.39	221.72	0.000
Days(D)	2	1.86	6.83	0.000
CR	4	0.91	3.35	0.011
CD	4	0.76	2.79	0.028
RD	4	2.35	8.63	0.000
CRD	8	0.43	1.59	0.132
Error	189	0.27		

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