A POSSIBLE ROLE BY CADMIUM INDUCED METALLOTHIONEIN IN RADIATION PROTECTION FOLLOWING GAMMA IRRADIATION IN RATS

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF NATURAL AND SOCIAL SCIENCES

DEPARTMENT OF BIOLOGY

BY

STEVEN THOMAS SMITH, B.S., M.S.

DENTON, TEXAS

AUGUST, 1986

DEDICATION

This dissertation is dedicated to Charles B. Smith MSgt. U.S.A. (Ret.). 1912-1984, who told me I could if I tried.

ABSTRACT

A POSSIBLE ROLE BY CADMIUM INDUCED METALLOTHIONEIN IN RADIATION PROTECTION FOLLOWING GAMMA IRRADIATION IN RATS

STEVEN THOMAS SMITH

AUGUST, 1986

Fisher strain F-344 rats, approximately seventy days of age, ± ten days, were used to determine what effect, if any, the metalloprotein, metallothionein, has on increasing the lethal dose of gamma radiation from a ^{60}Co source. Production of metallothionein was induced utilizing single injections of 1 mg $CdCl_2$ in water/ kg of body weight. Ultrastructural examination of three selected cadmium-sensitive tissues, liver, kidney, and testes was performed to determine if the dosage of cadmium given would cause demonstrable damage. No pathological changes were noted in any of the tissues examined. This information was correlated with a lethality study, in which a group of 10 animals was given a single subcutaneous injection of 1 mg $CdCl_2$/kg body weight and observed for 30 days. No deaths were observed in this group of animals after 30 days.

A determination of the level of induced metallothionein utilizing atomic absorption spectroscopy was made. There were approximately 3.50×10^{-6} umoles of metallothionein/mg of liver, 1.11×10^{-5} umoles/mg of kidney tissue, and 9.96 x

10^{-6} umoles/mg of testes.

A lethality study was performed on 3 groups of 10 animals each at 9, 9.5, and 10 Gy of gamma radiation from a ^{60}Co source. This procedure was performed in order to demonstrate an LD 50/30 dose of gamma radiation from a ^{60}Co source. All animals in both the 9.5 Gy and 10 Gy groups died within 10 days. Fifty percent of the animals in the 9 Gy group died after 14 days, with no further deaths after 30 days. The 9 Gy level was then determined to be the LD 50/30 dose. To determine to what extent metallothionein affects the LD 50/30 of gamma radiation, a group of animals was first injected with 1 mg $CdCl_2$/kg body weight then allowed to synthesize metallothionein for 4 hours. Then the animals were divided into two subgroups which were then exposed to 0.5 Gy and 1 Gy above the LD 50/30 level of gamma radiation from a ^{60}Co source. Fifty percent of the animals receiving 0.5 Gy above the LD 50/30 and ten percent of the animals receiving 1 Gy above the LD 50/30 level survived. Since $CdCl_2$ induces metallothionein synthesis, this would seem to indicate that metallothionein could have a role in protecting the animals from the effects of radiation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Metallothionein was first identified by Margoshes and Vallee (1957). This metal-containing protein was purified and characterized by Kagi and Vallee (1961), who found it to be a low molecular weight, cysteine-rich, metal-binding protein. Evidence that metallothionein is produced in response to elevated levels of cadmium was found by Piscator (1964).

It has been postulated that metallothionein has a role in metal detoxification or metal metabolism (Kagi and Nordberg, 1979). The protein has been found to be produced in response to several metals *in vivo*. Cadmium (Cd^{2+}), zinc (Zn^{2+}), mercury (Hg^{2+}), and copper (Cu^{2+}) are known inducers of metallothionein, a protein-like cellular substance with a molecular weight of approximately 6000 Daltons.

Cadmium and mercury tend to produce higher concentrations of metallothionein than do zinc or copper. Metallothionein acts as a binding protein for these metals, sequestering them in both the liver and blood stream before deposition in the kidney's renal cortex or more specifically, the liver and kidneys with little deviation in amino acid sequence between the two forms. There are two basic forms of

metallothionein produced irrespective of its origin, metallothionein-I (MT-I) being preferentially bound to Cu^{2+} and Zn^{2+} and metallothionein-II (MT-II) binding preferentially to Cd^{2+} and Hg^{2+}. Both forms, MT-I and MT-II, are produced in response to Cd^{2+} and both forms bind Cd^{2+} without difficulty.

Administration of Cd^{2+} by either intraperitoneal injection or by other routes causes production of MT-I and MT-II from the liver generally by the action of Cd^{2+} on the nucleus. The metallothionein thus produced will bind to the target metal with a ratio of approximately seven molecules of metal to one molecule of metallothionein. This, in effect, sequesters the metal preventing it from depositing in other tissues. Once bound to the metal, the complex is transported via the blood to the kidney where it is broken down in the proximal tubules and the metal then binds again to metallothionein of renal origin. This process obviously does not present a fool-proof mechanism for dealing with heavy metals by the body; however, the exact nature of metallothionein's function is unknown. Damage due to heavy metal intoxication may occur prior to production of metallothionein by the liver. Damage does occur to the kidneys due to the breakdown of bound metal from metallothionein of liver origin in the cells of the proximal tubules. Kidney function is greatly disrupted when metal concentration (i.e. Cd^{2+}) reaches 200 mg/gm of kidney tissue, exemplified by proteinuria. Fetal

levels of this protein are also shown to be higher than adult levels with zinc and copper as the metals associated with it (Bremner and Young, 1976; Evans and Johnson, 1978; Porter, 1974; Ryden and Deutsch, 1978; Whanger and Oh, 1979; Webb, 1979; and Bremner et al., 1977). In all this research metallothionein was postulated as having a role in zinc and copper metabolism.

It is possible that the apoprotein, not the metal-containing form, has the primary role in cellular metabolism. This "role" is described by Vallee (1979), as one in which the cadmium, zinc, copper or mercury, in binding to metallothionein, may inhibit the normal function of the protein. The increase in biosynthesis of the metallothionein would then be an attempt to overcome the inhibition due to metal-binding. This approach may be seen as being supported by the work of Bethune (1979). His research has shown that metallothionein, or rather its apoprotein, may have a specific biological function in selectively binding guanosine triphosphate. This might, in turn, indicate a relationship with nucleic acid synthesis. The role of metallothionein is, at best, questionable in respect to whether its purpose is metal metabolism or a more specific nuclear function. Whatever this ideal function, this unique protein allows an interesting use that being a "clean up" mechanism for highly charged molecules such as metal ions and perhaps that of free radi-

cals formed by the interaction of cells with ionizing radiation.

The possibility exists due to the chemical nature of metallothionein, for it to act as a radioprotective agent, first, due to the large number of cysteine residues present in the molecule and second, due to the compound's preferential binding to highly charged ions (i.e., Cd^{2+}, Zn^{2+}, and Hg^{2+}). The presence of an inducible radioprotective compound within tissues of the body affords an opportunity to speculate on whether this system may act as an early cleanup system for primary and secondary radicals formed due to irradiation. In this way, metallothionein may afford some protection for those individuals who receive multiple insults in areas of radiation exposure that may involve metals such as those found as part of nuclear reactor mechanisms (i.e. cadmium cladding around control rods). It is possible that exposure to small doses of metals which are known to induce metallothionein might be of some benefit in protecting against radiation exposure. Less toxic metals, such as zinc and copper, being much less toxic than cadmium or mercury might also be used to induce metallothionein.

Protection from the effects of radiation has been demonstrated for a number of compounds. Herve and Bacq (1949) demonstrated the protective effects of cyanide in mice exposed to lethal doses of x-irradiation. Other compounds,

such as sodium azide and metal enzymes, have also been shown to protect against the effects of radiation. The unfortunate aspect of these types of compounds is that they are also cytotoxic and as such, do not lend themselves well to employment in medical practice.

Patt et al. (1949) discovered the ability of cysteine to protect against the effects of x-irradiation. In this case, it was discovered that the protective substance must be present at irradiation if it was to be effective.

The mechanisms by which these types of compounds decrease the effects of ionizing radiation vary. They may act as absorbers for the various oxidizing compounds formed after irradiation, or possibly by competition for oxygen which promotes HO_2 radicals (Grosch and Hopwood, 1979). Bacq and Alexander (1961) postulated that peroxides were protected from becoming peroxyls, or it is possibly due to the donation of hydrogen atoms to free radicals.

In terms of overall biological effect, it makes little difference whether the action of ionizing radiation is taken as direct or indirect. Due to the nature of most biological systems, that being primarily aqueous, an indirect mechanism of radical formation is as deleterious as a direct path. While cells do contain some quantities of compounds possessing sulfhydryl groups, their concentrations are insufficiently high to compete with oxygen for the free radicals. This

results in the preferential formation of peroxyl compounds. In this respect, metallothionein may offer an advantage over other protective sulfhydryl containing compounds. Metallothionein has the ability to be either induced or to apparently be injected and be taken up by the cells. It also possesses a high concentration of cysteine residues which may be protective by one of two possible pathways, that of hydrogen ion donation as a resevoir of sulfhydryl groups or in the formation of disulfide cross-linkages (Eldjarn and Pihl, 1960). By this method, protein molecules would be protected via the interaction of a sulfhydryl group of an aminothiol with a sulfhydryl group of a protein forming a disulfide bridge.

The extent of the protection is afforded by various methods. Glutathione, a resevoir of sulfhydryl groups, protects certain enzymes such as catalase and invertase, by increasing the inactivation dose. The conversion of oxyhemoglobin to methemoglobin via irradiation is reduced by cysteamine and aminoethyl isothiuronium (AET). Aminothiols may also act by a mechanism of free radical removal or scavenging. Aminothiols are oxidized by free radicals produced by the radiolysis of water. By this method, other molecules are prevented from being attacked. Aminothiols and similar compounds have an increased affinity for hydroxyl and peroxyl radicals, due to their molecular configuration, as

demonstrated by electron spin resonance studies (Arena, 1971).

The study involved inducing metallothionein utilizing a single subcutaneous injection of cadmium. The injected cadmium given was in the form of an aqueous solution of $CdCl_2$. The injection was given four hours prior to sacrifice. Three cadmium sensitive tissues were removed from these animals. The tissues studied were the liver, kidney and testes. Subjecting these tissues to atomic absorption spectroscopy allowed the determination of metallothionein content based on the ratio of cadmium binding to metallothionein. This information was used later in the study to quantitate the amount of protection afforded by metallothionein's presence. The ultimate goal of this study was to demonstrate the possibility of increasing the dose of radiation required to kill fifty percent of a group of animals in thirty days (an LD 50/30 dose). Demonstration of this "radioprotective" aspect of metallothionein was attempted by its induction with $CdCl_2$ in two groups of animals. One group was exposed to 0.5 Gy of gamma radiation above the LD 50/30 level. The second group of animals was exposed to 1 Gy of gamma radiation above the LD 50/30 level. The animals of both groups received single injections of $CdCl_2$ in water four hours prior to exposure.

Thus the object of this experimental procedure was to

obtain as low a level of cadmium possible, yet elicit sufficient production from either liver or kidney to determine what possible effects metallothionein had on reducing the effects of free radicals in the tissues of animals which have been exposed to an LD 50/30 dose of gamma radiation from a ^{60}Co source. Since the functional amino acid residues involved in binding the metal in metallothionein are cysteine, it was thought that in the absence of large metal quantities and high quantities of free radicals metallothionein might be put to use by binding the free radicals and thus removing them from the tissues. In structure, metallothionein bears some functional resemblance to other aminothiol radioprotective agents. The preference in using metallothionein for its potentially radioprotective properties lies in 1) the similarity of all metallothionein, regardless of what species animal provides it; and, 2) the lack of toxicity and the ease of induction given a relatively low dose of metal thereby reducing the number of formed radicals.

CHAPTER II

REVIEW OF LITERATURE

Cadmium has long been known to exert various toxic effects in man and other species (Stokinger, 1963; Bonnell, 1965; Kazantzis et al., 1963; Kazantzis, 1956; and Friberg, 1950). In man, the ingestion of 15 mg results in immediate nausea and vomiting. Both chronic and acute effects are noted as a result of occupational exposure. Inhalation from either dust or exposure to the oxide of cadmium may result in death, if levels range from 3-100 mg/m^3. Non-fatal pneumonitis has resulted from exposure to concentrations of 0.5-2.5 mg/m^3. Some attention has been given to the idea that cadmium in the environment (atmosphere) might be related to cardiovascular disease. Abnormal excretion of amino acids in urine has been noted (Clarkson and Kench, 1956). A concentration of 200 ug/mg of kidney tissue resulted in proteinuria and renal tubule necrosis.

From a pathological viewpoint, cadmium may cause injury to the kidney via two pathways (Nriagu, 1981). The first mechanism would involve direct action on tubular epithelium after absorption of cadmium. This action is exemplified by renal glycosuria, aminoaciduria, proteinuria, impaired concentrating ability, and impaired acid excretion. These symptoms generally represent what is described as renal tubule mal-

function. The second mechanism involves action upon blood vessels. Vascular lesions may give rise to edema within the interstitial connective tissue. Both of these were clearly demonstrated to occur in early work done on epitheleal alterations (Kawai et al., 1976). The action of cadmium on the vasculature was seen as the most probable explanation for the morphological changes seen in testicular necrosis resultant from acute cadmium intoxication (Friberg et al., 1974). This concept was supported by the observation of interstitial edema concomitant with degenerative changes in blood vessel endothelium. Edematous interstitium may eventually convert to interstitial fibrosis and in the kidney may well develop into nephrosclerosis. This in turn, would lead to degeneration of tubular epithelium and result in long term renal dysfunction. In this respect, blood cadmium levels become important as opposed to retained tissue levels in the kidney or testes. Based on tissue culture work and on animal work, it has been estimated that levels of cadmium above 1 ug Cd^{2+}/ml may cause visible alterations in visceral organs (Kawai et al., 1976). Thus, duration of exposure as well as dose may be of equal importance in producing lesions of a chronic nature. Development of renal damage was a principal result of chronic exposure to Cd^{2+} (Friberg, 1974; Tsuchiya, 1978; and Shaikh and Hirayama, 1979). The mechanism through which this toxicity is induced is unclear. Onset of the damage is

delayed and this delay is believed to be due to the slow rate of accumulation of Cd^{2+} in renal tissue (Friberg, 1974). The other possibility also set forth by Friberg (1974) is that of a critical concentration above which the toxic effects of Cd^{2+} are exerted.

It has also been postulated that this renal damage may be resultant from increased circulation of metallothionein bound cadmium (Cd-MT) released from tissues, i.e. liver, when they become saturated (Shaikh and Hirayama, 1979; and Nordberg and Piscator, 1972). This speculation raises the possibility that the extracellular Cd-MT might be the direct toxic agent in the kidney as opposed to the ionic form of cadmium. Results of research by various groups (Shaikh and Hirayama, 1979; Nordberg et al., 1971; and Chang et al., 1980) showed a definite correlation between circulating Cd-MT levels and renal damage. The LD_{50} value for Cd-MT was one-tenth that seen for ionic Cd^{2+} (Webb and Etienne, 1977). Acute renal failure can be induced with 1.1 mg Cd^{2+} in its bound form (Nordberg et al., 1975). Cadmium-metallothionein is filtered through the glomerulus and reabsorbed through the renal proximal tubule cells (Foulkes, 1978). By this mechanism, Cd-MT is deposited in one specific cell type with this cell type as the target of Cd-MT toxicity. It has clearly been demonstrated in earlier studies (Squibb et al., 1979; Nordberg et al., 1972; and Cherian et al., 1976) that the

only structures in the kidney demonstrating morphological changes were the proximal tubules following Cd-MT treatment. These changes include vesiculation of the cells, an increase in apical lysosomes with a progression to cellular death, and desquamation.

The mechanism of Cd-MT nephrotoxicity is still unclear. Several researchers (Cherian et al., 1976; Cherian, 1981; and Rodgers and Cherian, 1981) have speculated on the toxicity of the Cd-MT molecule by exerting its influence on the brush border during reabsorption from the tubular lumen. However, research by other investigators (Webb and Etienne, 1977; Fowler and Nordberg, 1978; Squibb et al., 1979; and Squibb et al., 1982) has suggested that the Cd^{2+} ion is itself the toxic agent. The present data suggest that the release of the Cd^{2+} ion within the tubule cell, i.e. degradation of the reabsorbed Cd-MT, is the causitive agent of the nephrotoxicity. This view is supported by the data of Squibb et al., (1982) who demonstrated no inhibition of mitochondrial respiration due to Cd-MT. The proteinuria associated with Cd-MT treatment can be ascribed to the inhibition of normal heterolysosome formation in both the liver and kidneys by Cd^{2+}. Of the cellular functions studied, the most sensitive to Cd-MT was found to be renal RNA synthesis. This sensitivity was also demonstrated in liver by Hidalgo et al., (1976) and Stoll et al., (1976). In their work, RNA poly-

merase activity was inhibited shortly after injection of Cd^{2+}; however, this action was relieved as Cd^{2+} was "chelated" out of the nucleus by newly synthesized metallothionein groups. Nuclear RNA synthesis is thus inhibited prior to the induction of metallothionein, which decreases the effects on RNA synthesis. It becomes apparent that the most likely agent is not Cd-MT but rather the Cd^{2+} ion itself.

While the ultimate answer to the question of metallothionein's function is still not apparent, it does seem to fill the role of a metal transport protein which functions over a broad range of divalent cationic transition metals. As to what particular role metallothionein may have, other than sequestering the metals prior to their function in some aspect of cellular metabolism, is clearly speculative. Only two facets of the protein's nature are certain: 1) it normally associates with zinc at some point in that metal's function in cellular physiology; and 2) it binds guanosine triphosphate (Vallee, 1979). What makes the protein preferentially bind Cd^{2+} over zinc is unknown, but probably is related to the chemical and physical structure of its metal binding sites. It is this chemical and physical structure which may provide for more unique functions of this protein when applied to the prospect of free radical removal in irradiated animals.

With DNA as the most damaging target for ionizing radia-

tion, it is apparent that the effects of such radiation are felt not only at the cellular but also molecular levels (Dalrymple and Baker, 1973). Even considering other primary sites of action for radiation damage (Shenoy et al., 1974), the key to the effects of radiation damage must be at the molecular level.

Since the action of an ionizing radiation may be said to be of a direct or indirect type, the resultant effect may be of either an immediate of non-immediate nature with respect to cellular survival (Grosch and Hopwood, 1979). Different tissues in animals demonstrate different sensitivities to ionizing radiation (Grosch and Hopwood, 1979). This is based on, a priori, the mitotic activity of the cell. The more active in terms of cell replication, the sooner any effect due to damage occuring in the nucleus is seen. As an example, nerve tissue is said to be highly sensitive to radiation yet it requires a longer time period for such damage to be demonstrated (Grosch and Hopwood, 1979). This is due to the slow rate at which nerve tissue in general recovers.

With genetic damage appearing as the most damaging long term effect, it is not necessarily the most damaging overall. By production of free radicals, these being highly charged, highly reactive molecules such as $OH^{\cdot}$, within the cell damage may be done to more than one system simultaneously. Production of the radicals is incurred by the deposition of vast

amounts of energy by an ionizing radiation in a very small area (Arena, 1971; and Grosch and Hopwood, 1979). Free radicals diffuse in cells only a short distance, 30 Å on average (Hutchinson, 1961). The problem arising here is that while the radical may not travel far the primary component of cells is water with human tissues ranging up to 70-80% water (Grosch and Hopwood, 1979). The chemical products produced by the irradiation of water include $H^{\cdot}$, $OH^{\cdot}$, H_2O_2, and HO_2. These and other oxidizing agents can interfere with normal reactions by removing hydrogen ions from organic substances or by the formation of organic peroxides. In addition to these mechanisms, hydrogen bonds, double bonds in organic molecules, and sulfhydryl groups of other molecules may be split to give rise to more free radicals (Willson, 1983).

Free radicals are found to be increasingly associated with initiation and progression of various diseases and toxic reactions involving many drugs and chemicals (Willson, 1983). It appears that cells are constantly open to assault from free radicals whatever the source. It is only through various inherent features that cells survive. These features which include the cells compartmental structure and the presence of compounds such as catalase, glutathione peroxidase, ferritin, and transferrin act to minimize or prevent free radical formation (Willson, 1976, 1977, 1978, and 1979).

Should formation of free radicals occur, those of a

highly electrophilic nature would be picked up by nearby organic materials. Those radicals less reactive such as peroxy radicals may diffuse some distance before removal by scavanging compounds such as glutathione or one of the endogenous hydroquinones. In some cases a specific enzyme such as superoxide dismutase would act to remove it. Even through these protective measures, some radicals may reach what could be described as "vital" molecules and oxidize them. If such "target molecules" are not reduced quickly, they may be further oxidized allowing for serious damage to result. The reduction process of these targeted molecules is generally referred to as "free radical repair".

The concept of "free radical repair" by reduction or hydrogen ion transfer came about in the 1950's as an attempt to explain the protective actions of mercaptans on radiation-induced decomposition of polymers (Wall and Magat, 1953; and Prevot-Bernas, 1953). In later work, Alexander and Charlesby (1954) added support to the observations of Wall and Magat (1953) and those of Prevot-Bernas (1953) by noting that the addition of sulfhydryl compounds through the transferral of a hydrogen atom to the radicals formed prevented them from reacting with the substrate material. The radical formed by reaction of the protective compound is generally of low chemical reactivity. There are many compounds which function to remove radicals; these range from glutathione to vitamin C

(ascorbate). There have been various reports of ascorbate protecting against radiation induced damage (Bacq and Alexander, 1961; and O'Conner et al., 1977). However, in biological systems those compounds which are sulfhydryl in nature, such as cysteamine, demonstrate a greater effectiveness (Redpath and Wilson, 1973; and Willson, 1983). A similar compound which appears highly effective at removing radicals is glutathione. This is probably due to several factors unique to glutathione. In that glutathione is widely distributed in animal tissues its concentration is in the millimolar range. Glutathione functions in transport as a coenzyme and as a storage and transport form of cysteine. The protective functions of glutathione are probably due to the sulfhydryl group acting as an efficient intracellular reducing agent (Meister, 1983; and Harris, 1983).

It is for similar reasons that other inducible cysteine "resevoirs" may function in a useful fashion in treating or abating the production of cellular damage caused by radiation. Endogenous compounds have the advantage of position and concentration when compaired with exogenous protective agents. Exogenous agents by whatever route of administration must be transported inside the cell in sufficient quantities to be effective in terms of radical removal. Endogenous compounds have the capacity to be induced within the cell and are less likely to cause cellular disruption by their pre-

sence. Metallothionein similarly acts as a repository for cysteine, comprising 30-35% of its composition with approximately 20-21 sulfhydryl groups present per molecule (Vallee, 1979). For this reason along with its inherent inducibility metallothionein presents itself as an interesting possibility for playing a role in free radical removal thereby making it a potentially good radiation protection agent.

CHAPTER III

METHODS AND MATERIALS

The rats used in this study were Fisher strain F-344. All animals were maintained on Purina Laboratory Chow and water ad libitum. Only male rats were used so as to remove any sex related differences.

Radiation Lethality Study:

For this portion of the study the rats were divided into four groups of ten animals each. Three groups were exposed to either 9 Gy, 9.5 Gy, or 10 Gy of gamma radiation from a ^{60}Co source. The fourth group of animals were sham irradiated.

Electron Microscopic Observation:

This aspect of the study was an attempt to determine what effects could be demonstrated at the ultrastructural level resultant from the injection of 1 mg $CdCl_2$/kg body weight. The $CdCl_2$ was delivered as a single injection given subcutaneously to 5 animals. These animals were sacrificed after 3 days. Three selected cadmium sensitive tissues, liver, kidney, and testes, were examined electron microscopically to determine if any cadmium-induced pathology was present as compared with non-injected controls.

Selection of the dose of 1 mg/kg cadmium chloride injected subcutaneously was determined through literature

values (Berry, 1972; Nishizumi, 1972; Kajikawa et al., 1973; Kajikawa and Kuroda, 1974, 1976; Kawai et al., 1976; Suzuki, 1974; Nordberg and Piscator, 1972; and Nomiyama et al., 1977) and by experimentation to be below an LD 50/30 dose. Berry (1972) administered 0.75 mg Cd/g of body weight, subcutaneously, three times a week for 4 weeks to demonstrate changes in glomerular vasculature in rats. Nishizumi (1972) gave rats water containing 10, 50 and 300 ug Cd/g water for 42 weeks and demonstrated only slight changes at 24 weeks. These changes were localized in the proximal tubule epithelium and included mitochondrial swelling. Kajikawa et al., (1973) injected rats intraperitoneally with 0.61 mg Cd/kg of body weight once a week for 12 weeks and demonstrated only increased numbers of lysosomes and changes in the cytoplasmic matrix. Kajikawa and Kuroda (1976), after injecting rats with 1 mg Cd/kg body weight intraperitoneally weekly for 56 weeks, demonstrated only slight changes to the kidney at 38 weeks. At 56 weeks atrophy of tubular epithelium and interstitial fibrosis were present and no deaths were recorded. In the present study, a group of 10 animals received a single subcutaneous injection of 1 mg of $CdCl_2$ in water/kg of body weight. No deaths were recorded in this group after 30 days. This information correlated with values established by various authors (Berry, 1972; Nishizumi, 1972; Kajikawa et al., 1973; Kajikawa and Kuroda, 1974, 1976; Kawai

et al., 1976; Suzuki, 1974; Nordberg and Piscator, 1972; and Nomiyama et al., 1977) for determination of a lethal dose of $CdCl_2$.

Tissue Preparation for Electron Microscopy:

A segment of each tissue was placed in phosphate buffered glutaraldehyde (4% glutaraldehyde, 0.1 M sodium phosphate buffer, pH 7.4) and incubated for one hour at room temperature. Subsequently, the fixative was washed from the tissue by three changes of 0.1 M phosphate buffer. The tissues were postfixed in 1% OsO_4 in 0.1 M phosphate buffer for one hour at 4° C in complete darkness. The tissues were then washed with three changes of distilled water, dehydrated in a series of graded alcohols, and embedded in Epon-812.

The embedded material was thin-sectioned on a SorVall MT-2B ultramicrotome and sections 60-100 nm thick were placed on uncoated 200-mesh copper grids. The grids were routinely poststained in a solution of 1% uranyl acetate for 20 minutes and then in a solution of lead citrate (Reynolds, 1966) for 5 minutes. The grids were examined on a Seimens Elmiskop electron microscope at an operating voltage of 80 kV.

Cadmium Injected-Irradiated Study:

A group of 10 rats was injected with a single subcutaneous dose of 1 mg $CdCl_2$/kg body weight. These animals were then exposed to a sub LD 50/30 dose (8 Gy) of gamma irradiation at 4 hours post-injection (Cempel and Webb, 1976). This

was done in order to determine if the effect of cadmium was additive to that of the dose of gamma radiation. The animals were observed for 30 days to determine an increase in mortality above that seen in the controls. Controls consisted of a group of 10 rats receiving only the 8 Gy dose of gamma radiation.

Atomic Absorption Study:

A group of 10 rats was injected subcutaneously with a single dose of 1 mg $CdCl_2$/kg body weight. These animals were sacrificed after 4 hours to determine the quantity of metallothionein present as bound to cadmium by the use of atomic absorption spectroscopy.

Tissue Preparation for Atomic Absorption Spectroscopy:

Each tissue was weighed and then placed in a solution of 0.01 M tris-HCl buffer of pH 7.4 containing 0.25 M sucrose and homogenized for two minutes. A sample of this material was then subjected to atomic absorption spectroscopy using a Perkin Elmer model 370 atomic absorption spectrometer.

The atomic absorption spectroscopy data were then used to calculate the quantity of metallothionein present based on the procedure of Kimura et al. (1979) and Vallee (1979).

Extension of the LD 50/30 with Cadmium Treatment:

This portion of the experiment determined to what degree the LD 50/30 would be affected by treatment with cadmium. Two groups of 10 rats each were injected subcutaneously with

1 mg $CdCl_2$/kg body weight. After four hours, these animals were then irradiated with 0.5 Gy and 1.0 Gy above the LD 50/30 level of gamma radiation.

CHAPTER IV

RESULTS

The attempt to determine the LD 50/30 for this strain of rats resulted in the deaths of all rats exposed to 9.5 Gy and 10 Gy of gamma radiation within 10 to 7 days, respectively. Fifty percent of all animals receiving 9 Gy of gamma radiation were dead within two weeks and no further deaths were recorded during the 30 day period (Table 1).

Examination of those animals receiving a dose of 1 mg $CdCl_2$/kg body weight via electron microscopy demonstrated no obvious liver pathology at the ultrastructural level (Figure 1) compared to that of controls (Figure 2). As marker structures, the endoplasmic reticulum and mitochondria showed no apparent changes.

There also appeared to be no damage at the ultrastructural level in the kidneys of these animals. There appeared to be no degenerative changes to proximal tubules or associated tissues as demonstrated in Figure 3 compared to those of controls (Figure 4).

In the testes of this group of animals, there was no necrosis in the vasculature or to the spermatic epithelium (Figure 5) compared to that of the control (Figure 6).

To ascertain whether the effects of injecting $CdCl_2$ were additive to those of radiation, the following procedure

Table 1

Comparison of Lethality of Adult Male Rats Exposed to 9 Gy, 9.5 Gy and 10 Gy of Gamma Radiation

Dose	No. of Rats	No. of Rats Dying	Days Post-Irradiation	% Dead
9 Gy	10	1	10	
		2	12	
		2	14	50%
9.5 Gy	10	7	3	
		3	10	100%
10 Gy	10	10	7	100%
Control	10	0	0	0%

Figure 1. Cadmium treated rat liver showing no abnormal nuclear structure. Mitochondria (M) while numerous show no swelling or degradation. Endoplasmic reticulum (ER) appears normal without swelling or inclusions. Approx. X12,000.

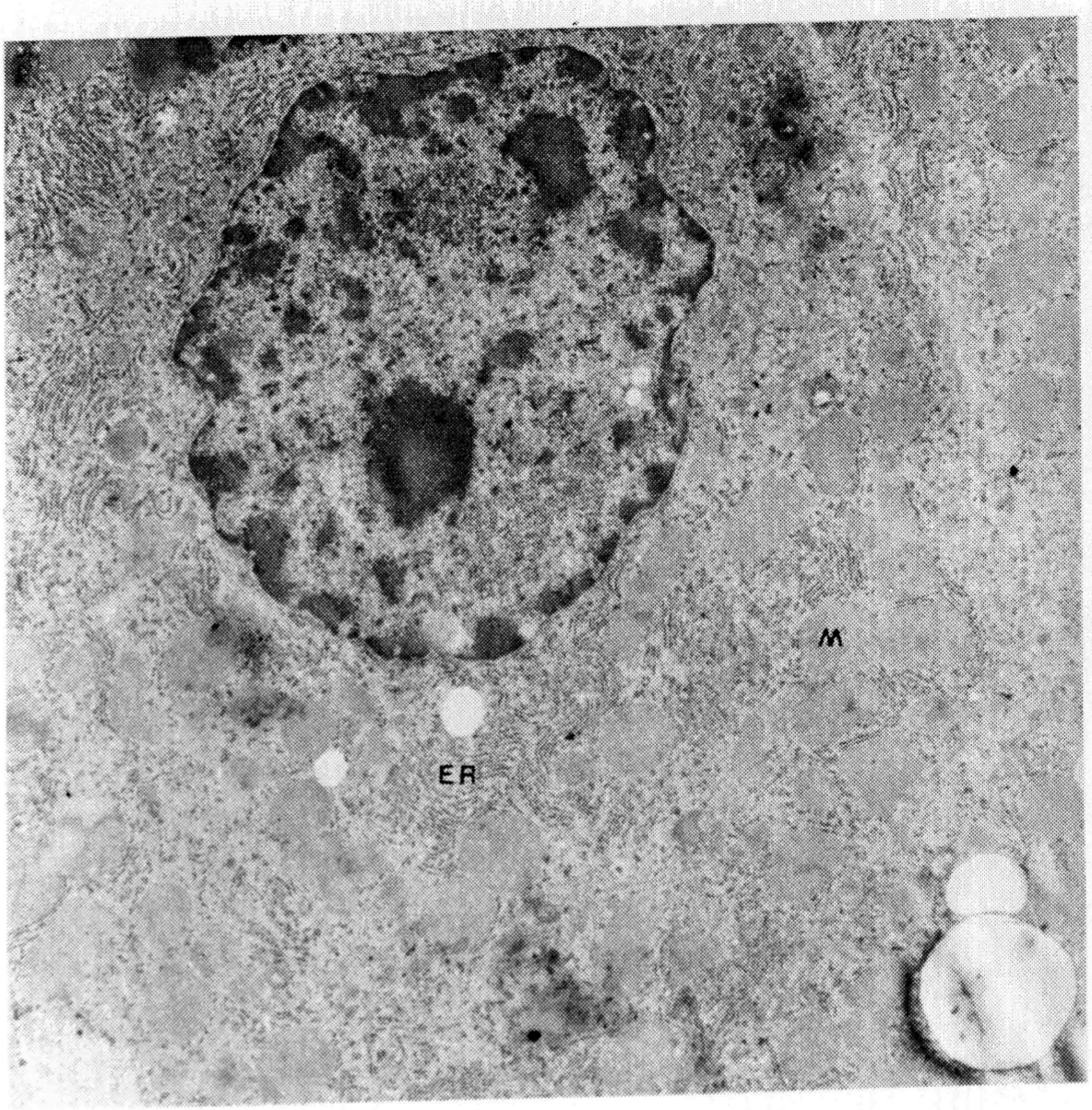

M
ER

Figure 2. Control rat liver showing apparently normal cytologic structures. The nucleus (N), endoplasmic reticulum (ER) and mitochondria (M) all appear unremarkable. Approx. X12,000.

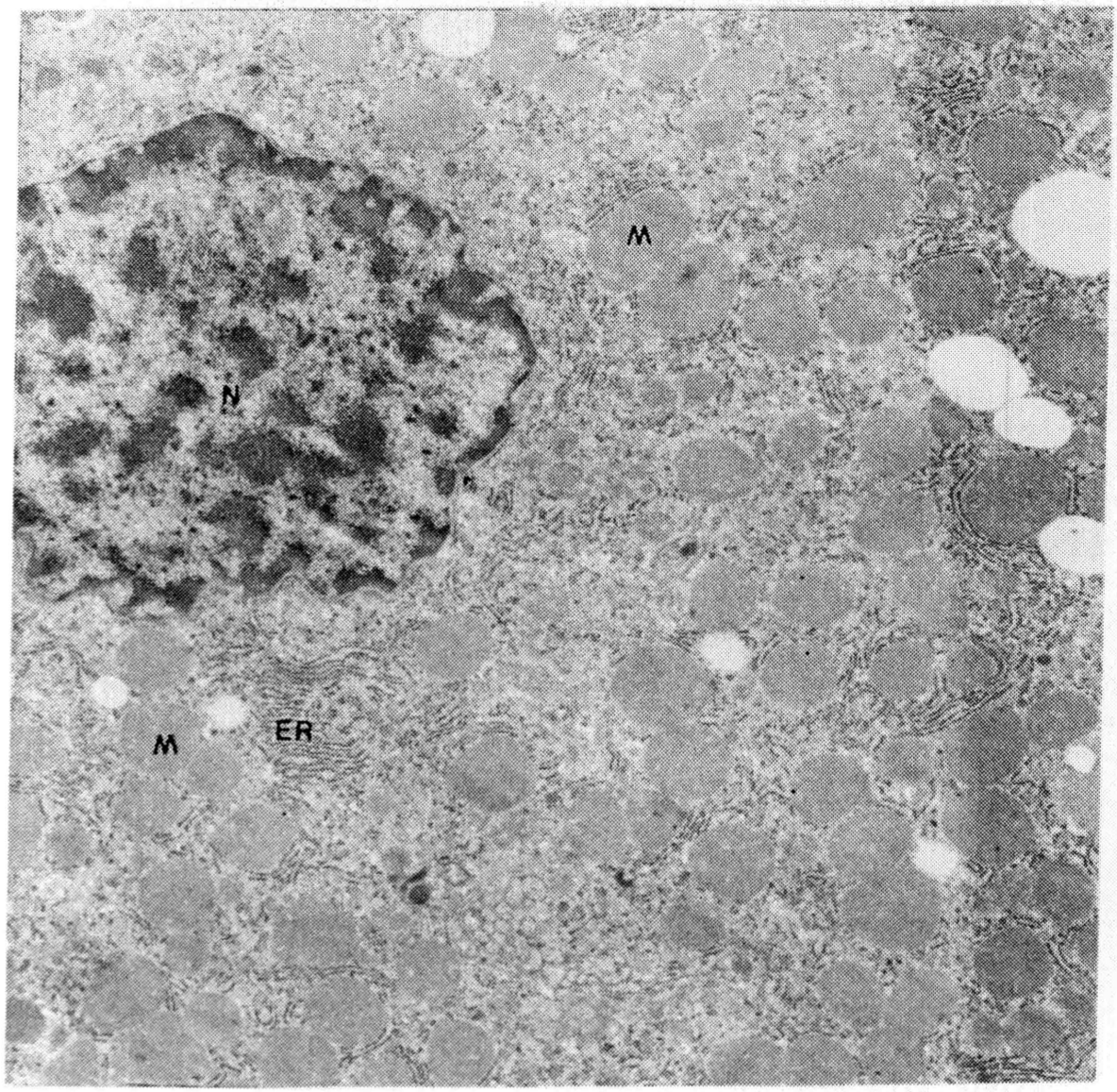

M
N
M
ER

Figure 3. Cadmium treated rat kidney structures shown are within the glomerulus. Slit pore (SP) structures appear normal. No apparent damage to basement membrane (BM). Approx. X12,000.

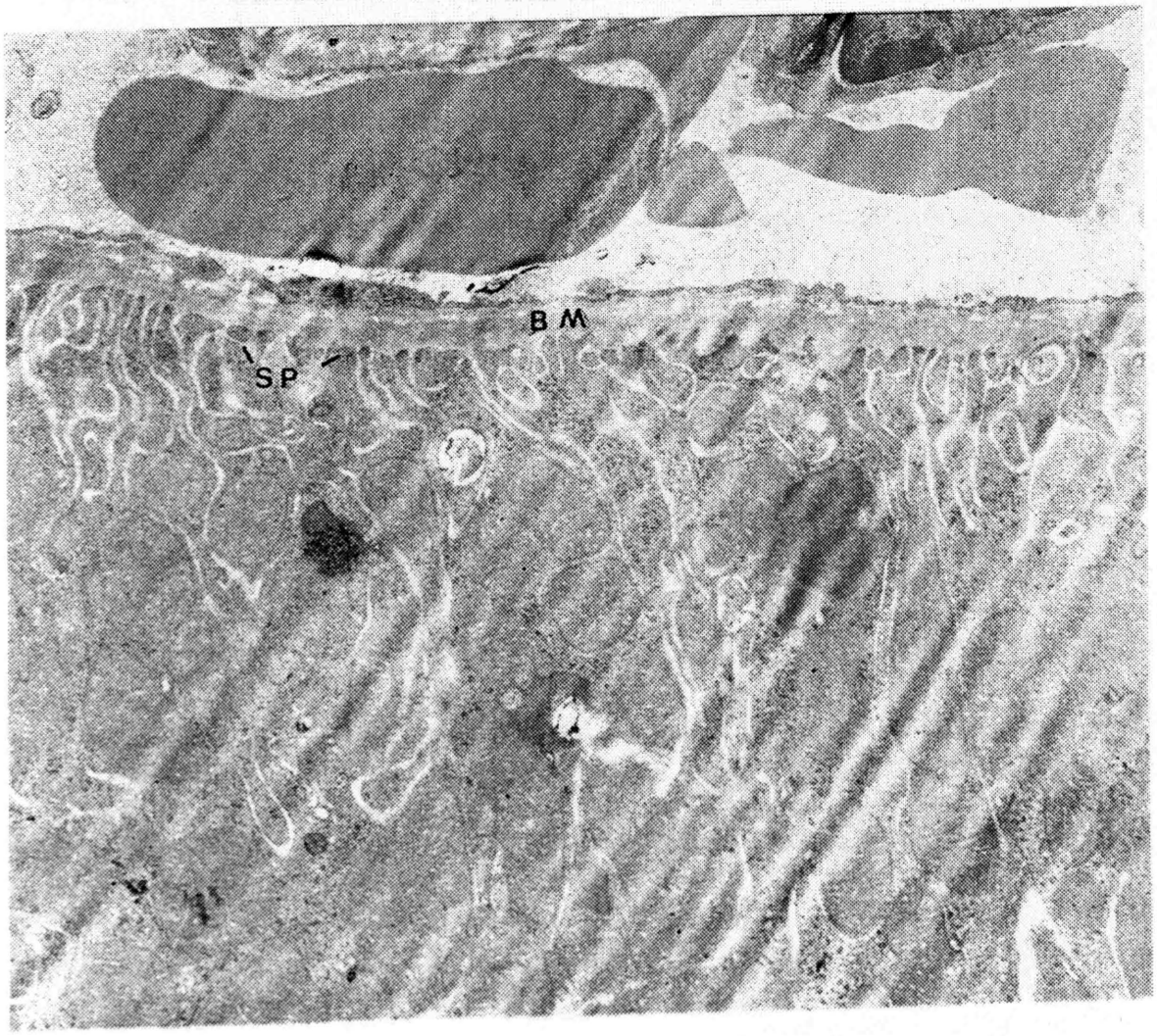

B M
S P

Figure 4. Close up of control kidney structures shown are in the vicinity of the Loop of Henley. No swelling or irregularly shaped mitochondrial membranes (M). Normal micropinocytotic vesicles (MPV) and secondary lysosomes (SL) present. Approx. X12,000.

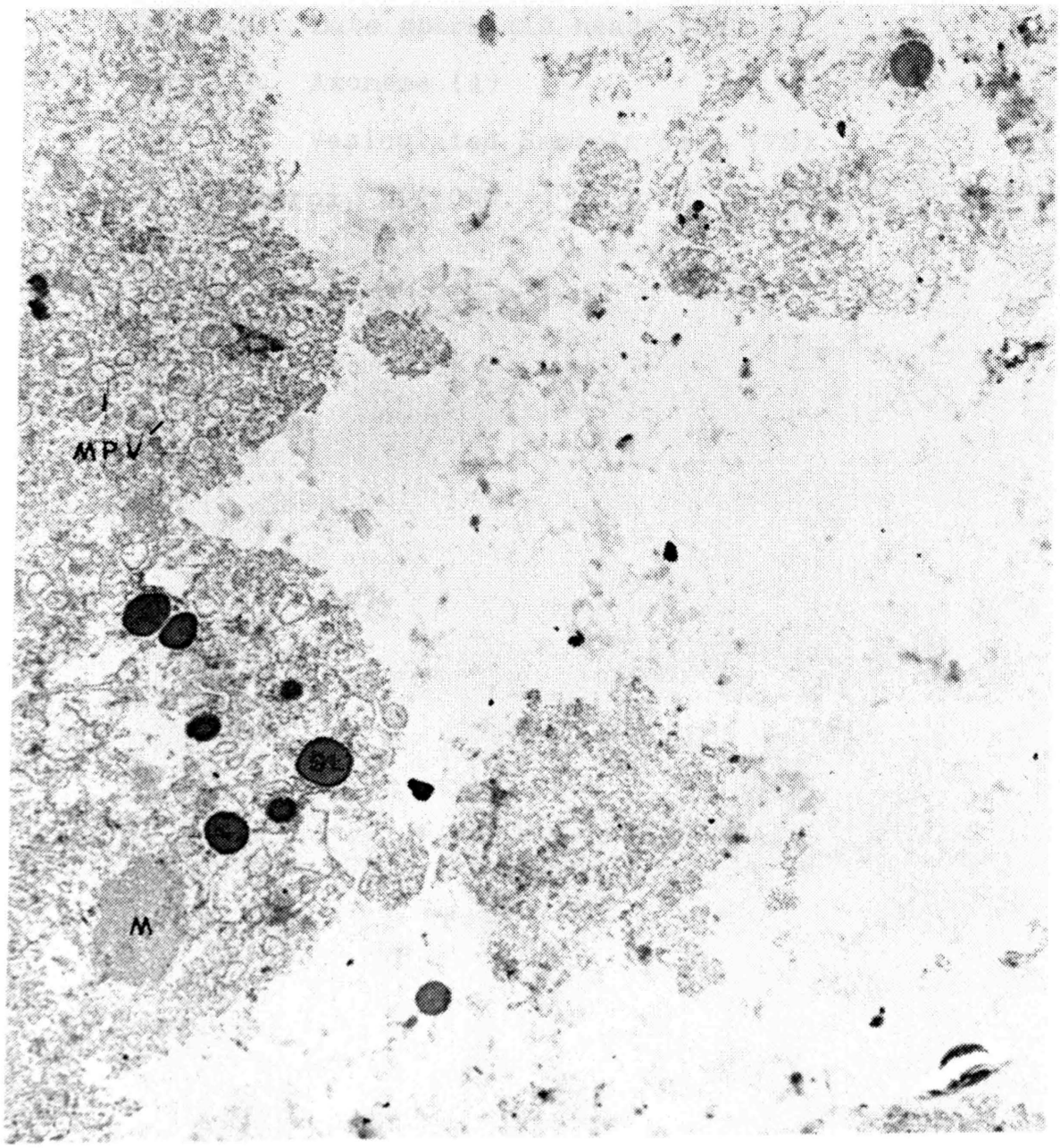

MPV
SL
M

Figure 5. Cadmium treated rat testes showing normal sperm development at several stages:

1. Mid-piece cross-section (MP)
2. Late spermatid heads (SH)
3. Axoneme (A)
4. Vesiculated Sertoli cell (VS)

Approx. X12,000.

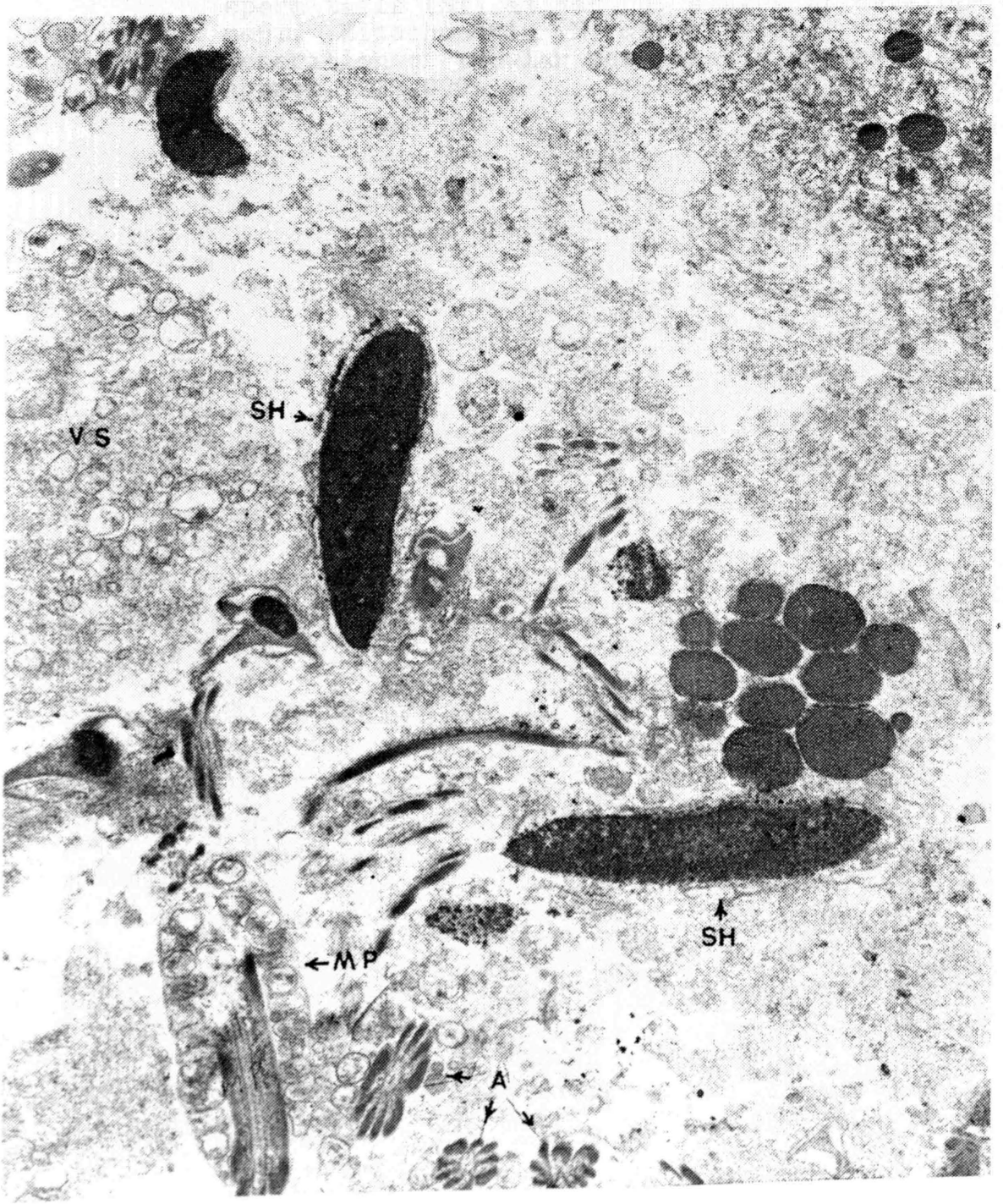
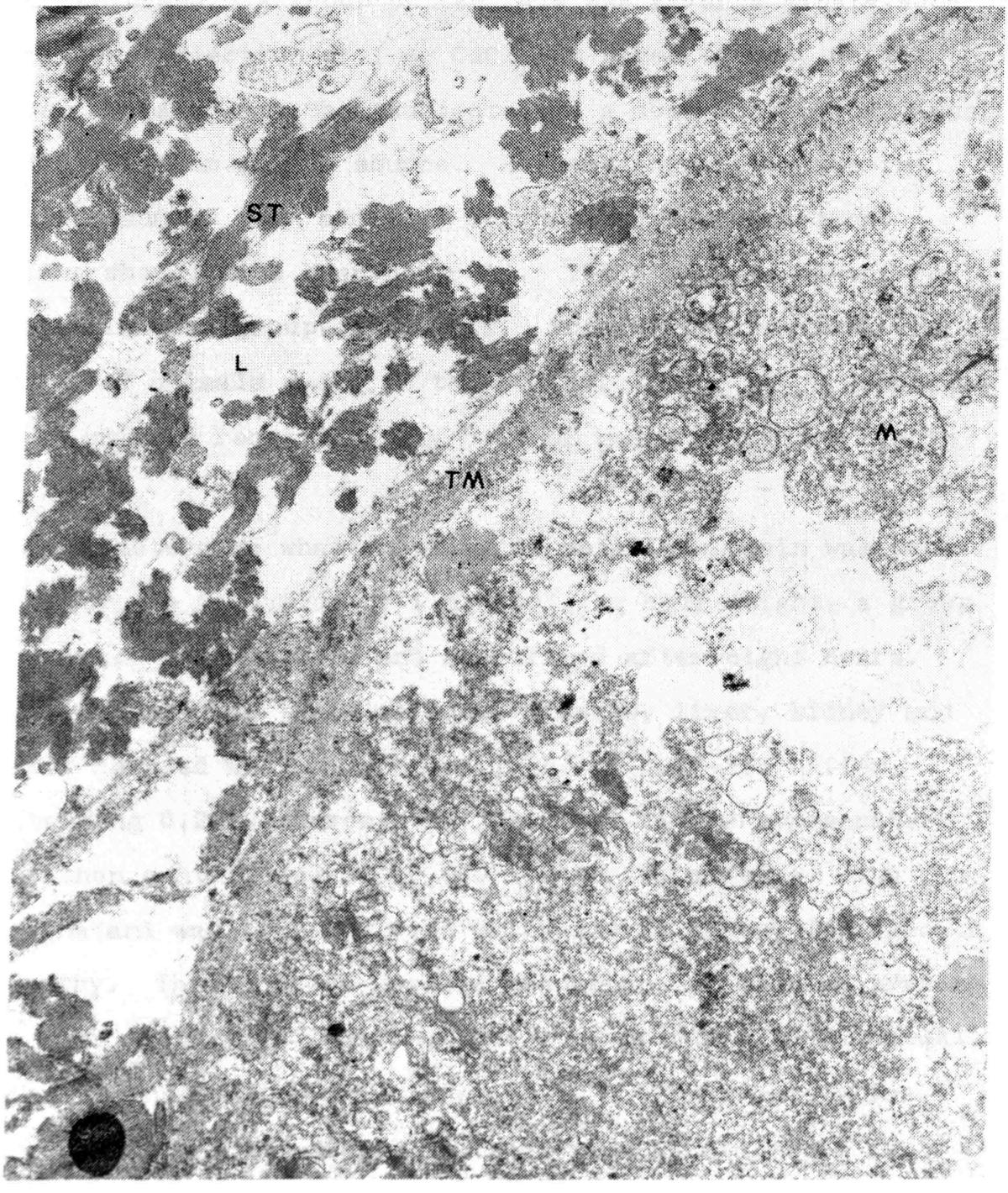

SH
V S
SH
MP
A

Figure 6. Control testes showing close up of seminiferous tubule membrane (TM). Lumen (L) demonstrates sperm tails (ST) at various stages of development. Mitochondria (M) appear normal. No degeneration of tubular membrane is observed. Approx. X12,000.


ST
L
TM
M

was performed. A group of ten rats was given a single subcutaneous injection of 1 mg $CdCl_2$ in water/kg body weight. These animals were then subjected to a dose of 8 Gy of gamma radiation from a ^{60}Co source. Had the effects of the injected cadmium been additive to those of the radiation, deaths should have been observed. There were no deaths recorded in this group of animals. This correlates with the number of animals dying in the control group, which consisted of 10 animals receiving only 8 Gy of gamma radiation (Table 2).

To determine what quantity of metallothionein was produced by the injection of 1 mg $CdCl_2$/kg body weight, a group of animals was injected and sacrificed after eight hours. These animals had three selected tissues, liver, kidney and testes removed and homogenized in 0.1 M tris-HCl buffer containing 0.25 M sucrose, pH 7.4. The tissue homogenates were then centrifuged at 10,000 x g for 60 minutes. The supernatant was then analyzed using atomic absorption spectrometry. This process yielded the amount of cadmium present per gram of tissue. Based on the work of Fuwa, 1982; Suzuki, 1982; Kimura et al., 1979; Bryan et al., 1976; and Vallee, 1979, the ratio of cadmium binding to metallothionein is known to be 7 moles of cadmium per 1 mole of metallothionein. Utilizing this information, an approximation of the amount of metallothionein is possible. Bryan and Hidalgo (1976)

Table 2

Comparison of Lethality for Irradiated, Cd-Injected and Cd-Injected-Irradiated Rats

	No. of Rats	No. of Rats Dying
8 Gy Gamma Irradiated	10	0
Cd-Injected*	10	0
Cd-injected 8 Gy Gamma Irradiated**	10	0

*Dose of Cd injected: 1mg $CdCl_2$ per kg body weight.

** Irradiated four hours post injection.

demonstrated that more than 90% of the cadmium administered by injection will be bound to metallothionein within 24 hours. Cempel and Webb (1976) demonstrated that following injection of a single dose of cadmium synthesis of metallothionein is complete within 8 hours. This approximation would indicate that, based on the concentration of cadmium determined by atomic absorption spectrometry, the tissues used in this study had the following metallothionein concentrations: liver, 3.50×10^{-6} umoles/mg; testes, 9.96×10^{-6} umoles/mg; kidney, 1.11×10^{-5} umoles/mg (Table 3).

Extension of the LD 50/30 dose of gamma radiation was observed in the injected irradiated group. In this portion of the experiment, to determine to what degree the LD 50/30 is affected by the presence of metallothionein, observation of a significant increase in survival at 0.5 Gy above the LD 50/30 dose was made. In this group, 5 out of 10 treated animals survived after 30 days. At 1 Gy above the LD 50/30 level only one animal out of the group of ten treated animals survived after 30 days (Table 4).

Table 3

Comparison of Tissue Cadmium Concentration and Yield of Metallothionein

	Average Cd Concentration ug Cd/ml (± S.E.)*	Average Cd Concentration ug Cd/mg	Average Concentration of MT**/mg of Tissue (1)
Treated			
Liver	0.91 ug/ml (± 0.034)	2.68×10^{-3} ug/mg	3.50×10^{-6} umol MT/mg
Kidney	0.62 ug/ml (± 0.019)	8.5×10^{-3} ug/mg	1.11×10^{-5} umol MT/mg
Testes	0.76 ug/ml (± 0.0855)	1×10^{-3} ug/mg	9.96×10^{-6} umol MT/mg
Control			
Liver	0.0	0.0	0.0
Kidney	0.0	0.0	0.0
Testes	0.0	0.0	0.0

*S.E.=Standard error of the mean
**MT-metallothionein
1) Based on binding ratio of 7 moles of metal per 1 mole of metallothionein (Vallee, 1979; and Kimura, 1979)

Table 4

Comparison of Survival of Irradiated Rats Treated with $CdCl_2$ vs. Non-Treated

Dose	% Survival $CdCl_2$ treated*	% Survival Non-Treated	Stat. Signif.
9 Gy	---	50	
9.5 Gy	50	0	$0.1>p>0.05$
10 Gy	10	0	N.S.

* Treated rats received 1mg $CdCl_2$/kg body weight 4 hours prior to irradiation.

CHAPTER V

DISCUSSION

The increased frequency of exposure to ionizing radiations makes it important to consider what treatments are possible to protect mammalian systems from its effects. This protection, aside from physical barriers such as distance or shielding, entails treatment with chemicals which react with or bind to the chemical moieties resultant from exposure to ionizing radiation (Arena, 1972). These moieties are produced by the primary interaction of the radiation with both organic and inorganic matter (Grosch and Hopwood, 1979). The compounds produced are either charged compounds or compounds whose reaction with other organic materials results in some form of damage. This damage may be mediated through enzyme inactivation, protein changes, alterations of the genetic complement or interruption of basic biochemical processes (Grosch and Hopwood, 1979). Production of such radicals, as the hydroxy and peroxy radicals and the superoxide radical $O_2^{\cdot -}$, may effectively alter organic material of primary importance to the cells survival (Willson, 1983).

The concept of protection from these radicals arose from work by Wall and Magat (1953), Prevot-Bernas (1953) and by Alexander and Charlesby (1954). The basic idea behind this protection is the reduction of oxidized compounds. This re-

duction in biological systems is most readily accomplished by compounds containing numerous sulfhydryl groups. The sulfhydryl group donates a hydrogen ion to the radical or to the compound which has been attacked by such a radical (Willson, 1983).

As already indicated, biological compounds which contain sulfhydryl groups lend themselves to the role of "repair" compounds, hopefully reacting with the radical prior to its oxidizing more important organic materials. In this role, a readily available compound, glutathione, plays an important part. Glutathione and its enzymatic relations glutathione reductase and peroxidase are extremely useful in coping with the insult produced from irradiation (Harris, 1983; Willson, 1983; and Meister, 1983). Glutathione's ability to protect against free radical attack is enhanced by its high concentration within the cell (Nygaard and Simic, 1983; Meister, 1983; Willson, 1983; and Harris, 1983). Any protective agent, to be of use, must <u>a priori</u> be present prior to the irradiation and also be in sufficient concentration to deal effectively with large numbers of free radicals (Arena, 1972; Grosch and Hopwood, 1979; and Willson, 1983). In this respect, a certain type of metalloprotein synthesized by all cells lends itself to use as a possible radioprotective agent. Metallothionein, a small molecular weight protein is synthesized in response to certain heavy metals. Metallo-

thionein is usually associated with zinc at some point in the metal's metabolism (Kagi and Vallee, 1961; Kagi, 1970; Bremner and Young, 1977; Kagi and Nordberg, 1979; Bethune, 1979; Webb, 1979; and Foulkes, 1982). Metallothionein has been shown to be both a storage and transport protein for this metal (Kagi and Vallee, 1961; Kagi, 1970; Bremner and Young, 1977; Kagi and Nordberg, 1979; Bethune, 1979; Webb, 1979; and Foulkes, 1982). Metallothionein is also induced by other metals, cadmium, mercury, copper, and bismuth. However, its synthesis is greatly stimulated by cadmium and mercury (Kagi and Vallee, 1961; Ryden and Deutsch, 1978; Webb, 1979; Whanger and Oh, 1979; and Kagi and Nordberg, 1979). Cadmium will displace zinc bound to metallothionein (Suzuki, 1982). The ability of metallothionein to bind large quantities of cadmium, 7 moles of metal per 1 mole of protein (Vallee, 1979) suggests that metallothionein may be useful in sequestering free radicals in a similar manner. Metallothionein's structure resembles that of other aminothiol radioprotective agents, in that it possesses a large number of cysteine residues. These residues are thought to be responsible for binding of metal ions to the protein (Vallee, 1979; and Kagi and Nordberg, 1979). Possessing the ability to form several sulfhydryl bonds, metallothionein resembles glutathione. The ability to induce the synthesis of this protein as opposed to waiting for a compound to diffuse into a cell also lends to

its usefulness as a protective agent.

A radiation lethality study performed on a group of rats exposed to 10 Gy, 9.5 Gy and 9 Gy resulted in the following. All animals exposed to 10 Gy of gamma radiation from a ^{60}Co source died within seven days. All animals exposed to 9.5 Gy died within ten days. In the 9 Gy exposure group, 50% of all animals exposed died within two weeks, with no further deaths recorded after 30 days. The 9 Gy dose of gamma radiation from the ^{60}Co source was accepted as the LD 50/30 dose.

Electron microscopic examination of the kidney, liver and testes provided good evidence that the dosage given (1 mg Cd^{2+}/kg body weight) was not injurious to the animals. In the kidney, the primary alterations due to cadmium intoxication are tubular and glomerular degeneration. In liver, the dose of cadmium required to induce acute injury is near the LD 50 level for cadmium. Morphological changes include abnormal mitochondrial alterations, dilation of the endoplasmic reticulum and in general, cellular necrosis, with increased collagen deposition. In the testes, it is reported that a single injection of cadmium results in testicular necrosis, with varying time of onset depending on which route is used. Examination of the three selected tissues demonstrated none of the reported pathological sequelae attributed to cadmium poisoning (Nriagu, 1983; and Nomiyama, 1977).

It was not possible in the electron microscopic study to

correlate the findings of this study with any pathological indicator (Figures 1-6). This provided clear evidence that no pathology due to cadmium was present. These data supported the idea that the only change attributable to the cadmium injection was the production of metallothionein.

A lethality study was performed on a separate group of animals to determine if the dosage of $CdCl_2$ given (1 mg/kg body weight) would induce mortality in any percentage of the study group after exposure to 8 Gy of gamma radiation from a ^{60}Co source. While the dosage of Cd^{2+} given is well below any reported in the literature as causing any deleterious effects, (Berry, 1972; Nishizumi, 1972; Kajikawa et al., 1973; Kajikawa and Kuroda, 1974, 1976; Kawai et al., 1976; Suzuki, 1974; Nordberg and Piscator, 1972; and Nomiyama et al., 1977), it was determined that in view of the problems inherent with using this strain of rats, it would be necessary to demonstrate that the dosage given did not induce mortality. The study demonstrated no lethal effects from the dose given.

Utilization of atomic absorption spectrometry on a group of animals injected with cadmium at a dose of 1 mg/kg body weight, demonstrated elevated levels of metallothionein as compared with controls (Table 3). In this study, the previous works of Bryan et al. (1976), Cempel and Webb (1976), Vallee (1979), Kimura et al. (1979), and Fuwa (1982) were

used to justify the determination of metallothionein content based on its binding characteristics with cadmium. Results from this study were correlated with a similar study on cadmium binding relationships with metallothionein by Suzuki (1982). He utilized atomic absorption spectrometry to analyze kidney homogenates. Injecting 300 ug $CdCl_2$/kg body weight subcutaneously, Suzuki was able to demonstrate a renal concentration of 3.33×10^{-4} ug Cd^{2+}/mg of kidney within six hours, in the present study the data indicates a twenty-eight-fold increase over Suzuki's concentration which may be attributed to the difference in concentration of $CdCl_2$ administered. The data obtained in this study demonstrated 8.5×10^{-3} ug Cd^{2+}/mg of kidney tissue. Based on the work of Vallee (1979), Kimura et al. (1979) and Kagi and Nordberg (1979), it is possible to extrapolate the quantity of metallothionein present in the kidney, liver and testes of the rats injected with $CdCl_2$ in this study. The values obtained, while not extraordinarily high, do indicate an increase over normal levels. Suzuki (1982) using 300 ug Cd^{2+}/kg body weight in rats obtained 4.36×10^{-7} umoles MT/mg of kidney. Kawai et al. (1976) found slight proximal tubular lesions and a cadmium concentration of 240 ug/g of kidney, after injecting 0.5 mg Cd^{2+}/kg body weight 6 days a week for 24 weeks.

Suzuki (1974) demonstrated proteinuria at a cadmium concentration of 225 ug/g in kidney after injecting 0.5 mg Cd^{2+}/kg body weight weekly for 25 weeks. Nomiyama et al. (1977) demonstrated cadmium levels of 225 ug/g of kidney after injecting 1 mg Cd^{2+}/kg body weight 5 times a week for 6 months.

The ultimate test of the hypothesis that metallothionein is a useful radioprotective agent, is the demonstration of an increase in the level of an LD 50/30 dose of radiation. In this study, an attempt was made to demonstrate an increase of 0.5 Gy and 1 Gy above the LD 50/30 level. The survival of 50% of the animals exposed to 9.5 Gy of gamma radiation is a clear indication of an extension above the LD 50/30 level. At the 10 Gy level, only one animal of the ten irradiated survived (Table 4). While this is by no means extraordinary, it does seem to indicate that some of the effects of the gamma radiation are being moderated by the presence of the induced metallothionein. Statistical analysis of the data on extension of the LD 50/30 using Student's t test shows a significant difference at the $p \leq 0.1$ level for treated animals receiving 0.5 Gy above the LD 50/30, but not for those animals receiving 1 Gy above the LD 50/30. These data would tend to indicate that metallothionein could be a useful radioprotective agent.

SUMMARY AND CONCLUSIONS

Fisher strain F-344 rats were injected subcutaneously with 1 mg/kg $CdCl_2$, in an effort to induce the metallo-protein metallothionein. This was done in an effort to determine what effect, if any, metallothionein would have on increasing the LD 50/30 dose of gamma radiation from a ^{60}Co source.

A group of ten animals was injected with 1 mg $CdCl_2$/kg body weight and allowed to synthesize metallothionein for 4 hours, this allowed complete synthesis (Cempel and Webb, 1976). Selected tissues of these animals, liver, kidney and testes were homogenized and analyzed for Cd-MT content utilizing atomic absorption spectrometry. Atomic absorption data indicated 3.50×10^{-6} umoles MT/mg of liver, 1.11×10^{-5} umoles MT/mg of kidney and 9.96×10^{-6} umoles MT/mg of testes. Analysis of electron micrographs of liver, kidneys and testes of animals given 1 mg $CdCl_2$/kg subcutaneously demonstrated no pathological changes attributable to cadmium intoxication. A group of 10 animals was injected subcutaneously with 1 mg $CdCl_2$/kg body weight. This was done to demonstrate that this dose was indeed below an LD 50/30 level for cadmium. No deaths were seen in this group after 30 days.

Administration of 1 mg $CdCl_2$/kg body weight increased

survivability by 50% at 0.5 Gy above the LD 50/30 level, and 10% at 10 Gy above the LD 50/30 level. Since the effects of the injected cadmium were negligible, as demonstrated by lethality studies and ultrastructural analysis of sensitive tissues, it is believed that the increase in the number of animals surviving at higher doses of radiation is attributable to the presence of higher than normal levels of metallothionein. It is possible that metallothionein is moderating the effect of the radiation by scavenging free radicals.

LITERATURE CITED

Alexander, P., A. Charlesby. Physico-chemical methods of protection against ionizing radiations. In: Radiobiology Symposium 1954. Z.M. Bacq, P. Alexander, eds. Academic Press, New York. Pp. 49-59. 1954.

Arena, V. Ionizing Radiation and Life. C.V. Mosby Co., Saint Louis. 1971.

Bacq, Z.M., P. Alexander. Fundamentals of Radiobiology, 2nd ed., Chapter 19. Pergamon Press, New York. 1961.

Berry, J.P. Renal lesions caused by cadmium: electron microscope studies and electron scan micro-analysis. Pathol. Biol. 20: 401-412. 1972.

Bethune, J. Metallothionein and other low molecular weight metal-binding proteins. In: Metallothionein. J.H.R. Kagi, M. Nordberg, eds. Birkhauser Verlag, Boston. P.36. 1979.

Bonnell, J.A. Cadmium poisoning. Ann. Occup. Hyg. 8: 45. 1965.

Bremner, I., B.W. Young. Isolation of (copper, zinc)-thioneins from the livers of copper-injected rats. Biochem. J. 157: 517-520. 1976.

Bremner, I., B.W. Young. Copper-thionein in the kidneys of copper-poisoned sheep. Chem. Biol. Interact. 19: 13-23. 1977.

Bryan, S.E., H.A. Hidalgo. Nuclear ^{115}cadmium: uptake and disappearance correlated with cadmium-binding protein synthesis. Biochem. Biophys. Res. Commun. 68: 858-866. 1976.

Cempel, M., M. Webb. The time-course of cadmium-thionein synthesis in the rat. Biochem. Pharmacol. 25: 2067-2071. 1976.

Chang, C.C., R.D. Van derMallie, J.S. Garvey. A radioimmunoassay for human metallothionein. Toxicol. Appl. Pharmacol. 55: 94-102. 1980.

Cherian, M.G., R.A. Goyer, L. Del Aquerriere Richardson. Cadmium-metallothionein induced nephrotoxicity. Toxicol. Appl. Pharmacol. 38: 399-408. 1976.

Cherian, M.G. Comparative studies on mechanism of cellular toxicity of cadmium metallothionein and $CdCl_2$ in rat kidney epithelial cell culture. Fed. Proc. (Abstr.) 40: 2767. 1981.

Clarkson, T.W., J.E. Kench. Urinary excretion of amino acids by men absorbing heavy metals. Biochem. J. 62: 361-372. 1956.

Dalrymple, G.V., M.L. Baker. Molecular biology. In: Medical Radiation Biology. G.V. Dalrymple, ed. W.B. Saunders Co., Philadelphia. Pp. 30-43. 1973.

Eldjarn, L., A. Pihl. Mechanisms of protective and sensitizing action. In: Mechanisms in Radiobiology. M. Errera, A. Forssberg, eds. Academic Press, New York. Vol. 2, Chapter 4. 1960.

Evans, G.W., P.E. Johnson. Copper- and zinc- binding ligands in the intestinal mucosa. In: Trace Element Metabolism in Man and Animals-3. M. Kirchgessner, ed. Pp. 98-105. 1978.

Foulkes, E.C. Renal tubular transport of cadmium-metallothionein. Toxicol. Appl. Pharmacol. 45: 505-512. 1978.

Foulkes, E.C., ed. Biological Roles of Metallothionein. Elsevier North Holland, New York. 1982.

Fowler, B.A., G.F. Nordberg. The renal toxicity of cadmium metallothionein: morphometric and x-ray microanalytic studies. Toxicol. Appl. Pharmacol. 46: 609-623. 1978.

Friberg, L. Health hazards in the manufacture of alkaline accumulators, with special reference to chronic cadmium poisoning. Acta. Med. Scand. 138: 1-124. 1950.

Friberg, L., M. Piscator, G.F Nordberg, T. Kjellstrom. Cadmium in the Environment, 2nd ed. CRC Press, Cleveland. 1974.

Fuwa, K. Metallothionein: Historical aspects of trace spectrochemical analysis. In: Biological Roles of Metallothionein. E.C. Foulkes, ed. Elsevier North Holland, New York. Pp. 1- 10. 1982.

Grosch, D.S., L.E. Hopwood. Biological Effects of Radiations, 2nd ed. Academic Press, New York. 1979.

Harris, J.W. Cellular thiols in radiation and drug response: Use of specific reagents. In: Radioprotectors and Anticarcinogens. O.F. Nygaard, M.G. Simic, eds. Academic Press, New York. Pp. 255-274. 1983.

Herve, A., Z.M. Bacq. Cyanure et dose lethale de rayons X.C.R. Seances Soc. Biol. Ses. Fil. 143: 881-883. 1949.

Hidalgo, H.A., V. Koppa, S.E. Bryan. Effects of cadmium on RNA polymerase and protein synthesis in rat liver. FEBS Lett. 64: 159-162. 1976.

Hutchinson, F. Sulfhydryl groups and the oxygen effect on irradiated dilute solutions of enzymes and nucleic acid. Radiat. Res. 14: 721-731. 1961.

Kagi, J.H.R., B.L. Vallee. Metallothionein: a cadmium and zinc-containing protein from equine renal cortex. II. Physiochemical properties. J. Biol. Chem. 236: 2435-2442. 1961.

Kagi, J.H.R. Hepatic metallothionein. Int. Cong. Biochem. Abstr., 8th, Switzerland, September, 1970. Pp. 130-131.

Kagi, J,H,R., S.R. Himmelhoch, P.D. Whanger, J.L. Bethune, B.L. Vallee. Equine hepatic and renal metallothioneins. Purification, molecular weight, amino acid composition, and metal content. J. Biol. Chem. 249: 3537-3542. 1974.

Kagi, J.H.R., M. Nordberg, eds. Metallothionein: Proceedings of the First International Meeting on Metallothionein and Other Low Molecular Weight Metal-binding Proteins. Zurich, July 17-22, 1978. Birkhauser Verlag, Boston. 1979.

Kajikawa, K., S. Katsuta, K. Kuroda. Electron-microscopic observation of the kidney of rats exposed to cadmium. Kankyo Hoken Rep. 24: 33. 1973.

Kajikawa, K., K. Kuroda. Electron-microscopic observation of the kidney of rats exposed to cadmium. Kankyo Hoken Rep. 31: 60. 1974.

Kajikawa, K., K. Kuroda. Light and electron microscopic findings in the kidneys of rats given cadmium for a long period. Kankyo Hoken Rep. 38: 146. 1976.

Kawai, K., K. Fukuda, M. Kimura. Morphological alterations in experimental cadmium exposure with special reference to onset of renal lesion. In: Effects and Dose-Response Relationships of Toxic Metals. G.F. Nordberg, ed. Elsevier, Amsterdam. Pp. 343-370. 1976.

Kazantzis, G. Respiratory function in men casting cadmium alloys. Part I. Assessment of ventilatory function. Brit. J. Ind. Med. 13: 30-36. 1956.

Kazantzis, G., F.V. Flynn, J.S. Spowage. Renal tubular malfunction and pulmonary emphysema in cadmium pigment workers. Quart. J. Med. 32: 165-192. 1963.

Kimura, M., N. Otaki, T. Kakefuda. Characterization of cadmium induced metallothionein in African green monkey kidney cells in vitro metallothionein. In: Metallothionein. J.H.R. Kagi, M. Nordberg, eds. Birkhauser Verlag, Boston. Pp. 187-196. 1979.

Margoshes, M., B.L. Vallee. A cadmium protein from equine kidney cortex. J. Am. Chem. Soc. 79: 4813-4814. 1957.

Meister, A. Glutathione metabolism and transport. In: Radioprotectors and Anticarcinogens. O.F. Nygaard, M.G. Simic, eds. Academic Press, New York. Pp.121-151. 1983.

Nishizumi, M. Electron microscopic study of cadmium nephrotoxicity in the rat. Arch. Environ. Health. 24: 215-225. 1972.

Nomiyama, K., H. Nomiyama, T. Taguchi. Effects of environmental temperatures on the chronic toxicity of cadmium in mice. Kankyo Hoken Rep. 41: 29-37. 1977.

Nordberg, G.F., M. Piscator, M. Nordberg. On the distribution of cadmium in blood. Acta. Pharmacol. Toxicol. 30: 289-295. 1971.

Nordberg, G.F., M. Nordberg, M. Piscator, O. Vesterberg. Separation of two forms of rabbit metallothionein by isoelectric focusing. Biochem. J. 126: 491-498. 1972.

Nordberg, G.F., M. Piscator. Influence of long-term cadmium exposure on urinary excretion of protein and cadmium in mice. Environ. Physiol. Biochem. 2: 37-49. 1972.

Nordberg, G.F., R. Goyer, M. Nordberg. Comparative toxicity of cadmium metallothionein and cadmium chloride on mouse kidney. Arch. Pathol. 99: 192-197. 1975.

Nordberg, M., G.F. Nordberg, M. Piscator. Isolation and characterization of a hepatic metallothionein from mice. Environ. Physiol. Biochem. 5: 396-403. 1975.

Nriagu, J.O., ed. Cadmium in the Environment Part II: Health Effects. Wiley and Sons, New York. 1981.

Nygaard, O.F., M.G. Simic, eds. Radioprotectors and Anti-carcinogens. Academic Press, New York. 1983.

O'Conner, M.K., J.F. Malone, M. Moriarty, S. Mulgrew. A radioprotective effect of vitamin C observed in Chinese hamster ovary cells. Br. J. Radiol. 50: 587-591. 1977.

Patt, H.M., E.B. Tyree, R.L. Straube, D.E. Smith. Cysteine protection against x-irradiation. Science. 110: 213-214. 1949.

Piscator, M. On cadmium in normal human kidneys together with a report on the isolation of metallothionein from livers of cadmium-exposed rabbits. Nord. Hyg. Tidskr. 45: 76-82. 1964.

Prevot-Bernas, A. Agents de transfert et protection chimique contre les rayonnements ionisants. J. Chem. Phys. 0: 445-446. 1953.

Redpath, J.L., R.L. Willson. Reducing compounds in radio-protection and radiosensitization: model experiments using ascorbic acid. Int. J. Radiat. Biol. 23: 51-65. 1973.

Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208. 1963.

Rodgers, K., M.G. Cherian. Toxicity of cadmium-metallothionein in rat kidney cell culture. The Toxicol. (Abstr.) 1: 300. 1981.

Ryden, L., H.F. Deutsch. Preparation and properties of the major copper-binding component in human fetal liver. Its identification as metallothionein. J. Biol. Chem. 253: 519-524. 1978.

Shaikh, Z.A., K. Hirayama. Metallothionein in the extracellular fluids as an index of cadmium toxicity. Environ. Hlth. Perspec. 28: 267-271. 1979.

Shenoy, M.A., B.B. Singh, A.R. Gopal-Ayengar. Enhancement of radiation lethality of E. coli B/r by procaine hydrochloride. Nature (London). 248: 415-416. 1974.

Squibb, K.S., J.W. Ridlington, N.G. Carmichael, B.A. Fowler. Early cellular effects of circulating cadmium-thioneins on kidney proximal tubules. Environ. Hlth. Perspec. 28: 287-296. 1979.

Squibb, K.S., J.B. Pritchard, B.A. Fowler. Renal metabolism and toxicity of metallothionein. In: Biological Roles of Metallothionein. E.C. Foulkes, ed. Elsevier North Holland. Pp. 181-192. 1982.

Stokinger, H.E. The metals (excluding lead). In: Industrial Hygiene and Toxicology, Vol. 2. Wiley, New York. 1963.

Stoll, R.E., J.F. White, T.S. Miya, W.F. Bousquet. Effects of cadmium on nucleic acid and protein synthesis in rat liver. Toxicol. Appl. Pharmacol. 37: 61-74. 1976.

Suzuki, K.T. Induction and degradation of metallothionein and their relation to the toxicity of cadmium. In: Biological Roles of Metallothionein. E.C. Foulkes, ed. Elsevier North Holland, New York. Pp. 215-235. 1982.

Suzuki, Y. Cadmium content in liver and kidney metallothionein by long-term cadmium administration. In: Proceedings of the 47th Annual Meeting of Japan Association for Industrial Health. Pp. 124-125. 1974.

Tsuchiya, K. Cadmium Studies in Japan: A Review. Biomedical Press, New York. 1978.

Vallee, B.L. Metallothionein: Historical review and perspectives. In: Metallothionein. J.H.R. Kagi, M. Nordberg, eds. Birkhauser Verlag, Boston. Pp. 19-40. 1979.

Wall, L.A., M. Magat. Degradation des polymeres par les rayons gamma et les neutrons. J. Chim. Phys. 50: 308-316. 1953.

Webb, M., A.T. Etienne. Studies on the toxicity and metabolism of cadmium-thionein. Biochem. Pharmacol. 26: 25-30. 1977.

Webb, M. Functions of hepatic and renal metallothioneins in the control of the metabolism of cadmium and certain other bivalent cations. In: Metallothionein. J.H.R. Kagi, M. Nordberg, eds. Birkhauser Verlag, Boston. Pp. 313-320. 1979.

Whanger, P.D., S.H. Oh. Nutritional and environmental factors affecting metallothionein levels. In: Metallothionein. J.H.R. Kagi, M. Nordberg, eds. Birkhauser Verlag, Boston. Pp. 281-292. 1979.

Willson, R.L. Electrophilic free radicals and nucleic acid damage: pulse radiolysis studies. Panminerva Med. 18: 391-402. 1976.

Willson, R.L. Iron, zinc, free radicals and oxygen in tissue disorders and cancer control. In: Iron Metabolism. Ciba Foundation Symp. 51. R. Porter, ed. Elsevier, Excerpta Medica, New York. Pp. 333-354. 1977.

Willson, R.L. Free radicals and tissue damage: mechanistic evidence from radiation studies. In: Biochemical Mechanisms of Liver Injury. T.F. Slater, ed. Academic Press, New York. Pp. 123-224. 1978.

Willson, R.L. Hydroxyl radicals and biological damage *in vitro*: What relevance *in vivo*? *In*: Oxygen Free Radicals and Tissue Damage. Ciba Foundation Series 65. D.W. Fitzsimons, ed. Excerpta Medica, New York. Pp. 19-42. 1979.

Willson, R.L. Free radical repair mechanisms and the interactions of glutathione and vitamins C and E. *In*: Radioprotectors and Anticarcinogens. O.F Nygaard, M.G. Simic, eds. Academic Press, New York. Pp. 1-22. 1983.