MINIREVIEW

Radiation Protection and Radiation Recovery with Essential Metalloelement Chelates (43939)

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Abstract. **Understanding essential metalloelement metabolism and its role in tissue maintenance and function, as well as the roles of essential metalloelement-dependent enzymes in responding to injury, offer a new approach to decreasing and/or treating radiation injury. This review presents the roles of some essential metalloelement-dependent enzymes in tissue maintenance and function, and their responses to radiation injury in accounting for radiation protection and recovery effects observed for nontoxic doses of essential metalloelement compounds. Effects of biochemicals including water undergoing bond radiolysis and the effects of free radicals derived from diatomic oxygen account for the acute and chronic aspects of radiation injury. Recognized biochemical roles of essential metalloelement-dependent enzymes and the observed pharmacological effects of small-molecular mass chelates predict the therapeutic usefulness of essential metalloelement complexes in decreasing and/or treatment of radiation injury. Copper chelates have radiation protection and radiation recovery activities and cause rapid recovery of immunocompetency and recovery from radiation-induced histopathology. Mice treated with $Cu(II)_2$(3,5-diisopropylsalicylate)$_4$ [$Cu(II)_2$(3,5-DIPS)$_4$] had increased survival and corresponding increases in numbers of myeloid and multipotential progenitor cells early after irradiation and earlier recovery of immune reactivity. Examination of radiation-induced histopathology in spleen, bone marrow, thymus, and small intestine also revealed $Cu(II)_2$(3,5-DIPS)$_4$-mediated rapid recovery of radiation-induced histopathology. Most recently, Fe, Mn, and Zn complexes have also been found to prevent death in lethally irradiated mice. These pharmacological effects of essential metalloelement chelates can be understood as due to facilitation of *de novo* synthesis of essential metalloelement-dependent enzymes which have roles in preventing the accumulation of pathological concentrations of oxygen radicals or repairing biochemical damage caused by radiation-induced bond homolysis. Essential metalloelement chelates offer a physiological approach to prevention and/or treatment of radiation injury.** [P.S.E.B.M. 1995, Vol 210]

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Essentiality of Cu, Fe, Mn, and Zn, and Their Speciation *in vivo*

Copper, iron, manganese, and zinc are essential metalloelements like sodium, potassium, calcium, magnesium, chromium, and cobalt. Just like essential amino acids, essential fatty acids, and essential cofactors (vitamins), these metalloelements are required by all cells for normal metabolic processes but cannot be synthesized *de novo* and dietary intake and absorption

are required to obtain them. Amounts of Cu, Fe, Mn, and Zn found in adult body tissues and fluids (1, 2) correlate with the number and kind of metabolic processes requiring them. However, there may be an aging-related decrease in tissues due to inadequate dietary intake and absorption.

Ionic forms of these metalloelements have particularly high affinities for organic ligands found in biological systems and rapidly undergo bonding interactions to form complexes or chelates in biological systems. Calculated amounts of ionic Cu (10^{-18} *M*), Fe (10^{-23} *M*), Mn (10^{-12} *M*), and Zn (10^{-9} *M*) in plasma (3) are extremely small and not measurable with any existing equipment and they are likely to be still smaller in solid tissues which contain many more bonding sites. Consequently, measured tissue Cu, Fe, Mn, and Zn contents reflect tissue contents of chelates, which are primarily metalloelement-dependent enzymes (Table I); proteins such as hemoglobin, ferritin, metallothioneines, β_2-macroglobulins, transferrin, transmanganin, and transcuprein; RNA and DNA chelates; small molecular mass amino acid, carboxylic acid, phosphate amine, diamine, and thiol chelates; and various small molecular mass peptide chelates (4). To study effects of essential metalloelements in a biological system, it is most *inappropriate* to use ionic forms and most *appropriate* to use relevant chelates, which have particularly important roles in mediating biochemical repair of radiation damage.

Essential Metalloelement Metabolism

Ingested foods and beverages contain these essential metalloelements in chelated forms that may yield other chelates as a result of ligand exchange in the digest or following absorption (4). However, some of the absorbed chelates may exist in the digest and in blood as the original chelate or a more stable ternary chelate. Absorbed metalloelement chelates undergo systemic circulation to all tissues and utilization by all cells following ligand exchange with small molecular mass ligands, apoproteins, and apoenzymes to form metalloproteins and metalloenzymes in *de novo* syntheses. These metalloelements are stored in the appropriate tissues as metallothioneins (MTs) or Fe-ferritin, or they are excreted in the event tissue needs have been met and stores replenished (4). There are no inducible excessive storage diseases known in normal individuals. However, there must be upper limits dictated by physiological requirements.

Stored essential metalloelements are released as chelates *via* ligand exchange to meet normal metabolic needs. This homeostatic release of relatively small amounts of essential metalloelement chelates meets normal physiologic requirements. Release of larger quantities in a pronounced mobilization of these metalloelements is a feature of interleukin-1 (IL-1)–mediated acute and chronic responses to many disease states (4 and cited references).

It is likely that the magnitude of these responses is adequate in normal, well-nourished individuals and allow them to overcome their disease. However, it is now generally recognized that dietary intakes of Cu, Fe, Zn (23, 24), and most likely Mn are less than the recommended daily intakes for the U.S. population and may be no better for individuals living in other developed countries. Individuals who have depleted stores of these essential metalloelements, such as cancer patients, may overcome radiation-induced pathology more slowly or not at all. The degree of radiation injury and the nutritional state of health of an individual may determine whether or not an individual will be able to overcome metalloelement-dependent repairable radiation damage. Treatment with essential metalloelement chelates either before and/or after radiation injury may support these essential metalloelement-dependent responses and facilitate recovery regardless of the state of nutriture.

Chemical Consequences of Ionizing Radiation

Since water represents 70% of the chemical composition of the adult body (90% of the infant body), its chemical transformation by ionizing radiation merits serious consideration. Ionizing radiolysis of water is well understood as producing very reactive aquated electrons, hydrogen atoms, hydroxyl radicals, hydrogen peroxide, and protonated water, as well as superoxide (O_2^-) and hydroperoxyl radical in the presence of oxygen. Hydroperoxyl radicals, hydroxyl radicals, hydrogen atoms, and aquated electrons have very short half-lives of the order of milliseconds and consequently react rapidly with cellular components in reduction, oxidation, initiation, insertion, propagation, and addition reactions causing loss of function and the need for biochemical replacement and/or repair. Ionizing radiation can also impart sufficient energy to all biochemicals to cause homolytic bond breaking and produce all conceivable organic radicals in considering C-C, C-N, C-O, C-H, P-O, S-O, etc. bond homolysis. These radicals will undergo the above listed radical reactions to cause further destruction and the need for replacement and/or repair. A third consequence of ionizing radiation is homolytic or heterolytic bond breaking of coordinate-covalent bonded metalloelements. These are the weakest bonds in biochemical molecules and potential sites of greatest damage which may be most in need of replacement and/or repair since many repair enzymes are metalloelement-dependent, as are the copper-dependent cytosolic and extracellular superoxide dismutases (CuSODs).

Recognizing that loss of essential metalloelement-dependent enzyme activity may at least partially account for lethality of ionizing radiation and that Cu-,

Table I. Copper-, Iron-, Manganese-, and Zinc-Dependent mammalian Enzymes

	Function
Copper-dependent enzymes (4–9)	
Cytochrome c oxidase	Reduction of oxygen
Extracellular and cytosolic Cu-dependent and Zn-modulated Superoxide Dismutases (Cu_2Zn_2SOD)	Superoxide disproportionation
Tyrosinase	Synthesis of dihydroxyphenylalanine
Dopamine-β-mono-oxygenase	Synthesis of norepinephrine and epinephrine
Neurocuprein	Synthesis of norepinephrine and epinephrine
Amine oxidases	Metabolism of primary amines
Factor V	Blood clotting
Ceruloplasmin	Cu transport, mobilization of stored Fe, and angiogenesis
α-Amidating mono-oxygenases	Synthesis of neuroendocrine hormones including: gastrin, choleocystokinin, melanocyte stimulating hormone, calcitonin, vasopressin, secretin, and some enkephalins
Procollagen and proelastin peptidyllysyl oxidases	Collagen and elastin cross-linking
Other possible copper-dependent enzymes (10–14)	
Adenylate cyclase	Synthesis of c-AMP
Guanylate cyclase	Synthesis of c-GMP
Lipolytic protein	Lipolysis
ACE1 and *CUP2*	Metallothioneine gene transcription regulatory proteins
Iron-dependent enzymes (5)	
Electron Transport cytochromes, including cytochrome c oxidase	Oxidation-Reduction
Catalase	Disproportionation of hydrogen peroxide
P-450s	Activation of oxygen
Lipoxygenase	Conversion of arachidonic acid to 5-HPETE and 5-HETE
Cyclo-oxygenase	Conversion of arachidonic acid to PGG_2
Xanthine oxidase	Purine metabolism
Peptidylprolyl and peptidyllysyl oxidases	Hydroxylation of procollagen and proelastin
Manganese-dependent enzymes (5, 15–20)	
Mn-dependent superoxide dismutase	Disproportionation of superoxide
Arginase	Syntheses of urea and ornithine
Pyruvate carboxylase	Synthesis of oxaloacetate
Pseudocatalase	Disproportionation of H_2O_2
Calmodulin-dependent protein phosphatase	Phosphate ester hydrolysis
α-Isopropylmalate synthetase	Synthesis of α-isopropylmalate
A brain adenylate cyclase	Synthesis of c-AMP
Catechol-O-methyltransferase	Synthesis of catechol methyl ether
Farnesyl pyrophosphate synthetase	Synthesis of farnesyl pyrophosphate
Glycosyl synthetase	Synthesis of glycosyl-phosphonucleotides
Extradiol-cleaving dioxygenase	Insertion of dioxygen
A ganglioside galactosyl-transferase	Galactose transfer
Saccharide polymerases	Synthesis of polymeric carbohydrates
Glycosyltransferases	Synthesis of glycosides
A galactosylhydroxylysyl connective glucosyltransferase	Glycosaminoglycan and glycoprotein tissue component syntheses
Zinc-dependent enzymes (5, 21, 22)	
Cu_2Zn_2SOD	Disproportionation of superoxide
Aminopeptidase	Protein hydrolysis
Aldehyde hydrase	Aldehyde hydration
Esterase	Ester hydrolysis
Methylmalonyl-oxaloacetate transcarboxylase	Transcarboxylation
Carboxypeptidases A and B	Protein hydrolysis
NAD-dependent dehydrogenases	Oxidations
Carbonic anhydrase	Dehydration of carbonic acid
α-Hydroxyacid dehydrogenase	Oxidation of α-hydroxy acids
Alkaline phosphatase	Phosphorylation
Purine and pyrimidine nucleoside kinases	Phosphorylation of nucleosides
DNA polymerase and gyrase	DNA synthesis
"Zinc-finger" proteins	Transcription regulating proteins

Fe-, Mn-, and Zn-dependent enzymes have roles in protecting against accumulation of O_2^- as well as facilitating repair (25) may explain the radiation protection and radiation recovery activities of Cu, Fe, Mn, and Zn compounds. It is suggested that the IL-1–mediated redistributions of essential metalloelements have a general role in responding to radiation injury. These redistributions may also account for subsequent *de novo* synthesis of metalloelement-dependent enzymes required for biochemical repair and replacement of cellular and extracellular components needed for recovery from radiolytic damage.

De novo syntheses of metalloelement-dependent enzymes required for utilization of oxygen and prevention of O_2^- accumulation as well as tissue repair processes including metalloelement-dependent DNA and RNA repair are key to the hypothesis that essential metalloelement chelates decrease and/or facilitate recovery from radiation-induced pathology. A widely held understanding is that O_2^- accumulation due to the lack of normal concentrations of CuSODs, reduction of O_2 leading to relatively large steady-state concentrations of O_2^-, or inappropriate release of O_2^- from oxygen-activating centers lead to the formation of much more reactive oxygen radicals. These more reactive oxygen radicals include: singlet oxygen and hydrogen peroxide produced as a result of acid-dependent self disproportionation or water-catalized disproportionation of O_2^-, hydroxyl radical, and hydroperoxyl radical (26, 27). Facilitated syntheses of CuSODs and catalase, and prevention of the formation of these oxygen radicals may account for some of the recovery from pathological changes associated with irradiation.

Radiation Protection and Recovery with Essential Metalloelement Chelates

There is a rich history of the limited use of essential metalloelements as radioprotective agents for which we are indebted to those who persisted in pursuing this area of research (28–29). Unfortunately, results of earlier studies could not be explained in terms of what was then known about essential metalloelement metabolism. Now, these results can be explained on the basis of roles of essential metalloelement-dependent enzymes in preventing the accumulation of toxic metabolic products, facilitating biochemical, cellular, and tissue repair in facilitating recovery from radiation injury.

Radiation Protection and Recovery with Copper Compounds

The SOD-mimetic and lipophilic complex, $Cu(II)_2$(3,5-diisopropylsalicylate)$_4$ [$Cu(II)_2$(3,5-DIPS)$_4$], was originally found to produce 58% survival in $LD_{100/30}$ γ-irradiated (10 Gy, 0.4 Gy/min) mice (30), when $Cu(II)_2$(3,5-DIPS)$_4$ was given subcutaneously (sc) at a dose of 80 μmol/kg of body mass 24 hr before irradiation (31). These results have been extended by Steel *et al.* (32) with the report that 25 or 50 μmol/kg $Cu(II)_2$(3,5-DIPS)$_4$ given sc are effective in increasing survival of $LD_{100/30}$-irradiated mice.

It was subsequently determined that a 20 μmol/kg dose of $Cu(II)_2$(3,5-DIPS)$_4$ was as effective as 80 μmol/kg when it was given sc 3 hr before an $LD_{50/30}\gamma$ irradiation (8 Gy, 1.55 Gy/min) producing 92% or 80% survival in male or female mice, respectively (33). Subcutaneous treatment with much smaller doses of 2.5 or 5.0 μmol/kg 3 hr after irradiation increased survival from 48% for vehicle-treated mice to 88% or 92%, respectively. These results have been confirmed and extended by Suriyachan *et al.* (34) with the report that $Cu(II)_2$(3,5-DIPS)$_4$ given intraperitoneally (ip) at a dose of 10 μmol/kg either before or after irradiation (8 Gy, 0.51 Gy/min) produced 60% or 75% survival, respectively, in male rats versus 40% for vehicle-treated rats. Male mice treated orally with 25 or 50 μmol/kg either 24 or 4 hr before irradiation had increased survivals of 75% or 95%, respectively, compared with 50% survival for the vehicle-treated group (33, 35). All doses of $Cu(II)_2$(3,5-DIPS)$_4$ used in these studies were nontoxic and ranged from 1/5 to 1/100 of acutely toxic dose for male or female mice (33).

It is of special interest to note that the $LD_{50/7}$ for $Cu(II)_2$(3,5-DIPS)$_4$ in female mice and rats, 261 ± 36 μmol/kg, is much higher than male mice and rats, 91 ± 13 μmol/kg (32–34). It is now known that acutely lethal doses of $Cu(II)_2$(3,5-DIPS)$_4$ produce marked central nervous system (CNS) depression resulting in loss of parasympathetic regulation of cardiac and pulmonary function (35). Smaller doses produce short duration hypnotic or sedative effects (35). Limited available data for $Cu(II)_2$(3,5-DIPS)$_4$ suggest that it may produce a sedative-hypnotic effect at about 30 μmol/kg (33, 35). Since hypnotics have some radioprotectant activity (28, 36) the hypoxia associated with hypnotic activity of Cu chelates may have some mechanistic implications concerning their radioprotectant application. A report that both $Cu(II)_2$(3,5-DIPS)$_4$ and Cu(II)Cl_2 produced kidney damage, "Cu-toxicosis", following a single sc injection of large lethal doses (32) was not substantiated with data for $Cu(II)_2$(3,5-DIPS)$_4$ treatment, nor was it supported by the published literature for $Cu(II)Cl_2$ treatment (37, 38), and this toxicity was not observed when 25 μmol $Cu(II)_2$(3,5-DIPS)$_4$/kg was given sc twice daily for up to 2.5 months (39).

$Cu(II)_2$(3,5-DIPS)$_4$ did not prevent hematopoietic and immunologic damage to irradiated mice, since 98%–99% of myeloid progenitor cells were destroyed and immune reactivity was ablated (40–42). However, $Cu(II)_2$(3,5-DIPS)$_4$ did accelerate the repopulating of bone marrow and spleen following irradiation. This was demonstrated as increased cellularity and the earlier recovery of myeloid progenitor cells responding to interleukin-3 or granulocyte-macrophage colony–stimulating factor (41, 42). The recovery of hematopoietic activity was accelerated whether mice were treated with the copper compound 3 hr before or 3 hr after irradiation. Mice treated with $Cu(II)_2$(3,5-DIPS)$_4$ had increased levels of the multipotential progenitor cells, endogenous CFU-S. Multipotential progenitor cells can generate both myeloid and lymphoid progenitor cells. Consistent with this, mice treated with this

compound recovered responses to T- and B-cell mitogens earlier than control irradiated mice and generated uniformly better antibody responses to a T-dependent antigen (40). $Cu(II)_2(3,5\text{-}DIPS)_4$ may act by inducing the generation of hematopietic cells, since it increases the number of myeloid progenitor cells in the spleen of nonirradiated mice (43).

The question still remained as to whether or not there was any correlation between these observations and recovery of radiation-induced tissue damage. Histopathological studies of spleen, bone marrow, thymus, and small intestine were conducted in parallel with immunological studies of animals exposed to $LD_{50/30}$ irradiation (8.0 Gy, 1.55 Gy/min) alone, 40 μmol/kg $Cu(II)_2(3,5\text{-}DIPS)_4$ alone, irradiation and a single dose of vehicle or $Cu(II)_2(3,5\text{-}DIPS)_4$ treatment. The protective effects of $Cu(II)_2(3,5\text{-}DIPS)_4$ treatment against irradiation-induced histopathology were apparent in these tissues. These effects can be best exemplified by changes that occurred in the spleen. Twenty-four hr after irradiation, total disintegration of splenic follicles (white pulp) and total depletion of lymphocytes were observed in animals unprotected by $Cu(II)_2(3,5\text{-}DIPS)_4$ (Fig. 1, A and B). Severe atrophy and fibrosis of this organ was observed on the 7th (Fig. 2) and 14th (Fig. 3) day, respectively, after irradiation. Both reduction of tissue damage and enhancement of tissue recovery were evident in animals treated with $Cu(II)_2(3,5\text{-}DIPS)_4$. While there were still signs of splenic follicle disintegration 24 hr after irradiation, many lymphocytes in these follicles were preserved (Fig. 4). Signs of recovery were also demonstratable by the 7th day after irradiation in $Cu(II)_2(3,5\text{-}DIPS)_4$-treated mice. A definitive recovery in splenic size and a gradual increase in splenic cellularity was evident by the 14th day after irradiation (Fig. 5).

Similar reductions in tissue damage and enhancement of tissue repair were observed in bone marrow, thymus, and intestine from animals treated with $Cu(II)_2(3,5\text{-}DIPS)_4$. These observations lead to the general conclusion that although $Cu(II)_2(3,5\text{-}DIPS)_4$ does not prevent tissue injury when it is given before irradiation, it facilitates rapid recovery from radiation-induced injuries. These recoveries from radiation-induced histopathology offer a biological marker for the re-establishment of the integrity of those tissues that are known to be most vulnerable to irradiation. It also serves as a basis for the increase in survival and recovery of immunological function following $Cu(II)_2(3,5\text{-}DIPS)_4$ treatment.

$Cu(II)_2(3,5\text{-}DIPS)_4$ has also been observed to inhibit radiation-induced carcinogenesis as a result of its anticlastogenic effect in C3H 10 T 1/2 cells (44). This anticlastogenic effect may, in part, be attributed to suppression of DNA replication as a result of blockade of cell growth in the S phase as observed in CHO cells

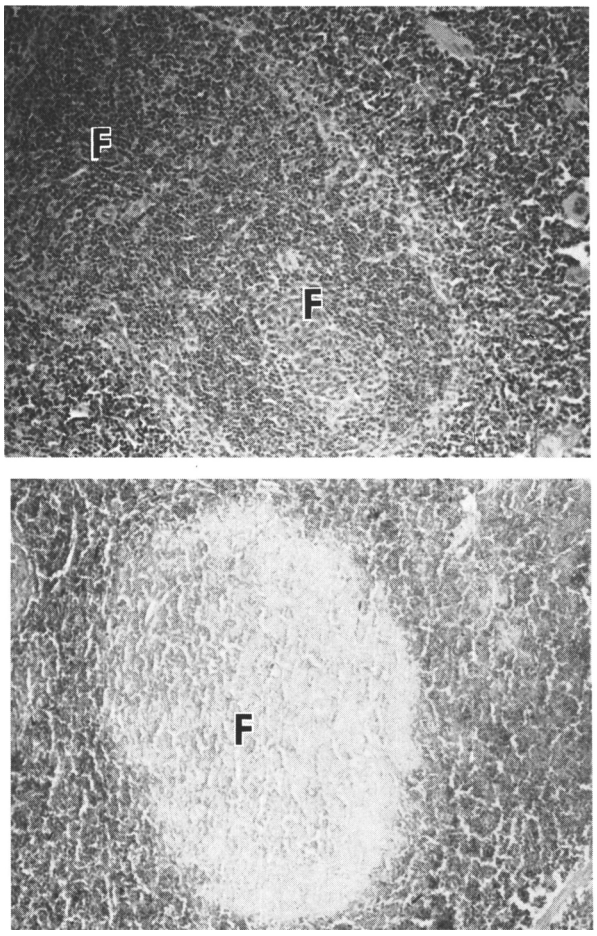

Figure 1. (A) Spleen, normal control. Note the abundance of cellalrity forming lymphocytic aggregates (follicles) in the spleen. ×400. (B) Spleen from vehicle-treated mouse 24 h after irradiation. Note total depletion of lymphocyte in the follicle (F) ×400.

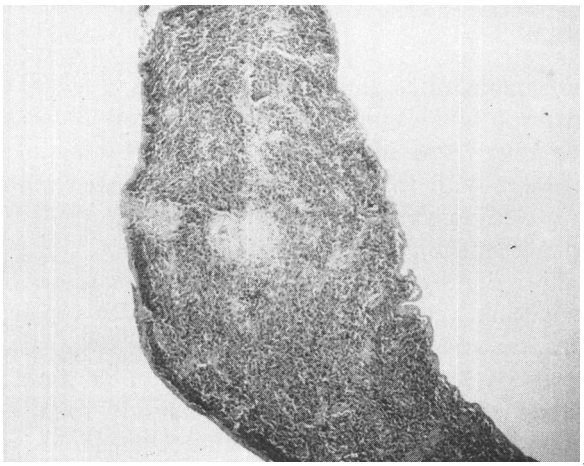

Figure 2. Spleen, from vehicle-treated mouse 7 days after irradiation. Note persistent severe atrophy of the organ. ×400.

treated with $Cu(II)SO_4$ (45). An earlier suggestion that $Cu(II)SO_4$ was clastogenic in mouse bone marrow cell experiments was refuted by Tinwell and Ashby (46),

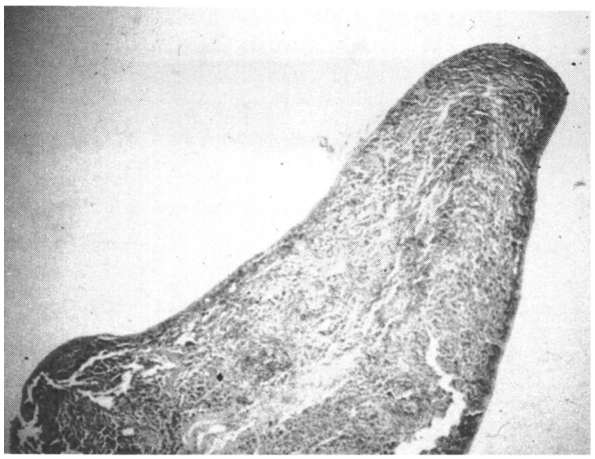

Figure 3. Spleen, from vehicle-treated mouse, 14 days after irradiation. The atrophied spleen also appeared to be fibrotic. ×400.

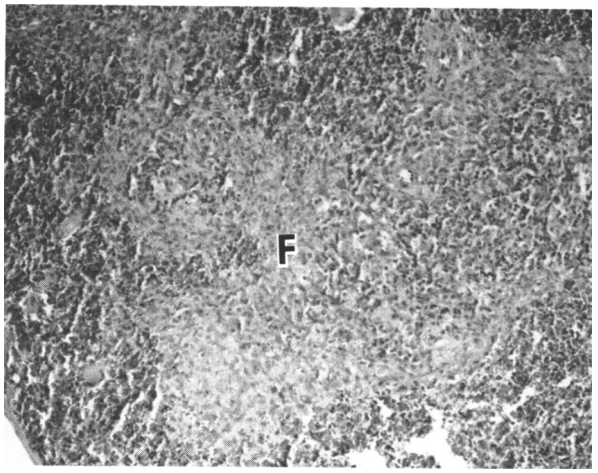

Figure 4. Spleen from $Cu(II)_2(3,5\text{-}DIPS)_4$-treated mouse 24 h after irradiation. While dispersion of the follicle (F) and reduction of cellularity were seen, many lymphocytes were still preserved. ×400.

who demonstrated that $Cu(II)SO_4$, which yields Cu chelates in *in vitro* and *in vivo*, was not clastogenic in two mouse bone marrow assays. These effects are consistent with the report that mice treated with 3 mmol $Cu(II)(glycinate)_2$/kg 15 min before irradiation with a clastogenic dose of irradiation (4.5 Gy, 0.88 Gy/min) rapidly recovered from bone marrow cell damage and recovery was associated with repair of DNA damage (47). Prevention or repair of DNA strand breaks by $Cu(II)_2(indomethacinate)_4$ in phorbol treated human leukocytes represents additional evidence supporting a Cu-dependent mechanism for prevention or repair of DNA damage (48).

Steel *et al.* (32) found that 24 to 48 μmol/kg doses of $Cu(II)_2(3,5\text{-}DIPS)_4$ and 49 to 96 μmol/kg doses of $Cu(II)Cl_2$ given sc 24 hr prior to irradiation were radioprotective in mice irradiated with 10 Gy (0.4 Gy/min), however, doses of 133 to 901 μmol/kg 3,5-DIPS acid produced no radiation protection, which has since

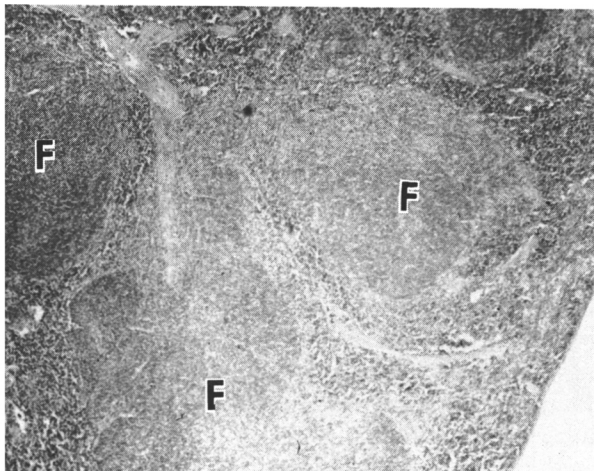

Figure 5. Spleen from $Cu(II)_2$ $(3,5\text{-}DIPS)_4$-treated mouse 14 days after irradiation. The spleen returned to full size and many follicles (F) were reforming. ×400.

been confirmed (35). In contrast, treatment with 10 or 20 μmol $Cu(II)_2(3,5\text{-}DIPS)_4$ after $LD_{50/30}$ irradiation revealed that $Cu(II)_2(3,5\text{-}DIPS)_4$ was more effective that $Cu(II)Cl_2$. These two doses of $Cu(II)Cl_2$ only increased survival 20% or 29% above vehicle-treated control female mice (56% survival), while the two copper equivalent doses of $Cu(II)_2(3,5\text{-}DIPS)_4$ increased survival 75% or 88% above vehicle-treated control mice (32% survival) (unpublished). It is most likely that Cu chelates formed *in vivo* following the administration of $Cu(II)Cl_2$ ultimately account for its radioprotectant effects. However, use of chelates avoids the irritation caused by the injection of $Cu(II)Cl_2$ or $Cu(II)SO_4$, which are strong Lewis acids. Copper chelates are less acutely toxic than these inorganic forms of Cu and the use of chelates also enables tissue targeting with regard to essential metalloelement distribution.

Immunological properties of the 3-morpholine-2-hydroxypropyl ether of dextran (49) led to the discovery of superior immunostimulant properties of the Cu chelate of the 3-mercapto-2-hydroxypropyl ether of dextran (50), which afforded nearly 100% survival of mice infected with *Shegella sonnei* dysentery bacilli (51), attenuated tumor growth and increased survival of sarcoma 180 implanted mice, suppressed spontaneous leukemia and prolonged survival of AKR-mice (52), and had some radioprotectant activity in 6 Gy–irradiated 129 Ao/Boy female mice (53).

It is of special interest that the report by Matsubara *et al.* (54) contains two footnotes, one reporting results of a preliminary radioprotection study using $Cu(II)SO_4$ and one reporting $Cu(II)SO_4$ induction of metallothioneine (MT). An 8 μmol/kg dose of $Cu(II)SO_4$ given sc to male mice irradiated with 5.5 Gy produced 50% survival versus 37% survival for control mice, which was correlated with an increase in liver MT synthesis. Induction of MT synthesis may provide

a reservoir of Cu for systemic responses enabling increased survival. Its presence in cells may also offer local protection of cytosolic enzymes by removing aquated electrons and oxygen radicals. The presence of Cu also offers an accounting for the observation that enzymatic activity, penicillinase, persisted in irradiated solutions (55).

A dose of 3992 μmol/kg WR-2721 [S-2-(3-aminopropylamino)ethylphosphorothioic acid] given ip to male mice produced 97% mortality, while this same dose of WR-2721 plus a 5 μmol/kg dose of $Cu(II)SO_4$ given ip 3 hr before the injection of WR-2721 decreased lethality to 10% (56). However, the same dose of $Cu(II)SO_4$ given 24 hr before treatment only decreased lethality to 30%. When this 3922 μmol/kg dose of WR-2721 was mixed with the 5 μmol/kg dose of $Cu(II)SO_4$, mortality was again 90%. This observation is consistent with the possibility that all of the Cu was lost as a result of the formation of Cu(II)sulfide or $Cu(II)_3(PO_4)_2$, which formed in our experience when Cu(II) salts and WR-2721 are mixed. The 5 μmol/kg dose of $Cu(II)SO_4$ given ip 2 hr before a 2713 μmol/kg dose of WR-2721 given orally 1 hr before irradiation (14 Gy, 1 Gy/min) increased survival from 15% with WR-2721 treatment alone, to 34%. When this dose of $Cu(II)SO_4$ was given 3 hr before irradiation and a 775 μmol/kg dose of WR-2721 given 0.5 hr before irradiation, survival was increased from 38% to 85%. These results support the combination treatment approach to radiation protection designed to overcome toxicities of existing radioprotectants and improve their radioprotective efficacy, as had been originally suggested by Doherty (57), and reduce tumor protection of existing radioprotectants (56). Treatment ip with a physical mixture of 775 μmol/kg WR-2721/kg and 5 μmol/kg $Cu(II)SO_4$ produced marked increases in survival of 14 or 15 Gy–irradiated mice. While formation of Cu(II) sulfide or $Cu(II)_3(PO_4)_2$ in this mixture may have resulted in loss of protection with regard to lethality of WR-2721, Cu(II)sulfide or $Cu(II)_3(PO_4)_2$ may still have had a beneficial effect with regard to radiation protection by facilitating de novo syntheses of Cu-dependent enzymes.

$Cu(II)_2(3,5\text{-DIPS})_4$ was originally examined for radioprotectant activity based upon reports that Cu_2Zn_2SOD had radioprotectant activity (58). However, two recent reports suggest that superoxide disproportionation by Cu_2Zn_2SOD does not account for its radioprotectant activity. Inactivated polyethyleneglycol derivatized Cu_2Zn_2SOD was as effective as the enzymatically active derivative (59). This result and the possibility that these proteins are in fact antigenic (60), although reduced antigenicity is always claimed, may lead to phagocyte elaborated IL-1 and/or some other cytokine that initiates immune and repair responses that account for increased survival. Another report by Scott et al. (61) demonstrated that increased radiation sensitivity of three *Escherichia coli* clones was related to increased FeSOD activity. However, radiation sensitivity in these clones may actually be due to O_2^- produced and allowed to accumulate in these clones following radiation-induced loss of Fe from this Fe-dependent enzyme and production of inactive apoenzyme. If differing concentrations of Fe-SOD in these clones is due to relative need to remove O_2^- produced under normal metabolic conditions, then more O_2^- is produced by the clone having the highest concentration of FeSOD and this clone is most labile to radiation-induced death with radiolysis of the coordinate-covalent bonded Fe in this enzyme.

The hypothesis that there is radiolytic loss of an essential metalloelement cofactor has merit and accounts for the 20% loss of both Cu-dependent and Mn-dependent SODs in intestinal smooth muscle of rats immediately (1 hr) following irradiation (15 Gy) (62). Activities of both enzymes increased over a 20-hr period to a level 45% higher than normal with a further increase in Mn SOD by 72 hr accounting for the further increase in total SOD activity, a likely result of de novo synthesis in response to injury.

Another possible mechanistic accounting for the radioprotectant/radiorecovery activity of Cu chelates involves Cu-dependent nuclear metabolism (63). It is known that 20% of cellular Cu is found in the nucleus. Copper has the highest bonding affinity for nucleic acids of all of the essential metalloelements. Nucleic acid phosphate and base donor sites yield Cu chelates that are much more stable than Cu chelates of most amino acids and near the stability of protein chelates. This stability has been used to support suggestions of intrastrand and interstrand DNA bridging, which was recognized early as having a role in stabilization of the Watson-Crick double helix. Higher physiologic concentrations account for inhibition of ribonuclease syntheses: initiation, nucleotide condensations, and release of newly formed mRNA. However, the original observation that injection of $Cu(II)Cl_2$ into rats induced the synthesis of mRNA coding for the synthesis of MT has been explained as due to the formation of a Cu-dependent DNA binding protein ACE1 or CUP2, the protein products of S. cerevisiae (*ACE1*) and E. coli (*CUP2*) genes, which recognize upstream activation sequences required for active transcription of CUP1, a yeast MT gene (64–67). Since radiation injury and injection of Cu chelates induce MT synthesis and increased MT synthesis correlates with increased survival, Cu-dependent transcription and translation are important nuclear responses in overcoming radiation injury.

Spassky and Sigman (68) recognized nuclease-mimetic activity for the $Cu(I)(o\text{-phenanthroline})_2$ chelate as a valuable footprinting reagent useful in reveal-

ing details of RNA polymerase bonding to the lac promoter. Predictable chromatin DNA cleavage by this chelate due to preferential bonding of the chelate to DNA spacer segments was also suggested to be a useful characterizing feature (69). Interpretations that these and other DNA strand scissions resulting from Cu(I) chelates are damaging (70, 71) without recognizing that these scissions may be due to expected Cu chelate (72) or other essential metalloelement chelate (73) catalyzed diphosphate ester hydrolyses, which may be viewed as endonuclease- (or exonuclease-) mimetic activity merit reconsideration.

Two genes, SoxR and SoxS, are involved in activating the*soxR* regulon gene (74–77). The predicted SoxR and SoxS proteins may constitute a novel two-compartment regulatory system in which these two proteins act sequentially to activate transcription of various regulon genes including *soxR* in response to O_2^- stress. Two soxR regulon proteins, endonuclease IV (*E. coli*) and Apn1 (*S. cerevisiae*), have recently been suggested to be metalloelement-dependent endonucleases (78). Both proteins were found to contain various essential metalloelements including: Cu, Fe, Mn, and Zn. The order of abundance in endonuclease IV is: Zn > Mn > Cu > Fe and in Apnl it is: Zn > Fe > Mn > Cu. Both proteins are active in removal of base or sugar damaged nucleotides, the penultimate steps in repair of damaged DNA.

Radiation Protection with Iron Compounds

Since radiolytic loss of Fe from Fe-dependent enzymes is also a possible consequence of ionizing radiation, the 3,5-diisopropylsalicylate chelate of Fe, Fe(III)-(3,5-DIPS)$_3$, was examined for radioprotectant activity (33, 35). Doses ranging from 0 to 280 μmol/kg produced 50% to 84% survival in $LD_{50/30}$-irradiated (8 Gy, 1.55 Gy/min) male mice. Treatment with 35, 70, or 140 μmol Fe(III)-(3,5-DIPS)$_3$ before a $LD_{100/30}$ irradiation revealed that only the 35 μmol/kg dose increased survival, 300% above vehicle-treated control mice (8% survival). Treatment with doses of 17.5, 35, or 70 μmol Fe(III)-(3,5-DIPS)$_3$ after an $LD_{100/30}$ irradiation failed to increase survival above vehicle-treated control mice (unpublished). Effective doses of Fe(III)-(3,5-DIPS)$_3$ were less than 1/4 of the acutely toxic dose for male mice, 1173 ± 213 μmol/kg (33). Acute toxicity of this complex was also due to CNS depression with a sedative-hypnotic dose of 407 μmol/kg (35). The IC_{50} for Fe(III)-(3,5-DIPS)$_3$ as an SOD-mimetic was found to be >650 μ*M* at pH 7.8 in 50 m*M* phosphate buffer using the xanthine/xanthine oxidase cytochrome C system (unpublished observation). It is known that Fe(III) chelates have very weak SOD-mimetic activity with rates four orders of magnitude slower than the corresponding Cu chelate as in the case of Cu(II) and Fe(III)-picolinates (79). Since it is unlikely that disproportionation of O_2^- accounts for the radioprotectant activity of this chelate, catalase-mimetic activity and facilitated syntheses of Fe-dependent enzymes required for tissue or cellular repair processes, including DNA repair, (74), and perhaps gene-regulating proteins may ultimately account for its mechanism of action as a radioprotectant or radiorecovery agent.

Radiation Protection with Manganese Compounds

Subcutaneous injection of 182 μmol Mn(II)Cl_2/kg in saline 24 hr prior to 6.3 Gy (0.7 Gy/min) irradiation produced 94% survival (54). These mice gained body mass throughout the 30-day period of these experiments, while the saline-treated control mice lost considerable body mass during the first 21 days of the postirradiation period. This dose of Mn(II)Cl_2 produced 87%, 72%, 75%, or 0% survivals as the total dose of irradiation was increased from 6.7 to 7.2, 7.6, or 8.1 Gy. This dose also increased liver MT synthesis 6-fold above nontreated and nonirradiated control values, demonstrating radiation-induced MT synthesis in association with host radioresistance, consistent with an earlier report by Shiraishi *et al.* (80) of radiation-induced MT synthesis in rat liver. Manganese(II)Cl_2 treatment was also reported to *prevent* the 90% decrease in leukocytes observed for control mice with 6.3 Gy irradiation, however, the decrease in leukocyte count following 8 Gy was not prevented. Mn(II)Cl_2-treated 8 Gy-irradiated mice had leukocyte counts that were 19% of those found for Mn-treated nonirradiated mice whereas nontreated irradiated controls had leukocyte counts of only 10% of normal. Leukocyte counts also recovered more rapidly in Mn-treated mice than in nontreated irradiated controls.

Administration of 182 μmol Mn(II)Cl_2 24 hr before a 7-Gy irradiation and either 130 to 1000 μmol/kg 2-aminoethylisothiouronium dihydrobromide (AET) or 200 to 3000 μmol/kg mercaptoethylamine (MEA) 5 min prior to irradiation was approximately twice as effective as treatment with AET or MEA alone (81).

A dose of 55 μmol $Mn(III)_2(II)(O)$(3,5-DIPS)$_6$/kg given sc to female or male mice 3 hr before the $LD_{50/30}$ irradiation (8 Gy, 1.55 Gy/min) produced 100% or 96% survival, respectively (33, 35).

Treatment with doses of 10, 20, or 40 μmol $Mn(III)_2(II)(O)$(3,5-DIPS)$_6$/kg 3 hr after a $LD_{50/30}$ irradiation revealed that the 40 μmol/kg dose increased survival 130% above vehicle-treated control mice survival (40% survival) (82). Repeating this experiment using doses of 30, 40, and 50 μmol $Mn(III)_2(II)(O)$(3,5-DIPS)$_6$ again demonstrated that the 40 μmol/kg dose was most effective producing a 229% increase in survival above vehicle-treated control mice (28% survival) while the 30 and 50 μmol/kg doses produced

200% or 214% increases in survival respectively (unpublished).

Treatment with 50 μmol $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_6$ before an $LD_{100/30}$ irradiation and then 10, 20, or 40 μmol/kg on three successive days after irradiation produced survivals of 28%, 12%, or 16%, respectively (unpublished). These are infinite increases in survival compared to the vehicle-treated control group which had no survivors. Treatment with 40, 80, or 160 μmol $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_6$ before a $LD_{100/30}$ irradiation revealed that only the 80 and 160 μmol/kg doses were effective, producing survivals of 16% or 27%, respectively, with no surviving vehicle-treated and irradiated control mice (unpublished). Treatment with doses of 20, 40, or 80 μmol $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_6$ after a $LD_{100/30}$ irradiation revealed that only the 40 μmol/kg dose was effective, increasing survival 333% above the vehicle-treated control mice (12% survival) (unpublished).

These doses of $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_6$ are 1/10 to 1/9 the acutely toxic dose of this chelate in male or female mice respectively (33). Acutely lethal doses of $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_6$, 353 ± 32 or 421 ± 46 μmol/kg for male or female mice, respectively, cause CNS depression and its sedative-hypnotic dose was found to be 130 μmol/kg (35).

The IC_{50} for $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_2$ as an SOD-mimetic is 1.5 μ*M* at pH 7.8 in 50 m*M* phosphate buffer using the xanthine/xanthine oxidase nitroblue tetrazolium system (unpublished observation). Either SOD-mimetic activity, facilitation of *de novo* synthesis of MnSOD, or synthesis of other Mn-dependent enzymes would seem to be plausible modes of action for $Mn(III)_2(II)(O)(3,5\text{-}DIPS)_6$ in accounting for its radioprotectant or radiorecovery activity. In this regard, it is of special interest that *Deinococcus radiodurans IR*, a bacterial strain known for its extremely high radiation resistance due to very efficient DNA repair, accumulates large amounts of Mn, which is required for the DNA repair enzyme, UV endonuclease β. Addition of $Mn(II)SO_4$ to medium of stationary phase deinococcal cultures unexpectedly caused a reduction in resistance to UV-and x-irradiation (83). Addition of 2.5 μ*M* of $Mn(II)SO_4$ or $Mn(II)Cl_2$ resulted in a 9-fold increase in cell number due to a triggering of at least three rounds of cell division in cells about to enter the stationary phase. This increase in cell division did not occur when these cells were in exponential growth or death phases. The Mn(II)-induced cell division produced daughter cells of reduced size, pigmentation, and radioresistance, however, they had increased activity and increased oxygen toxicity defense enzymes, SOD and catalase. This suggests that these cells are actively respiring and making more O_2^- as a consequence. Irradiation-induced loss of these protective enzymes as well as Mn-dependent endonuclease activity (74) and other as yet unknown Mn-dependent transcription- and translation-regulating proteins could account for an increase in cell death with concomitant induced cell division and irradiation.

Radiation Protection with Zinc Compounds

Anticipated mortality was not found for mice given drinking water containing 1000 μg (15.3 μmol)/mL Zn as $Zn(II)_2(acetate)_4$, and irradiated with an $LD_{50/30}$ dose of γ-radiation (6.53 Gy, 0.92 Gy/min) (84). ^{65}Zn turnover increased in these mice demonstrating a marked increase in metabolic redistribution. It was also found that sc injection of 39 or 153 μmol $Zn(II)_2(acetate)_4$/kg given in saline 24 hr before an $LD_{50/30}$ dose of radiation (6.3 Gy, 0.7 Gy/min) produced 62% or 81% survival, respectively, versus 25% for control mice given a sc saline injection (54). While the 39 μmol/kg dose of $Zn(II)_2(acetate)_4$ did not measurably increase liver MT synthesis the 153 μmol/kg dose increased synthesis 5-fold by 24 hr after injection. Both doses followed by 6.3 Gy irradiations increased MT synthesis 4 and 8-fold respectively by 24 hr after irradiation, consistent with an earlier report of irradiation-induced MT synthesis in rat liver (80).

Subcutaneous injection of 37 μmol of $^{65}Zn(II)Cl_2$/kg 1 and 3 days before irradiation with an $LD_{30/30}$ dose of radiation (6.5 Gy) produced 100% survival and injection 1 and 3 hr before 7.7 or 5.5 Gy produced 60% survival versus 40% survival for noninjected control mice (85). Treatment with a 5.9 μmol/kg dose of $Zn(II)Cl_2$ given ip 2 hr prior to a 2713 μmol/kg oral dose of WR-2721, given 1 hr before irradiation (14 Gy, 1 Gy/min), increased survival from 15% to 56% (56). This 5.9 μmol/kg dose of $Zn(II)Cl_2$ given ip 2.5 hr prior to the 775 μmol/kg dose of WR-2721 given ip 0.5 hr before irradiation increased survival from 38% to 68%. Mixtures of WR-2721 and $Zn(II)Cl_2$ were also effective in increasing radioprotectant activity of WR-2721 (56). Mice irradiated with 14 or 15 Gy (1 Gy/min) and treated with 775 μmol WR-2721/kg ip had survivals of 50% or 6% respectively. Treatment ip with a physical mixture of this dose of WR-2721 and 5.9 μmol/kg dose of $Zn(II)Cl_2$ gave survivals of 81% and 31%, respectively, following 14 or 15 Gy irradiations. However, 37 μmol $Zn(II)Cl_2$ injected sc at some unstated time after a 7.7-Gy iradiation produced only 5% survival. Postirradiation treatment with this dose of $Zn(II)Cl_2$ was more toxic than irradiation alone.

Since addition of Zn to culture medium (0.15 μmol/ml) *did not protect human melanoma cells* through a radiation dose range of 1.5 to 8.0 Gy (0.85 Gy/min), it was concluded that the protective effect of Zn was due to the facilitation of some homeostatic mechanism which was suggested to be induction of MT synthesis and some undefined cooperative biochemical role in facilitating the glutathione-dependent

reduction in radiosensitivity of normal cells (86). In support of this suggestion, it was pointed out that mouse fibroblasts having high levels of MT were more radioresistant. It was speculated that MT was either capable of scavenging O_2^- or hydroxyl radicals or it had a role in a glutathione (GSH) sparing mechanism. It was also pointed out that studies of MT gene regulation identified the involvement of bacterial lipopolysaccharide, IL-1, interferon, and other immunostimulants with control of MT synthesis in the liver (54). These observations coupled with the observation that IL-1 has radioprotectant activity (87) support the hypothesis that MT synthesis has a role in reducing radiosensitivity and that induction of MT synthesis is a physiological response to stress as described for IL-1 or endotoxin mediated responses to stress (87, 88).

Floersheim and Floersheim were the first to report the remarkable radioprotectant effects of Zn(II)(D,L-aspartate)$_2$ (89). Treatment with doses of 31, 61, 92, or 122 μmol/kg given ip to female mice 10, 30, or 120 min before irradiation (9, 10, 11, 12, or 13 Gy, 0.3 Gy/min) revealed that the maximally effective dose was 92 μmol/kg given 30 or 120 min before irradiation. This 92 μmol/kg dose given 10, 30, or 120 min before a 9 Gy irradiation produced 88%, 80%, or 90% survival, respectively, while treatment before the 12 Gy irradiation produced 3%, 57%, or 35% survival. Treatments with $Zn(II)Cl_2$, $Zn(II)SO_4(H_2O)_7$, Cu(II)D,L-aspartate, or Na aspartate were either less effective or ineffective. Treatment with Cu_2Zn_2SOD ip 10 min before irradiation failed to increased survival.

Zinc(II)(D,L-aspartate)$_2$ was also found to be more effective than GSH and cysteine, as effective as MEA, and slightly less effective than AET. A combination of 92 μmol Zn(II)(D,L-aspartate)$_2$/kg and 1974 μmol MEA/kg produced 100% survival of 12 Gy irradiated mice. The combination of 92 μmol Zn(II)(aspartate)$_2$/kg and 890 μmol AET/kg produced 100% survival of 13 Gy irradiated mice. These remarkable results were subsequently confirmed by Weiss *et al.* (90). Increasing doses of either agent and simultaneous administration decreased survival. Synergistic responses were only observed for combinations containing less than the maximally effective dose of each component. The fact that Zn(II)(D,L-aspartate)$_2$ and the combination protocols were ineffective when given after irradiation suggested a protective mechanism. Mechanistically, it was suggested that Zn(II)(D,L-aspartate)$_2$ provided Zn in an advantageous form which underwent cell membrane translocation and subsequent formation of Zn(II)-thiol chelates *in vivo*.

The LD_{50} values for Zn(II)(D,L-aspartate)$_2$, $Zn(II)Cl_2$, and $Zn(II)SO_4(H_2O)_4$ were found to be 168, 176, or 164 μmol/kg, respectively, following ip administration, and the effective dose of Zn(II)(D,L-aspartate)$_2$ was only one half of the acutely toxic dose. The advantage of Zn(II)(aspartate)$_2$ was suggested to be better toleration and longer duration of action, being effective when given up to 120 min before irradiation while MEA and AET were ineffective when given at this time. It was also pointed out that even low doses of these aminothiols are accompanied by intolerable side effects in humans. High nontoxic doses of Zn(II)(D,L-aspartate)$_2$ and aminothiols such as WR-2721, MEA, and AET were lethal when given in combination and the nature of this synergistic toxicity, while unknown, was suggested to be due to CNS depression resulting in cardiorespiratory failure. It remains to be determined as to whether or not Zn(II)(D,L-aspartate)$_2$ will protect neoplastic cells.

Following the earlier observation that $Zn(II)Cl_2$ enhanced normal tissue radioprotectant activity of WR-2721, it was found that ip injection of 61 μmol $Zn(II)Cl_2$/kg and 1415 μmol WR-2721 decreased tumor volume 8-fold and there was a 38% increase in tumor growth delay (91). Tumor protection by WR-2721 was reversed by this $Zn(II)Cl_2$ treatment and evidenced a therapeutic (anticancer) gain as well as a radiation protection gain since combination treatment was more effective than WR-2721 in protecting or enabling repair of normal tissues.

Treatment with 60 μmol $Zn(II)_2(3,5\text{-DIPS})_4$/kg produced 95% survival in $LD_{50/30}$ (8 Gy, 1.55 Gy/min) irradiated male mice (32, 34). Acute toxicity of this chelate was also due to CNS depression with a sedative-hypnotic dose of 123 μmol/kg (35). However, the effective dose of $Zn(II)_2(3,5\text{-DIPS})_4$ was only 1/6 of the acute toxic dose 381 ± 75 μmol/kg. (33).

Since Zn compounds including $Zn(II)_2(3,5\text{-DIPS})_4$ have no SOD-mimetic activity (92, 93), activation or synthesis of Cu_2Zn_2SOD or other Zn-dependent enzymes including endonucleases (78) and other known and suspected Zn-dependent transcription and translation processes (94, 95) must at least partially account for their radioprotectant or radiorecovery activity. Another partial accounting may be attributed to protection of spleen and bone marrow stem cells by decreasing DNA replication and blockade in the S growth phase as observed for CHO cells treated with $Zn(II)Cl_2$(45).

All of these data support the use of Cu, Fe, Mn, and Zn chelates as a physiological approach to preventing and/or treating radiation injury (35). Essential metalloelement chelates offer many advantages, including reduced toxicity, both for the study of mechanisms by which radiation kills cells and for drug development (96). Facilitation of *de novo* syntheses of antioxidant enzymes and site-directed hydrophilic or hydrophobic enzyme-mimetic chelates offer approaches to these ends (97).

Conclusions

Understanding essential metalloelement metabolism and its role in responding to radiation injury as mediated by immunomodulating cytokines offers a renewed and better understood approach to protection against hematopoietic and gastrointestinal syndromes and perhaps, cardiovascular and central nervous system syndromes. Copper, Fe, Mn, and Zn chelates have radioprotectant activity based upon results obtained with $Cu(II)_2(3,5\text{-DIPS})_4$, Cu-(3-mercapto-2-hydroxypropyl ether of dextran), $Cu(II)Cl_2$, $Cu(II)SO_4$; $Fe(III)(3,5\text{-DIPS})_3$; $Mn(III)_2(II)(O)(3,5\text{-DIPS})_6$, $Mn(II)Cl_2$; and $Zn(II)_2(3,5\text{-DIPS})_4$, $Zn(II)(aspartate)_2$, $Zn(II)_2(acetate)_4$, $Zn(II)SO_4$, and $Zn(II)Cl_2$. It is noteworthy that radiation protection with $Mn(II)Cl_2$ was not associated with radiation-induced weight loss during the postirradiation period. Multiple treatments, as observed with $Mn(III)_2(II)(O)(3,5\text{-DIPS})_6$ and $Zn(II)Cl_2$, also offer promise with regard to further increasing survival. It is likely that chelates of these essential metalloelements are more effective as radioprotectant and radiorecovery agents since they are less toxic than their inorganic forms and they represent more bioavailable forms of these metalloelements enabling tissue-targeting and site-directed approaches to protection and recovery. Existing radioprotectant aminothiols may form chelates *in vivo,* which may account for their beneficial effects, however, this chelate formation may result in the removal of metalloelements from required sites and partially account for the intolerable toxicities of these aminothiols. Administration of synthesized complexes of them would overcome this problem. Inorganic forms of these metalloelements form chelates *in vivo,* though indiscriminent bonding which may cause toxicity and require the use of larger quantities of these metalloelements to compensate for losses due to the formation of chelates that are not therapeutically useful.

Essential metalloelement chelates may also be useful in postirradiation treatment as radiation recovery agents with recovery of immunocompetency, based upon observations with $Cu(II)_2(3,5\text{-DIPS})_4$, $Mn(III)_2(II)(O)(3,5\text{-DIPS})_6$, and $Zn(II)Cl_2$. Postirradiation treatment remains to be examined thoroughly with individual as well as combination treatment using more than one essential metalloelement (i.e., Cu, Fe, Mn, Zn, etc. chelates).

Combination treatment with aminothiol radioprotectorants and essential metalloelement chelates is an established approach to improving radiation protection based upon results obtained with combinations of MEA, AET, or WR-2721 and $Mn(II)Cl_2$, $Zn(II)(DL\text{-aspartate})_2$, $Zn(II)Cl_2$, or $Cu(II)SO_4$, which produce marked increases in efficacy and reduced toxicity.

Copper complexes including $Cu(II)_2(3,5\text{-DIPS})_4$ have anticancer activity in animal models of solid tumor growth. These chelates have anticarcinogenic activity in *in vitro* and *in vivo* models of carcinogenesis using recognized carcinogens and in recognized models of initiation and promotion paradigms. They also have antimutagenic activity in model systems using polycyclic aromatic hydrocarbons or irradiation. All of these activities speak to the merits of using copper chelates in treating neoplastic diseases and in particular treating patients undergoing ionizing radiation therapy for their neoplastic disease. The question as to whether or not copper chelates protect neoplastic cells from effects of irradiation remains to be studied. $Zinc(II)Cl_2$ does not protect irradiated neoplastic cells grown in culture or *in vivo.*

Essential metalloelement salts and chelates also have antimicrobial activity, and these chelates may have value with regard to preventing or treating infection in LD_{100} irradiated individuals which is the ultimate cause of death.

Non-toxic doses of essential metalloelements decrease the toxicity of existing radioprotectants and their chelates have larger separations between effective and acutely toxic doses than existing radioprotectants. Also, combination radioprotectant therapy which is markedly more effective than single component therapy is achieved with less than maximal doses of each component. The principal acute toxicity of essential metalloelement chelates appears to be graduated central nervous system depression. Doses smaller than acutely toxic doses cause a decrease in physical activity, larger doses induce sleep, and still larger doses cause CNS depression with cessation of cardiac and pulmonary function.

Although essential metalloelement metabolism is still not completely understood—and this is especially true for nuclear metabolism, current understanding is sufficient to support the suggestion that pharmacological uses of essential metalloelement chelates offers a physiological approach to decreasing and/or treating radiation injury.

1. Tipton IH, Cook MJ. Trace elements in human tissue II: Adult subjects from the United States. Health Phys **1:**103–145, 1963.
2. Iyengar GV, Kollmer WE, Bowen JM. The Elemental Composition of Human Tissues and Body Fluids. New York: Springer, 1978.
3. May PM, Williams DR. Computer simulation of chelation therapy: Plasma mobilizing index as a replacement for effective stability constants. FEBS Lett **78:**134–138, 1977.
4. Sorenson, JRJ. Copper complexes offer a physiological approach to treatment of chronic disease. Prog Med Chem **26:**437–568, 1989.
5. Smith EL, Hill RL, Lehman IR, Lefkowitz RJ, Handler P, White A. Principals of Biochemistry: General Aspects and Mammalian Biochemistry (7th ed). New York: McGraw-Hill, 1983.
6. Prohaska JR. Biochemical functions of copper in animals. In:

Prasad AS, Ed. Essential and Toxic Trace Elements in Human Health and Disease. New York: Alan R. Liss, p105–124, 1988.
7. Mains RE, Eipper BA, Glembotski CC, Dores RM. Strategies for the biosynthesis of bioactive peptides. Trends Neuro Sci **6**:229–235, 1983.
8. Weber E, Esch FS, Bohlen P, Paterson S, Corbett AD, McKnight AT, Kosterlitz HW, Barchas JD, Evans CJ. Metrophamide: Isolation structure, and biologic activity of an amidated opiod octapeptide from bovine brain. Proc Natl Acad Sci USA **80**:7362–7366, 1983.
9. Liebisch DC, Seizinger BR, Michael G, Herz A. Novel opioid peptide amidorphin: Characterization and distribution of amidorphin-like immunoreactivity in bovine, ovine, and porcine brains, pituitary and adrenal medulla. J Neurochem **45**:1495–1503, 1985.
10. Baba A, Kihara T, Lee E, Iwata H. Activation of rat brain adenylate cyclase by copper plus dithiothreitol. Biochem Pharmacol **30**:171–174, 1981.
11. White A, Crawford KM, Patt CS, Lad PJ. Activation of soluble guanylate cyclase from rat lung by incubation or hydrogen peroxide. J Biol Chem **251**:7304–7312, 1976.
12. Gerzer R, Bohme E, Hofmann F, Schultz G. Soluble guanylate cyclase purified from bovine lung contains heme and copper. FEBS Lett **132**:71–74, 1981.
13. Halevy O, Sklan DA. A low molecular weight zinc-copper lipolytic protein from chick liver. Lif Sci **34**:1945–1951, 1984.
14. Culotta VC, Hsu T, Hu S, Furst P, Hamer D. Copper and the ACE 1 regulatory protein reversibly induce yeast metallothionein gene transcription in a mouse extract. Proc Natl Acad Sci **86**:8377–8381, 1989.
15. Lawrence GD, Sawyer DT. The chemistry of manganese. Coord Chem Rev **27**:173–193, 1978.
16. Korc M. Manganese homeostasis in human and its role in disease states. In: Prasad AS, Ed. Essential and Toxic Trace Elements in Human Health and Disease. New York: Alan R. Liss, p253, 1988.
17. Schramm VL, Wedler FC. Manganese in Metabolism and Enzyme Function. New York: Academic Press, 1986.
18. Neer EJ. Interaction of soluble brain adenylate cyclase with manganese. J Biol Chem **254**:2089–2096, 1979.
19. Leach RM Jr. Role of manganese in mucopolysaccharide metabolism. Fed Proc **30**:991–994, 1971.
20. Myllyla R, Anttinen H, Kivirikko KI. Metal activation of galactosylhydroxylysyl glucosyl-transferase, an intracellular enzyme of collagen biosynthesis. Eur J Biochem **101**:261–269, 1979.
21. Westin G, Shaffner W. Heavy metal ions in transcription factors from Hela cells: Sp 1, but not octamer transcription factor requires zinc for DNA binding and for activator function. Nucleic Acid Res **16**:5771–5781, 1988.
22. Klug A, Rhodes D. Zinc fingers: A novel protein fold for nucleic acid recognition. Cold Spring Harbor Symp Quant Biol **52**:473–482, 1987.
23. Klevay LM. Ischemic heart disease: Toward a unified theory. In: Lei KY, Carr TP, Eds. Role of Copper in Lipid Metabolism. Boca Raton: CRC Press, p233–267, 1990.
24. Wright HS, Guthrie HA, Wong M-Q, Bernardo V. The 1987–88 nationwide food consumption survey: An update on the nutrient intake of respondents. Nutr Today **26**:21–27, 1991.
25. Sorenson JRJ. An evaluation of altered copper, iron, magnesium, manganese, and zinc concentrations in rheumatoid arthritis. Inorg Perspect Biol Med **2**:1–26, 1978.
26. Fee JA, Valentine JS. Chemical and physical properties of superoxide. In: Michelson AM, McCord JM, Fridovich I, Eds. Superoxide and Superoxide Dismutases. New York: Academic Press, p19–60, 1977.
27. Corey EJ, Mehrota M, Khan AU. Water induced dismutation of superoxide anion generates singlet molecular oxygen. Biochem Biophys Res Commun **145**:842–846, 1987.
28. Foye WO. Radioprotective drugs. In: Wolf ME, Ed. Burger's Medicinal Chemistry. New York: John Wiley and Sons, p11–45, 1981.
29. Schubert J. Copper and Peroxides in Radiobiology and Medicine. Springfield, IL; Charles C. Thomas, 1964.
30. Sorenson JRJ. Bis(3,5-diisopropylsalicylato)copper(II), a potent radioprotectant with superoxide dismutase mimetic activity. J Med Chem **27**:1747–1749, 1984.
31. Sorenson JRJ, Soderberg LSF, Barnett JB, Baker ML, Salari H, Bond K. Radiation protection with Cu(II)$(3,5\text{-DIPS})_2$. Rec Trav Chim **106**:391, 1987.
32. Steel L, Seneviratne S, Jackson WE, III. Toxicity and radioprotective efficacy of bis(3,5-diisopropylsalicylate) copper(II) and $CuCl_2$. In: Cerutti P, Nygaard OF, Simic MG, Eds. Anticarcinogenesis and Radiation Protection. New York: Plenum Press, p355–360, 1988.
33. Sorenson JRJ, Soderberg LSF, Baker ML, Barnett JB, Chang LW, Salari H, Willingham WM. Radiation recovery agents: Cu(II), Mn(II), Zn(II), or Fe(III) 3,5-diisopropylsalicylate complexes facilitate recovery from ionizing radiation induced radical mediated tissue damage. In: Emerit I, Auclair C, Packer L, Eds. Antioxidants in Therapy and Preventive Medicine. New York: Plenum Press, Vol **264**:p69–78, 1990.
34. Suriyachan D, Patarakitvanit S, Ratananakorn P. Radioprotective effect of copper$(II)_2(3,5\text{-diisopropylsalicylate})_4$ in rats. Royal Thai Army Med J **42**:145–148, 1989.
35. Sorenson JRJ, Soderberg LSF, Chang LW, Willingham WM, Baker ML, Barnett JB, Salari H, Bond K. Copper, Iron, Manganese, and Zinc 3,5-diisopropylsalicylate complexes increase survival in gamma irradiated mice. Eur J Med Chem **28**:221–229, 1993.
36. Prasad KN, Acute Radiation syndromes. In: Pizzarelli DJ, Colombetti LG, Eds. Radiation Biology. Boca Raton: CRC Press, p205–235, 1982.
37. Haywood S. The effect of excess dietary copper on liver and kidney of the male rat. J Comp Pathol **90**:217–232, 1980.
38. Haywood S. Copper toxicosis and tolerance in the rat. I—Changes in copper content of the liver and kidney. J Pathol **145**:149–158, 1985.
39. Torregrosa, D, Kasemeier SL, Sorenson JRJ, Chang LW. Effects of Cu(II)$(3,5\text{-DIPS})_2$ on solid Ehrlich's cell tumor in mice: A pathological study. In: Sorenson JRJ, Ed. Biology of Copper Complexes. Clifton, NJ: Humana Press, p371–386, 1987.
40. Soderberg LSF, Barnett JB, Baker ML, Salari H, Sorenson JRJ. Copper(II)$(3,5\text{-diisopropylsalicylate})_2$ accelerates recovery of B and T cell reactivity following irradiation. Scand J Immunol **26**:495–502, 1987.
41. Soderberg LSF, Barnett JB, Baker ML, Salari H, Sorenson JRJ. Copper$(II)_2(3,5\text{-diisopropylsalicylate})_4$ stimulates hemopoiesis in normal and irradiated mice. Exp Hematol **16**:577–580, 1988.
42. Soderberg LSF, Barnett JB, Baker ML, Salari H, Sorenson JRJ. Post-Irradiation treatment with copper$(II)_2(3,5\text{-diisopropylsalicylate})_4$ enhances radiation recovery and hemopoietic regeneration. Exp. Hematol **18**:801–805, 1990.
43. Soderberg LSF, Barnett JB, Sorenson JRJ. Copper complexes stimulate hemopoiesis and lymphopoiesis. In: Keis C, Ed. Copper Bioavailability and Metabolism. New York: Plenum Press, p209–217, 1989.
44. Kennedy AR, Troll W, Little JB. Role of free radicals in the initiation and promotion of radiation transformation in vitro. Carcinogenesis **5**:1213–1218, 1984.
45. Costa M, Cantoni O, deMars M, Swartzendruber DE. Toxic metals produce an S-Phase-specific cell cycle block. Res Commun Chem Path Pharmacol **38**:405–419, 1982.

46. Tinwell H, Ashby J. Inactivity of copper sulfate in a mouse bone-marrow micronucleus assay. Mutat Res **245**:223–226, 1990.
47. Jagetia GC, Ganapathi NG. Effect of copper glycinate on the radiation induced micronuclei formation in mice bone marrow. Radiat Environ Biophys **29**:115–118, 1990.
48. Birnboim HC, Kanabus-Kaminska M. The production of DNA strand breaks in human leukocytes by superoxide anion may involve a metabolic process. Proc Natl Acad Sci USA **82**:6820–6824, 1985.
49. Wieczorek Z, Gieldanowski J, Zimecki M, Skibinski G, Mioduszewski JZ. Immunological properties of 3-morpholine-2-hydroxypropyl ether of dextran (H-2). Arch Immunol Therap Exp **31**:677–689, 1983.
50. Wieczorek Z, Gieldanowski J, Zimecki M, Skibinski G, Mioduszewski JZ. Immunobiological properties of the complex of bivalent copper with 3-mercapto-2-hydroxypropyl ether of dextran (C-79). Arch Immunol Therap Exp **31**:691–700, 1983.
51. Wieczorek Z, Kowalewska D, Gieldanowski J, Zimecki M, Mioduszewski JZ. Anti-infectious properties of the complex of bivalent copper with 3-mercapto-2-hydroxypropyl ether of dextran (C-79). Arch Immunol Therap Exp **31**:701–705, 1983.
52. Wieczorek Z, Gieldanowski J, Hraba T, Zimecki M, Mioduszewski JZ. Oncostatic properties of the complex of bivalent copper complex with 3-mercapto-2-hydroxypropyl ether of dextran (C-79). Arch Immunol Therap Exp **31**:707–713, 1983.
53. Wieczorek Z, Gieldanowski J, Zimecki M, Mioduszewski JZ, Szymaniec S, Daczynska R. Radioprotective activity of the complex of bivalent copper with 3-mercapto-2-hydroxypropyl ether of dextran (C-79). Arch Immunol Therap Exp **31**:715–729, 1983.
54. Matsubara J, Tajima Y, Karasawa M. Promotion of radioresistance by metallothionein induction prior to irradiation. Environ Res **43**:66–74, 1987.
55. Samuni A, Chevion M, Czapski G. Unusual copper-induced sensitization of the biological damage due to superoxide radicals. J Biol Chem **256**:12632–12635, 1981.
56. Weiss JF, Kumar KS, Walden TL, Neta R, Landauer MR, Clark EP. Advances in radioprotection through the use of combined agent regimens. Int J Radiat Biol **57**:1–14, 1990.
57. Doherty DG. Chemical protection to mammals against ionizing radiation. In: Hollaender A, Ed. Radiation Protection and Recovery. Oxford: Pergamon Press, p45–86, 1960.
58. Petkau A. Radiation protection by superoxide dismutase. Photochem Photobiol **28**:765–774, 1978.
59. Westman NG, Marklund SL. Inability of polyethylene-glycol substituted copper- and zinc-containing superoxide dismutase to protect mice against radiation lethality. Acta Oncol **26**:483–487, 1987.
60. Vaille A, Jadot G, Elizagaray A. Anti-Inflammatory activity of various superoxide dismutases on polyarthritis in the Lew rat. Biochem Pharmacol **39**:247–255, 1990.
61. Scott MD, Meshnick SR, Eaton JW. Superoxide dismutase amplifies organismal sensitivity to ionizing radiation. J Biol Chem **264**:2498–2501, 1989.
62. Summers RW, Maves BV, Reeves RD, Arjes LJ, Oberley LW. Irradiation increases superoxide dismutase in rat intestinal smooth muscle. Free Rad Biol Med **6**:261–270, 1989.
63. Agarwal K, Sharma A, Talukder G. Effects of copper on mammalian cells components. Chem-Biol Interact **69**:1–16, 1989.
64. Buchman C, Skroch P, Welch J, Fogel S, Karin M. The *CUP2* gene product, regulator of yeast metallothionein expression, is a copper-activated DNA-binding protein. Mol Cell Biol **9**:4091–4095, 1989.
65. Buchman C, Skroch P, Dixon W, Tullius TD, Karin M. A single amino acid change in *CUP2* alters its mode of DNA binding. Mol Cell Biol **10**:4778–4787, 1990.
66. Ecker DJ, Butt TR, Crooke ST. Yeast metallothionein: Gene function and regulation by metal ions. In: Sigel H, Sigel A, Eds. Metal Ions in Biological Systems. New York: Marcel Dekker, p147, 1989.
67. O'Halloran TV. Metalloregulatory proteins: Metal-responsive molecular switches governing gene expression. In: Sigel H, Sigel A, Eds. Metal Ions in Biological Systems. New York: Marcel Dekker, p105–146, 1989.
68. Spassky A, Sigman DS. Nuclease activity of 1,10-phenanthroline-copper ion. Conformational analysis and footprinting of the *lac* operon. Biochemistry **24**:8050–8056, 1985.
69. Skalka M, Cejkova M. DNA cleavage in chromatin spacer segments by a non-enzymatic probe, 1,10-phenanthroline-copper complex. Mol Biol Rep **10**:163–168, 1985.
70. Swauger JE, Dolan PM, Zeier JL, Kuppusamy P, Kensler TW. Role of benzoyloxyl radical in DNA damage mediated by benzoyl peroxide. Chem Res Toxicol **4**:223–228, 1991.
71. Reed CJ, Douglas KT. Chemical cleavage of plasmid DNA by glutathione in the presence of Cu(II) ions. Biochem J **275**:601–608, 1991.
72. Modak AS, Gard JK, Merriman MC, Winkler KA, Bashkin JK, Stern MK. Toward chemical ribonucleases. 2. Synthesis and characterization of nucleoside-bipyridine conjugates. Hydrolytic cleavage of RNA by their copper(II) complexes. J Am Chem Soc **113**:283–291, 1991.
73. Basile LA, Barton JK. Metallonucleases: Real and artificial. In: Sigel H, Sigel A, Eds. Metal Ions in Biological Systems. New York: Marcel Dekker, p31–102, 1989.
74. Greenberg JT, Chow JH, Monach PA, Demple B. Activation of oxidative stress genes by mutations at the *soxQ/cfxB/marA* locus of *Escherichia coli*. J Bacteriol **173**:4433–4439, 1991.
75. Ramotar D, Popoff SC, Gralla EB, Demple B. Cellular role of yeast *Apn1* apurinic endonuclease/3'-diesterase: Repair of oxidative and alkylation DNA damage and control of spontaneous mutation. Mol Cell Biol **11**:4537–4544, 1991.
76. Demple B. The *SOXR* superoxide-stress regulon: Inducible DNA repair and other functions. In: UCLA Symposia on Molecular and Cellular Biology—New Series Ionizing Radiation Damage to DNA: Molecular Aspects. New York: Wiley-Liss, Vol **136**:p297–307, 1990.
77. Amobile-Cuevas CF, Demple B. Molecular characterization of the *soxRS* genes of *Escherichia coli:* Two genes control a superoxide stress regulon. Nucleic Acids Res **19**:4479–4484, 1991.
78. Levin J, Shapiro R, Demple B. Metalloenzymes in DNA repair: *Escherichia coli* endo-nuclease IV and *Saccharomyces cerevisiae Apn1*. J Biol Chem **266**:2893–2898, 1991.
79. Trotman SM, Webb J, Sangster DF. A re-investigation of the reactions between superoxide anion and metal picolinate complexes. Inorg Chim Acta **153**:213–218, 1988.
80. Shiraishi N, Aono K, Utsumi K. Increased metallothionein content in rat liver induced by X-irradiation and exposure to high oxygen tension. Radiat Res **95**:298–302, 1983.
81. Matsubara J, Tajima Y, Karasawa M. Metallothionein induction as a potent means of radiation protection in mice. Radiat Res **111**:267–275, 1987.
82. Henderson TD, Burt RL, Kaufman SE, Willingham WM, Sorenson JRJ. Radiorecovery activity of manganese(III)$_2$(II)(μ_3-O)(3,5-DIPS)$_6$. Radiat Res **136**:126–129, 1993.
83. Chou FI, Tan ST. Manganese(II) induces cell division and increases in superoxide dismutase and catalase activities in an aging deinococcal culture. J Bacteriol **172**:2029–2035, 1990.
84. Matsubara J, Ishioka K, Shibata Y, Katoh K. Risk analysis of multiple environmental factors: Radiation, zinc, cadmium, and calcium. Envir Res **40**:525–530, 1986.
85. Matsubara J, Shida T, Ishioka K, Egawa S, Inada T, Machida K. Protective effect of zinc against lethality in irradiated mice. Environ Res **41**:558–567, 1986.

86. Matsubara J. Alteration of radiosensitivity in metallothionein induced mice and a possible role of Zn-Cu-thionein in GSH-peroxidase system. In: Kagi JHR, Kojima Y, Eds. Metallothionein II, Experientia Suppl. Basel: Birkhauser Verlag, Vol **52**:p603–612, 1987.
87. Neta R. The role of cytokines in radioprotection. Pharm Ther **39**:261–266, 1988.
88. Ainsworth EJ. From endotoxins to newer immunomodulators: Survival-promoting effects of microbial polysaccharide complexes in irradiated animals. Pharm Ther **39**:223–241, 1988.
89. Floersheim GL, Floersheim P. Protection against ionizing radiation and synergism with thiols by Zn(II)(aspartate). Br J Radiobiol **59**:597–602, 1986.
90. Weiss JF, McLean WA, Kumar KS. Radioprotection by combinations of WR-2721 and metals (Poster Presentation), Radiation Research Society Meeting, 1988, Philadelphia, PA, April 16–21.
91. Brown DQ, Pittock JW, Webb CL. Chemical modifiers of tumor radioprotection by WR-2721 (Poster Presentation), Radiation Research Society Meeting, 1988. Philadelphia, PA, April 16–21.
92. Kensler TW, Trush MA. Inhibition of oxygen radical metabolism in phorbol ester-activated polymorphonuclear leukocytes by an antitumor promoting copper complex with superoxide dismutase-mimetic activity. Biochem Pharmacol **32**:3485–3487, 1983.
93. Reiners JR Jr., Brott E, Sorenson JRJ. Inhibition of benzo[a]pyrene-dependent mutagenesis and cytochrome P-450 reductase activity by copper complexes. Carcinogenesis **7**:1729–1732, 1986.
94. Coleman JE, Giedroc DP. Zinc-Binding proteins involved in nucleic acid replication. In: Sigel H, Sigel A, Eds. Metal Ions in Biological Systems. New York: Marcel Dekker, p171–234, 1989.
95. Berg JM. "Zinc Fingers": The role of zinc(II) in transcription factor III A and related proteins. In: Sigel H, Sigel A, Eds. Metal Ions in Biological Systems. New York: Marcel Dekker, p235–254, 1989.
96. Skov KA. Modification of radiation response by metal complexes: A review with emphasis of nonplatinum studies. Radiat Res **112**:217–242, 1987.
97. Kumar KS, Vaishnav YN, Weiss JF. Radioprotection by antioxidant enzymes and enzyme mimetics. Pharm Ther **39**:301–309, 1988.