

# COPPER AND PEROXIDES IN RADIOBIOLOGY AND MEDICINE

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**CHARLES C THOMAS • PUBLISHER**  
*Springfield • Illinois • U.S.A.*

*Published and Distributed Throughout the World by*

CHARLES C THOMAS • PUBLISHER

BANNERSTONE HOUSE

301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

NATCHEZ PLANTATION HOUSE

735 North Atlantic Boulevard, Fort Lauderdale, Florida, U.S.A.

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Library of Congress Catalog Card Number: 64-11667

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## FOREWORD

OF ALL THE essential trace metals, the unique and widespread role of copper has become recognized. The purpose of this book is to explore and review the functions of copper in living organisms and to point out new areas of research. Especial emphasis is given to the involvement of copper in radiobiology. A study of the chelating properties of the drugs employed most successfully to counteract the lethal effects of ionizing radiation revealed that copper is uniquely involved in the mechanisms by which ionizing radiation affects living organisms. This opens the way to new experimental approaches for chemically counteracting radiation injury.

A critical analysis of the different chemical behavior of the two principal oxidation states of copper has led to new interpretations of its biochemical and medical behavior. Equally important has been the stimulus to the design of experiments which otherwise would not have been performed.

Among the topics related to radioprotection and medicine treated in this book are: (1) New approaches to the nature of and the treatment of Wilson's disease; (2) Elucidation of the copper catalyzed oxidations of ascorbic acid and the demonstration of enhanced destruction of ascorbic acid by small concentrations of a chelating agent; (3) New approaches to the role of copper in antipyresis and in the ageing processes; (4) Demonstration of a mechanism of action of small doses of ionizing radiation on a copper protein leading, among other things, to the elucidation of the protective action of hibernation; (5) Simple correlations between molecular structure and protective or sensitizing action of sulfhydryl and other compounds in radioprotection; (6) Explanation of the fact that the lethal effects of ionizing radiation on living organisms vary from a few hundred roent-

gens to hundreds of thousands of roentgens; (7) Inter-dependence and variation of the degree of radiation protection by cyanide and related cuprous chelating agents on both the dose of the agent and the radiation dose.

The book has been written with the needs of the chemist, biologist, and medical investigator in mind. I have attempted to present the chemical and biological aspects with sufficient background information so that these would be comprehensible and useful to both the chemist with little background in biological matters or radiation chemistry, and to the biologist with a limited background in chemistry. It is hoped that the book will serve to stimulate and excite the curiosity of the advanced student and those engaged in active research in radiobiology, medicine, and trace metal biochemistry.

I am highly appreciative for the information and advice given me by Prof. I. M. Klotz of the Department of Chemistry, Northwestern University, on many of the chemical aspects of copper chemistry; and Dr. E. J. Hart of the Chemistry Division, Argonne National Laboratory, who gave me help on many aspects of radiation chemistry. I am also grateful for helpful discussions I have had with former colleagues at the University of Buenos Aires in the Facultad de Ciencias Exactas y Naturales. Naturally, errors of fact, fancy, or concept are mine alone.

I am indebted to my former associates at the Argonne National Laboratory, Mrs. Joan Fried Markley, Dr. Marcia W. Rosenthal, Dr. Arthur Lindenbaum, and especially Mr. William M. Westfall for their help and advice.

JACK SCHUBERT

*Pittsburgh*

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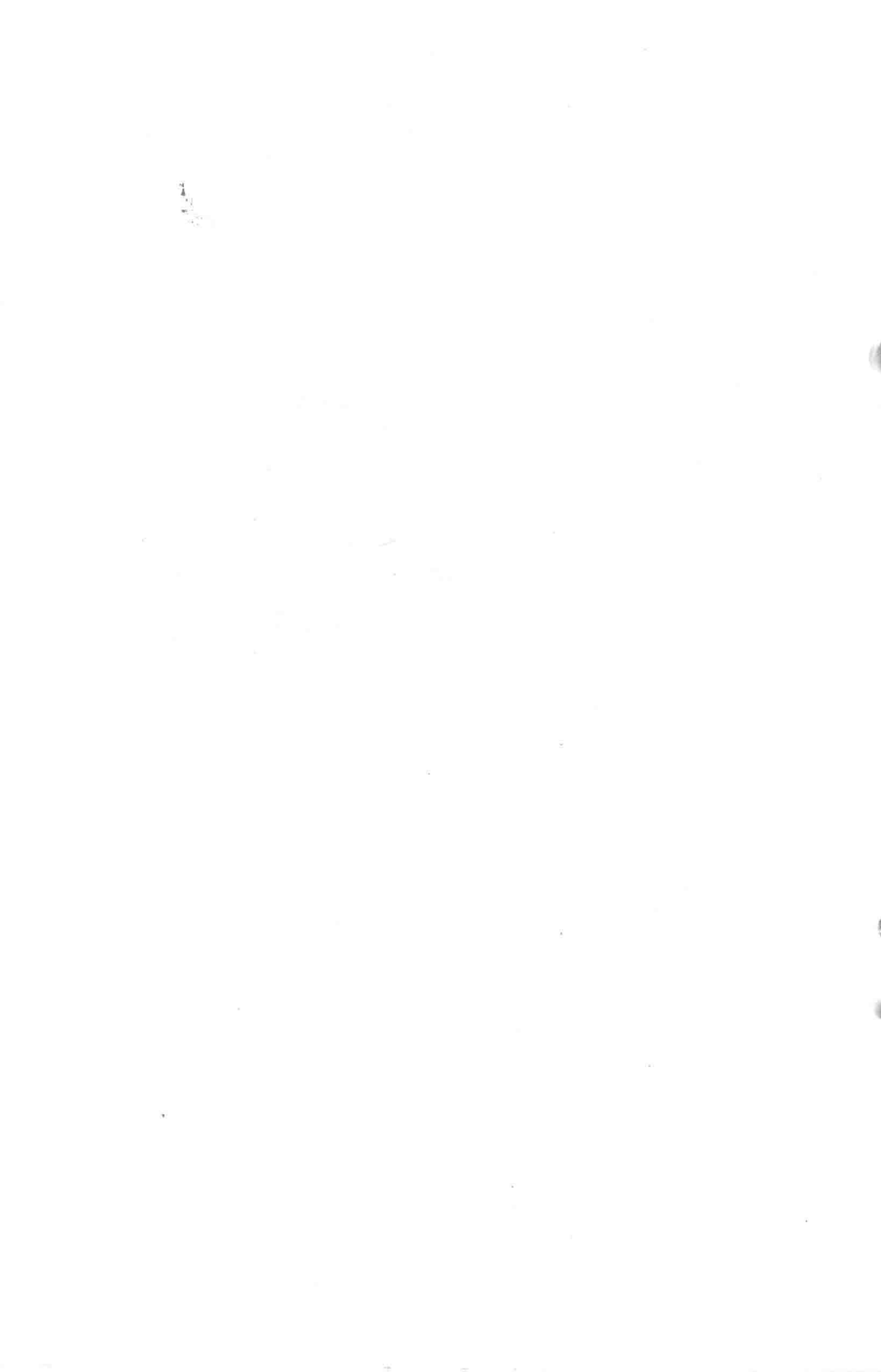
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# **COPPER AND PEROXIDES IN RADIOBIOLOGY AND MEDICINE**



## Chapter I

### INTRODUCTION

IT IS AN intriguing and curious fact that a large variety of chemical agents which modify radiobiological actions of ionizing radiation can react selectively in the physiological milieu with copper, and only copper in its distinctive oxidation states. Thus, chemical protective agents such as the mercaptans, dithiols, dithiocarbamates, cyanides, azides, and nitriles (E6, P5) complex or chelate preferentially with *cuprous* copper (M8, P10, S33) while chemical sensitizing agents (in mammals) such as the  $\alpha$ -alkyl alanines (L3) and quinoids (M25) favor *cupric* copper. This observation, together with conventional thermodynamic considerations relating redox potentials to complexing action (M8) suggest that retardation of the oxidizing action of acutely lethal doses of ionizing radiation could result from chemical stabilization by the protective agents of critical Cu(I) receptor sites, either *directly* through the formation of a dissociable complex or chelate or *indirectly* by electron transfer reactions. Corresponding reactions involving Cu(II) would enhance oxidation of Cu(I).

Irradiation of metal ions in solutions containing complexing or chelating agents has been demonstrated experimentally to favor the formation of that oxidation state which forms the most thermodynamically stable complex with a given ligand. For example, depending on the oxidation state stabilized and the concentration of the complexing agent, ionizing radiation can induce 100 per cent oxidation of ferrous salts or 100 per cent reduction of ferric salts (A9).

This monograph presents biological, radiochemical, and biochemical evidence for a new mechanism of radiobiological action involving aerobic organisms. It is postulated that ionizing radia-

tion produces organic peroxides which damage the genetic structure in the cell nucleus and oxidative processes in the cytoplasm. The cytoplasmic effect is assumed to involve an interference with the ability of copper enzymes—the 4-electron transfer oxidases—from interacting with molecular oxygen because of the radiation-induced oxidation of the cuprous component. Eventually there is produced adverse interactions on the cytoplasmic sites as a result of the nuclear damage and vice versa (D22). Normally, both the cytoplasm and nucleus are irradiated simultaneously in the organism so that a radiomimetic agent which acts only on the nucleus (nucleotoxic agent) or only on the cytoplasm (cytotoxic agent) cannot be expected to fully mimic radiation effects. It would be interesting to test suitable combinations of radiomimetic agents.

A plethora of theories of radiobiological protection and sensitization mechanisms exist (B6, 7, 12, 19, D20, E6, F2a, H12, L6, M1, 21, O2, P2, 4, 9, 17, S31, 44a, T7) which focus principally on radiation effects on the cell nucleus. However, their correlative and predictive value have been singularly disappointing.

The mechanism of radiobiological action described here is called the Cu(I,II)—Peroxy theory. It allows the modifying effects of apparently unrelated chemical agents to be evaluated from a chemical basis and makes it possible to correlate and clarify much of the existing experimental data. New experimental observations and correlations stemming from a preliminary formulation of the Cu(I,II)—Peroxy theory (S21) have already appeared on the action of ionizing radiation on a copper protein (S23), the radioprotective action of cyanide in mammals (S22), and the correlation of comparative radiosensitivity of animal species with copper content (S25).

In view of the central role occupied by copper, not only in the Cu(I,II)—Peroxy theory, but in biology in general (F11), a comprehensive review on selected and new aspects of copper chemistry and biochemistry and in medicine is included in this monograph along with chapters on topics necessary to a fuller understanding and application of the theory.

More and more the important and often unique role of copper in living organisms is becoming recognized. Yet it may be asked,

why should copper be considered to be a key metal in radiobiological action? Research is needed to uncover all the reasons but a few can be enumerated here, though details are given in subsequent chapters.

Copper ions form stronger complexes and chelates than other biologically essential transition cations with the exception of trivalent iron. However, at physiological pH's Fe(III) ions react very strongly with hydroxide ion so that the actual concentration of trivalent iron available for complexing reactions with a specific ligand is reduced by a factor of about 100 million (S20). The net result is that the "effective" constant of copper complexes are often stronger than those of iron and possess remarkable specificity, especially toward sulfhydryl, phenolic, and cyanide compounds.

It has been pointed out that the relative stabilities of Cu(I) and Cu(II) complexes depend much more than do the relative stabilities of oxidation states of other elements differing by one unit on the nature of the anions or other ligands present, on the dielectric constant of the solution, and on the nature of neighboring atoms in a crystal (C11a, p. 749).

Copper ions are uniquely sensitive to steric effects. In the case of the cupric ion, for example, which does not possess spherical symmetry, a remarkable flexibility in reactions is present (Williams, J2, p. 6). In one plane the cupric ion has a radius close to that of nickel and in the plane perpendicular to it there is a close resemblance to calcium.

Because of the enormous varieties of complexing substances present in biological systems no particular correlation between catalytic efficiency and complexing ability exists in such systems. However, as Frieden (F11) has noted, copper possesses unique catalytic properties: "No metal ion or other nonprotein catalyst surpasses copper salts in their versatility as catalysts for an impressive variety of reactions. . . . The recognition of the virtually unique catalytic features of copper ions and copper complexes occupies hundreds of pages in the chemical and the biochemical literature."

Another unique feature of copper is the observation that all the known four-electron transfer oxidases are copper enzymes.

These distinctive enzymes catalyze the reduction of molecular oxygen to water. Not only is copper the principal metal in these terminal oxidases, such as cytochrome oxidase, but *no alternative pathway exists by which the cell can catalytically reduce molecular oxygen to water* (M12). Hence, the copper oxidases provide a unique biochemical function.

The pragmatic applications of the Cu(I,II)—Peroxy theory are, in an empirical sense, independent of speculations concerning the primary biochemical role of copper in radiobiology and cell metabolism or of the specific radiochemical sequences by which organic peroxides are produced in the cell or how they interact to produce the postulated end results. More important, the theory enables the investigator to design experiments which can pinpoint the mechanism or combination of mechanisms by which a chemical protective or sensitizing agent acts.

The modification of radiation injury by radioisotopes deposited in tissues is essentially the same problem as that involved in externally delivered radiation. Indirectly, it is possible to minimize radiation damage from internal emitters by removing them from the body or from critical sites within the body, and thus reduce the radiation dosage (S16).



## Chapter II

### SELECTED ASPECTS OF COPPER CHEMISTRY

THE LITERATURE on copper, both on its chemical and biological properties is growing rapidly. Much information on the biochemistry of copper now available in relatively recent textbooks and in the literature has become obsolete or is incorrect as a consequence of the discoveries and investigations of the past few years. The extent to which copper is uniquely involved in the functioning of living matter is only now becoming appreciated and uncovered. Some of the general reference material used in the preparation of this chapter are cited here (A3, B8, C4, C11a, C14, F11, G15, M8, M12, M18, O4, S10, S15, S17, S29, S33, S34, and W9) while specific literature citations are given throughout. Emphasis is given to those aspects of chemical behavior of copper ions which bear directly on biological and medical problems.

#### STABILITY OF COPPER CHELATES

Qualitative and quantitative interactions involving copper, chemical protective and sensitizing agents, and enzymes can be surmised from a consideration of the known interactions involving Cu(I) and Cu(II) and simple molecules. Only the predominating structure of a complex or chelate is discussed. However, when a complexing agent contains several potential ligand atoms, several species of complex ions may and do exist simultaneously. Thus, in glutathione which has  $\alpha$ -amine, carboxyl, and sulfhydryl groups, copper may form a chelate ring with the -N, -O pair, or with the -N and -S pair (J2, p. 54). The relative amounts of each form depend on the relative affinities of the cations for the ligand atoms. These, and other factors, such as ligand basicity, hydra-