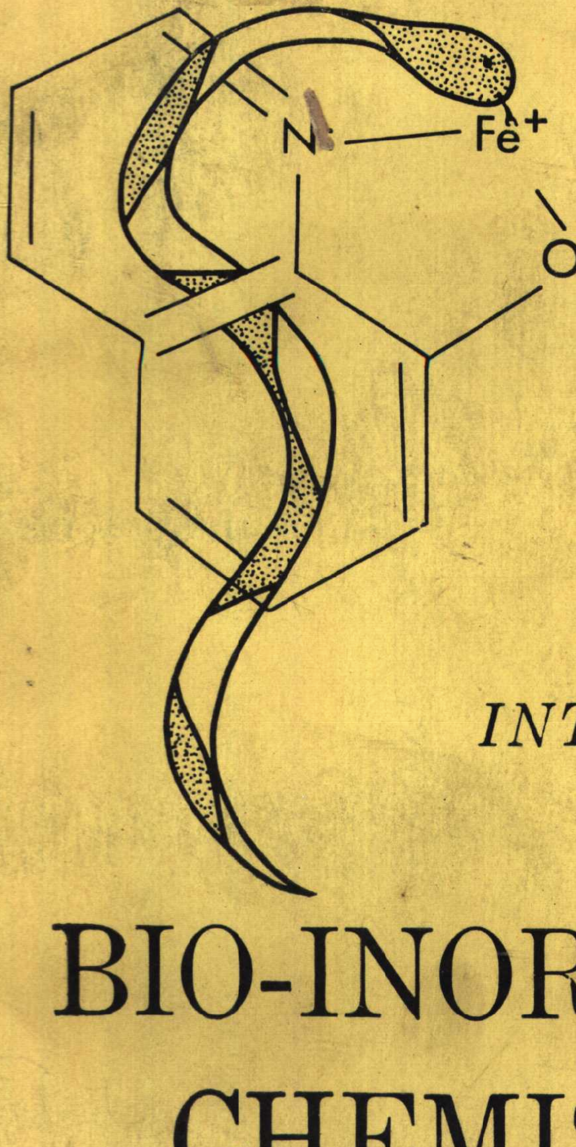

AN INTRODUCTION TO BIO-INORGANIC CHEMISTRY

Edited by

DAVID R. WILLIAMS

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PREFACE

THE IDEA OF A MULTIAUTHOR, interdisciplinary textbook of bio-inorganic chemistry was conceived in an Amsterdam bar by an *ad hoc* committee of bio-inorganic researchers delayed by a twenty-four-hour pilots strike: The book is designed to provide an introduction to graduates (and senior undergraduates) in inorganic chemistry, biochemistry and medicine to the field of metal ions in biological systems.

Recently there has been an avalanche of printed material concerning all aspects of the bio-metals. This book is designed as an introduction to scientists of any discipline who are interested in obtaining an overview of this emerging subject called bio-inorganic chemistry. Our aims are primarily to teach the *principles* of the subject but, in addition, we propose to draw the reader's attention to unanswered questions and to areas that have not yet been researched so that he or she may be tempted to tackle these problems.

Students and researchers are currently favoring topics that have altruistic overtones. This is leading them to interdisciplinary areas and therefore there is a concomitant need for intelligible communication across scientific boundaries—a need that will undoubtedly grow in the future. All biochemical, inorganic and medical students should have an understanding of bio-inorganic chemistry because much of our way of life depends upon bio-inorganic principles and the resulting technological applications of these concepts. Thus, it is not our intention to present streams of chemical facts unrelated to the world outside the lecture hall and the laboratory, but rather in compiling this book we have tried to portray bio-inorganic chemistry as a challenging field of research rather than as an established collection of factual knowledge. Creative science involves extrapolating beyond the information currently available.

In spite of the recent increase in cooperation between inorganic chemistry and biological chemistry, there is still a communications gap. Our aim is to collect the principles, and some subject matter, of this newly established discipline of bio-inorganic chemistry and to narrow this communications gap, chapter by chapter. The "biomass" (carbon, hydrogen, oxygen and nitrogen) is controlled and tantalized by the "trace elements"—a misnomer if it conveys the impression that the elements do not have an important role *in vivo*. Even if required as only one atom in ten million, life cannot exist without these essential trace elements. The literature on the subject is distributed throughout many journals from several disciplines and we have attempted a combination which forms a general picture of the growing interest in inorganic chemistry other than within the confines of reaction flasks and test tubes.

The transition from an isolated laboratory system to the real multiphase multicomponent phenomena of human life creates the possibility that this volume could contain an infinite number of chapters but we have limited our choice to sections describing the three areas, caused by two natural cleavage planes that divide the subject, I, of the general principles of bio-inorganic chemistry, II, the experimental methods used to produce the facts that gave rise to these principles, and, III, the applications of these principles to medicine. Within all three sections we have tried to take the reader to the most important of the frontiers of research within the constraints set by time lags in publishing schedules and by the limited size of this volume. We have not attempted to be encyclopaedic but rather to give a well-chosen sampling of material, the relative emphasis upon certain areas being dictated by a need to describe the majority of the principles carefully selected to comprise a coherent presentation of this very diffuse subject.

Workers at the interface between the disciplines of biological, inorganic and medical chemistry now talk in terms of atoms and nanometer units: It is hoped that our selection of the material for this book will persuade even more life scientists to devote their researches to the rational evolution of new drugs along the lines mentioned in Section III and to devote their teaching to new courses and practical training laboratories. (In this latter respect we must point out that, from students, bio-inorganic chemistry attracts added motivation since the subject offers ideals that can be realized.) During these last few years it has become apparent that academic growth either at pedagogic or research levels is no longer a sufficient goal in itself. The work has to have some meaning, some intellectual wealth, and some challenge. This text book initiates this process that leads to the more advanced reviews listed in the references and further reading. We hope that the interdisciplinary complexity of the subject stimulates rather than deters.

There will, of course, be opposition to the growth of this new subject–opposition from those intellectual Scrooges who react against the concept that a scientist trained in one discipline can ever do anything competent in another discipline. It is only human, for example, that inorganic chemists cling jealously to their prerogative to be the only teachers of inorganic chemistry. One can only hope that they too will soon appreciate (a) the fascination that interdisciplinary researches hold for men's minds, and (b) the many important achievements arising from multidisciplinary contributions.

I am indebted to a whole host of authors, advisers, critics, referees and publishers in helping me to edit this volume. We would also like to invite both students and teachers to advise us of constructive changes that will make an improved second edition. I hope that my prejudices and enthusiasms have not colored my presentation too much, and I hope that the readers will enjoy working with this book as much as I have enjoyed compiling it.

David R. Williams

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SECTION I

BIO-INORGANIC CHEMISTRY

CHAPTER 1

INTRODUCTION

David R. Williams

This book has been written to teach the principles and uses of bio-inorganic chemistry. It is neither a reference book nor a compendium of information. Our aim has been to be clear rather than exhaustive and we have attempted to demonstrate to the student reader that bio-inorganic chemistry is a living, expanding subject that offers both interest and challenge.

We have also designed this book with a wider, nonspecialist, research audience in mind: Within the scientific community, cross fertilization of ideas and information is currently very essential because an increasing number of discoveries are being made by multidisciplinary research teams working in the areas between the traditional disciplines of chemistry, biology and medicine. This situation has produced a requirement for intelligible communication across scientific boundaries and it is hoped that this book will bridge some of the gaps involved in bio-inorganic chemistry.

Why has bio-inorganic chemistry been chosen? Metal ions play a vital role in numerous, widely differing biological processes, and, of late, new developments in instrumental techniques have accelerated studies involving the uncharted territory between inorganic chemistry and biological sciences thus producing many exciting developments to such an extent that the subject is now one of the most rapidly expanding areas of science. It is as incorrect to divide nature into the sharply defined areas of chemistry, biology and medicine as it was to divide chemistry into organic and inorganic from a "vital force" standpoint.

Thus, we endeavor to introduce the reader to the principles of the thousands of processes, each being a collection of many metal-dependent reactions, that occur in the human body. (In order to restrict this book to a manageable size the plant and lower animal kingdoms have been omitted.)[1–3]

What Is Bio-inorganic Chemistry?

A star belonging to our galaxy disappeared 4.6 to 4.7 thousand million years ago in a blinding supernova explosion, the fragments of which formed the solar system of which our earth is a part. The periodic table of elements comprising our planet are those which coalesced as products of the explosion. Gravity compacted them and temperature and solar energy reacted these elements into a primitive hydrosphere. This prebiotic soup then underwent very many further reactions to form simple molecules which through evolution (the survival of the fittest or perhaps more correctly the prevalence of the most reproducible) led to metabolism—the organization of series of reactions from a wide selection of random reactions—and this eventually gave cellular, plant, animal and human life as we know it today.

The majority of chemical elements can be found in minute quantities in the human body, their concentrations depending upon their concentrations in food, soil and the atmosphere. Some of these elements have been termed "essential" or "beneficial" in that homeostatic control mechanisms exist to govern their concentrations in various organs or body fluids. The twenty-five elements that are currently accepted as being necessary for healthy human life are shown in the Figure 1-1.[4] As expected, these elements follow the abundances of the elements in the earth's crust and in sea water since the process of natural selection has removed organisms dependent upon less readily available elements. Biochemistry is the study of compounds of these elements—their structures, reactions and mechanisms *in vivo*. However, traditional biochemistry has investigated the elements present in bulk *in vivo*—the non-

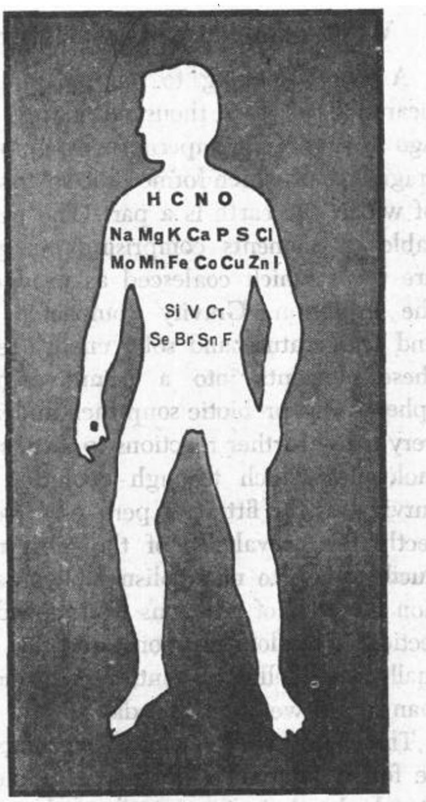

Figure 1-1. Elements essential for healthy human life.

metals—but as our experimental abilities have improved to be capable of examining smaller concentrations, a new subject—bio-inorganic chemistry—has evolved, a subject which is intent upon modernizing biochemistry by redressing the balance to consider metal-dependent reactions. Furthermore, more heavy element compounds are being used by our civilization and so our world is becoming more polluted. The interactions between pollutants and *in vivo* chemicals is also a part of bio-inorganic chemistry as is our evolution to future generations requiring essential elements in addition to the ones shown in Figure 1-1. Thus, *bio-inorganic chemistry is a branch of natural philosophy whose aims are to understand the chemistry of reactions involving the essential metals, and other trace elements,* in vivo *and to apply this knowledge altruistically.*

If the view that evolution and adaptation permit elements to traverse the scheme: *poisons* → *tolerable impurities* → *useful elements* → *essential elements* is accepted then one can understand how the seven elements printed in light type in Figure 1-1 have recently been shown to be essential for health because they occupy periodic table positions adjacent to the eighteen essential elements whose biochemical properties are well known—dietary deficiencies produce animal growth rates as low as two-thirds the normal growth rates.[5]

All three groups of metals *in vivo*—those essential for life, those essential for *healthy* life, and the polluting metals, have a cycle equivalent to the familiar nitrogen or carbon cycles. For example, Figure 1-2 shows the cycle recently composed for mercury in the biosphere.[6]

The basic elements of biochemistry, hydrogen, carbon, nitrogen and oxygen, comprise 99 percent of the atoms in the human body—chiefly as water, protein and fat.

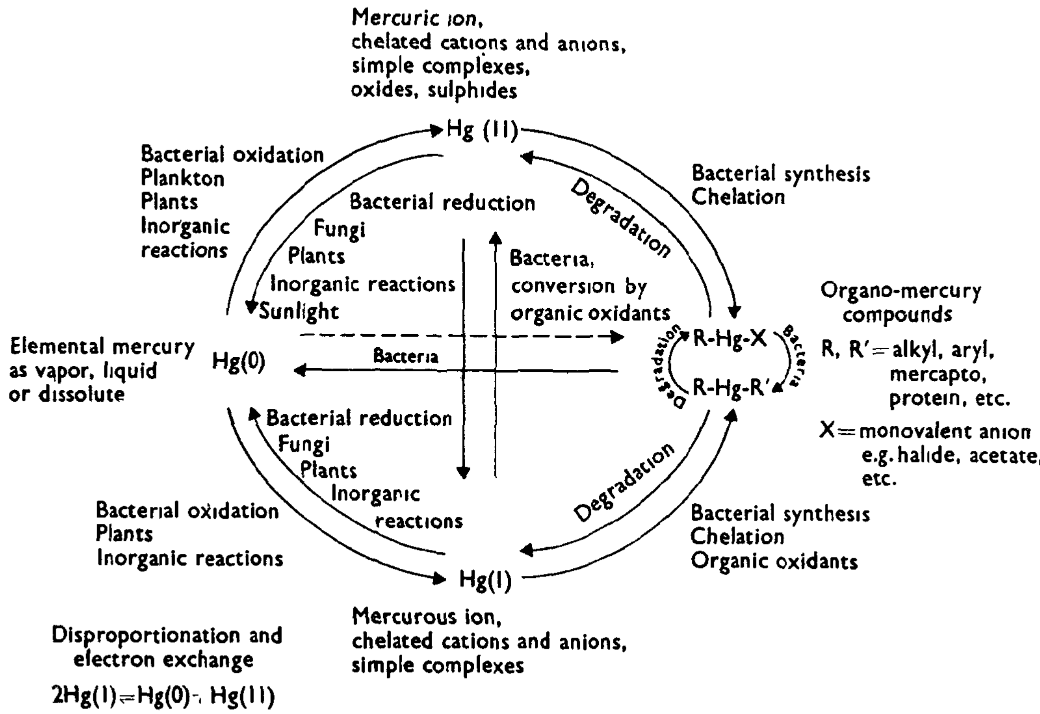

Figure 1-2. Cycle suggested for mercury in the biosphere.

Other *nonmetals* exist as anions in biological fluids. The total *metallic* component of our bodies is composed almost entirely of the main group metals including sodium, potassium, magnesium and calcium. The trace metals of the transition series—either as essential trace elements or as polluting ones—usually weigh less than 10 g *in toto*. Nevertheless, many volumes have already been published concerning the fascinating roles of these trace elements.

Historical Discoveries of Trace Metals

The history of the development of the idea that minute amounts of trace metals were indispensible for human life is intriguing. The term "trace" arose because early researchers, although aware of small amounts of mineral elements in living tissue, were unable to measure their precise concentrations. The adjective "essential" is only dubbed if the element is present in all healthy tissues, has a fairly constant concentration range between different animals, and when excluded from a body produces reproducible physiological abnormalities which are reversible upon readmittance of the element. Such definitions have excluded some two dozen trace elements which are ubiquitous to tissues but whose concentrations vary and whose physiological roles could not be determined. These must be assumed to be contaminants, some of which are toxic in small amounts (*all trace elements are toxic in larger amounts*).

Every element, essential, beneficial or polluting, has a spectrum of biological actions which depends upon the concentration of the element, or its compounds, in any particular organ or body fluid. Figure 1-3

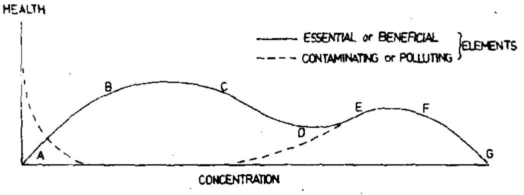

Figure 1-3. The effects of varying the concentration of the trace element in the organ *in vivo*.

schematically shows how the state of health of an organ is dependent upon the concentration of an element in that organ.[7] As the concentration is increased from A to B the organ becomes progressively more normal in its reactions until a homeostatically buffered plateau B to C is reached. Clearly this "concentration for optimum health" plateau varies from essential element to essential element and from organ to organ. However, it would be hasty to assume that the only concentration of nonessential elements giving optimum health is zero: First, our bodies are skilled at tolerating quite high concentrations of polluting elements, especially if these elements are carried into the organs by the same mechanism that provides an essential element. For example, it is important to have sufficient zinc *in vivo* even if this means having to accept some cadmium as well. Secondly, regions D to G depict the property of a nonessential element to stimulate the body's defense mechanisms. Region C to D depicts the decline in health as excessive concentrations build up in an organ (for example, the siderosis effects associated with too much iron) until the curve reaches trough D. This is a passive protective intermediate concentration between C and E.

Some diseases, especially those caused by invading organisms such as viruses or bacteria, do not respond adequately to merely correcting elemental *in vivo* concentrations to plateau B to C. In these instances there may be grounds for administering a higher concentration of a metal or of its complex. Region D to E shows the pharmacological effect of administering this element as doses of drugs. Such drugs stimulate the host's defense mechanisms. Naturally there is a limit to this process at plateau E to F. The presence of this plateau is fortunate in that it means an 80 kg man can be reasonably safely prescribed the same quantity of drug as a 50 kg girl. All therapies eventually change from excitation to a toxicological inhibition of the living process. F to G depicts this drug poisoning. Eventually, large doses of the element—as an essential element, as a polluting element, or as a medically administered compound of the element—cause an irreversible reaction, a complete decline in the living systems, leading to death.

Such curves differ from element to element, some having better homeostatic capacities than others and some having but a brief safety margin between optimum and toxic concentrations. Further, such *curves* ought really to be *surfaces* enclosed in three (or multi-) dimensional axes since healthy concentrations of elements are sometimes dependent upon the prevailing concentration of other elements. (For example, there are widely known mutual antagonisms between Fe and Co, Cu and Mn, Cu and Mo, Cu and Zn, and Ca and K concentrations.) Finally, we must remember that these curves or surfaces may have a varying amplitude according to prevailing circadian rhythms.

The first realization that trace elements were important *in vivo* came more than a century ago but it was not until the first quarter of this century that emission spectrophotography was applied to the study of iron and iodine in human health. Nuclear fission in World War II gave a boost to such studies from the viewpoint of assessing the maximum "safe" concentrations of radionuclides and their involvement in cycles such as the mercury example given. Then came the purified diet techniques for establishing "essentiality to health" criteria, such approaches helping to uncover many nutritional maladies in animals and humans and later chronic metal poisoning diseases were revealed. During the last two decades radiotracers and improved analytical techniques have helped to establish the metabolic movements and biochemical roles of these trace elements. Finally, the growing concern for human environmental health has boosted trace element biochemistry even further.

In summary: The absence of an attack upon living systems by the inorganic chemist on a scale comparable to that launched by the organic chemist has left us with a totally false impression of living things. Biochemistry is as much inorganic as organic.[8]

Outline of Chapter Contents

Music for the tone deaf can carry no message and so we have adopted the following three-part disciplining of our bio-inorganic receptors: Section I describes the principles of the subject and sufficient facts to illustrate them; Section II lists the instrumental techniques used to determine these facts; Section III puts the "bio-" into "bio-inorganic" by describing some of the useful products of the exercise—new drugs for Wilson's disease, for cancerous or polluted humans.

Before describing these sections more fully, an apology is in order since some aspects of bio-inorganic chemistry have been omitted from this book. This is usually because the topic has not yet reached the degree of maturity such that a simple survey at the level of the present book would achieve anything.

Section I describes some specific examples to illustrate the general principles of bio-inorganic chemistry. From ancient times until the late nineteenth century, most great thinkers subscribed to the cult of Spontaneous Generation. Chapter 2 discusses modern twentieth century concepts of how we actually evolved from the elements distributed over the surface of the Earth and its environs. Although only a small fraction of this planet's atoms are involved in the fascinating set of processes we call "life," what this collection lacks in numbers it makes up for in activity and complexity. Yet, our evolution, like all chemical processes, could proceed no faster than the rate at which the scarcest elements became available.

Chapter 3 describes how the trace metals—once regarded simply as contaminants—are now known to be uniquely matched to ligand donor groups (and *vice versa*—the groups are matched to the available trace elements). Chapters 2 and 3 were not included for purely historical or academic interest: Man is both a part of, and a product of, the environment (a point reemphasized in Chapter 20) and so Chapters 2 and 3 are not only mere studies of how we came to be what we are, but also, they will help us to extrapolate to the future, to see the possible effects upon future generations of overpopulation, of increased material prosperity, of pollution and of agricultural practices which overstress natural systems.

Chapter 4 takes this specificity of metal ion-ligand bonding even deeper by studying a range of metalloenzymes—a description of the complete matching of a metal to its enzyme, to the enzyme substrate, and to the catalytic mechanism. Chapter 5 is also concerned with enzymology in general, and in particular, the inorganic chemistry of the activation of molecular oxygen and the description of the struggle involved as we human macroorganisms try to mimic the efficiency of microorganisms at nitrogen fixation.

In vivo we have learned to expect the unusual or the exceptional and this is found to be true for the bio-inorganic chemistry of *Homo sapiens* and bacteria in which the oxygen and nitrogen activating systems sometimes exhibit unexpected oxidation states and bond configurations. Much of Chapter 5 has been researched on model systems or on the simpler biological ligand (for example, amino acid) systems. Nucleic acids marshall the conjoining of these simple units into polymers and so it is appropriate that five chapters in Section I mention nucleic acid-metal ion interactions.

Man's ability to manipulate metal ion concentrations and bond configurations is currently limited to the use of *single* multidentate ligands as tools. Nature invokes *several* such ligands competing for a mutual metal ion. Chapter 6 describes our current understanding of these mixed ligand complexes. Each of Chapters 2 to 6 covers important subjects in their own rights: Too frequently students want to know the purpose of life before they have discovered what the chemistry of life is all about. Although it is possible to train young students in interdisciplinary work it is far more desirable to train them in specific topics (for example, those covered in Chapters 2 to 6) with the idea that they will eventually apply this expertise to other disciplines.

To illuminate the effectiveness of combining expertises from several disciplines to researches into one particular topic, Chapters 7 and 8 are overviews of our present state of knowledge of the reactions and roles of just three bio-metals—transition metal *iron*, and main group metals *calcium* and *magnesium*, the latter emphasizing the essential interdependence of metal ion concentrations *in vivo*.

Section II stresses the *uses* of a variety of techniques rather than being a description of the exact instrumental approach in the belief that if a person is sufficiently motivated to apply a method he can learn the rigors of the method from one of the source books listed.

Although life is predominantly aqueous, there are no direct methods for determining the structures of complexes present in aqueous solution and so indirect (or as Tobe calls them, "sporting") methods are necessary. For example, extrapolations from crystal structures or of thermodynamic reasoning.

As in Section I, we have tried to concentrate upon patterns of behavior and to produce generalized views of the usefulness and applicabilities of the instrumental techniques described. Through these means we aim to bridge the gap between the standard inorganic or biochemical textbook and the research paper in another discipline. Understandably, there are techniques which attract only occasional glances in this book since, although their potentials may be immense, useful bio-inorganic achievements to date have been modest.

All life is based upon two important chemical reactions—the capture of solar energy for producing oxygen from carbon dioxide and water, and the reversion of this oxygen to carbon dioxide, water and muscular energy. Life has been defined as an organism continually reducing its entropy (and death is the reassertion of the claims of thermodynamic equilibria). Another unique feature of life is that organisms exist because of their kinetic stability—all living forms being thermodynamically unstable. Finally, life functions in cycles (cir-