ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 48

PROTEIN–METAL INTERACTIONS

Edited by
Mendel Friedman

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Western Regional Research Laboratory
Agricultural Research Service
U. S. Department of Agriculture
Berkeley, California

PLENUM PRESS • NEW YORK AND LONDON

Preface

Metal ions and proteins are ubiquitous. Therefore, not surprisingly, new protein-metal interactions continue to be discovered, and their importance is increasingly recognized in both physical and life sciences. Because the subject matter is so broad and affects so many disciplines, in organizing this Symposium, I sought participation of speakers with the broadest possible range of interests. Twenty-two accepted my invitation. To supplement the verbal presentations, the Proceedings include five closely related invited contributions.

The ideas expressed are those of the various authors and are not necessarily approved or rejected by any agency of the United States Government. No official recommendation concerning the subject matter or products discussed is implied in this book.

This book encompasses many aspects of this multifaceted field. Topics covered represent biochemical, immunochemical, bioorganic, biophysical, metabolic, nutritional, medical, physiological, toxicological, environmental, textile, and analytical interests. The discoveries and developments in any of these areas inevitably illumine others. I feel that a main objective of this Symposium, bringing together scientists with widely varied experiences yet with common interests in protein-metal interactions, so that new understanding and new ideas would result has been realized. I hope that the reader enjoys and benefits from reading about the fascinating interactions of metal ions and proteins as much as I did.

Although an adequate summary of the Symposium is not possible in a brief preface, I wish to express particular interest in the ideas reported by Professor Frieden: that the relative occurrence and participation of the various metals as essential elements in enzyme action and other life processes is an adaptive relationship to their relative abundance in the ocean. Undoubtedly, this adaptation is a continuing process. A more immediate practical concern voiced by D. K. Darrow and H. A. Schroeder that has received widespread publicity and debate is that children are highly susceptible to lead poisoning and that their exposure to lead nowadays comes mainly from automobile exhaust.

Of the invited contributions supplementing the Symposium, the paper by J. T. MacGregor and T. W. Clarkson deserves special mention. Dr. MacGregor collaborated with Dr. Clarkson, his former major professor, in this thorough review while the latter was out of the country dealing directly with an episode of mercury poisoning described in their paper. I believe their critical compilation of tissue distribution and toxicity of mercury compounds will greatly benefit the medical and other scientific communities in dealing with this useful but dangerous element.

I am confident that the Proceedings of the Symposium on "Protein-Metal Interactions" will be a valuable contribution to the literature. I am particularly grateful to Dr. I. A. Wolff, Chairman of the Protein Subdivision of the Division of Agricultural and Food Chemistry of the American Chemical Society, who invited me to organize this Symposium, to all contributors and participants for a well realized meeting of minds, and to R. N. Ubell, Editor-in-Chief, Plenum Publishing Company, for arranging publication as a volume in the series, ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY.

Mendel Friedman
Moraga, California
April 1974

Contents

THE EVOLUTION OF METALS AS ESSENTIAL ELEMENTS [with special reference to iron and copper]

Earl Frieden

Department of Chemistry

Florida State University

Tallahassee, Fla. 32306

The past few years have witnessed exciting progress in our understanding of the elements required for the growth and survival of the higher animal. As anticipated, these new discoveries have been exclusively among the trace elements, those elements which in minute quantities are essential for growth and development. As shown in Table I, after the discovery of the requirement for cobalt in 1935, there was a hiatus of about two decades before the essentiality of molybdenum (1953), chromium (1957) and selenium (1959) was confirmed. This was the end of an era in trace element research. From here on a major change in the research technique had to be devised. The animals, their food and their entire environment had to undergo a complete trace element decontamination, using special plastic houses, highly purified diets and filtered air. After a decade of painstaking research, Dr. Klaus Schwarz of the Veterans Administration and others added three new elements to the essential list: fluorine, tin and vanadium. In 1972, Dr. Edith Carlisle, University of California, Los Angeles, proved that silicon also was required for the growth and development of chicks. We now know that at least twenty-five of the 96 elements found on earth are required for some form of life (Figure 1; see also Table III).

Two-thirds of the lightest elements, 22 out of the first 34, are essential. These 22 elements plus molybdenum (no. 42), tin (no. 40) and iodine (no. 53) complete the list of 25 essential elements. It is an impressive fact that despite our current sophistication in biochemistry and molecular biology, there remain many fundamental, unanswered questions concerning the elements required for animal or plant life. Moreover, there has been little effort to relate the evolution of the dependence on specific elements to the past and present status of living organisms.

TABLE I

DISCOVERY OF TRACE ELEMENTS REQUIREMENTS OF ANIMALS

Iron . . . . .17th century	
Iodine 1850	Chatin, A.
Copper 1928	Hart, E.B., H. Steenbock, J. Waddell, and C.A. Elvehjem
Manganese . .1931	Kemmerer, A.R., and W.R. Todd
Zinc 1934	Todd, W.R., C.A. Elvehjem, and E.B. Hart
Cobalt 1935	Underwood, E.J., and J.F. Filmer Marston, H.R. Lines, E.W.
Molybdenum . .1953	deRenzo, E.C., E. Kaleita, P. Heytler, J.J. Oleson, B.L. Hutchings, and J.H. Williams Richert, D.A., and W.W. Westerfield
Selenium . . .1957	Schwarz, K., and C.M. Foltz
Chromium . . .1959	Schwarz, K., and W. Martz
Tin 1970	Schwarz, K., and D.B. Milne, and E. Vinyard
Vanadium . . .1971	Hopkins, L.L., and H.E. Mohr Schwarz, K., and D.B. Milne
Fluorine . . .1971	Schwarz, K., and D.B. Milne
Silicon . . .1972	Carlisle, E.M. Schwarz, K., and D.B. Milne

Adopted from K. Schwarz in "Trace Element Metabolism in Animals", C. F. Mills, Ed., E85 Livingstone, Edinburgh, p. 25 (1970). See this article for appropriate references.

In his classic text, The Fitness of the Environment, published in 1913, Lawrence J. Henderson concluded:

> The properties of matter and the course of cosmic evolution are now seen to be intimately related to the structure of the living being to its activities; they become, therefore, far more important in biology than has been previously suspected. For the whole evolutionary process, both

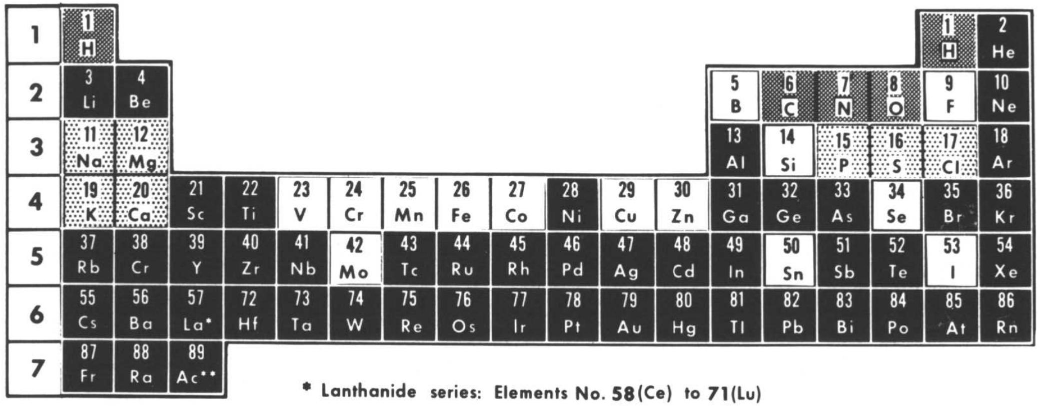
> cosmic and organic, is one, and the biologist may now rightly regard the universe in its very essence.

Despite the title of this far reaching work, Henderson put as much emphasis on the organism fitting to the environment through Darwinian evolution as on the milieu fitting the organism. Henderson wrote that the fitness of the environment was just one part of a reciprocal relationship of which the fitness of the organism is the other. This condition of life was expressed in the characteristics of water, carbonic acid and the compounds of carbon, hydrogen, and oxygen. These combined to provide "the best of all possible" environmnets for life. Henderson's basic approach is none the less applicable today and provides us with the starting point for our consideration of the basis for the selective dependence of life on the 25 natural elements. To fit their environment, living systems were involved in two major adaptations--first to the origin of life in the oceans and second to the utilization of oxygen in the atmosphere. These two adaptations have established the major guidelines for the requirement of the elements and the determination of the chemical structures accompanying the development of life on earth.

EVOLUTION OF LIFE IN THE SEA

It is widely accepted that life on earth first evolved in the sea or in saline tidal pools. This has led to an indelible imprint of the composition of the oceans in the chemistry of all cells and organisms. The oceans provided a convenient medium in which the primitive organism could float freely in a solution of the basic chemicals required to maintain life. For these primitive organisms the fluidity of a medium like water was indispensible. A denser matrix (e.g., solids) would have been too restrictive on the mobility of dissolved ions or metabolites. Finally, after millions of years, the metamorphic transition from aquatic to terrestrial existence began. This development was successful only if the transforming organisms were able to carry part of their marine environment with them. The distribution of salts in the blood and other body fluids reflects the oceanic nature of their evolutionary antecedents. The choice of sodium, potassium, calcium, magnesium and chloride ions for the major ionic environment of the cell, both inside and outside, stems from their availability in the primeval seas.

All this has been a widely recognized facet of comparative biochemistry (Prosser and Brown, 1961). What has not been fully appreciated is that the nature of the trace element requirement, particularly the metal ions, appears to have been limited by their availability in the early oceans. This is evident upon an examination of the concentrations in the oceans of the five bulk ions and the fourteen trace elements, (Table II; compare with Table III).



* **Lanthanide series: Elements No. 58(Ce) to 71(Lu)**

** **Actinide series: Elements No. 90(Th) to 103(Lw).**

ESSENTIAL LIFE ELEMENTS, 24 by the latest count, are clustered in the upper half of the periodic table. The elements are arranged according to their atomic number. The four most abundant elements that are found in living organisms (hydrogen, oxygen, carbon and nitrogen) are indicated by the grey cross-hatched areas. The seven next most common elements are shown in dotted areas. The 14 elements that are shown in white are needed only in traces.

Adapted from E. Frieden, Sci. Amer., July, 1972

Figure 1

Table II

Concentration of Some Crucial Elements in Sea Water

Element	Parts per Million	Element	Parts per Billion
K	380	Fe	10
Mg	1,350	Cu	3
Ca	400	Zn	10
Cl	19,000	Mn	2
Na	10,500	Mo	10
B	4.6	Co	.27
F	1.3	Se	.09
Si	3.0	Cr	.05
I	.06	V	2
		Sn	3

Taken from: E. D. Goldberg in The Sea (ed. by M. N. Hill), pp. 4-5, (1963), J. Wiley, New York.

The macro ions are present in concentrations 10^5 - 10^6 times those of the trace metals. The ten essential trace metals are present in 10 parts per billion or less. Three required non-metals, boron, fluorine, and silicon are present in 1-5 parts per million, but are still only trace elements in most systems, possibly due to the lack of the unique binding properties exhibited by these non-metals. Finally iodine proves to be an essential trace element to mainly non-marine forms. The special properties of these trace elements in both simple and complex organisms will be discussed later.

AEROBIC CELLS

Another major evolutionary adaptation met by living systems arises from changes in the atmosphere leading to an accumulation of and a dependence on oxygen. My colleague, Hans Gaffron (1960), of Florida State University, has summarized the major stages of biogenesis in terms of the transition from a highly reducing atmosphere to our present oxygen rich world. There was first an anaerobic era of excess hydrogen with ammonia, methane and water vapor and an accumulation of highly reduced organic substances. This was followed by a hydrogen poor period with the beginnings of inorganic or organic catalysis and traces of oxygen. In the third period, the anaerobic organisms deplete their useful reservoir of organic substances dooming the earth to essential dependence on photoreduction as the major initial source of energy. This leads to today's era of photosynthesis and ascendancy of cells dependent on oxidative energy, eliminating or driving the obligate anerobes "underground". Oxygen itself then becomes a major evolutionary force. As we shall see later the essentiality of numerous metals arises from their ability to promote the effective utilization of oxygen.

How environmental circumstances may determine whether an organism will survive such drastic changes as the step from anaerobic to aerobic conditions was shown in an actual laboratory model. In 1954, Jensen and Thofern found a micrococcus strain which grew well heterotrophically under anaerobic conditions. On contact with oxygen the cells die because they have neither an efficient mechanism for destroying the hydrogen peroxide which might be formed nor a respiratory system. But, when the nutrient medium contained a simple iron porphyrin, ordinary protohemin, these bacteria survived in air. The hemin was taken up into the cells and there combined with the right kind of protein to provide the bacteria with catalase and respiratory enzymes. The presence or absence of hemin in the surroundings at the time oxygen reaches the cells determines their fate.

THE THREE GROUPS OF ESSENTIAL ELEMENTS

The current state of knowledge about the 25 elements now recognized to be essential to the various types of organisms is summarized in Table III. In this table the elements are classified in three categories: (++) is used to describe the elements which have been shown to be essential to numerous representative animals in that group and are presumed to be required for life and survival by virtually every member of that group, (+) designates those elements for which a requirement has been demonstrated but the number of examples or evidence is so sparse as to limit any generalization of requirement (although the "unity" of nature strongly suggests a group requiremtent), and (-) indicates the lack of any definitive data supporting the essentiality of that element for the particular class of organism. This may be due to the absence of any experiments or to the failure to show a requirement in those experiments reported.

Each of the first group of six elements is required because they provide most of the atoms for the primary molecular building blocks for all forms of living matter: amino acids, sugars, fatty acids, purines, pyrimidines and their nucleotides. These molecules not only have independent biochemical roles but also are the respective constituents of the major categories of large molecules: proteins, cellulose, glycogen, starch, lipids and nucleic acids. Several of the 20 amino acids contain sulfur in addition to carbon, hydrogen, and oxygen. Phosphorus plays an important role in the nucleotides, such as adenosine triphosphate (ATP), which is central to the energetics of the cell. Adenosine triphosphate includes components that are also one of the four nucleotides needed to form

TABLE III

ESSENTIALITY OF THE ELEMENTS FOR LIVING ORGANISMS

Element	Bacteria	Algae	Fungi	Higher Plants	Inverte-brates	Verte-brates
H,C,N, O,P,S	++	++	++	++	++	++
K	++	++	++	++	++	++
Mg	++	++	++	++	++	++
Ca	+	++	+	++	++	++
Cl	++	++	-	++	++	++
Na	+	+	-	+	++	++
Fe	++	++	++	++	++	++
Cu	++	++	++	++	++	++
Zn	++	++	++	++	++	++
Mn	++	++	++	++	++	++
Mo	++	++	++	++	++	++
Co	++	+	+	+	++	++
Se	+	-	-	+	-	++
Cr	+	-	-	-	-	+
V	+	+	-	-	+	+
Sn	-	-	-	-	-	+
Ni	-	-	-	-	-	+
B	-	+	-	++	-	-
F	-	-	-	-	-	+
Si	-	+	-	+	-	+
I	-	+	-	+	+	++

KEY: (++) - Essential for several and, probably, all species in the group.
(+) - Essential for one or a few species in the group.
(-) - Not known to be essential for any species.

the double helix of deoxyribonucleic acid. Polysaccharides, such as cellulose, form the major structural component of plants and trees. Both sulphur and phosphorus are present in many of the small indispensible molecules called coenzymes. In bony animals phosphorus and calcium help to create strong supporting structures.

The second group of essential elements play a critical role in the electrochemical properties of cells from all forms of life. The principal cations are potassium and magnesium with a somewhat less versatile role for calcium and sodium. The principal anions or negative charges are provided by chloride, sulfate and phosphate and are widely distributed with an anomalous feature of fungi in not requiring chloride. These seven ions maintain the electrical neutrality of body fluids and cells and also play a part in maintaining the proper liquid volume of the blood and other fluid systems. Whereas the cell membrane serves as a physical barrier to the exchange of large molecules, it allows small molecules to pass relatively freely. The electrochemical functions of the anions and cations serve to maintain the appropriate relation of osmotic pressure and charge distribution on the two sides of the cell membrane. One of the striking features of the ion distribution is the specificity of these different ions. Cells are rich in potassium and magnesium, and the surrounding plasma is rich in sodium and calcium. It seems likely that the distribution of ions in the plasma of higher animals reflects the oceanic origin of their evolutionary antecedents.

The third and most numerous group of essential elements is those required only in very small, trace amounts. This latter fact in no way diminishes their great importance to the survival and proper functioning of all organisms. For the purpose of this discussion we are going to make a* distinction within this group between the metals and the non-metals.

The non-metals, boron, fluorine, silicon and iodine; will be mentioned briefly for several reasons. The first is that we don't know much about their requirement or biochemical role except for iodine. The indispensability of fluorine and silicon in the mammal has been reported in the last 18 months although the role of fluorine in preventing tooth decay was well known. Boron is required only in some plants and algae with no positive role in animals. Iodine, the heaviest required element presents a more interesting case. It is, of course, best known in the vertebrates as an essential part of the molecules which comprise the thyroid hormones, iodothyronine. These hormones play two probably related roles in the vertebrates (Frieden and Kent, 1974): (1) the initiation and control of metamorphosis and development in amphibia and in the higher vertebrates and (2) the adaptation of animals to homeothermy, which utilizes the calorigenic properties of these hormones. Yet the precise function of iodine in hormonal activity is still not understood. The role of iodine in invertebrates and plants is spotty and uncertain--there is no certainty of a group requirement for this element. The trace non-metals may be a more interesting group than imagined, but we will need much more information than is currently available.

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*Boron and silicon are sometimes considered as metalloids, since they are at the borderline in the delineation of metals and non-metal properties.

The indispensible elements which most excite the bio-chemists are the trace metal ions, the most numerous category listed in Table III. They are especially interesting because of their widespread distribution, importance, and unique chemical properties. Five of these metal ions, Fe, Cu, Zn, Mn, Mo, appear to be generally required by all organisms. Cobalt will undoubtedly end up in this category. The other metals are required in such small amounts and the chances of contamination at this level are so great that highly refined experimental techniques were required to show their indispensibility. These metals include selenium, vanadium, chromium, and tin ions. Recently selenium has been identified as an essential component of an enzyme in the red blood cell, glutathione peroxidase, which is involved in the protection of the erythrocyte against the toxic, oxidative effects of hydrogen peroxide. There is growing evidence that nickel is also a required trace element. When we learn how to cope with the experimental conditions in the various media required for proper testing of different kinds of organisms, these four metals may prove to be needed by all cells. This experimental approach may also reveal additional new essential metals, the most likely being aluminum or germanium.

DEVELOPMENT OF CATALYSIS

What more can be said about the development of the need of cells for these 25 elements? The literature is replete with discussions of proteins, nucleic acids and informational macromolecules. Accordingly let us focus our attention on the development of the need for certain elements, in particular the metal ions. The six metals, iron, copper, zinc, manganese, molybdenum and cobalt, which are required by every major group of organisms have a reasonably well defined biological function. These six metals belong to a group known as the transition elements. The transition metals serve as essential constituents of a wide range of metalloproteins, usually enzymes, which participate in important oxidative, hydrolytic or transfer processes. Most of them undergo reversible changes in oxidation state which make them especially useful as intermediates in electron transfer or oxidation reactions. They also tend to form strong complexes with a variety of ligands of the type present in the side chains of proteins, and which gave extra stability to metalloprotein complexes.

How a typical catalytic activity of a transition metal enzyme might have evolved has been described by Melvin Calvin (1969) of the University of California (Berkeley). He pointed out the similarity between the enzymic reactions and the reactions known to the inorganic chemist. The model which he chose centers around the element iron, particularly in catalase, peroxidase and the cytochromes. Here a quantitative comparison was made between the ability of the simplest iron compounds to perform the same catalytic function as it has evolved in biological systems. Thus the accompanying (Figure 2)