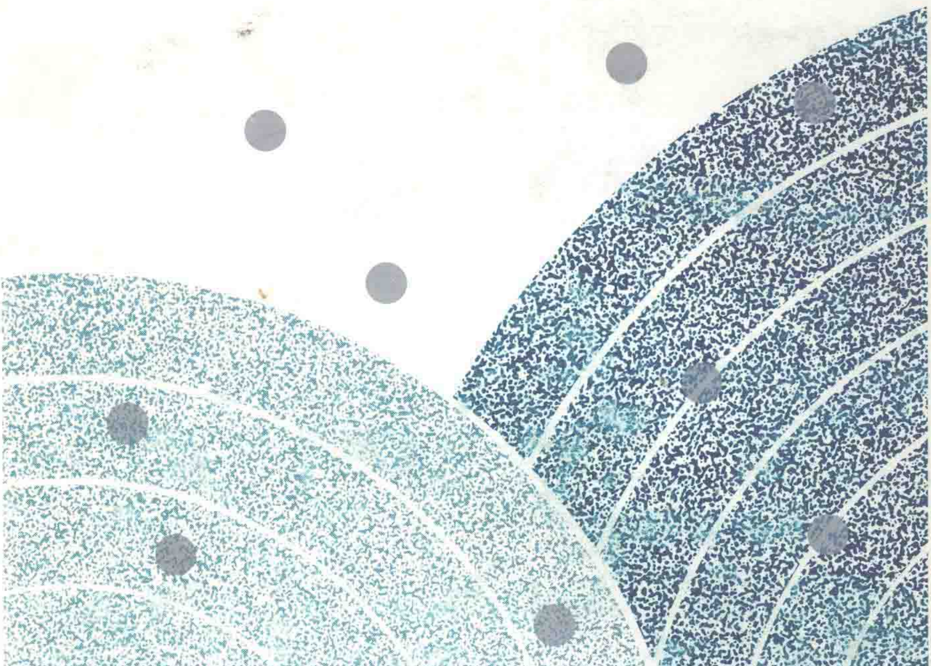
Ultratrace Metal Analysis in Biological Sciences and Environment

Terence H. Risby



ADVANCES IN CHEMISTRY SERIES 172

Ultratrace Metal Analysis in Biological Sciences and Environment

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A symposium sponsored by the Division of Analytical Chemistry at the 174th Meeting of the American Chemical Society, Chicago, Illinois, August 29–30, 1977.

ADVANCES IN CHEMISTRY SERIES **172**

AMERICAN CHEMICAL SOCIETY
WASHINGTON, D. C. 1979

Library of Congress CIP Data

Ultratrace metal analysis in biological sciences and environment.
(Advances in chemistry series; 172 ISSN 0065-2393)

Includes bibliographies and index.

1. Metals—Physiological effect—Congresses. 2. Metals—Analysis—Congresses. 3. Trace elements—Analysis—Congresses.
I. Risby, Terence H. II. American Chemical Society. Division of Analytical Chemistry. III. American Chemical Society. IV. Series.

QD1.A355 no. 172 [QP532] 540'.8s [574.1'921]
78-31903
ISBN 0-8412-0416-0 ADCSAJ 172 1–264 1979

PRINTED IN THE UNITED STATES OF AMERICA

Advances in Chemistry Series

Robert F. Gould, *Editor*

FOREWORD

Advances in Chemistry Series was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented, their papers, distributed among several journals or not published at all. Papers are reviewed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications. Volumes in the Advances in Chemistry Series maintain the integrity of the symposia on which they are based; however, verbatim reproductions of previously published papers are not accepted. Papers may include reports of research as well as reviews since symposia may embrace both types of presentation.

PREFACE

Metals at various concentrations play significant roles in many biological processes and these levels are dictated by the concentration of the protein, enzyme, etc. contained in the organism. Often the concentrations of the high molecular weight biochemical moieties are low and therefore the concomitant metal concentrations will be very low. With the increasing capability for the separation and purification of enzymes, etc. and with the improving sensitivities of analytical methodologies, the knowledge of the roles of metals in biochemical processes is continually expanding. The concentration of the metal in the organism is also critical since it is possible that at one concentration the metal can be essential whereas at a higher level this same metal can cause a serious metallic imbalance in the organism. Often the range between required and excessive concentration of a metal is not well defined. In addition to the discovery of new essential metals, other metals with known requirements are found to be involved in multiple processes.

In the area of the environment, metals are considered to be significant since they are, by definition, nonbiodegradable and are retained in the ecosystem indefinitely. Metals are found in air, water, and soil as a result of both natural and anthropogenic sources. Therefore organisms can receive body burdens via various assimulation routes. The symptoms of acute metal toxicity are generally well known, although often misdiagnosed, but the symptoms of long-term exposure to low-level concentrations of metals have not been established. This latter problem currently is being studied by many researchers since these data will enable environmental epidemiological studies to be performed.

This preamble exemplifies the need for cooperation between biochemists or environmental chemists with analytical chemists. This book attempts to show areas in which the collaboration between various disciplines will be the most productive.

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September 1978

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CONTENTS

1

Present Status and Future Development of Trace Element Analysis in Nutrition

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This discussion examines the recent progress of nutritional trace element research and its implications for trace element analysis. Elements recently identified as essential are present in low concentrations for which analytical methods are not yet reliable. Biological availability of trace elements depends on chemical form and on interactions with other inorganic and organic constituents of the diet. Therefore, information on elemental species is required, in addition to quantitative data. Finally, the demonstration of essential functions of trace elements previously known only for their toxicity necessitates establishing safe ranges of intake, free from danger of chronic toxicity but sufficient to meet human needs.

Trace element nutrition research is in a phase of rapid development. This is documented by the discovery of essential functions for the "new" trace elements in the past seven years (*1*) and by the increasing knowledge of marginal or pronounced deficiencies in humans of such elements as iron (*2*), zinc (*3*), chromium (*4*), and perhaps copper (*5*) and selenium (*6*). Although some of these deficiencies occur only in special age and sex groups or under unusual conditions, their existence has aroused public and scientific concern for the exact definition of human trace element needs and for the assessment of trace element nutritional status. These two major challenges for human trace element research cannot be met without proper analytical support. I will attempt to describe the present status of trace element analysis as it relates to nutrition research and to point out some new concepts that might well influence the future direction of trace element analysis.

0-8412-0416-0/79/33-172-001$05.00/0

Present Status

Trace elements fluorine, chromium, iron, copper, zinc, arsenic, selenium, molybdenum, cadmium, iodine, mercury, and lead are of public health concern because of suspected under-exposure or over-exposure (*7, 8*). Demands on analytical chemists for reliable analyses range from concentrations of micrograms/gram (e.g., iron) to less than 1 ng/g (e.g., chromium). Chemically defined aqueous solutions of trace elements within this range can be analyzed reliably by many different laboratories. The reliability with regard to accuracy, precision, and specificity declines drastically when foods or biological specimens (human and animal tissues) are analyzed. The steps involved in collection, preparation, and digestion of biological samples increase the probability for contamination of samples and for loss of the element under analysis by formation of refractory species or by volatilization. Each of the thousands of food and tissue samples has different matrix effects; even the urine of one individual can differ substantially in its concentration of salts from one day to another. An interlaboratory comparison on a bovine liver Standard Reference Material by the International Atomic Energy Agency revealed substantial differences in the results reported by various laboratories, ranging from 1 to 500 for the lowest and highest value for chromium to ± 30% around the certified value for zinc (*9*). Even the relatively small degree of imprecision for zinc is unacceptable to nutritionists. For example, the zinc content of typical daily diets in the United States apparently ranges between 10 and 12 mg (*10*). The Recommended Dietary Allowances, the amount considered adequate for most healthy human subjects, is 15 mg (*10*). In the analysis of the daily food intake, a 30% error in one direction would suggest that average intake is right at the Recommended Allowances, and the same error in the other direction would suggest that the average intake is deficient and would call for remedial measures such as zinc enrichment. Such errors also make it difficult, if not impossible, to compare data obtained by different investigators or to obtain agreement among nutritionists on which blood, urine, or tissue levels should be considered adequate or deficient. These problems do not impair the validity of comparative measurements obtained within one laboratory, but they are not compatible with large national or international studies. The only feasible solution to this problem would be the universal use of Certified Standard Reference Materials for the elements in question. Ideally, the composition of the material under analysis should be similar to that of the standard material. Ideal standards are not yet widely available, but the number and diversity of Standard Reference Materials probably will increase through the efforts of the National Bureau of Standards. At that time, scientific journals should insist on the proper use of these reference materials whenever absolute analytical trace element data are to be published. Some biological reference materials are available from the National Bureau of Standards (*11*), the International Atomic Energy Agency (*12*), and the U. K. National Physical Laboratory (Division of Chemical Standards), Teddington, England.

Food Tables

The only essential trace element for which food compositional data of some extent and reliability exist is iron (*13*). However, even assuming that the existing data are reliable, they are of limited use to nutritionists because biological availability differs greatly among iron species in foods (*14*). This will be discussed in more detail in a subsequent chapter. Food composition data for iodine are very limited. Environmental exposure of man to iodine is increasing, in part because of the iodization of table salt but also because of the high iodine intake from adventitious sources. Concern has been expressed about potential over-exposure to this element with the concomitant risk of marginal toxicity. Systematic studies of the iodine content in modern foods are clearly needed for assessment of a potential risk (*15*).

The 1974 edition of the Recommended Dietary Allowances established an allowance for zinc of 15 mg/day for adults (*10*). Several preliminary studies of daily zinc intakes suggested that this recommended allowance is not normally met by ordinary diets unless inordinate amounts of food are consumed; yet, comprehensive composition tables for zinc do not exist. Zinc concentrations in foods differ widely among laboratories (*16*). Data are too variable to serve as a basis for important decisions now being discussed (for example, whether or not zinc fortification of certain foods should be initiated). The U.S. Recommended Daily Allowances for copper are stated as 2 mg for adult persons. Early analyses suggested that copper intakes from U.S. diets ranged from 2 to 5 mg per day, but more recent analyses of diets have detected intakes of no more than 1 mg per day (*17, 18*). Systematic tables of copper in foods are lacking. For iron, iodine, zinc, and copper, problems of analysis are not insurmountable, and our knowledge of those elements in foods should continue to progress.

In contrast, the remaining trace elements of nutritional interest are silicon, vanadium, chromium, manganese, nickel, arsenic, selenium, molybdenum, tin, and perhaps cadmium. These elements present serious problems of analysis in the concentration range that is of interest to the nutritionist. Only a few specialized laboratories have developed expertise for the analysis of these elements, considerable discrepancies still exist among laboratories, and standardized reference materials are available for only a few elements. Consequently, food composition data are few and, except for selenium and manganese, of dubious value. An appraisal of the nutritional status of the population for these elements would depend upon the development of reliable methods so that valid compositional data could be compiled. For work with those trace elements whose concentrations in biological materials are very low, "clean room" techniques may become necessary for routine work. Finally, it is likely that the demands on the analytical chemist will increase as new trace elements are added to the list of those that are now known or suspected to be essential. It is reasonable to assume that essential trace elements still to be discovered are active in very low concentrations and, therefore, could present a new set of problems to the analytical chemist.

New Concepts Influencing Trace Element Analysis

Four new concepts that have emerged from recent trace element nutrition research could influence the future development of trace element analysis.

Concept of Optimal Range. Before the discovery of their essential roles, selenium, chromium, and arsenic were known for their toxic effects. The toxicology, acute and chronic, of cadmium was thoroughly investigated; on the other hand, recent work suggests that cadmium at low concentrations might be essential to animal species (*19*). Even lead might have a growth stimulating effect in animals that are kept in an ultra-clean environment, according to preliminary work (*1*). These observations are incompatible with the concept of "toxic" and "beneficial" trace elements. Toxicity is not an attribute of any one element, but rather of an excessive concentration range. Every nutrient in sufficiently excessive concentrations can be toxic; conversely, any of the trace elements now known for their toxicity might be shown in the future to have an essential function at low concentrations (*20*). Low tolerances have been established to limit the accumulation of "toxic" elements in the environment to "safe" levels; for selenium and arsenic (potential carcinogens), zero tolerances are considered desirable by some. Selenium enrichment of feedstuffs for some animal species is prohibited by law, even in regions with a substantial incidence of selenium deficiency. The demonstration of essential functions for selenium and arsenic has made it necessary to replace the concept of "zero tolerance" by the more scientific idea of "safe ranges of exposure" which takes into account the fact that a deficiency of an essential element is as incompatible with health as is toxicity. The range of safe intakes is defined as meeting the requirements of individual species without causing even the slightest signs of toxicity. Physiologically, this safe range comprises intakes that fall well within the ability of homeostatic control mechanisms to maintain optimal tissue concentrations through decreased intestinal absorption and increased excretion of intakes in excess of that required. The range of safe intakes is much lower than the range of even mild chronic toxicity so application of safe intake puts substantial demands for increased sensitivity and precision on the analytical chemist.

Reliable measurements of arsenic and cadmium in a concentration range of concern to the toxicologist might be of little interest to the nutritionist. Investigation of the mechanisms of essentiality of these elements would require increases of sensitivity of several orders of magnitude over those required for the mechanisms of toxicity.

Implications of Trace Element Interactions for Analytical Chemistry. Underwood stated that one and the same copper intake of animals can result in deficiency, a normal state, or toxicity, depending on the occurrence of molybdenum in the diet. That statement might well apply to other trace elements and might be valid for all animal species and man. The interaction of copper, molybdenum, and sulfur has been studied extensively in animals; it is of substantial importance in molybdenum-rich provinces in the USSR where a gout-like syndrome in man has been reported when the copper intake is relatively low but not when it is high (*21*). The new knowledge of the interaction of selenium with arsenic, cadmium, and mercury is another example, not only for its toxicological but also its nutritional implications (*22*). Selenium protects experimental animals against cadmium poisoning. The mechanism of this protection is unknown, but it does not function through increased excretion of cadmium. This latter metal is accumulated and retained by the organism as a consequence of selenium administration to a much higher degree than in the absence of selenium. Consequently, analytical data for cadmium alone have little biological meaning, unless they are accompanied by values for selenium. These latter values determine whether or not a given cadmium concentration in a food or tissue is toxic. Although much of our present knowledge of a selenium–cadmium interaction is derived from acute studies, recent work demonstrated similar effects in long-term chronic studies with very low dietary levels of these trace elements. A similar interaction was shown for selenium and mercury. The above examples indicate that toxic effects of heavy metals depend on the concentrations of selenium present; conversely, they also indicate that the selenium requirement is determined by the amount of heavy metals in diet and environment. Many other interactions are known (*7, 8*), and all of these directly affect the interpretation of analytical data.

Biological Implications of Chemical Forms. The biological availability of many trace elements is influenced by their valence state. Ferrous iron is believed to be more readily available than the ferric form, and selenium is better absorbed in its high oxidation state than in its lower ones. The organism is able to oxidize or reduce some, but not all, trace elements to their biologically active form. It is important, therefore, to determine the valence state in biological material, particularly in those cases where great differences of availability or toxicity exist, as in the case of chromium or of mercury.

Even more important than valence is the chemical form in which a trace element is present. This is clearly evident when functional aspects in the organism are considered. The zinc species in albumin, in macroglobulin or low molecular weight ligands, in zinc enzymes, or in bones all represent different pools of greatly divergent biological characteristics and significance. The iron in hemoglobin in the blood has an entirely different diagnostic meaning than the iron in serum ferritin. The total cobalt concentration in serum is of little interest in monogastric species whereas a small fraction of the total that is present as vitamin B_{12} has great importance for health. One of the great challenges of nutritional trace element analysis is the identification of those chemical species of trace elements that are meaningful indicators of the nutritional status of the organism. These species have been identified for iodine, cobalt, and iron but not for the remaining essential elements. The lack of adequate indicator species for that latter group is a major obstacle to the assessment of nutritional status of individuals and population groups.

Similar demands for speciation of trace elements exist for food analysis. Substantial differences in the biological availability are known for several essential elements and depend on the form in which they are present in the diet. The chemical bases for these differences are known for cobalt, iron, and chromium but not for zinc, copper, and selenium. The importance of speciation in food analysis is best demonstrated by the example of iron. That element, when part of heme compounds, is well absorbed, and there is little influence on the absorption by other factors in the diet. Nonheme iron, on the other hand, is not readily absorbed and, in addition, is subject to many influences from dietary ingredients; those influences are poorly understood and probably not completely known (*14*).

Calculations of heme and nonheme iron and dietary manipulations of these two categories are believed to offer the means to improve the iron nutritional status of a large number of people in the United States and abroad. Yet, no information concerning the concentration of heme and nonheme iron in foods is available, and the calculations have to be based on the estimate of an average iron content of meats of approximately 40% heme and 60% nonheme iron.

Work on the speciation of iron and zinc in vegetable products is just beginning (*23*), and some progress is being made on the speciation of chromium (*24*) but not enough data have been accumulated to be of interest to the nutritionist. Speciation analysis has produced impressive advances in the toxicology of heavy metals. Similar advances can be expected in trace element nutrition if the difficulties of methodology can be overcome.

Influence of Food Composition on Trace Element Action. The relation between food composition and the action of trace elements is perhaps not an immediate part of trace element analysis, but it provides the background for the interpretation of analytical data, i.e., for the ultimate judgement of whether the result of an analysis represents a deficient, optimal, or toxic concentration. Even if all the criteria previously discussed have been met (the analysis is reliable, interacting elements are known, and the chemical species have been identified), the determination of the biological implication still must take into account one additional factor—the composition of the diet. The toxicity of many substances is greater in purified diets than in those containing natural materials and fiber, whereas the biological availability of zinc and iron is decreased by the fiber content. Iron, chromium, and zinc, but not selenium, are better available from animal than from vegetable products. The availability for absorption of nonheme iron is enhanced significantly by the presence of ascorbic acid in an iron-containing meal, and a similar enhancement of nonheme iron absorption is produced by the presence of meat, attributed to an unidentified "meat factor."

Although the identification and analysis of such modifying factors go beyond the scope of trace element analysis, the importance of these factors should be recognized. The complexity of the process that leads from the first step of trace element analysis to the final statement of biological implication necessitates the close collaboration between the analytical chemist and the life scientist. The chemist should not be considered the provider of data, nor the biologist the interpreter of results; rather, both scientists must be aware of the whole process—its complexity and its difficulties (*25*). Only through this collaboration can the enormous amount of trace element analytical data be put in order and be interpreted properly. On this whole process depends the progress of trace element nutrition research and the improvement of the nutritional status of man.

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RECEIVED December 5, 1977.