CRC

ELEMENTAL ANALYSIS *of* BIOLOGICAL SYSTEMS

Volume I
Biomedical, Environmental, Compositional, and Methodological Aspects of Trace Elements

G. Venkatesh Iyengar

CRC PRESS

Elemental Analysis of Biological Systems

Volume I

Biomedical, Environmental, Compositional, and Methodological Aspects of Trace Elements

Author

G. Venkatesh Iyengar, Ph.D., D.Sc.
Principal Investigator
U.S. National Institute of Standards and Technology
(formerly The National Bureau of Standards)
Gaithersburg, Maryland

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging-in-Publication Data

Iyengar, G. Venkatesh.
Elemental Analysis of Biological Systems / G. Venkatesh Iyengar.
p. cm.
Bibliography: p.
Includes index.
ISBN 0-8493-5423-4
1. Trace elements — Analysis. 2. Biochemistry — Technique.
I. Title.
QP534.I94 1989
599'.019214—dc19 88-19474

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-5423-4

Library of Congress Card Number 88-19474
Printed in the United States

PREFACE

"Der Klebstoff der nicht klebt, verfehlt seinen Zweck."
(A glue that does not stick defaults its purpose)

This simple sentence, which I learned during my exposure to the German language, neatly sums up what I have to say in this preface. The goal of this book is to reach all those concerned with current developments in the area of biological trace element research (BTER) — analytical scientists and biomedical researchers alike, and also the scores of bench workers generating the analytical data. The chapters in Volume I which were conceived with the above notion are presented in the following sequence.

Chapter 1 focuses on the need for a multidisciplinary approach in BTER. Because this basic requirement was not recognized in time, progress has been severely hampered in this field, and the outcome has not been proportional to the efforts expended.

Chapter 2 deals with chemical elements in biological systems. Here, the reader's attention is drawn to the practical benefits of applying trace element analysis to solve some public health problems.

Chapter 3 is intended to help the analyst get acquainted with the basic physiological properties of biomedical specimens, analysis of which occupies so much of an analyst's time. Without this kind of introduction, it is likely that an analyst becomes a passive participant in the overall context of a scientific investigation. Analytical chemists must recognize that the processes of trace element chemistry in biological systems must be examined at morphological, biochemical, and physiological levels.

Chapter 4 summarizes the biological basis of various biomedical specimens. This section addresses the crux of one of the key BTER problems, namely, choice of a specimen as a meaningful representative of the problem under investigation.

Chapter 5 provides an account of situations confounded in dealing with elemental analysis of biological systems. The analyst's attention is drawn to the fact that in these cases one is dealing with a dynamic system unlike the analysis of static inorganic systems.

Chapter 6 identifies the applicability of numerous analytical methods to the determination of various elements. In order to cover all the elements of biological significance, one needs a combination of several analytical approaches, and this point is stressed from a practical point of view.

Chapter 7 emphasizes the role of quality control (QC) in BTER. There are no shortcuts to the QC requirements. Well-established QC procedures are a way of life for generating reliable analytical results, and the analyst's attention is drawn to this demand.

Chapter 8 provides insights into presentation and interpretation of analytical data in BTER. It should be recognized that findings that cannot be meaningfully interpreted are of little value. In exploring an unknown territory, the soundness of the analytical data should be safeguarded to facilitate meaningful explanation at a later date.

Chapter 9 attempts to sift through literature data to provide reliable reference values for trace elements in biological specimens, especially for routine clinical specimens. For many elements, there are still some unresolved analytical problems and defining reference values is still an unfinished task. Several perplexing problems are illustrated to clarify what will be needed in order to generate the missing information.

Chapter 10 is a catalog of available biological, clinical, and dietary reference materials for use in bioinorganic analytical QC programs. As an aid for analysts, a list of analytical results of various reference materials is provided. This should be a useful reference source for day-to-day use by all those interested in quality assurance endeavors.

It is now left to the readers to judge to what extent the information glue in this book has stuck to their minds!

G. V. Iyengar
Gaithersburg, Maryland

AUTHOR

Venkatesh Iyengar, D.Sc., Ph.D., obtained his Ph.D. in analytical chemistry (Uppsala, Sweden) and his D.Sc. in zoology (Mysore, India). He worked for several years at the Institute of Medicine of the Nuclear Research Center, Juelich, West Germany. He was also a consultant to the Medical Applications Section of the International Atomic Energy Agency (IAEA) in Vienna. He has been an active participant in several international trace element research studies, and a member of international committees. He is an adjunct Associate Professor at the Swedish University of Agricultural Sciences, Uppsala. He authored a book in 1978, has been commissioned to write reviews, and has published extensively in the area of trace element analytical chemistry and biological trace element research. Presently he is with the National Institute of Standards and Technology (NIST; formerly The National Bureau of Standards) as the principal investigator of an international project on the daily dietary intakes of minor and trace elements by human subjects, a joint undertaking of the IAEA, NIST, U.S. Food and Drug Administration, and the U.S. Department of Agriculture.

ACKNOWLEDGMENTS

I am grateful to friends and colleagues at the National Institute of Standards and Technology, the Food and Drug Administration (FDA), and the U.S. Department of Agriculture who helped me in several ways, allowed me to draw on their vast experience in various fields, and offered their comments and criticism on parts of the text.

I am particularly indebted to Dr. William Horwitz from the FDA, Dr. Robert Parr from the International Atomic Energy Agency, and Dr. Vasantha Iyengar, a consultant to the Department of Human Genetics, University of Michigan at Ann Arbor. I had several discussions with Drs. Horwitz and Parr that are reflected in Chapter 7 on quality assurance. Dr. Vasantha Iyengar was instrumental in preparing Chapter 3 on physiological aspects of biomedical specimens. Her combined expertise as a physician, biochemist, and trace element researcher has been of great help in presenting that chapter in very clear language.

I am also thankful to Dr. James Smith of the U.S. Department of Agriculture for reviewing Chapter 9, and to Ms. Sudha Rao for the artwork in Chapter 3.

Permission to reproduce several illustrations and tables from other publications is gratefully acknowledged. Some of these are presented with slight modifications, but in each case reference is made to the original source.

This work was started during my tenure at the Institute of Medicine (IME), Nuclear Research Center, Juelich, West Germany. I warmly recall the support of Professors H. Glubrecht and A. R. Gopal-Ayengar who motivated me to take up this assignment.

To A. R. Gopal-Ayengar and Helmut Glubrecht

TABLE OF CONTENTS

Chapter 1

BIOLOGICAL TRACE ELEMENT RESEARCH

"The lack of a multidisciplinary approach has been the Achilles heel of biological trace element research."

I. INTRODUCTION

Many chemical elements are indispensable for the proper functioning of biological systems, and investigations aimed at understanding their mode of action in biochemical processes are engaging the attention of researchers in various laboratories. A better understanding of these biochemical processes, especially those linked to elements which are present in extremely minute amounts and are essential to the maintenance of life in dynamic equilibrium, has been made possible by the advent of newer analytical techniques. However, a lack of multidisciplinary approach, characterized by the absence of teamwork between analytical scientists and life sciences researchers, has severely hampered progress in the area of biological trace element research.[1] Attention is devoted to these points in the following chapter.

II. NEED FOR A MULTIDISCIPLINARY APPROACH

It should be recognized that the paramount issue in biological trace element research is the importance of a multidisciplinary approach. This is a crucial parameter that combines a sound basis in analytical chemistry with a substantial knowledge of biological, physiological, pathological, and biochemical factors related to fundamental biological functions. An understanding of these basics is necessary to appreciate the changes taking place in the mean composition of samples before and even during the course of an analysis. It therefore becomes clear that, unlike analysis of static inorganic materials, analytical aspects of biological systems become more difficult because of these diverse factors.

It is essential to establish firm baseline values for elemental concentrations in tissues and body fluids in normality so that alterations during disease states can be identified. In practice, however, this is a difficult task requiring consideration of and compensation for a number of possible concurrent phenomena, and correlations are very complex. Baseline data studies can be deceptive, and the question of what is normal is by no means easy to answer.

The sources responsible for the wide variation of orders of magnitude in reported data are schematically presented in Figure 1.[2] As can be inferred from this, a reliable conclusion depends on the quality of the analytical result. Among the components shown in Figure 1, identification of analytical errors has received considerably more attention by the analytical community.[3-14] However, the role of presampling factors (defined as events encompassing biological variations, postmortem changes, and a host of inadvertent sources of errors leading to uncontrollable internal contaminations and endogenous losses associated with a biological specimen *in situ*) has not been sufficiently understood. These are discussed in detail under a separate chapter on presampling factors in Volume II.

Human bodies recovered from accidental deaths are the main sources for obtaining various tissues for analysis. However, postmortem events play a very significant role in causing elemental shifts within an organ, depending upon the degree of cell swelling, extent of imbibition undergone, and the stage of tissue autolysis (see chapter on presampling factors in Volume II). It is difficult to account for these changes if a thorough documentation has not been maintained. A review of the analytical data related to autopsy and biopsy specimens[15] exemplifies such a situation (Figure 2). As can be seen from Figure 2, a rather broad range of

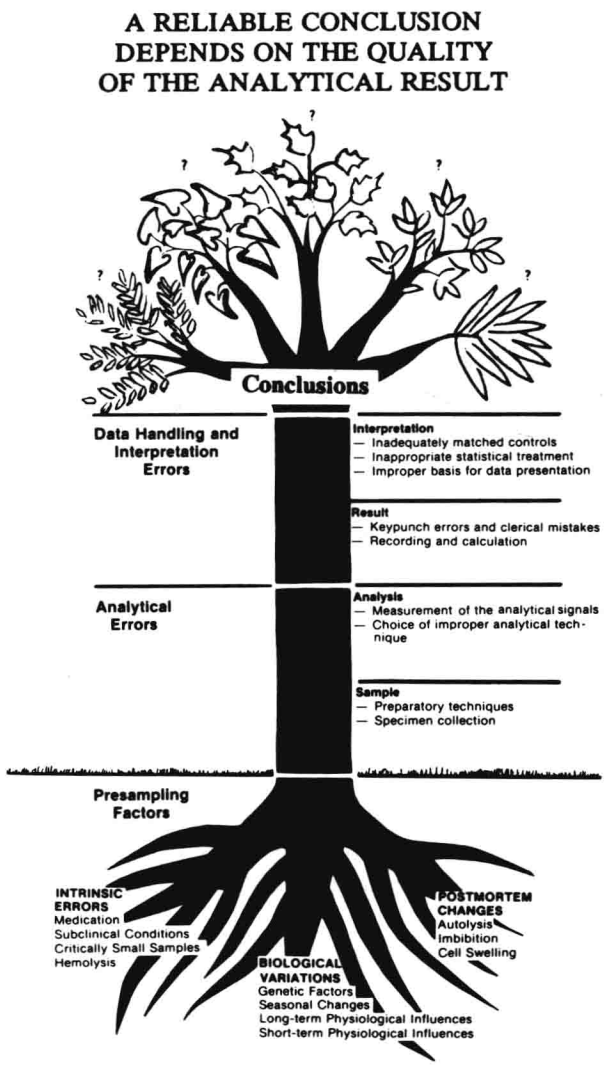

FIGURE 1. Essential components of the elemental analysis of biological systems. (From Iyengar, G. V., *Anal. Chem.*, 54A, 554, 1982. With permission.)

results, even for calcium, magnesium, potassium, and sodium, have been reported. These are clearly unacceptable situations since these elements are known to be under well-defined physiological control in living organisms and indicate some predominant changes that have taken place in the quality of the sample due to postmortem events such as cell swelling and autolysis. In the case of blood and serum specimens from living subjects, the variations observed are not as great as in samples collected at autopsy. However, an overall variation factor of 1.8 seen for zinc in blood serum (Figure 2) is clinically unacceptable as normal for reference value, since the spread of the results could be due to a combination of analytical errors and biological variations.

The need for a multidisciplinary approach in biological trace element research is effectively reflected in one of our investigations dealing with sampling of human platelets for trace element analysis.[16] As shown in Figure 3, the scheme involves sample size, retention of viability in the isolated product, cell purity, trapped plasma, and the strategies needed to deal with contamination by trace elements.

Element / Samples	Ca ×10	Cl ×10³	K ×10³	Mg ×10²	Na ×10³	Cu	Fe ×10²	Zn ×10
	3 9 15 21	0 2 4 6	0 2 4 6	0 2 4 6	0 2 4 6	0 4 8 12	0 2 4 6	0 4 8 12
Brain	(4·3)	(1·7)[b]	(2·2)	(5·4)	(1·1)[b]	(5)	(5)	(4·1)
Heart	(3·9)	[c]	(2·1)	(1·9)	(1·4)[b]	(2·2)	(5·3)	(5)
Kidney	(2·3)	[c]	(1·7)	(2·5)	(1·1)[b]	(3·5)	(4)	(3·6)
Liver	(3)	(2·4)	(1·8)	(2·2)	(3·1)	(7·3)	(5·6)	(4)
Lung	(2·2)	(1·5)[b]	(1·8)	(3)	(1·2)[b]	(4·5)	(5·3)	(2·5)
Muscle	(3·8)	(3·5)	(3·1)	(1·7)	(4·5)	(5·7)	(2·5)	(2)
Blood	(1·2)	(1·3)	(1·4)	(1·47)	(1·2)	(1·5)	(1·4)	(1·5)
Serum	(1·2)	(1·15)	(1·2)	(1·2)	(1·1)	(1·5)	(1·87)	(1·8)
	3 9 15 21	0 2 4 6	0 2 4 6	0 2 4 6	0 2 4 6	0 4 8 12	0 2 4 6	0 4 8 12

[a] only whole brain considered [b] limited number of results [c] single values

FIGURE 2. Variations in the elemental concentations in normal adult human tissues and body fluids. Figures in parentheses are maximum/minimum ratios. Concentrations expressed in milligrams per kilogram or per liter of fresh tissue or fluid. Data from Iyengar et al.[15]

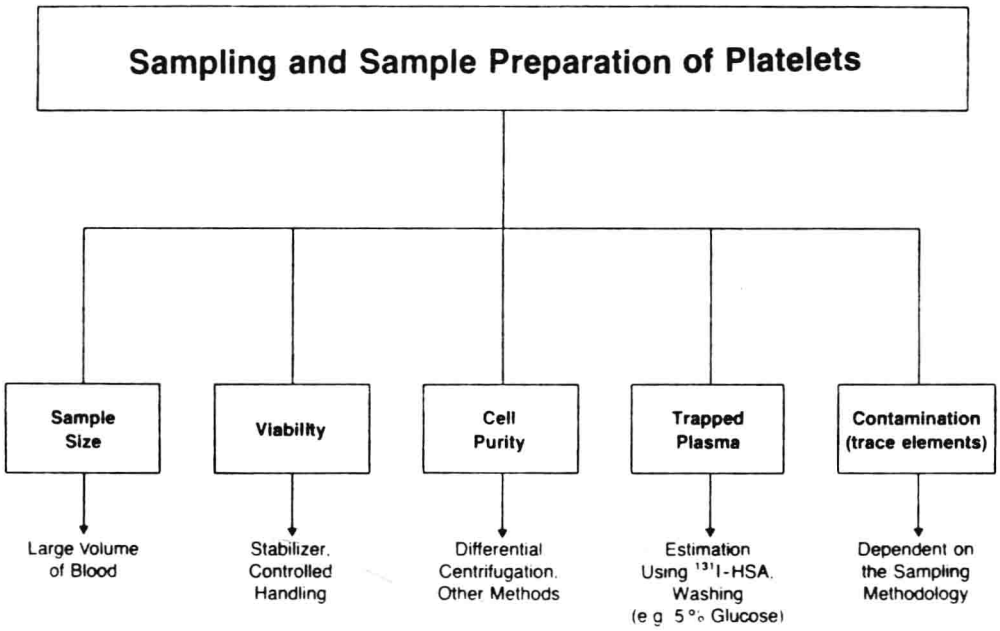

FIGURE 3. An example of multidisciplinary components involved in a biomedical trace element investigation of cells.

Sample size — Because the relative number of platelets in blood is very small, a large blood sample is necessary to obtain the quantity needed to express elemental data on a weight basis rather than only on the number of platelets. The main disadvantage in relating element content to platelet number is the difficulty in obtaining a standardized mean value of the single platelet. Hence, intercomparison of values from different investigations is difficult.

Viability — Viability of platelets means the retention of their functional integrity. Platelets are very sensitive and easily damaged, even by washing with isotonic solutions, so it is difficult to preserve their viability. Loss of viability results in significant changes of certain components, such as a decrease in potassium concentration.[17] It is reasonable to assume that trace elements will also be affected by any loss of viability. It is therefore necessary, insofar as possible, to