
MASS
SPECTROMETRY
in CANCER
RESEARCH

John Roboz



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MASS SPECTROMETRY *in* CANCER RESEARCH

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Dedication

*For my daughters Gail and Joanne,
with love and respect*

About the Author



John Roboz is a Professor in the Department of Medicine (Division of Medical Oncology) of the Mount Sinai School of Medicine, New York. He holds a B.S. degree (1955) from Eötvös University, Budapest, Hungary, and M.S. (1960) and Ph.D. (1962) degrees in Physical Chemistry from New York University.

After immigrating to the U.S. from Hungary in 1957, he worked as a Senior Research Chemist for General Telephone & Electronics Research Laboratories (Bayside, NY) and then at the Central Research Laboratories of the Air Reduction Company (Murray Hill, NJ). In 1969, he joined the Mount Sinai School of Medicine as an Associate Professor in the Department of Pathology and in 1974 also joined the faculty of the Biomedical Sciences Doctoral Program. In 1980, he became Professor of Neoplastic Diseases in the Department of Neoplastic Diseases, which was later incorporated within the Department of Medicine.

His primary research interests have been in the development of mass spectrometric techniques in analytical

pharmacology and biochemistry and the application of these methods in many collaborative studies with basic scientists developing new antineoplastic agents and clinicians conducting Phase I clinical trials. He has also conducted research on the occurrence of hyaluronic acid in mesothelioma, and on the role of D-arabinitol as a marker for the early diagnosis and monitoring of candidiasis in cancer patients. Current areas of interest include the use of surface-enhanced laser desorption time-of-flight and electrospray mass spectrometry for the detection and identification of unique proteins as potential markers for several malignancies.

Dr. Roboz has more than 130 publications in mass spectrometry, including several review chapters. One of these was the first review on mass spectrometry in cancer research (1978). In 1968 (reprinted 1979) he published a textbook entitled *Introduction to Mass Spectrometry: Instrumentation and Techniques* (Wiley-Interscience, New York). It was subsequently reprinted in 2000 by the American Society for Mass Spectrometry as Volume 3 in the *Classic Works in Mass Spectrometry* series.

Foreword

Over the past decade mass spectrometry (MS) has experienced a remarkable growth, spurred primarily by the introduction of ionization techniques such as electrospray and matrix-assisted laser desorption, and the development of relatively user-friendly instrumentation. Nowhere is this growth more evident than in the biological sciences where MS-related techniques (e.g., LC/MS, GC/MS, MALDI) are considered an essential analytical arsenal and are used routinely.

While the focus of this book is on mass spectrometry in cancer research, there is material in it for almost everyone. For the biological scientist who is a novice in mass spectrometry, the first chapter provides a comprehensive summary of the principles of the technique with emphasis on their applicability to the analysis of biomolecules. For the “purist” in mass spectrometry who is becoming involved in analytical biochemistry or cancer research, many of the essential fundamentals in the biological sciences are introduced with sufficient detail such that they allow one to see the broader picture and gain an appreciation of how valuable the technique can be. Finally, current researchers in the field will benefit from the generally extensive list of citations, which should enable them to further expand their research endeavors.

The reader is treated to a wide spectrum of MS applications to cancer research. These applications range from topics related to the use of MS techniques for the detection of biomarkers arising from environmental exposure to the elucidation of the mechanism of action of chemopreventive agents, both synthetic drugs and naturally occurring compounds. The coverage of the different topics is accompanied by presentation of well-selected examples with a clear discussion of the subject matter and figures illustrating the finer points of the analysis. The final chapter, which discusses techniques and strategies in cancer biochemistry and biology, provides a broad perspective of the field and introduces a variety of thought-provoking concepts that can serve as a guide in the design of future research projects.

Paul Vouros

*Professor of Chemistry
Northeastern University
Boston*

Mass Spectrometry: An Oncologist's Viewpoint

Yesterday's oncologist might question the role of mass spectrometry in cancer research. The early trial and error methods, the indiscriminate screening of every compound on the shelf, and acceptance of partial tumor inhibition in the mouse, no longer suffice to initiate a clinical trial hoping for eventual cures or prevention. The explosive expansion of knowledge about DNA, proteins, and intracellular chemistry have provided a new calculus for understanding the cancer process. Understanding the normal pathways of signal transduction, from ligand to receptor to the cascade of enzymes that lead to gene expression or repression, is necessary to illuminate the relationships of cells in a multicellular organism. Aberrations in these complex processes exist in cancer. Although the altered patterns may be mechanistically operative in bringing about cancer cell survival and growth, they may well be epiphenomena. Altered DNA function brought about by loss, mutation, or viral insertion or usurpation appear with today's knowledge to underlie the cancer process.

Every one of the involved molecules must be identified. To understand the kinetics of reactions they must be quantified. Many analytic techniques provide qualitative or even semi-quantitative indications of the compounds involved. Precision analysis is required for certainty and this book elegantly sets forth the contributions that mass spectrometry makes to that precision and certainty.

Cancer therapies still deal primarily with surgery and radiotherapy. Both these modalities are concerned predominantly with local and regional disease. The control of disseminated cancer cells relies on systemic therapies, chemical, and immunologic. Precise measurements of all aspects of chemotherapeutic compounds during synthesis and studies of pharmacokinetics and pharmacodynamics are essential. Metabolic products of the administered drug, both anabolic and catabolic, must be identified and quantified to arrive at optimal dosing regimens. Individuals may vary widely in their disposition of drugs. Mass spectrometry is unsurpassed as the gold standard in these areas.

Cancers secrete large and small molecules of many known and countless unknown structures. Enzymes that allow cancers to invade and metastasize and surface molecules and compounds of unknown function often serve as critical parameters of cancer behavior. Discovery of trace compounds of unique nature that could indicate the presence of early cancer is still theoretically possible, and still hoped for. Identification of such a compound in extremely small quantities in biologic fluids containing hundreds of other compounds is a classic undertaking for mass spectrometry. Coordinated immunologic assay, isotopic, spectroscopic, nuclear-magnetic resonance, and mass spectrometric analyses of such putative markers could advance our diagnostic acumen so that we might recognize pre-cancerous changes, or cancers so early in their course that a cure could be readily achieved.

Cancer is a vast collection of diseases with some common characteristics that usually represent abnormalities in somatic cell genetics, not germ line genetics. Most causes of cancer are environmental, not genetic. A treasure trove of possibilities in cancer prevention and detection awaits in the field of molecular epidemiology. Identification of changes in DNA that arise from interaction with individual environmental agents is of profound interest. Contributions of mass spectrometry to this infant field are largely untapped.

This volume displays the rich understanding of cancer that a brilliant mass spectrometrists has absorbed by proximity to and collaboration with physicians and biological scientists working on cancer. The advances in mass spectrometry have kept pace over the past 25 years with the advances in molecular biology and clinical cancer research. It is a safe bet that continuing advances in all three areas are imminent, and that mass spectrometry can contribute to the future of both laboratory and bedside cancer investigations. This book provides an admirable foundation for understanding the field and for perceiving the challenges that lie ahead.

James F. Holland, MD

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Preface

Modern cancer research is inevitably and increasingly becoming multidisciplinary with the realization of the interwoven complexity of the biochemical, structural, therapeutic, and clinical questions that must be answered. Such problems require integrated, synergistic approaches that employ an assortment of biochemical or immunological manipulations, chromatographic or electrophoretic separations, sequencing strategies and ... more and more, mass spectrometry.

The diversity of the disciplines in which mass spectrometry is now a critical component can be seen by inspecting the ever increasing number of publications dedicated to “real world” applications as opposed to those dealing with methodology development. In these applications the expertise of the co-authors often cover multiple disciplines including biochemistry, molecular biology, immunology, pharmacology, microbiology, and even reach areas such as surgery and molecular modeling. Accordingly, it is particularly relevant that a book on mass spectrometry in cancer research gives an overview of how mass spectrometry provides an analytical link between all these fields.

This book is intended for: (a) mass spectrometrists involved in cancer research or in providing core services; (b) “customers,” i.e., researchers in the biological, medical, pharmaceutical, or environmental sciences who use mass spectrometry in their work; (c) potential clients contemplating how to solve their intractable analytical problems; and (d) academic and industrial managers anxious to understand the approaches and results of the mass spectrometry being used in the projects they direct or administer.

The Overview (Chapter 1) is intended for those who just want to obtain a quick understanding of how mass spectrometers work, what the differences are between the various ion sources and analyzers, and what are the potential applications for the available analytical techniques in cancer research and in support of therapy. Chapter 2 is intended to provide the scientist and physician, not expert in the field, with an understanding of mass spectrometric instrumentation and pertinent methodologies. In contrast, Chapter 3 presents relevant concepts of cancer medicine and biology. They are intended for mass spectrometrists who are, *horribile dictu*, not also experts in both medicine and molecular biology. The intent of Chapters 4, 5, and 6 on occupational and environmental carcinogens, antineoplastic and chemopreventive agents, and proteins, lipids, nucleic acids, and glycoconjugates, is to provide a broad rather than exhaustive examination of current strategies and techniques illustrated with relevant applications. In a book designed to provide an overview, it is likely that experienced practitioners may be disappointed by the coverage of their specialty. If so, I ask for their indulgence for my naive and superficial, but, hopefully, not erroneous, approach.

It is emphasized that the length of a section does not automatically indicate the importance of the subject. In fact, it is often the case for new and developing methods and areas of application that they may be potentially of major importance but are covered only briefly. This is because there were few publications, albeit often pioneering, available at the time this manuscript was completed. In addition, it often felt as if new and relevant publications, especially in areas such as tumor proteomics and targeted therapies, were appearing every week. Similarly, the relative importance of specific mass spectrometric applications keeps changing, particularly with respect to the clinical uses of chemotherapeutic agents. An arbitrary halt had to be imposed to the search for new and relevant articles and therefore the literature was covered through August 2001. The selection of one reference over another is inevitably subjective. Those references selected should be of value to the

reader in that they describe original research, are particularly instructive, or provide detailed reviews of a particular subject. Intentionally there are no references in the Overview. In addition, only a brief list of general texts is provided at the end of Chapter 3. My indebtedness to the quoted references is obvious. Sometimes the original text was so succinct and well-written that it was difficult to recompose. In such cases I can do no more than honor the original authors.

Mass spectrometry grew almost unbelievably in the final quarter of the 20th century. According to industrial sources, instrument sales reached the \$2 billion level in 2000. During the last decade there was an astonishingly rapid development of technology and methodology for the determination of the molecular masses, sequences, and higher structures of large, hitherto untouchable molecules, including proteins, nucleic acids, and glycoconjugates. Publications describing and/or proposing new and novel strategies, approaches, schemes, and techniques abound. If one has to add a caveat it should be said that many of these reports are “proof of concept” studies in which the applicability of the new technique is demonstrated in “model” systems, i.e., in carefully selected situations where the solutions are known or easily predicted. Conclusions drawn usually include a catalogue of true and/or perceived advantages, often exaggerated, with few, if any, limitations listed. These reports play an essential role in the transition from innovative ideas to practical applications. Therefore, when such papers are included in this text, it is done without critical evaluation, using only the criterion that the technique has potential applicability to problems in cancer research.

The reader will find some annoying inconsistencies in the naming of compounds and their abbreviations, in concentration units, spelling, etc. When confronted with widely differing designations in the copyrighted figures and tables, I found that, regretfully, I had to give up my feeble attempts at uniformity when describing work on the same subject by different authors.

It has been said that predictions are dangerous, particularly when they concern the future. After several attempts, I abandoned the inclusion of a section on general prospects and personal prognostications. The sage suggestion of the great New York Yankee catcher and philosopher/poet Yogi Berra applies directly to the apparently endless series of breathtaking breakthroughs in the technology of mass spectrometry: “The future ain’t what it used to be.”

Acknowledgments

Throughout the preparation of the manuscript, I have profited from the intellectual input of many of my colleagues in the scientific and medical community. Foremost, my heartfelt thanks go out to Dr. John Greaves, Director, Mass Spectrometry Facility, University of California at Irvine, for the time he has invested, and for the care with which he has reviewed almost every sentence of this manuscript. Dr. Greaves has shown an extraordinary talent in reading the text from the point of view of an informed mass spectrometrists who intends to use this book as background material in preparing to apply a particular mass spectrometric technique to research not yet pursued by others. It was a pleasure, and provided satisfaction, to observe the text becoming visibly improved as I made modifications based on his suggestions, often by changing just a word or the structure of a sentence. Equally frequently, his challenging questions helped materially in the illumination of the strategy, technique, or result being discussed.

I am strongly indebted to Dr. Lawrence Phillips, who read several chapters of the manuscript and offered numerous suggestions for improvements, and to Dr. Jozsef Lango, for his useful criticism of the sections on basic principles and instrumentation. Acknowledgments and recognition are due to Drs. Ramu Avner, Stephen Carmella, Steven Dikman, Stephen Hecht, Joel Graber, James Holland, James Maggs, Mark McKeage, James-Gilmour Morrison, Kevin Park, Gail Roboz, Paul Vouros, Rong Wang, Martin Winkler, and Ralph Zimmermann, who read various parts of the manuscript and contributed in significant ways. I wish to thank the many individuals with whom I have conversed over the years and whose ideas and thoughts can be found in many corners of the book. As I did not always heed the good advice offered, I am alone responsible for the shortcomings of this book.

I am grateful to the many authors who provided copies of selected illustrations from their papers and reprints of their work. Thanks are due to Micromass Co. and Agilent Technologies Inc. for permission to include illustrations from their publications. The courtesy of Dr. George Wright, Jr., is acknowledged for providing a figure that shows unpublished results of his research on biomarkers of prostate cancer. Among members of my staff, I would like to thank Longhua Ma, for his superb help in making new illustrations, Demetra Silides, for spending long hours entering references into a database, and Lin Deng, for helping with many tasks.

Special thanks are due to James F. Holland, M.D., Distinguished Professor of Medicine at Mount Sinai School of Medicine, New York, for his interest and steadfast support of my research for almost 30 years. He has been an incomparable boss, collaborator, and friend. I wish to express my indebtedness to the T.J. Martell Foundation for Leukemia, Cancer, and AIDS Research, and to Mr. Derald Ruttenberg for their generous support of my research over several decades.

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Finally, I would like to express very special thanks to my wife, Julia, for her understanding and encouragement, and all too often, for just tolerating me.

John Roboz

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