



Nuclear Medicine in Vitro

second edition

Benjamin Rothfeld

NUCLEAR MEDICINE *IN VITRO* SECOND EDITION

Edited by

BENJAMIN ROTHFELD, M.D.

Chief, Nuclear Medicine, Baltimore City Hospital, Baltimore, Maryland
Assistant Professor of Medicine, The Johns Hopkins
University School of Medicine, Baltimore, Maryland

With 40 Contributors



J.B. Lippincott Company
PHILADELPHIA

London Mexico City New York
St. Louis São Paulo Sydney

Acquisitions Editor: William Burgower
Sponsoring Editor: Darlene D. Pedersen
Manuscript Editor: Carol M. Kosik
Indexer: Gene Heller

Art Director: Maria S. Karkucinski
Designer: Rita Naughton
Production Assistant: George V. Gordon
Compositor: Ruttle, Shaw & Wetherill, Inc.
Printer/Binder: Halliday Lithograph

Second Edition

Copyright © 1983, by J. B. Lippincott.

Copyright © 1974, by J. B. Lippincott Company. All rights reserved. No part of this book may be used or reproduced in any manner whatsoever without written permission except for brief quotations embodied in critical articles and reviews. Printed in the United States of America. For information write J. B. Lippincott Company, East Washington Square, Philadelphia, Pennsylvania 19105.

1 3 5 6 4 2

Library of Congress Cataloging in Publication Data

Rothfeld, Benjamin.

Nuclear medicine—in vitro.

Includes bibliographical references.

1. Radioisotopes in medicine. I. Title.

[DNLM: 1. Nuclear medicine. WN440 R846n]

RM858.R67 616.07'575 74-14921

ISBN 0-397-50505-1

The authors and publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new or infrequently employed drug.

To Fil, Alan, Barbara, Ed, Debbie, Sean, and Megan Lynn

CONTRIBUTORS

Stephen R. Abbott, Ph.D.

Celltech Limited
Berkshire, England

Solomon N. Albert, M.D.

Greater Southeast Community Hospital
Washington, DC

Claude D. Arnaud, M.D.

University of California and
Veterans Administration Medical Center
San Francisco, California

Thomas J. Cali, Pharm.D.

Assistant Professor of Clinical Pharmacy
University of Maryland School of Pharmacy
and University Hospital
Baltimore, Maryland

Tapan K. Chaudhuri, M.D.

Professor of Nuclear Medicine
Eastern Virginia Medical School
Norfolk, Virginia

Michael Cobb, B.S.

Syva Company
Palo Alto, California

Kenneth Cowan, Ph.D.

Medical Breast Cancer Section
Medicine Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Laurence M. Demers, Ph.D.

Professor of Pathology
Department of Pathology
The M.S. Hershey Medical Center
The Pennsylvania State University
Hershey, Pennsylvania

Carol A. Dorsch, M.D.

Attending Physician
Baptist Hospital
Pensacola, Florida

Henry A. Feldman, Ph.D.

School of Public Health
Harvard University
Boston, Massachusetts

Maguelone G. Forest, M.D.

Pediatric Endocrine Clinic
Johns Hopkins University Hospital
Baltimore, Maryland
Unité de Recherches Endocriniennes et
Métaboliques chez l'Enfant, INSERM
Hôpital Debrousse, Lyon, France

Signe Gotcher, B.A.

Syva Company
Palo Alto, California

John Griffiths, M.D.

Professor of Laboratory Medicine
Medical University of South Carolina
Charleston, South Carolina

Victor Herbert, M.D.

VA Hospital
Bronx, New York

Tah-Hsiung Hsu, M.D.

Associate Professor of Medicine
Johns Hopkins University
Baltimore, Maryland

Edward James

Clinetics Corporation
Tuftin, California

Alfonso H. Janoski, M.D.

Chief, Section of Endocrinology
Franklin Square Hospital
Baltimore, Maryland
formerly,
Assistant Professor
Department of Medicine
Division of Endocrinology and
Metabolism
University of Maryland
School of Medicine
Baltimore, Maryland

Raymond S. Koff, M.D.

Professor of Medicine
Boston University School of Medicine
Chief, Hepatology Section
VA Medical Center and
Boston University Medical Center
Boston, Massachusetts

Joseph R. Kraft, M.D.

St. Joseph Hospital
Chicago, Illinois

Kingsley R. Labrosse, Ph.D.

Chief, Clinical Chemistry
Department of Pathology
St. Paul–Ramsey Medical Center
St. Paul, Minnesota

John H. Laragh, M.D.

Hilda Altschul Master Professor of Medicine
Cardiovascular Center
New York Hospital–Cornell Medical Center
New York, New York

Marc Lippman, M.D.

Medical Breast Cancer Section
Medicine Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

John W. McBride, M.D.

Section of Cardiology
St. Paul–Ramsey Medical Center
Assistant Professor
School of Medicine
University of Minnesota
St. Paul, Minnesota

Harry G. McCoy, Pharm.D.

Section of Clinical Pharmacology
St. Paul–Ramsey Medical Center
Chief of Cardiovascular Section and
Assistant Professor
College of Pharmacy
University of Minnesota
St. Paul, Minnesota

Claude J. Migeon, M.D.

Pediatric Endocrine Clinic
Johns Hopkins University Hospital
Baltimore, Maryland

Eileen L. Nikoloff, Ph.D.

Director, Clinical Assay Development
E.R. Squibb & Sons, Inc.
Princeton, New Jersey

Jon Pehrson, M.D.

Assistant Professor of Medicine
Boston University School of Medicine
Section of Endocrinology and Metabolism
Thorndike Memorial Laboratory
Boston City Hospital
Boston, Massachusetts

Jacek J. Preibisz, M.D.

Assistant Professor of Medicine
Cardiovascular Center
New York Hospital–Cornell Medical Center
New York, New York

Salvatore Raiti, M.D.

Director, National Pituitary Agency
Director, Pediatric Endocrine Clinic
University of Maryland Hospital
Baltimore, Maryland

Hyman Rochman, M.D., Ph.D.

Department of Pathology
University of Chicago
Chicago, Illinois

Benjamin Rothfeld, M.D.

Assistant Professor of Medicine
School of Medicine
Johns Hopkins University
Baltimore, Maryland

Ellis Samols, M.D.

Department of Medicine
Veterans Administration Medical Center and
University of Louisville School of Medicine
Louisville, Kentucky

Jean E. Sealey, D.Sc.

Research Professor of Physiology in Medicine
Cardiovascular Center
New York Hospital–Cornell Medical Center
New York, New York

Dean S. Skelley, Ph.D.

Memorial Hospital
Houston, Texas

Marvin J. Stone, M.D.

Department of Internal Medicine
(Cardiovascular and Hematology Divisions)
University of Texas Health Science Center
and the Charles A. Sammons Cancer Center
Baylor University Medical Center
Dallas, Texas

Craig K. Svensson, Pharm.D.

Research Associate
Clinical Pharmacokinetics Laboratory
Buffalo General Hospital
Buffalo, New York

Judith Vaitukaitis, M.D.

Professor of Medicine and Physiology
Boston University School of Medicine
Head, Section of Endocrinology and Metabolism
Thorndike Memorial Laboratory
Boston City Hospital
Boston, Massachusetts

James T. Willerson, M.D.

Department of Internal Medicine
(Cardiovascular and Hematology Divisions)
University of Texas Health Science Center
and the Charles A. Sammons Cancer Center
Baylor University Medical Center
Dallas, Texas

Lynn R. Witherspoon, M.D.

Ochsner Medical Institutions
New Orleans, Louisiana

Donald G. Wood, M.D.

Department of Medicine
Veterans Administration Medical Center and
University of Louisville School of Medicine
Louisville, Kentucky

PREFACE

The purpose of *Nuclear Medicine In Vitro*, as in the first edition, is to more widely disseminate knowledge of immunoassay techniques that are valuable in clinical medicine. These methods offer to physicians highly specific and sensitive techniques to help with difficult diagnostic and therapeutic problems.

Due to the rapid progression in the field, it has been necessary to completely revise the previous volume. All the previous chapters have been redone. In addition, a chapter on statistics has been added. There are also chapters on reagent and sample quality controls, bile acids, CPK and myoglobin, immune complexes, therapeutic drug monitoring, and re-

ceptor assays. Finally, the nonisotopic modalities involving enzyme coupling and fluorescent immunoassay have been added.

As in the previous edition, attempts have been made to minimize technical details and stress the basic principles involved. Again, the book is addressed to internists, clinical pathologists, and nuclear medicine specialists. The book is divided into five parts as follows: Fundamentals, Endocrine Studies, Gastrointestinal Studies, Cardiac Studies, and Other Studies, and an Afterword is devoted to future directions.

Benjamin Rothfeld, M.D.

PREFACE TO FIRST EDITION

The purpose of this book is to more widely disseminate knowledge of isotopic techniques that are valuable in clinical medicine. These methods offer to physicians highly specific and sensitive techniques that can be of immeasurable help with difficult diagnostic and therapeutic problems.

In Vitro Nuclear Medicine may be defined as that area of nuclear medicine in which the results may be expressed in a quantitative fashion. This is in contrast to the field in which cameras and scanners are used to make interpretations on a qualitative (subjective) basis. In this latter field, interpretations are made for the most part by examining an image. It may be argued that even in this field results are expressed in quantitative terms at times because of the increasing use of computers and on-line data. However, in the vast majority of cases these additional tools are not used. By contrast, *in vitro* nuclear medicine expresses the results almost invariably in numerical terms.

Qualitative and quantitative nuclear medicine have both grown rapidly in the last 20 years. At first nuclear medicine was almost entirely quantitative with blood volumes, Schilling tests, and iodine uptakes predominating. Then in the mid-1950s there was a rapid growth in imaging techniques, first with the scanner and then with the gamma camera. At the present time in most nuclear medicine laboratories, the bulk of the work is done by means of imaging techniques. More recently, with the development of immunoassay and protein-binding techniques, quantitative nuclear medicine has begun to catch up.

While the qualitative area is becoming a more and more expensive field in which to keep up to date, with the increasing complexity of the imaging devices and the introduction of computer techniques, the quantitative area has remained a relatively less expensive area in which to participate.

As previously mentioned, *in vitro* nuclear medicine continues to be a rapidly growing

field with its quantitative techniques presenting useful tools for clinical situations. For instance, the ability to do assays for digitalis fairly rapidly by isotopic techniques has greatly facilitated the handling of persons suspected of being either over- or underdigitalized. Instead of proceeding, as previously, in a cautious manner with a therapeutic trial, it is now possible to get helpful therapeutic guidance from the serum digitalis level. Another example is the test for Australia antigen, a test of prognostic value in serum hepatitis. In addition, the value of isotopic techniques for detecting Australia antigen in prospective blood donors is becoming increasingly recognized. Also, the entity of T_3 toxicosis is being diagnosed with increased frequency by means of these isotopic techniques. There are numerous other examples of valuable clinical applications of these techniques throughout this book. It was for this reason that it was felt appropriate to prepare a book covering these techniques employed at the present time.

It is impossible, of course, to be all-inclusive in this field, discussing every possible technique used in *in vitro* nuclear medicine. It has been the effort of the editor, therefore, to cover those areas of greatest interest to internists, clinical pathologists and nuclear medicine specialists.

The book is appropriately arranged by subject matter in five parts with related chapters being grouped together as follows: Methods—Chapters 1 through 4; Blood: Volume and Production—Chapters 5 through 7; Radioassays of Compounds Having Naturally Occurring Binding Substances—Chapters 8 through 12; Radioassays of Compounds Without Naturally Occurring Binding Substances—Chapters 13 through 22; Gastrointestinal Diagnostic Tests—Chapters 23 and 24; Infection—Chapters 25 and 26; and Chapter 27 suggesting and predicting Future Directions of this highly important field of *in vitro* nuclear medicine.

BENJAMIN ROTHFELD, M.D.

Acknowledgments

I would like to thank Eileen Nikoloff, Ph.D. of Squibb Laboratories, Princeton, New Jersey and Henry N. Wagner, Chief of the Division of Nuclear Medicine, Johns Hopkins Hospital for their encouragement and many helpful suggestions in the production of this book.

CONTENTS

PART I. FUNDAMENTALS

1. FUNDAMENTALS OF RADIOIMMUNOASSAY COUNTING

EDWARD JAMES 3

Principles of Detection	3
Gamma Energy Spectrum	4
System Components	5
Performance Parameters	5
New Developments	8

2. STATISTICS

HENRY A. FELDMAN 10

Models of Error	11
Empirical, Standard Binding Curves	15
Biomathematical Binding Curves	20
Cooperative Binding	24
Graphical Methods	28

3. RADIOIMMUNOASSAY METHOD SELECTION

EILEEN L. NIKOLOFF 35

Components of the System	35
Parameters in Kit Selection	36
Kit Evaluation	36
Sample Control	42

4. BASIC PRINCIPLES OF RADIOIMMUNOASSAYS

DEAN S. SKELLEY 45

Original Work by Berson and Yalow	46
Studies by Other Investigators	48
Kinetics of Radioligand Assays	49
The Assay-Standard Reaction Mixtures (Assay Reactants)	51
The Scatchard Plot	76
Evaluation of Radioimmunoassay Kits	79
Automation	86

5. ENZYME IMMUNOASSAY AND FLUORESCENT IMMUNOASSAY— BASIC PRINCIPLES AND INSTRUMENTATION

MICHAEL COBB AND SIGNE GOTCHER 101

Heterogeneous Methods of Enzyme Absorbance Detection	102
Heterogeneous Methods of Fluorescence Detection	106
Homogeneous Methods of Absorbance Detection	107
Homogeneous Methods of Fluorescence Detection	112

PART II. ENDOCRINE STUDIES

6. PLASMA AND URINARY HYDROXYCORTICOSTEROIDS	
TAH HSIUNG HSU AND BENJAMIN ROTHFELD	119
Regulation of Cortisol Secretion	119
Chemistry of Steroids	120
Determination of Corticosteroids	120
Competitive Radioassay of Cortisol	121
Specificity of Various Assays for Cortisol	122
Cushing's Syndrome	122
Adrenal Insufficiency (Addison's Disease)	125
7. ESTROGENS	
ALFONSO H. JANOSKI	127
General Considerations	128
Measurement of Estrogens Prior to Radioimmunoassay	128
Radioimmunoassay of Estrogens	129
Radioimmunoassay of Estrone and Estradiol-17 β	129
Radioimmunoassay of Estriol	135
Collection of Samples in Clinical Studies	137
Clinical Use of Estrogen Radioimmunoassays	137
Estrogen-Receptor Assays in Breast Carcinoma	140
8. ANDROGENS IN BIOLOGICAL FLUIDS	
CLAUDE J. MIGEON AND MAGUELONE G. FOREST	145
General Considerations	146
Determination of Neutral Urinary 17-Ketosteroids	148
Evolution of Plasma Testosterone Measurement	150
Radioimmunoassay of Testosterone and Its Precursors	152
Physiological Levels of Androgens	155
Androgens and Various Disorders	162
9. GONADOTROPINS AND PROLACTIN	
JON PEHRSON AND JUDITH VAITUKAITIS	171
Gonadotropins	171
Human Chorionic Gonadotropin	182
Prolactin	184
10. THYROID STIMULATING HORMONE	
DAVID G. WOOD AND ELLIS SAMOLS	189
Biochemistry and Physiology	189
Radioimmunoassay Methodology	193
TRH-TSH Test	194
Clinical Application	194
Conclusions	197
11. GROWTH HORMONE	
SALVATORE RAITI	202
Measurement of Growth Hormone	203
Radioimmunoassay Problems	203
Metabolic and Physiological Growth Hormone Activity	206
Factors Controlling Release and Actions	206
Clinical Tests	207

	Clinical Types of Deficiency	208
	Therapeutic Uses	209
12.	INSULIN AND GLUCAGON	
	JOSEPH R. KRAFT	211
	Classification of Diabetes	211
	Obesity and Diabetes Mellitus	213
	Glucose Tolerance Tests	213
	Pathology of Diabetes Mellitus	219
	Pancreatic Glucagon	221
13.	CALCIUM REGULATING HORMONES—PARATHYROID HORMONE, CALCITONIN, VITAMIN D	
	STEPHEN R. ABBOTT AND CLAUDE D. ARNAUD	224
	Parathyroid Hormone	225
	Calcitonin	233
	Vitamin D	237
14.	MEASUREMENT OF SERUM THYROID HORMONES	
	LYNN R. WITHERSPOON	246
	Control of Thyroid Hormonogenesis and Hormone Secretion	248
	Peripheral Effects of Thyroid Hormones	248
	Measurement of Serum Thyroid Hormones	249
	Free Thyroxine Methods of Normalizing Serum Thyroxine-Binding Protein Effects	251
	Direct Measurement of Free Thyroxine	256
	Clinical Applications of Thyroid Hormone Measurements	258
	Thyroid Function and Pregnancy	263
	Nonthyroid Illness	263
PART III. GASTROINTESTINAL HORMONES		
15.	HEPATITIS MARKERS	
	RAYMOND S. KOFF	273
	Hepatitis A Markers	274
	Hepatitis B Markers	278
	Non-A, Non-B Markers	283
16.	GASTRIN	
	TAPAN K. CHAUDHURI	287
	Chemistry	287
	Anatomy and Histology	287
	Physiology	288
	Applied Physiology	291
	Biochemistry	291
	Pharmacology	291
	Radioimmunoassay	291
17.	BILE ACIDS IN SERUM AND URINE	
	LAURENCE M. DEMERS	297
	Bile Salts	298
	Measurement of Serum Bile Acids	300

Serum Bile Acids in Hepatobiliary Disease	304
Conclusions	307

PART IV. CARDIAC STUDIES

18. DIGITALIS

HARRY G. McCOY, KINGSLEY R. LABROSSE, AND JOHN W. McBRIDE	313
---	-----

Clinical Application of Digoxin Assays	313
Clinical Pharmacokinetics of Digoxin	314
When to Measure Digoxin Concentration	315
Modification of Digoxin Dosage	316
Argument Against Routine Clinical Assay for Digoxin	316
Radioimmunoassay of Digoxin	317
Digoxin Assay—Present and Future	322

19. CREATINE KINASE AND MYOGLOBIN

JAMES T. WILLERSON AND MARVIN J. STONE	327
--	-----

Radioimmunoassay for Creatine Kinase-B Isoenzyme	327
Normal Serum CK-B Levels	329
Radioimmunoassay for Myoglobin	330
Conditions Causing Increases in Serum Myoglobin	332

PART V. OTHER STUDIES

20. FOLIC ACID AND VITAMIN B₁₂

VICTOR HERBERT	337
----------------	-----

Diagnosis of Vitamin B ₁₂ Deficiency	338
Radioassay Technique	341
Red Cell Vitamin B ₁₂ Levels	342
Diagnosis of Pernicious Anemia: Assay of Intrinsic Factor in Gastric Juice and Antibody to Intrinsic Factor in Serum	342
Diagnosing Malabsorption of Vitamin B ₁₂	343
Diagnosis of Folate Deficiency	346
Studies of Folate Absorption Using Radioactive Folate	347
Therapeutic Trial in the Test Tube: The dU Suppression Test	347
Serum Unsaturated Vitamin B ₁₂ Binding Capacity	348

21. BLOOD VOLUME MEASUREMENT

SOLOMON N. ALBERT	355
-------------------	-----

Blood Volume	355
Indices of Blood Volume	357
Principles of Measurement	360
Shortcomings of Computers Using Single Tracers	365
Contraindications to Blood Volume Measurements	366
Conclusions	367

22. MEASUREMENT OF ANTI-DNA ANTIBODIES AND IMMUNE COMPLEXES

CAROLE A. DORSCH	369
------------------	-----

Measurement of Anti-DNA Antibodies	369
Radioimmunoassays for Anti-DNA	369
Clinical Use of Anti-DNA Antibody Determination	374
Measurement of Immune Complexes	375

Problems With Immune Complex Assays	382
Usefulness of Immune Complex Assays	383
23. THERAPEUTIC DRUG MONITORING	
CRAIG K. SVENSSON AND THOMAS J. CALI	389
Pharmacokinetics	390
Basic Principles of Therapeutic Drug Monitoring	395
Theophylline	396
Phenytoin	398
Gentamicin	401
24. TUMOR MARKERS	
HYMAN ROCHMAN	407
Carcinoembryonic Antigen	407
Alpha-Fetoprotein	410
Measurement of Alpha-Fetoprotein	411
Human Chorionic Gonadotropin	411
Hormone Secretion by Nonendocrine Tumors	414
Ectopic Corticotropin Syndrome	416
Calcitonin and Human Chorionic Somatomammotropin	417
25. STEROID RECEPTOR ASSAYS IN CANCER	
KENNETH COWAN AND MARC LIPPMAN	421
Breast Cancer	422
Endometrial Cancer	429
Prostate Cancer	429
Leukemia and Lymphoma	430
Other Neoplastic Diseases	431
26. RENIN-ANGIOTENSIN	
JEAN E. SEALEY, JACEK J. PREIBISZ, AND JOHN H. LARAGH	434
The Renin-Angiotensin-Aldosterone System	434
Summary	443
27. PROSTATIC ACID PHOSPHATASE	
JOHN GRIFFITHS	446
Acid Phosphatases in Serum	447
Preparation of Purified Enzyme	447
Preparation of Antiserum	449
Labeling of PAcP Protein	449
Specificity of PAcP Antiserum	449
Stability of the Serum Sample	450
Radioimmunoassay Procedure	450
Clinical Interpretation and Use	450
AFTERWORD	452
INDEX	453

PART 1

Fundamentals

1

Fundamentals of Radio-immunoassay Counting

Edward James

State-of-the-art technology in radioimmunoassay (RIA) has refined labeling almost exclusively to low-energy nuclides such as ^{125}I and ^{57}Co and, therefore, has directed gamma counting instrumentation toward smaller, lighter, self-contained systems that are more and more foolproof.

This chapter provides a general understanding of scintillation counting principles and discusses a practical approach to the day-to-day use of the counter in the present-day RIA laboratory. With such a limited scope it is impossible to cover all subjects in detail, so suggested readings are provided at the end of the chapter.

PRINCIPLES OF DETECTION

Thallium-activated sodium iodide [$\text{NaI}(\text{Tl})$] is the basis for all modern gamma scintillation counting systems. Optically coupled to a photomultiplier tube (PMT), it forms the beginning of the detection system that translates, ana-

lyzes, and records gamma emissions into meaningful form.

Gamma rays commonly interact with the crystal in one or more of three ways: the photoelectric effect, which predominates at energy levels below 300 keV; Compton scatter, which occurs most frequently in the range of 300 keV to 1.5 or 2 MeV; and pair production, which occurs above 1 MeV (Fig. 1-1).

Pair production is a phenomenon occurring only at energies higher than those encountered in the laboratory and, for the purposes of this text, will be disregarded.

Photoelectric Effect

For ease of understanding, the photoelectric effect can be thought of as an elastic collision in which the gamma ray transfers all of its energy to an orbital electron of a sodium or iodine atom. This photoelectron finally comes to rest and the energy lost during the slowing process is emitted as a light photon.