

Contents

CHAPTER 1

Radioimmunoassay: Historical Aspects and General Considerations

R. S. YALOW. With 1 Figure	1
A. Historical Aspects	1
B. Principle, Practices, and Pitfalls	1
I. Principle	2
II. Practices	2
III. Pitfalls	4
C. Conclusions	5
References	5

CHAPTER 2

Basic Principles of Antigen-Antibody Interaction

F. CELADA. With 4 Figures	7
A. Introduction to the Immune System	7
B. Antigens	10
I. Chemical Nature	10
II. Antigenic Determinants (Epitopes)	11
III. Haptens and Carriers	11
IV. Size of Determinants	11
V. Sequential and Conformational Epitopes	11
C. Antibodies	12
I. The Ab Combining Site (Paratope)	13
D. The Immune Interaction	14
I. The Forces Involved	14
II. Paratope-Epitope Fit	15
III. Specificity	16
IV. Affinity	16
E. Effects of Ab-Ag Interaction	19
I. Free Ag Binds Ig Receptor	19
II. Free Ag Binds Free Ab	19
III. Free Ab Binds Cell-Associated Ag	20
F. Immune Interaction as a Signal: Role of Conformational Changes	20
I. Antibody	20
II. Antigen	21
References	21

CHAPTER 3

Production of Antisera by Conventional Techniques

G. CIABATTONI. With 7 Figures	23
A. Introduction	23
B. Substances Which Are Able to Evoke Immunogenic Responses Per Se	24
C. Special Problems with Small Peptides	28
D. Haptens Covalently Coupled to Protein Carriers	29
I. Preparation of Derivatives Containing Reactive Groups	29
II. Covalent Coupling to Protein Carriers	34
E. Immune Response to Hapten-Protein Conjugates	40
I. Effect of Method and Duration of Immunization	40
II. Role of Adjuvants in Antibody Production	43
III. Effect of Carrier Protein on Characteristics of Antisera	44
IV. Effect of Hapten: Protein Molar Ratio on Characteristics of Antisera	45
V. Effect of Site of Hapten Linkage to Protein on Characteristics of Antisera	46
F. Characterization of Antisera	48
I. Titer	48
II. Affinity	50
III. Sensitivity	53
IV. Specificity	54
References	61

CHAPTER 4

Production of Monoclonal Antibodies for Radioimmunoassays

K. BRUNE and M. REINKE. With 1 Figure	69
A. Introduction	69
I. Rationale for the Production of Monoclonal Antibodies	69
II. Applications of Monoclonal Antibodies	70
III. Problems	70
B. Techniques	71
I. Materials	71
II. Methods	73
C. Results and Outlook	79
References	83

CHAPTER 5

Radioiodination and Other Labeling Techniques

J. GRASSI, J. MACLOUF, and P. PRADELLES. With 5 Figures	91
A. Labeling for Immunologic Assay: Introduction	91
B. Radioiodination	92

I. General Considerations	92
II. Chemistry	103
C. Nonisotopic Labeling	111
I. General Considerations	111
II. Labeling with Enzymes	113
III. Labeling with Fluorescent Compounds	121
IV. Labeling with Luminescent Compounds	124
V. Other Labeling Methods	126
D. Conclusions	128
References	130

CHAPTER 6

Strategies for Developing Specific and Sensitive Hapten Radioimmunoassays

E. EZAN, S. MAMAS, C. ROUGEOT, and F. DRAY. With 23 Figures	143
A. Introduction	143
B. Hapten-Carrier Conjugation	143
I. Haptens with Amino Groups	145
II. Haptens with Carboxyl Groups	147
III. Haptens with Other Functional Groups	149
C. Strategy for Increasing Specificity	152
I. General Considerations: From Hapten Size to Epitope Size	152
II. Steroids	152
III. Other Small Haptens	157
IV. Peptides	158
V. Transformation of Immunogen	161
D. Immunoassay Sensitization	163
I. Increase in Tracer Specific Activity	164
II. Modification of Reagent Concentrations and Assay Procedures	169
E. Validation	171
I. Nonantigenic Materials	172
II. Antigen-like Materials	172
III. Example of Validation: Peptide Radioimmunoassay	173
References	175

CHAPTER 7

How to Improve the Sensitivity of a Radioimmunoassay

G. CIABATTONI. With 2 Figures	181
A. Introduction	181
B. Basic Considerations	182
C. The Influence of Labeled Tracer	184
D. The Influence of Antiserum Dilution	186
E. The Role of Incubation Volume	187
F. Temperature and pH Effects	188

G. Disequilibrium Conditions	189
H. Other Factors Affecting Sensitivity	190
References	191

CHAPTER 8

Statistical Aspects of Radioimmunoassay

D. RODBARD, V. GUARDABASSO, and P.J. MUNSON. With 8 Figures	193
A. Introduction	193
B. The Logit-Log Method	194
C. Is the Logit-Log Method Failing?	194
D. The Four-Parameter Logistic	196
E. Examples of Problems	198
I. Evaluating Goodness of Fit	199
II. Pooling of Information Over Assays	202
F. Strategy to Deal with Failure of Logistic Models	203
I. Does it Matter?	203
II. Choice of Other Methods	204
III. A "Universal" Approach	209
G. Concluding Remarks	210
References	210

CHAPTER 9

Validation Criteria for Radioimmunoassay

C. PATRONO. With 3 Figures	213
A. Introduction	213
B. Conditions Necessary, but not Sufficient for Establishing the Validity of RIA Measurements	214
C. Appropriate Biological Behaviour of the Measured Immunoreactivity	215
D. Limited Cross-Reactions of Structurally Related Substances.	216
E. Use of Multiple Antisera	218
F. Identical Chromatographic Behaviour of Standards and Unknowns	219
G. Comparison with an Independent Method of Analysis	221
H. Are we Measuring the Right Compound, in the Right Compartment?	222
J. How to Evaluate an RIA Kit Critically?	223
References	224

CHAPTER 10

Measurement of Opioid Peptides in Biologic Fluids by Radioimmunoassay

F. NYBERG, I. CHRISTENSSON-NYLANDER, and L. TERENIUS. With 1 Figure	227
A. Introduction	227
B. General Aspects	228
I. Distribution and Processing of Opioid Peptides	228
II. Characterization of Opioid Receptors	230

C. Methodological Aspects	231
I. Sampling Protocol	231
II. Sample Workup (Preseparation)	231
III. Radioimmunoassay of Opioid Peptides from Individual Systems	232
D. Functional Aspects	239
I. Opioid Peptides in Biologic Fluids	239
E. Clinical Aspects	241
I. Pain	241
II. Stress, Physical Exercise, and Shock	241
III. Narcotic Dependence	242
IV. Psychiatry	242
V. Neuroendocrinology and Endocrine Tumors	243
F. Concluding Remarks	244
References	245

CHAPTER 11

Radioimmunoassay of Pituitary and Hypothalamic Hormones

D. COCCHI	255
A. Introduction	255
B. General Principles of Determination	255
I. The Antibody	255
II. The Labeled Antigen	256
III. Reference Preparations	256
IV. Separation of Bound from Free Labeled Hormone	257
V. Future Development of RIAs	257
C. Radioimmunoassay of Anterior Pituitary Hormones	258
I. Prolactin	259
II. Growth Hormone	261
III. Glycoprotein Hormones	264
IV. Adrenocorticotropin, Endorphins, and Related Peptides	267
D. Radioimmunoassay of Hypothalamic Hypophysiotropic Hormones	270
I. Premises	270
II. General Principles of Determination	272
III. Features Peculiar to the Determination of a Single Hypophysiotropic Hormone	274
IV. Significance of Hypophysiotropic Peptides in Biologic Fluids	278
References	281

CHAPTER 12

Radioimmunoassay of Nonpituitary Peptide Hormones

G. NILSSON. With 7 Figures	291
A. Hormones Involved in Regulation of Carbohydrate Metabolism	291
I. Insulin	291
II. C-Peptide of Insulin	298
III. Glucagon	301

B. Hormones Regulating Calcium Homeostasis	305
I. Physiology of Calcium Homeostasis	305
II. Parathyroid Hormone	305
III. Calcitonin	308
References	310

CHAPTER 13

Radioimmunoassay of Gastrointestinal Polypeptides

J. H. WALSH and H. C. WONG. With 18 Figures	315
A. Introduction	315
B. Gastrin	316
I. Physiologic Relevance	316
II. Chemical Composition	316
III. Characteristics of Antibodies and Labeled Peptides	317
IV. Other Published Assays	317
C. Cholecystokinin	317
I. Published Radioimmunoassays	318
D. Secretin	318
I. Chemistry	319
II. Antibody Production	319
III. Radioiodination	320
IV. Radioimmunoassay Procedure	321
V. Characterization of Antibodies	322
VI. Sample Preparation for Radioimmunoassay	322
VII. Other Published Radioimmunoassays	323
E. Somatostatin	324
I. Antibody Production	324
II. Radioiodination	324
III. Sampling and Treatment of Serum	325
IV. Serum Extraction	325
V. Radioimmunoassay Procedure	325
VI. Detection Limit	326
VII. Other Published Radioimmunoassays	326
F. Motilin	327
I. Chemistry	328
II. Antibody Production	328
III. Radioiodination	328
IV. Radioimmunoassay Procedure	330
V. Characterization of Antibodies	331
VI. Other Published Radioimmunoassays	331
G. Gastric Inhibitory Peptide	331
I. Antibody Production	331
II. Characterization of Antisera	332
III. Radioiodination	332
IV. Radioimmunoassay Procedure	333
V. Other Published Radioimmunoassays	334

H. Neurotensin	334
I. Chemistry	334
II. Preparation of Antigen	335
III. Immunization of Animals	335
IV. Radioiodination	335
V. Radioimmunoassay Procedure	337
VI. Characterization of the Antibody	338
VII. Stability of Neurotensin	339
VIII. Other Published Radioimmunoassays	339
J. Vasoactive Intestinal Polypeptide	340
I. Chemistry	340
II. Antibody Production	340
III. Radioiodination	341
IV. Radioimmunoassay Procedure	342
V. Characterization of Antibodies	343
VI. Sample Preparation for Radioimmunoassay	344
VII. Other Published Radioimmunoassays	344
K. Pancreatic Polypeptide and Peptide YY	345
I. Pancreatic Polypeptide	345
II. Peptide YY	345
References	346

CHAPTER 14

Radioimmunoassay of Atrial Peptide Blood and Tissue Levels

M. L. MICHENER, D. SCHWARTZ, M. G. CURRIE, D. M. GELLER, and P. NEEDLEMAN. With 2 Figures	351
A. Introduction	351
B. Development of Atriopeptin Radioimmunoassay	352
I. Immunization	352
II. Iodination of Peptides	353
III. Titering and Sensitivity	353
C. Assay of Tissues and Plasma	354
I. Atrial Extracts	354
II. Plasma Immunoreactivity	354
III. Brain Atriopeptin	357
D. Processing of the Atriopeptin Prohormone	358
E. Summary	359
References	359

CHAPTER 15

Immunochemical Methods for Adrenal and Gonadal Steroids

M. PAZZAGLI and M. SERIO. With 8 Figures	363
A. Chemistry and Nomenclature	363
B. Immunoassay Methods for Steroids	364

I. Basic Principles	364
II. Synthesis of the Immunogen Derivative	365
III. The Radioactive Tracer	366
IV. Sample Preparation Before Immunoassay	367
V. Bound/Free Separation Systems	368
VI. Monoclonal Antibodies to Steroids	368
VII. Quality Assessment	368
VIII. Alternative Immunoassay Methods	369
C. The Adrenal Cortex	373
I. Physiology	373
II. Control Mechanisms for the Release of Cortisol	376
III. Control Mechanisms for the Release of Aldosterone	378
IV. Biosynthesis of Adrenal Steroids	378
V. Metabolism of Adrenal Steroids	378
VI. Immunochemical Methods for Adrenal Steroids	379
D. The Testis	384
I. Physiology	384
II. Transport of Testosterone in Blood and Its Action at the Target Level.	385
III. Immunochemical Methods for Androgens	386
E. The Ovary	388
I. Physiology	388
II. Immunochemical Methods for Ovarian Steroids	388
References	393

CHAPTER 16

Radioimmunoassay of Thyroid Hormones

L. BARTALENA, S. MARIOTTI, and A. PINCHERA. With 1 Figure	401
A. Introduction	401
B. Iodometric Techniques	403
I. PBI	403
II. BEI	403
III. T_4I_C	404
C. Radioligand Assays of Total T_4 (TT_4) and Total T_3 (TT_3)	404
I. TT_4 Assays	404
II. TT_3 Assays	406
III. Factors Affecting the Diagnostic Accuracy of Total Thyroid Hormone Determination	408
D. Free Thyroid Hormone Assays	412
I. Indirect Methods for the Estimation of Free Thyroid Hormone	413
II. Direct Methods for Free Thyroid Hormone Determination	416
III. Factors Affecting the Diagnostic Accuracy of Free Thyroid Hormone Measurements	420
E. Radioimmunoassay of Other Iodothyronines, Iodotyrosines, and Products of Thyroid Hormone Degradation	423

I. rT_3 RIA	423
II. RIA of Diiodothyronines	424
III. RIA of Monoiodothyronines	424
IV. RIA of Tetraiodothyroacetic Acid (TETRAC) and Triiodothyroacetic Acid (TRIAC)	424
V. RIA of Iodotyrosines (MIT and DIT)	424
References	425

CHAPTER 17

Radioimmunoassay of Catecholamines

E. KNOLL and H. WISSER. With 6 Figures	433
A. Introduction	433
B. Radioimmunoassay of Catecholamines and Their Metabolites	434
I. Antibodies to Catecholamines	434
II. Antibodies to Metabolites of Catecholamines	438
C. Summary	445
References	445

CHAPTER 18

Radioimmunoassay of Prostaglandins and Other Cyclooxygenase Products of Arachidonate Metabolism

B. A. PESKAR, TH. SIMMET, and B. M. PESKAR. With 2 Figures	449
A. Introduction	449
B. Biosynthesis and Metabolism of Prostaglandins and Thromboxanes	449
C. Development of Radioimmunoassays for Prostaglandins and Thromboxanes	452
I. Preparation of Immunogens	452
II. Immunization	453
III. Labeled Ligands	453
IV. Separation of Antibody-Bound and Free Fractions of Ligands	455
D. Validation of Radioimmunoassays for Cyclooxygenase Products of Arachidonic Acid Metabolism	455
E. Factors that Affect the Validity of Prostaglandin and Thromboxane Radioimmunoassay Results	456
I. Extraction and Purification Procedures	456
II. The Blank Problem	458
III. Problems Associated with the Determination of Tissue and Plasma Concentrations of Prostanoids	458
F. Radioimmunoassay for Various Prostaglandins and Thromboxanes	459
I. Prostaglandin $F_{2\alpha}$	459
II. 15-Keto-13,14-dihydro-prostaglandin $F_{2\alpha}$	459
III. Prostaglandin E_2	460
IV. 15-Keto-13,14-dihydro-prostaglandin E_2	460
V. Prostaglandin E_1	461

VI. Prostaglandin D ₂	462
VII. Indirect Determination of Prostaglandin Endoperoxides and Thromboxane A ₂	462
VIII. Thromboxane B ₂ and 11-Dehydro-thromboxane B ₂	463
IX. 15-Keto-13,14-dihydro-thromboxane B ₂	464
X. 6-Keto-prostaglandin F _{1α}	464
XI. Metabolites of Prostaglandin I ₂ and 6-Keto-prostaglandin F _{1α}	465
XII. Main Urinary Metabolites of Prostaglandin F _{2α} and Prostaglandin E ₂	466
G. Comparison of Radioimmunoassay with Other Methods for Quantitative Determination of Cyclooxygenase Products of Arachidonic Acid Metabolism	467
H. Radioimmunoassay of Cyclooxygenase Products of Arachidonic Acid Metabolism in Basic and Clinical Pharmacology	469
J. Concluding Remarks	470
References	471

CHAPTER 19

Radioimmunoassay of Leukotrienes and Other Lipoxigenase Products of Arachidonate

H. J. ZWEERINK, G. A. LIMJUCO, and E. C. HAYES. With 6 Figures	481
A. Introduction	481
B. Biosynthesis and Metabolism of Lipoxigenase Products	482
C. Biologic Activities of Lipoxigenase Products	483
D. Biologic Assays to Measure Lipoxigenase Products	484
E. Physicochemical Separation and Identification of Lipoxigenase Products	485
F. Radioimmunoassays for Lipoxigenase Products	485
I. Introduction	485
II. Radioimmunoassays to Measure LTC ₄	486
III. Radioimmunoassays to Measure LTD ₄	491
IV. Radioimmunoassays to Measure LTB ₄	492
V. Radioimmunoassays to Measure 12-HETE	493
VI. Radioimmunoassays to Measure 15-HETE	494
G. Sample Preparation for Radioimmunoassays	494
H. Combination of Radioimmunoassays with Physicochemical Separation Methods	495
J. Conclusions	496
References	497

CHAPTER 20

Radioimmunoassay of Cyclic Nucleotides

C. W. PARKER. With 6 Figures	501
A. Introduction	501
B. Preparation of the Immunogen	502

C. Immunization	504
D. Preparation of the Radioindicator Molecule	504
E. Evaluation of Antisera and Routine Immunoassay Procedures	507
I. Double-Antibody Procedure	509
II. Ammonium Sulfate Precipitation	509
III. Charcoal	509
F. Tissue Extraction	510
G. Derivatization of Tissue Samples	510
H. Pitfalls in Immunoassay Measurements	511
J. Validation of the Immunoassay	512
K. Special Applications of the Immunoassays	513
References	514

CHAPTER 21

Radioimmunoassay of Platelet Proteins

D. S. PEPPER. With 1 Figure	517
A. Introduction	517
B. Sample Collection and Processing	520
C. Assay of β -Thromboglobulin	524
D. Assay of Platelet Factor 4	526
E. Assay of Other Platelet Proteins	528
F. Applications of Platelet-Specific Protein Radioimmunoassays	529
G. Discussion	533
References	535

CHAPTER 22

A Competitive Binding Assay for Heparin, Heparan Sulphates and Other Sulphated Polymers

J. DAWES and D. S. PEPPER. With 8 Figures	543
A. Introduction	543
B. Radiolabelling of Glycosaminoglycans	544
I. Derivatisation	544
II. Iodination	545
C. Synthesis of Binding Reagent	546
D. Assay for Therapeutic Heparins and Heparinoids	546
I. Method	546
II. Sensitivity	547
III. Specificity	548
IV. Studies in Human Volunteers	549
E. Assay for Endogenous Heparan Sulphate	552
I. Method	552
II. Sensitivity	554
III. Specificity	554
IV. Results of Rat and Human Studies	554

F. Other Applications	557
G. Summary	557
References	558

CHAPTER 23

Radioimmunoassay of the Somatomedins/Insulin-like Growth Factors

L. E. UNDERWOOD and M. G. MURPHY. With 6 Figures	561
A. Introduction and Nomenclature of the Somatomedins	561
I. Methods for Measuring Somatomedins Before the Development of Radioimmunoassays	562
B. Radioimmunoassay for Sm-C/IGF-I	563
I. Antisera Production, Sensitivity, and Specificity	563
II. Influence of Binding Proteins	563
III. Clinical Utility of Measurements of Sm-C/IGF-I in Plasma	567
IV. Heterologous Radioimmunoassays for Sm-C/IGF-I	570
V. Measurement of Sm-C/IGF-I in Tissues and Media Extracts . . .	571
C. Radioimmunoassay for IGF-II	571
References	572

CHAPTER 24

Radioimmunoassay of Drugs and Neurotransmitters

S. SPECTOR. With 3 Figures	575
A. Introduction	575
B. Methods for Coupling Neurotransmitters to Carrier Proteins	576
I. Acetylcholine	576
II. Serotonin	578
III. Melatonin	579
C. Methods for Coupling Drugs to Carrier Proteins	580
I. Barbiturates	580
II. Curare	580
III. Clonidine	581
IV. Phenothiazines	582
V. Benzodiazepines	584
VI. Butyrophenones	585
VII. Atropine	586
VIII. Opiates	586
References	588

Subject Index	591
--------------------------------	------------