

Methods of Hormone Analysis

H. Breuer, D. Hamel, and H. L. Kruskemper

Methods of Hormone Analysis

EDITED BY

HEINZ BREUER

University of Bonn

DAISY HAMEL

University of Bonn

HANS LUDWIG KRÜSKEMPER

University of Düsseldorf

GEORG THIEME VERLAG, Stuttgart

JOHN WILEY & SONS

New York • Sydney • Toronto

Copyright © 1976 by Georg Thieme Verlag, Stuttgart, Germany

English-language edition of *Methoden der Hormonbestimmung*
Copyright © 1975 by Georg Thieme Verlag, Stuttgart, Germany

All rights reserved. Published simultaneously in Canada.

No part of this book may be reproduced by any means,
nor transmitted, nor translated into a machine language
without the written permission of the publisher.

Library of Congress Cataloging in Publication Data:

Main entry under title:

Methods of hormone analysis.

Translation of *Methoden der Hormonbestimmung*.

Includes bibliographical references and index.

1. Hormones—Analysis. I. Breuer, Heinz,

II. Hamel, Daisy. III. Kruskemper,

Hans Ludwig.

RB48.5.M4713

616.4

75-19225

ISBN 0-471-10333-0 (Wiley)

ISBN 313 525801 7 (Thieme)

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Preface

The evaluation of the reliability and usefulness of the methods for measuring hormones is always difficult—mostly because of the considerable differences in methodological principles and of the increasing number of methods continually being published. From the abundance of these methods we have here compiled those procedures that are internationally recognized as valid standard methods in clinical chemistry and experimental endocrinology. In addition to the methods that have proved reliable for routine estimation, such as radioimmunoassay techniques, other determinations are included which, although laborious and time consuming, are indispensable as reference methods. All contributions are arranged so that the methods can be directly followed. Also, particular value has been attached to the presentation of reliability criteria and to detailed discussions of individual methods. For the measurement of some hormones more than one method appears; one can thus choose the most suitable method for the hormone in question depending on the available apparatus.

This book could not have been written without the cooperation of the contributing authors and their willingness to follow the guidelines of the editors. Special thanks go to Dr. Lieselotte Nocke-Finck (Bonn), Dr. H. K. Kley (Düsseldorf), and Dr. B. Fillmann (Bonn) for their valuable suggestions and criticism, and to Miss Evelyn Dymars and Mrs. Lieselotte Yahraes for their participation in the preparation of the final copy of the manuscript.

HEINZ BREUER
DAISY HAMEL
HANS LUDWIG KRÜSKEMPER

Bonn, Germany
Düsseldorf, Germany
October 1975

Authors

ABRAHAM, G. E., M.D.
Reproductive Biology Section
Department of Obstetrics
and Gynecology
UCLA School of Medicine
Harbor General Hospital Campus
Torrance, California

ADLERCREUTZ, H., M.D.
Department of Clinical Chemistry
University of Helsinki
Meilahti Hospital
Helsinki, Finland

BAULIEU, E. E., Dr.
Département de Chimie Biologique
Faculté de Médecine
Université de Paris-Sud
94 Bicêtre, France

BETTENDORF, G., Dr. med.
Abteilung für klinische und
experimentelle Endokrinologie
Universitäts-Frauenklinik
Hamburg-Eppendorf, F.R.G.

BÖTTGER, I., Dr. med.
Institut für Diabetesforschung
Städtisches Krankenhaus
München-Schwabing, F.R.G.

BORTH, R., D.Sc.
Department of Obstetrics
and Gynaecology
St. Michael's Hospital
University of Toronto
Toronto, Ontario, Canada

BOWMAN, R. E., Ph.D.
Department of Psychology
University of Wisconsin and
Wisconsin Regional Primate
Research Center
Madison, Wisconsin

BRECKWOLDT, M., Dr. med.
Universitäts-Frauenklinik
Freiburg, F.R.G.

BREUER, H., Dr. rer. nat.
Institut für Klinische Biochemie
der Universität
Bonn-Venusberg, F.R.G.

BROWN, J. B., Ph.D.
Department of Obstetrics
and Gynaecology
University of Melbourne
Melbourne, Australia

COLLINS, W. P., Dr.
Department of Biochemical
Endocrinology
Chelsea Hospital for Women
London, United Kingdom

CORPÉCHOT, COLETTE
Département de Chimie Biologique
Faculté de Médecine
Université de Paris-Sud
94 Bicêtre, France

DÖLLEFELD, E.
Klinisch-Chemisches Laboratorium
Bremer Str. 14
Hamburg, F.R.G.

DUFAU, MARIA L., M.D., Ph.D.
Reproduction Research Branch
National Institute of Child Health
and Human Development
Bethesda, Maryland

EISENTRAUT, A. M.
Nuclear Medical Laboratories
Dallas, Texas

EXLEY, D., Ph.D.
Department of Biochemistry
Queen Elizabeth College
University of London
Kensington, London, United Kingdom

FALOONA, G. R., Ph.D.
Department of Internal Medicine
University of Texas Southwestern
Medical School
Dallas, Texas

FEHM, H. L., Dr. med.
Abteilung für Endokrinologie
und Stoffwechsel
Zentrum für Innere Medizin
und Kinderheilkunde der Universität
Ulm (Donau), F.R.G.

FOTHERBY, K., Ph.D.
Department of Steroid Biochemistry
Royal Postgraduate Medical School
Hammersmith Hospital
London, United Kingdom

FRANCHIMONT, P., Dr.
Institut de Médecine
Université de Liège
Liège, Belgium

HAMEL, DAISY, Dr. phil. nat.
Institut für Klinische Biochemie
der Universität
Bonn-Venusberg, F.R.G.

HERRMANN, J., Dr. med.
2. Medizinische Klinik und Poliklinik
der Universität
Düsseldorf, F.R.G.

HESCH, R. D., Dr. med.
Abteilung für Klinische Endokrinologie
Department Innere Medizin
Medizinische Hochschule Hannover
Hannover, F.R.G.

HUFNER, M., Dr. med.
Medizinische Universitäts-Poliklinik
Heidelberg, F.R.G.

HUNTER, W. M., Ph.D.
Medical Research Council
Radioimmunoassayteam
Edinburgh, United Kingdom

JOHANSSON, E. D. B., M.D.
Kvinnokliniken
Akademiska Sjukhuset
Uppsala, Sweden

DE JONG, F. H., Dr.
Afdeling Biochemie
Instituut voor Chemische Endocrinologie
Erasmus Universiteit
Rotterdam, The Netherlands

KARG, H., Dr. med. vet.
Süddeutsche Versuchs-
und Forschungsanstalt
für Milchwirtschaft Weißenstephan
Institut für Physiologie
Technische Universität München
Freising b. München, F.R.G.

KELLIE, A. E., Ph.D.
Courtauld Institute of Biochemistry
The Middlesex Hospital
Medical School
London, United Kingdom

KLEY, H. K., Dr. med.
2. Medizinische Klinik und Poliklinik
der Universität
Düsseldorf, F.R.G.

KLOPPER, A. M.D.
Department of Obstetrics
and Gynaecology
Medical School
University of Aberdeen
Aberdeen, United Kingdom

KNORR, D., Dr. med.
Kinderklinik der Universität
München, F.R.G.

KÖDDING, R., Dr. med.
Abteilung für Klinische Endokrinologie
Department Innere Medizin
der Medizinischen Hochschule
Hannover, F.R.G.

KORENMAN, S. G., M.D.
Division of Endocrinology
Department of Internal Medicine
University of Iowa Hospitals and Clinics
Iowa City, Iowa

KRÜSKEMPER, H. L., Dr. med.
2. Medizinische Klinik und Poliklinik
der Universität
Düsseldorf, F.R.G.

KUSS, E., Dr. med., Dr. rer. nat.
I. Frauenklinik und Hebammenschule
der Universität
München, F.R.G.

LEMARCHAND-BÉRAUD, THÉRÈSE, Dr.
Division de Biochimie Clinique
Hôpital Cantonal Universitaire
Lausanne, Switzerland

LEYENDECKER, G., Dr. med.
Abteilung für Gynäkologische
Endokrinologie
Universitäts-Frauenklinik
Bonn-Venusberg, F.R.G.

LÖFFLER, G., Dr. med.
Forschergruppe Diabetes
Städtisches Krankenhaus
München-Schwabing, F.R.G.

MIGEON, C. J., M.D.
Pediatric Endocrine Clinic
The Johns Hopkins Hospital
and University
Baltimore, Maryland

VAN DER MOLEN, H. J., Dr.
Afdeling Biochemie
Instituut voor Chemische Endocrinologie
Erasmus Universiteit
Rotterdam, The Netherlands

VON ZUR MÜHLEN, A., Dr. med.
Abteilung für Klinische Endokrinologie
Department Innere Medizin
Medizinische Hochschule Hannover
Hannover, F.R.G.

MÜLLER, O. A., Dr. med.
II. Medizinische Klinik der Universität
München, F.R.G.

NIESCHLAG, E., Dr. med.
2. Medizinische Klinik und Poliklinik
der Universität
Düsseldorf, F.R.G.

NOCKE, W., Dr. med.
Abteilung für Gynäkologische
Endokrinologie
Universitäts-Frauenklinik
Bonn-Venusberg, F.R.G.

NOCKE-FINCK, LIESELOTTE, Dr. med.
Institut für Klinische Biochemie
der Universität
Bonn-Venusberg, F.R.G.

OAKEY, R. E., Ph.D.
Division of Steroid Endocrinology
Department of Chemical Pathology
School of Medicine
University of Leeds
Leeds, United Kingdom

PFEIFFER, E. F., Dr. med.
Abteilung für Endokrinologie
und Stoffwechsel
Zentrum für Innere Medizin und
Kinderheilkunde der Universität
Ulm (Donau), F.R.G.

O'RIORDAN, J. L. H., D.M., F.R.C.P.
The Middlesex Hospital
Medical School
London, United Kingdom

RIVAROLA, M. A., M.D.
Servicio de Endocrinología
Hospital de Niños
Buenos Aires, Argentina

SCHAUMAN, K.-O., Dr.
Department of Clinical Chemistry
University of Helsinki
Meilahti Hospital
Helsinki, Finland

SCRIBA, P. C., Dr. med.
II. Medizinische Klinik der Universität
München, F.R.G.

SOMMERVILLE, I. F., M.D., Ph.D.
Department of Biochemical
Endocrinology
Chelsea Hospital for Women
London, United Kingdom

STERLING, K., M.D.
Department of Medicine
Columbia University College of
Physicians and Surgeons
New York, New York
and
The Protein Research Laboratory
Bronx Veterans Administration Hospital
Bronx, New York

VON STUDNITZ, W., Dr. med., Dr. rer. nat.
Medizinisch-Diagnostisches Institut
Nussbaumstr. 14
München, F.R.G.

THIJSEN, J. H. H., Dr.
Kliniek voor Inwendige Geneeskunde
Stichting Academisch Ziekenhuis
Utrecht
Utrecht, The Netherlands

THOMAS, H., Dr. med.
Abteilung für Biochemie
der Universität
Ulm (Donau), F.R.G.

VERMEULEN, A., Dr.
Afdeling Endocrinologie, Hematologie,
Stofwisselingsziekten
Rijksuniversiteit Gent
Akademisch Ziekenhuis
Gent, Belgium

VOIGT, K. D., Dr. med.
Klinisch-Chemische Abteilung der
II. Medizinischen Universitätsklinik
Hamburg-Eppendorf, F.R.G.

VOIGT, K. H., Dr. med.
Abteilung für Endokrinologie und
Stoffwechsel
Zentrum für Innere Medizin und
Kinderheilkunde der Universität
Ulm (Donau), F.R.G.

WARDLAW, SHARON, B. Sc. (Biochemistry)
Abteilung für Gynäkologische
Endokrinologie
Universitäts-Frauenklinik
Bonn-Venusberg, F.R.G.

WEISS, L., Dr. med.
Klinisch-Chemisches Institut des
Städtischen Krankenhauses
München-Schwabing, F.R.G.

WOODHEAD, J. S., M.D.
Department of Chemical Pathology
The Welsh National School of Medicine
Heath Park, Cardiff, United Kingdom

WOTIZ, H. H., Ph.D.
Boston University
School of Medicine
Boston, Massachusetts

Contents

1. Radioimmunoassay of Growth Hormone (HGH, Somatotropin, STH) in Plasma 1

by W. M. HUNTER

Introduction	1
Standard Preparation of Human Growth Hormone	1
Preparation and Storage of Antisera	1
Iodination	2
Separation of Antibody-Bound from Free [125 I] HGH	3
Testing and Selection of Suitable Antisera to HGH	6
Testing of [125 I]HGH Preparations	7
Determination	8
Concentration of HGH in Plasma in Various Situations	10
References	11

2. Radioimmunoassay of Adrenocorticotrophic Hormone (ACTH) in Plasma 12

by K. H. VOIGT, H. L. FEHM, and E. F. PFEIFFER

Principle	12
Materials	12
Collection and Preparation of Samples	14
Determination	15
Discussion	15
Normal Range	16
References	16

3. Bioassay of Adrenocorticotrophic Hormone (ACTH) in Plasma 18

by H. L. FEHM, K. H. VOIGT, and E. F. PFEIFFER

Principle	18
Materials	18
Collection and Preparation of Samples	19
Determination	19
Discussion	20
Normal Range	21
References	21

4. Radioimmunoassay of Thyroid-Stimulating Hormone (TSH) in Plasma 22

by THÉRÈSE LEMARCHAND-BÉRAUD

Introduction	22
Principle	22
Materials	23
Collection and Preparation of Samples	24
Determination	24
Analysis of Assay	26
Reliability	29
Discussion	29
Concentration of TSH in Plasma in Various Situations	30
References	34

5. Radioimmunoassay of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in Serum	36
by P. FRANCHIMONT	
<i>5.1 Radioimmunoassay of Follicle-Stimulating Hormone in Serum</i>	<i>36</i>
Labeling of FSH	36
Specific Anti-FSH Serum	37
Methods of Separating Free from Antibody-Bound FSH*	38
Procedure for Incubating Standards and Unknowns	38
Choice of Reference Preparation	39
Normal Serum FSH Values	39
<i>5.2 Radioimmunoassay of Luteinizing Hormone in Serum</i>	<i>40</i>
Labeling of LH	40
Specific Antiserum	41
Methods of Separating Free from Antibody-Bound LH*	42
Reference Preparation	42
Application of LH Radioimmunoassay to Serum	42
Normal Serum LH Values	43
References	43
6. Radioimmunoassay of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in Urine	47
by P. FRANCHIMONT	
Passive Inhibition of Agglutination	47
Radioimmunoassay	48
Iodination	49
Antiserum Specificity	49
Reference Preparation	49
Determination	49
Urinary Excretion of FSH and LH	50
References	51
7. Bioassay of Luteinizing Hormone (LH) in Plasma and in Urine	52
by H. KARG	
Principle	52
Materials	52
Gonadotropin Preparations	54
Collection and Storage of Samples	54
Procedure of Plasma Extraction According to Walter	54
Procedure of Urine Extraction According to Albert et al.	55
Ovarian Ascorbic Acid Depletion Test	56
Reliability	57
Discussion	58
Normal Range	59
References	59
8. Bioassay of Total Gonadotropins in Urine	61
by G. BETTENDORF and M. BRECKWOLDT	
Introduction and Principle	61
Materials	61

Collection and Preparation of Samples	62
Determination	62
Reliability	63
Discussion	63
Normal Range and Values in Pathological Conditions	64
References	65

9. Immunoradiometric Assay of Parathyroid Hormone in Serum 66

by J. L. H. O'RIORDAN and J. S. WOODHEAD

Principle	66
Materials and Methods	66
Collection of Blood Samples for Assay	73
Determination	73
Reliability	74
Normal Range and Values in Various Conditions	75
References	75

10. Radioimmunoassay of Calcitonin (HCT) in Serum 77

by R. D. HESCH, M. HÜFNER, and A. VON ZUR MÜHLEN

Introduction and Principle of Method	77
Materials	77
Antibody Production	78
Labeling HCT with ¹²⁵ I	78
Preparation of Samples	80
Determination	80
Reliability	82
Discussion	82
Normal Range (Preliminary)	84
References	84

11. Radioimmunoassay of Insulin in Serum 85

by G. LÖFFLER and L. WEISS

Principle	85
Materials	85
Storage of Samples	89
Determination	90
Reliability	95
Discussion	97
Test Kits	97
References	98

12. Assay of Insulin-Like Activity (ILA) in Serum with Isolated Cells of Rat Epididymal Adipose Tissue 101

by L. WEISS and G. LÖFFLER

Introduction and Principle of Method	101
Materials	101
Preparation of Samples	103
Determination	103
Reliability and Discussion of Method	107
Normal Range	109
References	109

13. Radioimmunoassay of Glucagon in Plasma	111
by G. R. FALOONA, I. BÖTTGER, and A. M. EISENTRAUT	
Principle	111
Materials	111
Collection and Preparation of Plasma Samples	112
Antisera	112
Determination	113
Reliability	115
Normal Range	116
References	117
 14. Determination of Total Thyroxine in Serum by Competitive Protein Binding	 118
by J. HERRMANN and H. L. KRÜSKEMPER	
Principle	118
Materials	118
Preparation of Samples	119
Determination	120
Reliability	121
Discussion	123
Normal and Pathological Ranges	124
Diagnostic Relevance of Determination of Total T ₄	124
References	124
 15. Determination of Free Thyroxine in Serum by Equilibrium Dialysis	 126
by J. HERRMANN and H. L. KRÜSKEMPER	
Introduction	126
Principle	126
Materials	127
Samples	128
Determination	128
Reliability	130
Discussion	131
Normal and Pathological Ranges	132
References	132
 16. Determination of Total Triiodothyronine in Serum by Competitive Protein Binding	 134
by K. STERLING	
Introduction	134
Principle	134
Materials	135
Determination	136
Reliability	140
Normal Range and Pathological Findings	140
Physiological Implications	145
Addendum	146
References	147

17. Radioimmunoassay of Total Triiodothyronine in Plasma 150

by R. D. HESCH, A. VON ZUR MÜHLEN, and M. HÜFNER

Principle	150
Materials	150
Preparation of Samples	151
Determination	151
Reliability	153
Discussion	154
Normal and Pathological Ranges	154
References	154

18. Determination of Free Thyroxine and Free Triiodothyronine in Serum by Equilibrium Dialysis 156

by K. STERLING

Introduction	156
Materials	157
Determination	157
Normal Range of Free T_4	159
Free Triiodothyronine	160
References	161

19. Semiautomated Determination of Protein-Bound Iodine (PBI) in Serum 163

by R. KÖDDING and H. L. KRÜSKEMPER

Principle	163
Materials	163
Preparation of Samples	164
Determination	164
Reliability	168
Discussion	169
Normal Range and Values in Pathological Conditions	170
References	171

20. Determination of Noradrenaline (Norepinephrine) and Adrenaline (Epinephrine) in Urine by Fluorimetry 172

by W. VON STUDNITZ

Principle	172
Materials	172
Collection and Preparation of Urine Samples	174
Determination	174
Reliability	176
Normal Range	177
Comments	177
References	177

21. Determination of 4-Hydroxy-3-methoxymandelic Acid ("Vanillylmandelic Acid") in Urine by Spectrophotometry 178

by H. THOMAS

Principle	178
Materials	178

Collection and Preparation of Samples	179
Influence of Diet and Drugs	179
Determination	180
Reliability	181
Discussion	182
Normal Range and Values in Pathological Conditions	182
References	183
 22. Determination of Total 17-Hydroxycorticosteroids in Urine by Spectrophotometry	 184
by LIESELOTTE NOCKE-FINCK and H. BREUER	
Principle	184
Materials	184
Collection and Storage of Urine Specimens	185
Determination	185
Reliability	186
Normal Range	186
Notes	186
References	187
 23. Determination of Total 17-Hydroxycorticosteroids in Urine as Porter-Silber Chromogens	 189
by K. D. VOIGT	
Principle	189
Materials	189
Collection and Preparation of Urine Samples	191
Determination	191
Reliability	192
Discussion	193
Normal Range	193
References	194
 24. Determination of Cortisol in Plasma by Competitive Protein Binding	 195
by A. VERMEULEN	
Principle	195
Materials	195
Determination	197
Reliability	198
Discussion	201
Normal Range	201
References	202
 25. Determination of Cortisol in Serum by Fluorimetry	 203
by P. C. SCRIBA and O. A. MÜLLER	
Principle	203
Materials	203
Collection and Preparation of Samples	205
Determination	205
Reliability	206
Discussion	207

Normal Range and Values in Pathological Conditions	209
References	209

26. Determination of Cortisol in Plasma as a Porter-Silber Chromogen 211

by R. BOWMAN

Principle	211
Materials	211
Collection and Preparation of Plasma Samples	213
Determination	214
Reliability	216
Normal Range	217
References	217

27. Determination of Cortisol and 11-Deoxycortisol in Plasma by Competitive Protein Binding 219

by H. K. KLEY and H. L. KRÜSKEMPER

Introduction	219
Principle	219
Materials	220
Collection and Preparation of Sample	221
Determination	221
Reliability	224
Discussion	227
Normal Range	228
References	228

28. Determination of Aldosterone in Urine by Gas-Liquid Chromatography 230

by H. ADLERCREUTZ

Principle	230
Materials	230
Collection and Preparation of Urine Samples	232
Determination	232
Reliability	234
Addendum	235
Normal Range	236
References	236

29. Determination of Aldosterone in Urine by Double Isotope Derivative Method 238

by E. DÖLLEFELD and DAISY HAMEL

Principle	238
Materials	238
Collection and Preparation of Urine Samples	239
Determination	240
Reliability	242
Addendum	243
Normal Range	244
References	244

30. Determination of Progesterone in Plasma by Competitive Protein Binding	245
by E. D. B. JOHANSSON	
Principle	245
Materials	245
Preparation and Storage of Blood Samples	246
Determination	247
Reliability	249
Normal Range	251
References	251
31. Determination of Progesterone in Plasma by Gas-Liquid Chromatography	253
by H. J. VAN DER MOLEN and F. H. DE JONG	
Principle	253
Materials	253
Methods	256
Determination	259
Reliability	261
Normal Range	265
References	265
32. Radioimmunoassay of 17-Hydroxyprogesterone in Serum	266
by G. LEYENDECKER, ^o SHARON WARDLAW, and W. NOCKE	
Principle	266
Materials	266
Determination	268
Reliability	271
Addendum	271
Normal Range	272
References	272
33. Determination of Pregnanediol in Urine by Spectrophotometry or Gas-Liquid Chromatography	273
by A. KLOPPER	
Principle	273
Materials	274
Collection and Preparation of Urine Samples	275
Determination	275
Reliability	277
Modified Methods Suitable for Nonpregnancy Urine	277
Normal Range	279
References	280
34. Determination of Pregnanediol in Urine by Gas-Liquid Chromatography	281
by H. H. WOTIZ	
Introduction	281
Principle	281
Materials	281

	Contents	xix
Collection and Preparation of Urine Samples		282
Determination		283
Reliability		285
Discussion		286
Addendum		286
Normal Range		286
References		287
35. Determination of Pregnanetriol in Urine by Spectrophotometry or Gas-Liquid Chromatography		288
by K. FOTHERBY		
Principle		288
Spectrophotometric Measurement after Alumina Column Chromatography		288
Materials		289
Collection and Preparation of Urine Samples		289
Determination		289
Notes Regarding Methodology		290
Reliability		291
Normal Range		292
Estimation by Gas-Liquid Chromatography		292
References		294
36. Determination of Pregnanediol and Pregnanetriol in Urine by Spectrophotometry		295
by LIESELOTTE NOCKE-FINCK		
Principle		295
Materials		295
Collection and Preparation of Urine Samples		297
Determination		297
Reliability		299
Normal Range		300
References		300
37. Determination of 17-Oxosteroids in Urine by Spectrophotometry		301
by LIESELOTTE NOCKE-FINCK and H. BREUER		
Principle		301
Materials		301
Collection and Storage of Urine Specimens		302
Determination		302
Reliability		304
Normal Range		304
Notes		304
References		305
38. Differential Determination of 17-Oxosteroids in Urine		306
by A. E. KELLIE		
Principle		306
Materials		306
Determination		308
Identity of Urinary 17-Oxosteroids		310