

RECENT  
PROGRESS  
IN  
HORMONE  
RESEARCH

VOL. IX



RESEARCH  
PRESS

# Recent Progress in HORMONE RESEARCH

The Proceedings of the Laurentian Hormone Conference

*Edited by*  
GREGORY PINCUS

VOLUME IX

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Recent Progress in  
HORMONE RESEARCH  
The Proceedings of the Laurentian Hormone Conference  
VOLUME IX



*Konrad Dobriner*

KONRAD DOBRINER

1902-1952

THIS VOLUME IS DEDICATED TO THE LATE

KONRAD DOBRINER,

PIONEER INVESTIGATOR IN THE STEROID FIELD, STAUNCH  
SUPPORTER OF THE LAURENTIAN HORMONE CONFERENCE,  
RESPECTED BY ALL AS A SCIENTIST, LOVED AS A SELFLESS  
FRIEND.

## P R E F A C E

A survey of articles concerned with steroid physiology and biochemistry in past volumes of *RECENT PROGRESS IN HORMONE RESEARCH* and of a great number published elsewhere demonstrates the extraordinary dependence of much of the work upon analytical methods. In fact, with the revision and improvement of these methods, ideas and interpretations have had to be revised and corrected. The Committee on Arrangements of the Laurentian Hormone Conference is therefore very much pleased to have had the opportunity to conduct a special interim conference on "Methods of Steroid Determination in Blood and Urine."

This conference was made possible by a generous grant from the National Science Foundation. The grant was made to the Worcester Foundation for Experimental Biology in order that the meetings might be held at the Worcester Foundation's laboratories in Shrewsbury, Massachusetts. With the resources made available to it by the grant, the Committee was able to invite several visitors from abroad as well as investigators from various parts of the North American Continent. Membership was of necessity restricted to a limited number of laboratory and clinical investigators actively concerned with the development and application of the significant analytical methods. The result was a compact, highly interested and informed group. The membership of this group is published herein.

The Committee on Arrangements is indebted to the chairmen of the various conference sessions, Drs. Astwood, Dorfman, Hechter, Kritchevsky, Savard, Schneider, and White, whose skillful leadership resulted in the critical and largely relevant discussion. A special debt of gratitude is owed to the faithful, industrious secretaries to the conference: Miss Joanne Sanford, Miss Ruth Kirby and Miss Phyllis Gallento. We extend to the trustees of the Worcester Foundation, to its Executive Director, Dr. Hudson Hoagland, and to its administrative staff our sincere thanks for hospitality and for invaluable assistance in the conduct of the conference.

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Shrewsbury, Massachusetts

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# CONTENTS

	PAGE
PREFACE	v
<b>I. Introductory Remarks</b>	
1. Conference Problems	
BY GREGORY PINCUS	1
2. Neutral Steroid Hormone Metabolites	
BY RALPH I. DORFMAN	5
Discussion by <i>Katzman, Samuels, Bush, Fukushima, Gallagher and Dorfman</i>	24
<b>II. Progesterone and Metabolites</b>	
3. Recent Experiences in the Detection, Estimation and Isolation of Progesterone and Related C <sub>21</sub> Steroids	
BY WILLIAM H. PEARLMAN	27
Discussion by <i>Atwood, Szego, Horwitt, Engel, Samuels, Pincus, Smith, Venning, Marrian, Cohen, Stimmel, Gornall, Bradlow, and Pearlman</i>	40
<b>III. Estrogens</b>	
4. Hydrolysis of Conjugated Estrogens	
BY PHILIP A. KATZMAN, RICHARD F. STRAW, HENRY J. BUEHLER, AND EDWARD A. DOISY	45
5. The Chromatographic Fractionation and Identification of Compounds Related to the Estrogens	
BY LEONARD R. AXELROD	69
Discussion by <i>Marrian, Stimmel, Werthessen, Engel, Gallagher, Hechter, Savard, Bates, Katzman and Axelrod</i>	89
6. Spectrophotometric and Fluorometric Methods for Determination of Estrogenic Steroids	
BY ROBERT W. BATES	95
Discussion by <i>Axelrod, Engel, Goldzieher, Migeon, Bush, Savard and Bates</i>	107
<b>IV. Neutral Ketosteroids</b>	
7. Hydrolysis of Urinary Ketosteroid Conjugates	
BY SEYMOUR LIEBERMAN, BERTRAND MOND AND ELEANORE SMYLES	113
Discussion by <i>Fukushima, Cohen, Katzman, Wolfson, Migeon, Rubin and Lieberman</i>	130
8. Colorimetric Analytical Methods for Neutral 17-Ketosteroids of Urine	
BY PAUL L. MUNSON AND ALEXANDER D. KENNY	135
Discussion by <i>Wolfson, Wilson, Pincus, Robinson, Horwitt, Goldzieher, Engel and Munson</i>	153
9. The Uses and Limitations of Adsorption Chromatography for the Separation of Urinary Ketosteroids	
BY ALICE M. ROBINSON	163
Discussion by <i>Kritchevsky, Lakshmanan, Bush, Savard and Meyer</i>	177
10. Some Theoretical and Some Practical Aspects of Partition Chromatography of Ketosteroids	
BY KENNETH SAVARD	185
Discussion by <i>Wolfson, Axelrod, Kritchevsky, Bush, Richardson, Kochakian and Savard</i>	208

	PAGE
11. Quantitative Determination of Seven Urinary 17-Ketosteroids	
BY BETTY L. RUBIN, RALPH I. DOREMAN AND GREGORY PINCUS .....	213
Discussion by <i>Kochakian, Lieberman, Axelrod, Kritchevsky, Engel, Hechter, Jailer, Wolfson, Dorfman and Rubin</i> .....	231
12. Neutral 17-Ketosteroids in Human Plasma	
BY CLAUDE J. MIGEON AND JOHN E. PLAGER .....	235
Discussion by <i>Werthessen, Pincus, Katzman, Savard, Hechter, Venning, Bush, Szego, Prunty, Kochakian, Meyer, Lieberman, Samuels and Migeon</i> .....	245
<b>V. Non-Ketonic Neutral Steroids</b>	
13. Estimation of Alcoholic Steroids	
BY LEWIS L. ENGEL AND BILLY BAGGETT .....	251
Discussion by <i>Munson, Pincus, Heard, Kochakian, Pearlman, Bush, Lieberman, Kritchevsky and Engel</i> .....	262
<b>VI. Corticosteroids</b>	
14. Hydrolysis of Conjugates and Extraction of Urinary Corticosteroids	
BY HAROLD L. MASON .....	267
Discussion by <i>Marrian, Robinson, Horwitt, Pincus, Wilson, Nelson, Schneider, Wolfson, Forsham, Baggett, Jailer, Venning, Cohen, Engel and Mason</i> .....	278
15. Observations on the Determination of Urinary Formaldehydogenic Substances	
BY G. F. MARRIAN .....	303
Discussion by <i>Wilson, Mason, Prunty, Stimmel, Gornall, Bush and Marrian</i> ...	310
16. The Possibilities and Limitations of Paper Chromatography as a Method of Steroid Analysis	
BY IAN E. BUSH .....	321
Discussion by <i>Savard, Hechter, White, Pincus, Axelrod, Samuels and Bush</i> ....	330
17. The Fractionation of Corticosteroid Metabolites in Human Urine	
BY LOUISE P. ROMANOFF AND ROSALIE S. WOLF .....	337
Discussion by <i>Baggett, Nelson, Richardson, Schneider, Bush, Engel, Katzman, Horwitt and Romanoff</i> .....	352
18. Methods of Isolating Corticosteroids from Blood	
BY HAROLD LEVY AND STANLEY KUSHINSKY .....	357
Discussion by <i>Hechter, Axelrod, Nelson, Meyer, Bush, Kritchevsky, Schwenk, Samuels and Levy</i> .....	377
<b>VII. Steroids as Tracers</b>	
19. The Application of C <sup>14</sup> to the Study of the Metabolism of the Sterols and Steroid Hormones	
BY R. D. H. HEARD, R. JACOBS, V. O'DONNELL, F. G. PERON, J. C. SAFFRAN, S. S. SOLOMON, L. M. THOMPSON, H. WILLOUGHBY AND C. H. YATES ...	383
Discussion by <i>Gallagher, Pearlman, Savard and Heard</i> .....	408
20. Studies of the Metabolites of Isotopic Steroid Hormones in Man	
BY T. F. GALLAGHER, H. LEON BRADLOW, DAVID K. FUKUSHIMA, CHARLES BEER, T. H. KRITCHEVSKY, MADELEINE STOKEM, MAXWELL LEIGH EIDIN-OFF, LEON HELLMAN AND KONRAD DOBRINER .....	411
Discussion by <i>Katzman, Samuels, Levin, Smith, Pearlman and Gallagher</i> ....	433

	PAGE
21. Summarizing Discussion	
A. Introductory Remarks by Pincus	435
B. Estrogen Hydrolysis and Extraction discussed by <i>Cohen, Pincus, Marrian, Odell, Gallagher, Smith, Heard</i> and <i>Stimmel</i>	436
C. The Hydrolysis and Extraction of Neutral Ketosteroids and Non-Ketonic Steroids discussed by <i>Schneider, Archibald, Pincus, Gallagher, Mason, Cohen, Lieberman</i> and <i>Samuels</i>	439
D. Corticosteroid Hydrolysis and Extraction discussed by <i>Pincus, Heard, Migeon, Forsham, Hechter, Cohen</i> and <i>Marrian</i>	441
E. Methods of Measurement of Estrogens discussed by <i>Pincus, Smith, Bush</i> and <i>Archibald</i>	442
F. Ketosteroid Measurement discussed by <i>Mason, Kochakian, Prunty</i> and <i>Cohen</i>	443
G. Pregnanediol Measurement discussed by <i>Pincus, Cohen</i> and <i>Smith</i>	445
H. The Measurement of Corticosteroids discussed by <i>Pincus, Marrian, Hechter, Gornall, Nelson</i> and <i>Forsham</i>	445
I. Corticosteroids in Blood discussed by <i>Nelson, Hechter, Bush, Cohen</i> and <i>Axelrod</i>	448
J. The Separation of Estrogens, Neutral Ketosteroids and Non-Ketonic Steroids discussed by <i>Pincus, Engel, Bush, Goldzieher, Axelrod</i> and <i>Rubin</i>	450
K. Isotopic Steroids discussed by <i>Heard, Schwenk</i> and <i>Gallagher</i>	451
L. Corticosteroid Fractionation discussed by <i>Savard, Hechter</i> and <i>Bush</i>	452
AUTHOR INDEX	455
SUBJECT INDEX	465

## I. INTRODUCTORY REMARKS

### Conference Problems

GREGORY PINCUS

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There is no need to speak to the members of this conference on the need for a thorough and critical review of current methods for the determination of steroids in blood and urine, but, since with the publication of our proceedings we shall have a larger audience, it may be apropos to stress several significant considerations.

The isolation and chemical identification of estrone in 1929 may be truly said to have initiated modern steroid biochemistry. Although naturally occurring estrogenic substance was recognized before 1929, its chemical nature was unknown. The recognition of the steroidal character of this hormone led to a search for steroids in plants and animals that is still going on in the face of numerous brilliant discoveries in this field in the past 24 years. An examination of the index of Chemical Abstracts for 1930 discloses approximately 100 references to the hormones of the ovaries, testes, and adrenal cortices; there is an index of 29 titles under "sterols" and no index for "steroids." The 1951 index has close to 1,000 references on steroids and the various steroidal hormones plus 57 on "sterols." This burgeoning of interest and investigation is based on an increasing recognition of the ubiquity of these substances within the living organism and of the manifold processes they affect. Seven years ago\* I stated: "From conception to death the steroid hormones regulate, control, arbitrate, and defend." I don't know what can be truer than true, but, in the vernacular, this statement is "even more true" today.

With this manifold interest and consequent investigation there has come as a basic study the chemical determination of these substances in the tissues, the blood, and the excreta of animals. Such determinations are essential not merely to the familiar questions concerning biologically active substances: How are they made? Where do they go? How do they act? These determinations are also consequential to severely practical questions in clinical endocrinology involving diagnoses of numerous conditions, prob-

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\*In D. E. Green. 1946. Currents in Biochemical Research, p. 320. Interscience Publishers, New York.

lems of steroid therapy, judgments of reactions to stress, even analysis of aging processes in men and women. With the advancement of our knowledge of the chemistry, biochemistry, physiology, and clinical endocrinology of the steroids have come accompanying advances in biochemical methodology. Much of the work in this field is scattered. This conference is an attempt to bring it together and to subject it to critical scrutiny. Since this biochemical methodology has for the most part in recent years been applied to blood and urine, it was considered wisest to concentrate on these two fluids. Nonetheless, the Committee, in arranging the program, has sought to have covered the theory as well as the practice of steroid analytical methods.

The program is designed rather simply. A survey of the basic principles and implications of steroid metabolism studies is to be followed by considerations of analytical methods applicable to five major groups of steroids occurring in blood and urine: (a) progesterone and related substances, (b) the estrogens and their metabolites, (c) the neutral 17-ketosteroids, (d) the neutral nonketonic steroids, and (e) the corticosteroids. A final section on studies of steroids as tracers is designed to present a new and unique approach to steroid analysis that covers all types and classes of steroids, and which offers an attack on problems hitherto unsolvable by classical methods.

No speaker has been asked for an all-inclusive, completely comprehensive coverage. Pertinent and informative data for the purposes of this conference are anticipated not only from the papers but also from the discussions of the papers. For this reason discussants have been encouraged to present factual material. This factual material is sought from all fields. We need the data on the physical chemistry and organic chemistry of steroids essential to rigorous analytic methods; but we require also experiences from physiological, biochemical, and clinical investigation to highlight fundamental problems.

Steroid analytical methods may be considered as a double-edged sword. This sword must with one edge cut to the roots of the most searching of laboratory investigations and with the other cut a usable, easily trod path for certain types of clinical pathological research and hospital laboratory routine. Sometimes it is extremely difficult to make the distinction between what is really needed and what is unessential for germane observations in these major fields. I recall many attempts to devise quick and easy methods for urinary estrogen or 17-ketosteroid determinations; and I ask myself if the time and energy spent in taking what have so often eventuated as fruitless short-cuts might not have been better spent in applying more laborious but fundamentally sound methods. We shall see that the methods

one employs are determined chiefly by the information one seeks. But both simple and complex methodologies require validation, and one of the chief objectives of this meeting is to examine the criteria used to establish any usable method.

Following any presentation of specific problems the give-and-take of discussion may lead to overconcentration on certain aspects and the slighting of others. Often the main theme may be obscured. This conference will devote its final session to a summarizing discussion intended to round out what has gone before, to correct important omissions, and to anchor significant judgments. We hope that this ultimate discussion will be as pertinent to the objectives of the conference as the preceding matter. In order that it may be so the active cooperative interest of all conference members is needed. I know that I can count on such interest. I hope too that that drive which makes working steroiders of all of us will be sustained to the last minute of the last day.





# Neutral Steroid Hormone Metabolites

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## INTRODUCTION

This conference is concerned with the methods of steroid determination in blood and urine, and it is therefore proper, at this time, to examine the origin and significance of the substances to be analyzed. This can be done within the framework of our knowledge of the metabolism of the steroid hormones. More specifically, we may say that we are interested in the substances produced in specific tissues, the substances circulating in the blood, the substances that are excreted in the urine, and the interrelationships that exist among the steroids in the gland, in the blood, and in the urine.

The reviewer takes the responsibility for the following philosophy of steroid metabolism. The presence of a given steroid is not a function of the state of health or disease of an individual. There exists in the glands, in the body, and in the urine an array of steroids which may differ quantitatively, but not qualitatively, in health and disease. Interconversion of steroids is a rule not an exception. The metabolism of steroids, particularly the catabolic aspects, may be described by a dynamic state. Finally, certain relationships between tissue and urine steroids can be established and in fact now are recognized.

In this review I propose to (1) indicate the metabolism of the principal biologically active steroids from the viewpoint of the steroids produced in the special glands, the steroids circulating in the blood, and the steroids excreted in the urine; (2) indicate certain generalizations of steroid hormone metabolism in humans; and (3) present a method for the calculation of quantity of endogenously produced neutral steroid hormones based on the present knowledge of steroid hormone metabolism in humans.

## METABOLISM OF NEUTRAL STEROID HORMONES

Significant studies on steroid metabolism started in 1937 with the introduction of the classical technic of administering large doses of known crys-