RECENT PROGRESS IN HORMONE RESEARCH

Proceedings of the
1969 Laurentian Hormone Conference

Edited by
E. B. ASTWOOD

VOLUME 26

COMMITTEE ON ARRANGEMENTS

E. Alpert
E. B. Astwood
R. W. Bates
J. Beck
J. Fried
G. A. Grant
R. O. Greep
E. C. Reifenstein, Jr.
J. F. Tait
A. White

1970

ACADEMIC PRESS, New York and London

ACADEMIC PRESS INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W1X6BA

LIBRARY OF CONGRESS CATALOG CARD NUMBER: MED. 47–38

PRINTED IN THE UNITED STATES OF AMERICA

Recent Progress in

HORMONE RESEARCH

The Proceedings of the Laurentian Hormone Conference

VOLUME 26

PREFACE

This volume records the twenty-seventh annual meeting of the Laurentian Hormone Conference which was held at Mont Tremblant, Quebec, Canada on August 24–29, 1969. The 190 registered participants filled the meeting hall to capacity.

At the conclusion of one of these annual meetings, including this last one, it is not unusual to hear the comment that it was the best one yet. It is, of course, patently impossible for the program to become better every year, but at least a continuing and perhaps growing enthusiasm for the meeting prevails. The success of the Conference is dependent first-of-all upon the speakers; over the years they have set a high standard of excellence in the subject matter presented, in the meticulous preparation of this material, and in the clarity of presentation. The discussions, aided by the chairmen, add greatly to the interest of the meeting and contribute to the value of this publication by giving the reader a perspective of the state-of-the-art.

The Committee on Arrangements is grateful to the authors and coauthors of the papers and to the many discussants who, having contributed to a lively meeting, then painfully pared their remarks and carefully prepared them for publication. For the deft handling of the discussions we are grateful to the chairmen, Drs. A. Albert, G. A. Bray, L. L. Engel, J. Furth, R. Gaunt, V. P. Hollander, C. W. Lloyd, J. E. Rall, and R. J. Ryan. For the past 2 years the names of the chairmen of the sessions have been included on the program. By identifying the chairmen ahead of time, the speakers had the opportunity to suggest individuals who might be called upon to comment, and prospective discussants were given the opportunity to have their remarks introduced at the proper juncture. The discussants were able to obtain permission to show slides and to illustrate their discussion in the printed volume.

An innovation this year was the inclusion of a symposium of four short papers on the final morning of the Conference. The subject was hormones and tissue culture and included the action of hormones on growing cells as well as the formation of hormones by such cells. Each presentation was limited to 20 minutes with a period for discussion at the end of the symposium. The discussion proved to be unusually lively, to say the least, and the session was without question the most successful final session of the Conference to date. This symposium is recorded in somewhat expanded form in the last four chapters of this volume, followed by the discussion, correspondingly somewhat condensed.

The Committee wishes to thank the following companies for voluntary contributions: Abbott Laboratories; Armour Pharmaceutical Company; Ayerst Laboratories; Ciba Pharmaceutical Company; Hoffmann-LaRoche Inc.; Lederle Laboratories; The Lilly Research Laboratories; Mead John-

son Research Center; Merck Sharp & Dohme Research Laboratories; Wm. S. Merrell Company; Organon; Ortho Research Foundation; Parke, Davis & Company; Chas. Pfizer & Co., Inc.; Schering Corporation; Schering, A. G.; G. D. Searle & Co.; Smith Kline & French Laboratories; Smith, Miller and Patch, Inc.; The Squibb Institute for Medical Research; Sterling-Winthrop Research Institute; Research Division of Syntex Corporation; The Upjohn Company, Warner-Lambert Research Institute; and Wyeth Laboratories Inc.

For invaluable help in arranging the meeting and for the careful recording and editing of the discussions we are most grateful to the executive secretary of the Committee, Miss Joanne Sanford, and to the secretaries of the Conference, Mrs. Mina Rano and Miss Lucy Passalapi.

Recent Progress in Hormone Research has established a notable reputation as a valuable record of authoritative new work in endocrinology and as a most useful reference source. With this volume it enters its second quarter century of publication, and for its high standard of printing and publication much credit is due to the staff of the Academic Press, who through hard work and diligence and a patient understanding of scientists, always succeeds in bringing out an immaculate volume.

ERRATUM

Volume 25

Page 66, line 2 should read:

Andreoli (1965) found that 5 of 6 women hysterectomized in the early luteal phase of the menstrual cycle still showed luteal function on days 30–36. In a further 4 women hysterectomized in the late luteal phase, there was luteal regression at days 26–28.

CONTENTS

Pituitary and Gonadal Hormones in Women during Spontaneous and Induced Ovulatory Cycles[1]

G. T. Ross, C. M. Cargille, M. B. Lipsett, P. L. Rayford, J. R. Marshall,[2] C. A. Strott, and D. Rodbard

Endocrinology Branch, National Cancer Institute, Bethesda, Maryland

I. Introduction

The president of the World Bank, Robert S. McNamara, recently observed:[3] "The greatest single obstacle to the economic and social advancement of the majority of the people in the underdeveloped world is their rampant population growth." In appealing for appropriate solutions, Mr. McNamara asked: "Are we to solve this problem by famine? Are we to solve it by riot, by insurrection, by the violence that desperately starving men can be driven to? Are we to solve it by wars of expansion and aggression? Or are we to solve it rationally and humanely in accord with man's dignity?"

In responding to the questions raised by Mr. McNamara, my colleagues and I have the conviction that appropriate solutions will be based upon insights gained by scientific investigation of the physiology of reproduction. We know that Dr. Pincus shared our opinion (Pincus, 1965), and we are privileged to present for the second Gregory Pincus Memorial Lecture our studies of hormonal events during the human menstrual cycle. On this occasion we recall that Dr. Pincus was one of the first and foremost in applying the scientific method to the study of reproductive physiology and in the search for solutions to problems of overpopulation: rational and humane solutions, in accord with man's dignity.

Prior to the development of radioimmunoassays for gonadotropins (Bagshawe *et al.*, 1966; Midgley and Jaffe, 1966; Odell *et al.*, 1966), relatively insensitive methods of measurement coupled with low concentrations of these substances in blood and urine of normal men and women made relatively large quantities of urine or plasma requisite for accurate measurement (Apostolakis, 1960; Becker and Albert, 1965; Igarashi *et al.*, 1967; Keller, 1966; Kulin *et al.*, 1968; McArthur *et al.*, 1964; Yokota *et al.*, 1965). Consequently specimens had to be pooled either vertically within days between subjects or horizontally within subjects between days, so that changes in hormone concentrations occurring at short intervals were obscured. Alternatively, the large numbers of samples from many subjects

---

[1] The Gregory Pincus Memorial Lecture.

[2] Present address: Surgery Branch, Harbor General Hospital, Torrance, California.

[3] Excerpt from address to the University of Notre Dame, May 1, 1969.

required in order to detect subtle changes made the undertaking logistically formidable.

Similarly, more sensitive and practical methods for measuring gonadal steroid hormones in plasma were essential, and these methods have been developed (Baird and Guevara, 1969; Kirschner *et al.*, 1965; Korenman, 1968; Murphy, 1964, 1967; Neill *et al.*, 1967; Riondel *et al.*, 1965; Strott and Lipsett, 1968; Yoshimi and Lipsett, 1968). Thus, it is now possible for the first time to consider interactions of components of the hypothalamic-hypophyseal-gonadal axis in individual men and women studied at intervals of 24 hours or less. Development of some of these methods and studies using them have been the principal research interest of investigators in the Endocrinology Branch, National Cancer Institute, for almost a decade (Bardin *et al.*, 1967; Cargille *et al.*, 1968b; T. Davis *et al.*, 1965; Jacobson *et al.*, 1968a, b).

For this study, a series of spontaneous cycles were selected on the basis of two presumptive indicators for ovulation: (1) a biphasic basal body temperature curve (Hartman, 1962), and (2) a postovulatory interval of 13 days or more (Rock, 1949: Vande Wiele and Turksoy, 1965) measured from the day of the LH peak until the onset of the following menses. From daily determinations of plasma concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17-hydroxyprogesterone, and progesterone, we have described mean and modal patterns of changes for these hormones. Results of similar studies made during cycles in which ovulations were induced with clomiphene citrate were examined in an attempt to define the features that were common to spontaneous and induced ovulations. We then considered the hormonal events in some cycles during which characteristic features failed to occur and attempted to interpret the phenomena in terms of a hypothesis concerning interactions of the hypothalamic-hypophyseal-ovarian axis in normal women. Discrepancies and similarities between our data and the data others have obtained by measuring hormonal activities in plasma and urine have been described. Explanations have been advanced to account for discrepancies where these were found.

II. Materials and Methods

A. Subjects

The subjects studied were mostly young women, ranging in age from 18 to 35 years, admitted either as normal controls recruited under the aegis of the Normal Volunteer Patient Program, Clinical Center, National Institutes of Health, or as patients referred for evaluation of hypogonadism, menstrual abnormalities, or infertility. In addition, a few women, some of whom were postmenopausal, admitted either for follow-up or treatment of malignancies,

agreed to participate in studies such as measurement of metabolic clearance rates. Most of the normal volunteers were unmarried and most of the patients were married. Fully informed consent was obtained for every procedure undertaken and the safety and scientific relevance of all procedures were reviewed in accordance with standard practice at the Clinical Center, National Institutes of Health.

Basal body temperatures (BBT) were measured either orally or rectally (using thermometers specially designed for such measurements) and recorded daily. No restrictions were placed upon physical activities or diet of either normal volunteers or patients.

Culdoscopy was performed by the method of Marshall and Hammond (1966), for visualization and biopsy of the ovary when these procedures were indicated for diagnostic evaluation.

Endometrial biopsies were examined histologically and dated according to the criteria of Noyes *et al.* (1950).

For attempts at induction of ovulation in women with oligoovulatory or anovulatory infertility, clomiphene citrate was given orally in divided doses ranging from 25 to 250 mg per day for 5 days (Jacobson *et al.*, 1968a,b).

B. Assays

Venous blood was usually taken in the fasting state before 9:00 AM, anticoagulated with heparin, and centrifuged; plasma was stored at —15°C until assayed. Aliquots of the same samples were used for all measurements, and all samples from a single study period in a given subject were measured in the same assay to take advantage of increased within assay precision. In the case of steroid hormones, pooling of aliquots of several daily specimens was sometimes necessary in order to obtain adequate samples.

Plasma FSH and LH were measured by radioimmunoassays (Cargille *et al.*, 1968a; Odell *et al.*, 1966, 1967a), and progesterone and 17-hydroxyprogesterone by competitive protein binding assays (Strott and Lipsett, 1968; Yoshimi and Lipsett, 1968). Results of all determinations of FSH and LH were expressed in terms of units of biological activity of the Second International Reference Preparation for Human Menopausal Gonadotrophin (IRP 2 HMG). This material was shown to have the requisite criteria of similarity for use as a reference preparation in assays of plasma samples using our reagents for both FSH and LH. Dose response relationships obtained from assays of varying doses of solutions of this reference preparation dissolved in buffer were the basis for graphic interpolations for potencies of unknown samples.

For LH, a minimal detectable quantity varied from 0.75 to 1.9 milli

International Units (mIU) per assay tube, more commonly the latter. In terms of a maximal volume of 300 μl for plasma samples, the lower limits of detection usually ranged from 6 to 8 mIU/ml. The standard deviation of within assay precision averages 1.5 mIU for values ranging from 15 to 40 mIU/ml. The corresponding value for between assay precision is 4 mIU (Rodbard *et al.*, 1968).

For FSH, the minimal detectable quantity varied from 0.57 to 1.93 mIU per assay tube. A 200-μl volume for plasma samples usually permitted detection of at least 4.7 mIU/ml. Based upon duplicate measurements of six plasma samples in 24 assays, within assay precision was ±0.9 mIU/ml and between assay precision was ±2.0 mIU/ml for the range of 5–11 mIU/ml (Rodbard *et al.*, 1968).

The measurements of progesterone and 17-hydroxyprogesterone have a coefficient of variation of 8% at a plasma concentration greater than 30 ng/100 ml. The smallest detectable concentration is usually 15 ng/ml.

C. Data Analysis

The onset of menses has been used as a reference point for beginning and ending *sampling* periods despite the fact that, in common with Brewer, we believe menses to mark the end rather than the beginning of a menstrual cycle (Brewer and Jones, 1947). However, since many clinically useful data have been obtained using this traditional external marker, it is not easily abandoned.

While onset of menses has been used to delineate sampling periods, another event has been used as a reference point for pooling data, for calculating means and for defining intervals: namely, the day on which the maximal plasma LH concentration is observed. We have elected to use it as a marker since we believe it to be the most accurate indicator of the phases of the ovulatory cycle.

Hereafter, this maximal LH concentration will be referred to as the LH peak, and the day on which it occurs will be designated as day zero in graphic displays of the data. In Fig. 1 this format is shown together with definitions of intervals used. Unless otherwise specified, numbers on the ordinate represent milli International Units (mIU) of IRP 2 HMG per milliliter of plasma for FSH and LH, or nanograms per milliliter of plasma for 17-hydroxyprogesterone and progesterone.

For convenience, we have designated the period from the day following onset of flow until 1 day prior to the LH peak as the follicular or preovulatory phase of the cycle and the interval from 1 day following the LH peak until and including the day of the onset of next menses as the luteal or postovulatory phase. This formulation may be too restrictive for the follicular phase, particularly if this period be reckoned to terminate with

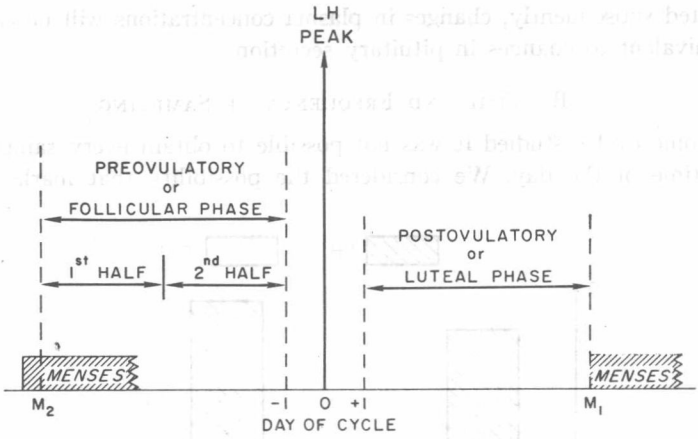

FIG. 1. Schema for synchronizing cycles, calculating means, and determining intervals.

rupture of the follicle and extrusion of an ovum since these two events are thought to occur rarely, if ever, prior to a midcycle gonadotropin surge. However, the designation for luteal phase is probably accurate since a distinct rise in progesterone, thought to indicate early function of the corpus luteum, will be shown to occur regularly by the day following the LH peak in each of these cycles.

III. Results

A. Metabolic Clearance Rates

Metabolic clearance rates (MCR) for FSH (Coble *et al.*, 1969) and LH (Kohler *et al.*, 1968) were similar for pre- and postmenopausal women whether determined by continuous infusion of labeled hormone to constant specific activity or by the single injection technique (Table I). The MCR for LH is greater than that for FSH when the two are measured either separately as shown in Fig. 2, or simultaneously in a single subject as shown in Fig. 3. Neither MCR is altered by large differences in concentration of the hormone so that variations in plasma concentrations of FSH and LH must be proportional to changes in pituitary secretion. In the data to be

TABLE I

Metabolic Clearance Rates for Plasma FSH and LH in Pre- and Postmenopausal Women

Women	FSH (ml/min)	LH (ml/min)
Premenopausal	14.2 ± 1.1	24.4 ± 1.8
Postmenopausal	12.6 ± 1.1	25.6 ± 4.1

presented subsequently, changes in plasma concentrations will be considered as equivalent to changes in pituitary secretion.

B. Time and Frequency of Sampling

In some cycles studied it was not possible to obtain every sample at the same time of the day. We considered the possibility that marked diurnal

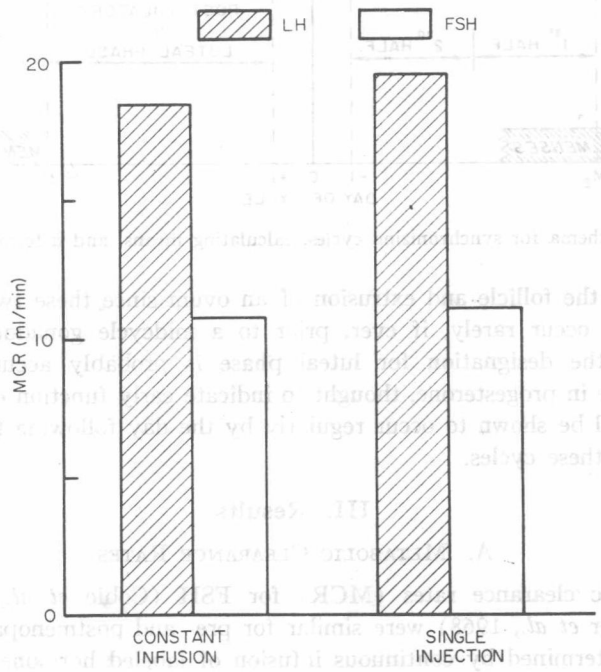

FIG. 2. Average metabolic clearance rates (MCR) for FSH and LH determined by a method based upon continuous infusion and by a method based upon a single injection of isotopically labeled hormone.

variation might introduce another variable into the results obtained in these cycles. Accordingly, concentrations of FSH and LH were measured in samples obtained at 8:00 AM, 4:00 PM, and 10:00 PM daily throughout a spontaneous presumptively ovulatory cycle in each of three young women. One of these cycles is shown in Figs. 4 and 5.

No consistent pattern of variation was seen in either FSH or LH concentrations which was related to time of day. Values for samples taken at any given time appeared to vary randomly in relation to the mean of all values for the day. The changes in pattern were equally apparent in the curves

generated when the 8:00 AM, 4:00 PM, and 10:00 PM values were plotted separately. We conclude that variations in time of sampling introduced no significant bias in these data for either FSH or LH.

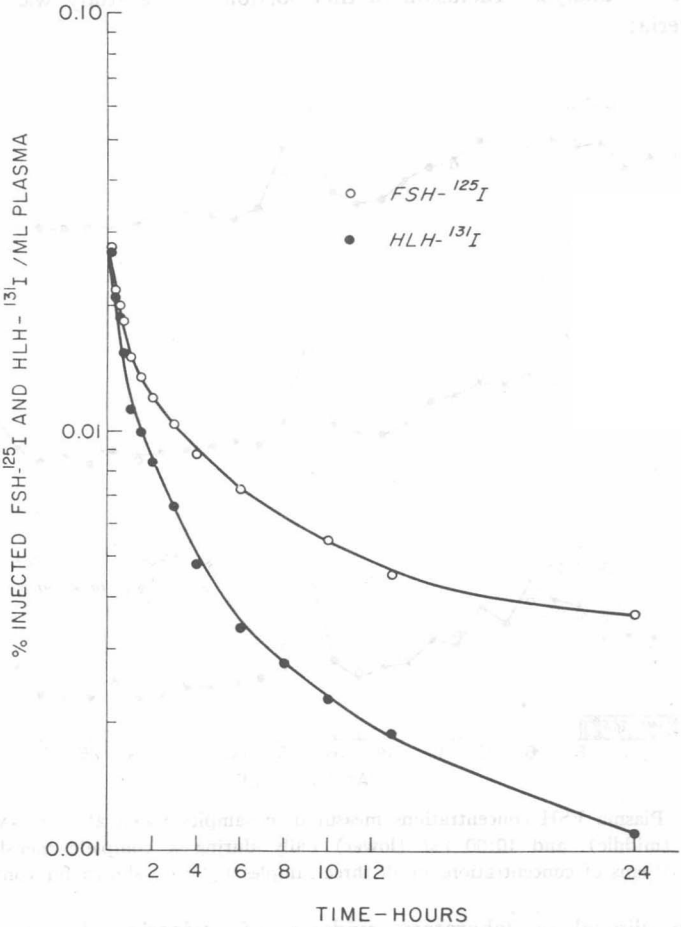

FIG. 3. Percent of injected dose of FSH-^{125}I and LH-^{131}I remaining in plasma at varying times following simultaneous injection of the two preparations in the same subject. Reprinted from Coble *et al.* (1969).

The extent to which areas under portions of the curve around the midcycle peak would vary in relation to frequency of sampling was examined. Detailed analysis indicated that the area under the plasma concentration curves was not systematically biased by frequency of sampling.