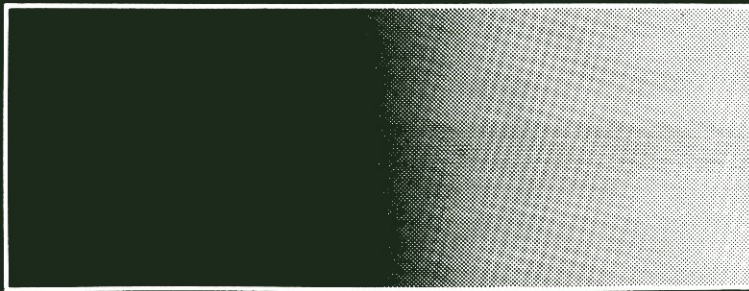


# **THE ANTIPROGESTIN STEROID RU 486 and HUMAN FERTILITY CONTROL**



**Edited by**  
**Etienne-Emile Baulieu**  
**and**  
**Sheldon J. Segal**

**THE ANTIPROGESTIN STEROID  
RU 486 and HUMAN  
FERTILITY CONTROL**

# **REPRODUCTIVE BIOLOGY**

Series Editor: Sheldon J. Segal

*The Rockefeller Foundation*

*New York, New York*

---

## **GOSSYPOL: A Potential Contraceptive for Men**

Edited by Sheldon J. Segal

## **THE ANTIPROGESTIN STEROID RU 486 AND HUMAN FERTILITY CONTROL**

Edited by Etienne-Emile Baulieu and Sheldon J. Segal

---

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

# **THE ANTIPROGESTIN STEROID RU 486 and HUMAN FERTILITY CONTROL**

**Edited by**

**Etienne-Emile Baulieu**

University of Paris-Sud

Paris, France

and

**Sheldon J. Segal**

The Rockefeller Foundation

New York, New York

**PLENUM PRESS • NEW YORK AND LONDON**

---

Library of Congress Cataloging in Publication Data

Conference on the Antiprogestational Compound RU 486 (1984: Bellagio, Italy)  
The antiprogestin steroid RU 486 and human fertility control.

(Reproductive biology)

"Proceedings of a Conference on the Antiprogestational Compound RU 486, held October 23–25, 1984, in Bellagio, Italy"—T.p. verso.

Includes bibliographies and index.

1. Mifepristone—Congresses. 2. Abortifacients—Congresses. 3. Fertility—Effect of drugs on Congresses. I. Baulieu, Etienne-Emile. II. Segal, Sheldon J. (Sheldon Jerome) III. Title. IV. Series. [DNLM: 1. Abortifacient Agents—pharmacodynamics—congresses. 2. Estrenes—pharmacodynamics—congresses. 3. Fertility—drug effects—congresses. QV 175 C7486 1984a]

RG137.6.M53C66 1985

615'.766

85-19815

ISBN-13: 978-1-4684-1244-4

e-ISBN-13: 978-1-4684-1242-0

DOI: 10.1007/978-1-4684-1242-0

---

Proceedings of a Conference on the Antiprogestational Compound RU 486,  
held October 23–25, 1984, in Bellagio, Italy

© 1985 Plenum Press, New York

Softcover reprint of the hardcover 1st edition 1985

A Division of Plenum Publishing Corporation

233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

## PREFACE

Advances in basic biological research have proceeded rapidly in recent years. The fields of molecular genetics and immunology have experienced dramatic breakthroughs, capturing the imagination of both the scientific community and the general public. With less public notice, receptor biology has brought a cascade of new discoveries and insights. The entire science of pharmacology has been virtually rewritten in terms of receptor phenomenology. In particular, the discovery of specific receptors for steroid and protein hormones has been of seminal importance. With this new information, we have advanced our understanding of the mechanism and specificity of hormone action. We can now explain how hormones interact selectively with specific target cells and how hormones alter biochemical events within the target cells.

These facts have already impacted on applied problems of clinical medicine, particularly in diagnosis and treatment of cancer and some metabolic diseases. Now, a new and important application of basic receptor biology and chemistry looms ahead. Within a few short years since the discovery of the progesterone receptor, chemists have synthesized molecules with a greater affinity for the receptor than progesterone itself and which, while occupying the receptor, fail to trigger the events which transform a target cell from the unstimulated to the stimulated state. This is the basis of the competitive inhibitory action of the anti-progestational agent, synthesized by the chemists at Roussel Uclaf, Paris, and designated RU 486.

In October, 1984 scientists working on the chemistry, pharmacology, and clinical applications of this interesting compound came together to review their results at the Rockefeller Foundation's Conference and Study Center in Bellagio, Italy. As co-chairmen, we issued invitations to colleagues from seven countries after reviewing comprehensively both published research and on-going research projects world wide. Roussel Uclaf kindly provided financial support for the RU-486 conference and for the publication of this volume, which represents the proceedings of the Bellagio conference. We gratefully acknowledge this financial support, as well as the interest and encouragement of Dr. Edouard Sakiz and his associate, Ms. Catherine Euvrard. As scientific editors, we reviewed all manuscripts for scientific content. The general editor, who had the responsibility for copy and style editing as well as page-formatting, is Amy R. Segal. We are grateful to her for her talented efforts and diligence. The process of preparing this volume for publication was facilitated by the cooperation of Evelyn Majidi and Carol Mensah, at the Rockefeller Foundation, and the staff of Plenum Press, particularly Linda Piccinino and John Matzka. We acknowledge, as well, the cooperation of all chapter authors in bringing this volume to print with a minimum of delay.

Sheldon J. Segal  
Etienne Baulieu

## CONTENTS

RU 486: An Antiprogestin Steroid with Contragestive Activity in Women Etienne-Emile Baulieu	1
Analogues of RU 486 for the Mapping of the Progestin Receptor: Synthetic and Structural Aspects Georges Teutsch	27
Pharmacological Profile of RU 486 in Animals D. Philibert, M. Moguilewsky, I. Mary, D. Lecaque, C. Tournemine, J. Secchi, and R. Deraedt	49
The Use of the Antiprogesterone Compound RU 486 to Control Timing of Parturition in Rats M. J. Bosc, G. Germain, A. Nicolle, D. Philibert, and E. E. Baulieu	69
In Vivo Assessment of Antiprogesterone and Antigluccorticoid Activities of RU 486 in Rats: Efficacy in Terminating Early Pregnancy in the Rat C. C. Chang, Sheldon J. Segal, and C. Wayne Bardin	71
Histopharmacology of RU 486 J. Secchi, D. Lecaque, C. Tournemine, and D. Philibert	79
Biochemical Profile of RU 486 M. Moguilewsky and D. Philibert	87
Radioimmunoassay of RU 486 J. Salmon and M. Mouren	99
Pharmacokinetics of RU 486 Roger Deraedt, Claude Bonnat, Monique Busigny, Pierre Chatelet, Christian Cousty, Michel Mouren, Daniel Philibert, Jacques Pottier, and Jean Salmon	103
Toxicological Study on RU 486 Roger Deraedt, Bernard Vannier, and Robert Fournex	123
Non-human Primate Studies with RU 486 David L. Healy and Gary D. Hodgen	127
Studies on the Antireproductive Mechanism of Action of RU 486 Francisco J. Rojas, James L. O'Conner, and Ricardo H. Asch	141

Effects of the Antiprogestosterone Agent RU 486 on the Natural Cycle and Gestation in Intact Cynomolgus Monkeys G. Germain, D. Philibert, J. Pottier, M. Mouren, E. E. Baulieu, and C. Sureau	155
Behavioral and Endocrine Consequences of Long-Term Antiprogestosterone (RU 486) Administration to Cynomolgus Monkeys: Preliminary Results Ronald D. Nadler, Christian Roth-Meyer, and Etienne-Emile Baulieu	169
Effects of the Antiprogestosterone RU 486 in Early Pregnancy and During the Menstrual Cycle W. L. Herrmann, A. M. Schindler, R. Wyss, and P. Bischof	179
Interruption of Early Pregnancy by the Antiprogestational Compound RU 486 A. A. Haspels	199
Clinical Study of RU 486 in Early Pregnancy David Elia	211
Termination of Very Early Pregnancy with Different Doses of RU 486: A Phase I Controlled Clinical Trial Laszlo Kovacs	221
Clinical Effects of RU 486 Administered for Seven Days in Early Pregnancy Lars Birgersson, Viveca Odling, and Elov Johansson	235
The Use of RU 486 as an Abortifacient in Early Pregnancy R. Sitruk-Ware, L. Billaud, I. Mowszowicz, H. Yaneva, P. Mauvais-Jarvis, C. W. Bardin, and I. M. Spitz	243
Pharmacokinetic and Clinical Studies of RU 486 for Fertility Regulation M. L. Swahn, S. Cekan, G. Wang, V. Lundstrom, and M. Bygdeman	249
RU 486 Stimulation of PGF <sub>2</sub> $\alpha$ Production in Isolated Endometrial Cells in Short Term Culture R. W. Kelly, D. L. Healy, M. J. Cameron, I. T. Cameron, and D. T. Baird	259
The Demonstration of the Antiprogesterone Effects of RU 486 when Administered to the Human During HCG-Induced Pseudopregnancy Horacio B. Croxatto, Irving M. Spitz, Ana Maria Salvatierra, and C. Wayne Bardin	263
RU 486 in Women with Normal or Anovulatory Cycles Gilbert Schaison, Martine George, Nelly Lestrat, and Etienne-Emile Baulieu	271
Use of Single Doses of the Antiprogestosterone Steroid RU 486 for induction of Menstruation in Normal Women Lynnette K. Nieman, David L. Healy, Irving M. Spitz, George R. Merriam, C. Wayne Bardin, D. Lynn Loriaux, and George P. Chrousos	279

Endocrinologic Effects of the Antiprogesterone RU 486 in the Luteal Phase of Normal Women	285
Donna Shoupe, Daniel R. Mishell, Jr., Maria Lacarra, Elia Gutierrez, Pekka Lahteenmaki, and Irving M. Spitz	
Endometrial and Pituitary Responses to the Steroidal Antiprogesterin RU 486 in Postmenopausal Women	295
Paul Robel, Achille Gravanis, Gilbert Schaison, Martine George, Jean de Brux, Pondichery G. Satyaswaroop, and Etienne-Emile Baulieu	
RU 486: A Full Progestin Antagonist in Human Breast Cancer Cell Lines	307
Henri Rochefort and Dany Chalbos	
Effect of RU 486 on the Pituitary-Adrenal Axis in the Dog	315
Irving M. Spitz, Charles E. Wade, Dorothy T. Krieger, Pekka Kahteenmaki, and C. Wayne Bardin	
RU 486: Studies of its Antiglucocorticosteroid Activity in Man	331
R. C. Gaillard, A. Riondel, A. F. Muller, W. Herrmann, and E. E. Baulieu	
Use of the Glucocorticoid Agonist RU 486 in the Treatment of Cushing's Syndrome	339
Lynnette K. Nieman, George P. Chrousos, Charles Kellner, Irving M. Spitz, Bruce C. Nisula, Gordon B. Cutler, Jr., George R. Merriam, C. Wayne Bardin , and D. Lynn Loriaux	
Clinical Update	347
E. Baulieu and A. Ulmann	
Index	349

RU 486: AN ANTIPROGESTIN STEROID WITH  
CONTRAGESTIVE ACTIVITY IN WOMEN

Etienne-Emile Baulieu

Université de Paris Sud  
Lab Hormone INSERM U33  
94270 Bicetre, France

ABSTRACT

We summarize the basic principles and the main experimental data that have led to the clinical use of RU 486's antiprogestin activity in human fertility control ("contragestion"). The structural features of the receptor binding properties of this steroidal antihormone are reported, as well as experiments at molecular, cellular and physiological levels in rodents and primates. RU 486 (17 $\beta$ -hydroxy-11 $\beta$ -(4-dimethylaminophenyl-1)-17 $\alpha$ -(prop-1-ynyl)-estra-4, 9-dien-3-one) is the first potent antiprogestin. Its preferential target cells are those of the endometrium or decidua; it also acts on the gonadotropic cells of the pituitary. Acting reversibly at the molecular level of receptor binding, RU 486 irreversibly interrupts target cell integrity if these cells are dependent on the continuity of progesterone action. In the case of the uterus, increased myometrium contractility and effects on the uterine cervix facilitate evacuation of the products of conception. In women, luteolysis appears to be secondary to the decrease of LH production (pituitary effect) or of hCG production, which is consequent to alteration or detachment of the trophoblast. Available clinical results indicate that RU 486 can be an efficient and safe contragestive agent, especially for the medical termination of early pregnancy, and has significant potential as a post-coital menses inducer or menstrual regulator. No significant systemic side effect has been recorded, including those events that seemed possible because of the compound's antiglucocorticosteroid capacity. Cases of incomplete uterine evacuation after using RU 486 alone may be avoided when efficient forms of administration and/or adjunct therapy with uterotonic agents are utilized. Preliminary results obtained with the addition of a small amount of prostaglandin (which by itself would be inactive) at the end of a course of RU 486 treatment are highly satisfactory. This additive treatment may decrease the potential risk of excessive bleeding. As a drug with a twofold basis of action, physiological and molecular, the antiprogesterone RU 486 is prototypic of the second generation of methods to achieve effective control of human fertility.

INTRODUCTION

Progesterone (pro gestare) is named after its supporting effect in pregnancy, and is believed to be essential in mammals, including women

(Csapo, 1979). In the fifties, Gregory Pincus, undisputed pioneer of the use of hormonal derivatives in human fertility control, championed hormonal contraception, essentially based on the inhibition of ovulation (Pincus, 1965). We describe here RU 486, a compound designed to achieve "contragestion" in women through its antiprogesterin activity (Herrmann et al., 1982; Sakiz et al., 1984). The term contragestion (for contra-gestation) is proposed to cover all aspects of fertility control interfering with the establishment or continuation of an early pregnancy. It applies as soon as sperm and egg have fused, and it pertains to effects on tubal transport of fertilized ova, blastocyst formation, preparation for nidation, or disruption of the nidation process itself. Similarly, S. Segal and C. Tietze (1971) and S. Segal and L. Atkinson (1973) have suggested a "contraprogestational pill" or "contragestational agents" as possible means of fertility control in women.

C. Djerassi in "Birth control after 1984" (1970) defined, "as an important example of future contraceptive methodology in the female, a "once-a-month" pill with luteolytic or abortifacient properties, or both... since such an agent has at least four advantages over agents now being used. 1) Administration of one pill a month is clearly more convenient than daily administration of pills. This is true both for major fertility control programs in developing countries and for highly motivated individuals in advanced countries. 2) Periodic short-term administration of a drug may be expected to give rise to fewer long-term side effects, primarily because the agent is intended to act more specifically on a well-defined biological process. 3) Since the agent will be effective in incapacitating the corpus luteum regardless of whether fertilization has or has not occurred, it does not matter whether the woman is pregnant or not. 4) Ideally, the agent might be active any time during the first 8 weeks after fertilization, so that it could also act as an abortifacient."

Such a compound "may well turn out to be a steroid," Djerassi said. However, while he praised the properties of prostaglandins (Bergstrom et al., 1972; Wilks, 1983), the concept of antiprogesterin was not mentioned. Indeed, at that time, the key biological component had not yet been revealed. Not until 1970 did we present the actual definitive description of the progesterone receptor in the uterus (Milgrom et al., 1970). This was based on work in the guinea pig, where it is easier to demonstrate the receptor than in the rat, where the progesterone receptor can be obscured by the presence of high doses of transcortin (Milgrom and Baulieu, 1968). While it is probably impossible to depend on specific suppression of progesterone biosynthesis<sup>1</sup> or on a practical method interrupting progesterone transport and delivery to target organs,<sup>2</sup> antagonization of progesterone action at the receptor level seemed a more obtainable objective.

Contrary to other steroid hormone receptors, the progesterone receptor is confined to a few specific organs. It is found in relatively high concentrations after estrogen exposure and in early pregnancy in the relevant target cells (Milgrom et al., 1970; Levy et al., 1980 (Fig. 1)). A high receptor concentration make them more likely to selectively respond to hormones or antihormones. During the menstrual cycle, there is an increase of progesterone receptor in the endometrium before ovulation. It decreases during the luteal phase, while a larger proportion appears in the "nuclear fraction" of the homogenate. In the decidua (obtained on the occasion of voluntary pregnancy interruption), there is more receptor, almost totally occupied by progesterone, activated and found in the nuclear fraction (Fig. 1).

The progesterone receptor is essential for progesterone action, and it is the molecular target for antiprogesterin. It has been used for in vitro binding tests of many compounds, since the most easily conceived

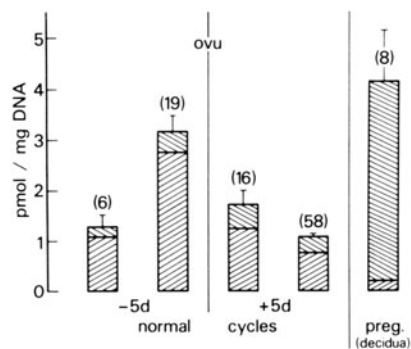


Fig. 1. Progesterone Receptor in Endometrium (Decidua) During the Menstrual Cycle and Early Pregnancy in Women. Progesterone receptor is the main physiological-molecular target of progesterone, progestine derivatives. ▨ receptor in cytosol. ▩ receptor in nuclear fraction. Patients during hormonal cycles: 5th-9th days; 10th-14th days; 15th-19th days; 20th-28th days.

antiprogesterone drugs would exclude progesterone and progestins from binding sites (Fig. 2). However, hormone binding assays cannot establish by themselves whether a given binding steroid is a progestin agonist or antagonist; they only indicate whether the compound has the potential to be active one way or another. Only a biological test can reveal the agonistic or antagonistic activities of the compound.<sup>3</sup> Since there was no convenient *in vitro* biological test, the work was tedious and expensive, necessitating relatively large amounts of compounds for *in vivo* testing of progestin or antiprogesterin activities.

Indeed, it took years before a progesterone antagonist was found. Certainly not all available scientific resources were used; the trend in most pharmaceutical companies was not toward contraceptive research at that time. In the 1970s, bio-pharmaceutical priorities did not favor steroids, even at Roussel-Uclaf, where some of the best steroid chemists in the world worked (and are still working). E. Sakiz, as president, and I, serving as a consultant, persisted in maintaining that potential... and on using it. At Roussel-Uclaf, as at other companies, there was little confidence in the commercial future of a novel method of fertility control. In addition, some colleagues were negatively influenced on this issue by their religious and social backgrounds.

Scientifically, the progesterone binding site of the receptor, naturally designed for the flat and rather "simple" progesterone steroid, was found to loosely bind a number of steroids of different structures (Milgrom et al., 1970; Smith et al., 1974). In most cases, these compounds did not have any assayable progesterone-like or antiprogesterone activity. For many years, I found intriguing the fact that the triphenylethylene antiestrogens bind to the estradiol receptor, and sometimes with very high affinity. This is true, even of the most potent pure antagonist, devoid of agonistic property (Binart et al., 1979). The estrogen receptor is very "strict" in that it has very narrow binding specificity for estrogens. The estradiol molecule also is flat and simple. These antiestrogens, with their extra-cycle

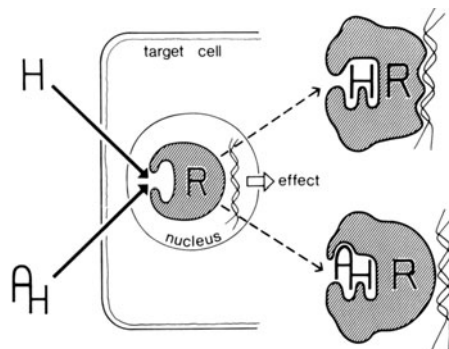


Fig. 2. Hormone and antihormone at the receptor level. Steroidal hormone (H) and antihormone (AH) penetrate into the target cell and reach their corresponding receptor originally present in the nucleus (Gasc et al., 1983). Hormone (agonist) provokes the "transformation-activation" of the receptor under the intracellular conditions; this includes transconformation of the receptor protein that acquires higher affinity for DNA and triggers the transcriptional response of specific genes. Alternately, when antihormone binds to the receptor, there is a "transformation," but the antihormone-receptor complexes are not active, have less affinity for DNA than the hormone-receptor complexes (Bourgeois et al., 1984), and there is no biological response. Competition between hormone and antihormone for the binding site of the receptor is the basic physical mechanism for explaining reversible antihormonal activity of antihormone.

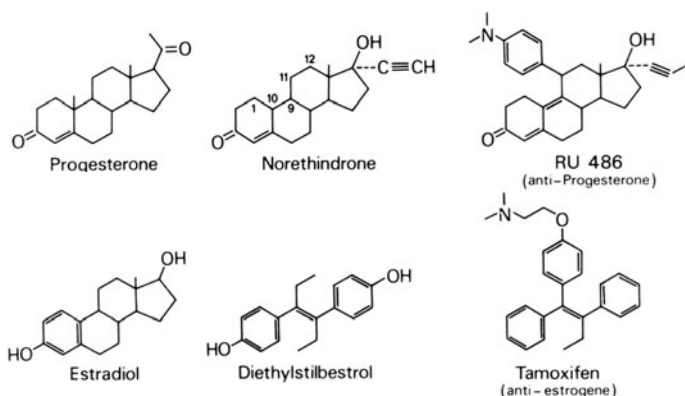


Fig. 3. Progestins and antiprogestin RU 486. Estrogens and antiestrogen tamoxifen. Norethindrone, a 19-nortestosterone, is a synthetic progestin. Note the main structural difference of RU 486: the presence of 11 $\beta$ -extra cycle and that the 3rd cycle of tamoxifen is situated at a position corresponding to the  $\beta$ -side of the steroid overall plane, just off the C 11 carbon. "RU 486" is short for RU 38486, the number used internally by Roussel-Uclaf people. Diethylstilbestrol is a synthetic analog of estradiol.

pointing out of the overall plane of the estrogen-like stilbene ring system (Hospital et al., 1972), compete well with estrogens for the receptor (Fig. 3). The physico-chemical characteristics of steroid receptors also suggested that they all belong to the same class of proteins and might have similar features in common (Joab et al., 1984), including those in the binding site region. It seemed reasonable, therefore, that the progesterone receptor could accommodate an extra cycle placed off the polycyclic ring system of a progestagen steroid, close to the region of carbons C12-C11-C9-C10-C1 (Fig. 3), with the possible consequence that the conformation of the corresponding receptor complexes would differ from that of agonist-receptor complexes (Rocheffort and Borgna, 1981; Geynet et al., 1983) and be inactive biologically.

A Roussel-Uclaf program was undertaken at my request with the declared aim of finding an antiglucocorticosteroid drug that could have more potential for general medical applications than would an anti-sex steroid derivative. However, all steroids, even those possessing distinct biological activities, have so much in common that I knew that screening should be performed with all receptor systems, including sex steroids (Baulieu and Raynaud, 1970). The synthesis of new analogs by Teutsch (see chapter 2) permitted the verification of both the initial intuitive concept and the research strategy. RU 486 is 17 $\beta$ -hydroxy-11 $\beta$ -(4-dimethylaminophenyl-1)-17a-prop-1-ynyl)-estra-4, 9-dien-3-one (Fig. 3). It can be regarded as a norethindrone derivative, that is an "historical" synthetic progestagen, bearing an additional side-chain at C17alpha that may be related to increased affinity to receptor, and an extra cycle at C11 $\beta$  which, in analogy with antiestrogens as described above,<sup>4</sup> is probably responsible for inducing or stabilizing a biologically inert receptor conformation.

#### Binding to Steroid Receptors (Table I)

RU 486 binds to the rabbit uterus progesterone receptor with an affinity superior to that of progesterone (mostly due to its slower rate of dissociation) (Philibert et al., 1982). Its affinity for the human progesterone receptor is approximately identical to that of progesterone itself (see details in Gravanis et al., 1984); and, curiously, it does not bind to the chick progesterone receptor (M. Renoir, unpublished). Among steroid receptors, progesterone receptors are those for which relative affinities of different ligands vary the most among species (Baulieu, 1983).

RU 486 also binds to other steroid receptors (Moguilewsky and Philibert, 1984). Because of a slow dissociation rate, it binds very strongly to the glucocorticosteroid receptor, even more so than dexamethasone and triamcinolone acetonide, which are the strongest synthetic agonists (Moguilewsky and Philibert, this volume; Jung-Testas and Baulieu, 1983; Bourgeois et al., 1984). It also binds strongly to the chick glucocorticosteroid receptor (Groyer et al., 1982). RU 486 binds to the androgen receptor but with lower affinity than testosterone (Moguilewsky and Philibert, this volume; Jung-Testas and Baulieu, 1984). RU 486 does not bind either to the mineralocorticosteroid receptor or to the estrogen receptor (Moguilewsky and Philibert, this volume). It binds to both native ("8S") and activated ("transformed") forms of progesterone receptor and glucocorticosteroid receptor (as do other steroid antagonists to their corresponding receptors). Available evidence also indicates that RU 486 "transforms" the receptor to which it binds, so that the complexes are found in the nuclear fraction of homogenates, even if "activation" is quantitatively inferior to what is observed with the corresponding agonist (for the glucocorticosteroid receptor see: Jung-Testas and Baulieu, 1983; Bourgeois et al., 1984; Moguilewsky and Philibert, 1984; S. Chasserot-Golaz and G. Beck, personal communication. For the rabbit progesterone receptor

Table I. Binding of RU 486 to Steroid Receptors and Plasma Proteins

Progesterone receptor	rat/rabbit +++(>>P)\	human/monkey ++(√P)	chick -
Glucocorticosteroid receptor	rat/mice +++(>>dex)	+++(>>dex)	+++(>>dex)
Androgen receptor	+(√1/3T)		
Mineralocorticosteroid receptor	-		
Estrogen receptor	mice -		
Sex steroid binding plasma protein SBP		-	-
Transcortin		-	-
Oocyte membrane receptor	Xenopus laevis +(<P) <sup>2</sup>		

Binding affinities are indicated by crosses ( $K_D$   $10^{-10}$  M +++,  $10^{-9}$  M ++,  $10^{-8}$  M +, - no affinity).

Experiments have been performed in the indicated species.

Between parentheses is a comparison with the affinity of the corresponding agonists, progesterone (P), dexamethasone (dex) and testosterone (T). <sup>2</sup>The binding to the oocyte membrane receptor, completely different from intracellular steroid receptors (Blondeau and Baulieu, 1984), is slightly inferior to that of progesterone; the + indicates  $K_D = 10^{-5}$ - $10^{-6}$ M). RU 486 is an agonist in this system (Schorderet-Slatkine, 1982, personal communication; Sadler et al., 1983).

see M. Rauch et al., 1985, who observe a small difference between progestin- and RU 486-induced activation. These results may be related to the parallel decrease in binding of antagonist-receptor complexes (as compared to agonist-receptor complexes) to both non-specific and so called high affinity DNAs (the latter is necessary to the hormonal regulation of transcription observed in the mouse mammary tumor virus (MMTV) system (Bourgeois et al., 1984).

No binding of RU 486 to transcortin (corticosteroid binding globulin (CBG), sex steroid binding plasma protein (SBP), or testosterone estradiol binding globulin (TEBG)) has been found (unpublished results). A high affinity binding system of yet unknown significance has been observed specifically in human plasma (Philibert et al., this volume).

#### Metabolism

Preliminary experiments have indicated clearly that RU 486, active after oral administration in all animal species tested (rat, mice, rabbit, dog,

Table II. Antiprogesterone Activity of RU 486

RU 486: Antiprogesterone

Endometrium: estradiol-pretreated rabbit (uteroglobin)  
implanted castrated cynomolgus monkey

Progesterone-induced giant mitochondria in endometrium:  
castrated rat

Deciduoma: castrated rat

Progesterone-induced increase of LH: castrate rat,  
in vivo and in vitro

Pseudo-pregnancy: rat

Progesterone-supported pregnancy: rat

Progesterone-facilitated sex behavior:  
estradiol-pretreated guinea pig

RU 486: Contragestive

rat - mice - guinea pig - monkey

The Table enumerates the various experimental systems that have been studied. References are found in the text.

cynomolgus monkey) and in man, is however extensively metabolized. Even though no complete study is yet available, both the antiprogesterone and the antigluccorticosteroid activities are very much inferior after oral administration than after intramuscular or subcutaneous injections. In man, the plasma half life of disappearance is of the order of 10-25 hours, and the apparent initial volume of distribution is small (approx. 10.1) (Deraedt et al., this volume a). These results are possibly due to the binding system described above. In the rat, eight metabolites have been found in the bile, essentially formed by demethylation of the dimethylamine group and/or hydroxylation of the 17 $\alpha$ -propynyl chain (Fig. 4, see details in Deraedt et al., this volume b). Four of them also have been identified in human beings, and all those that were tested (thanks to enough synthetic material) were weak analogs of RU 486 in terms of biological activity.

Current experiments in monkeys (Germain et al., this volume) and human beings (Gaillard et al., in preparation) will soon determine the appropriate plasma level of RU 486-like immunoreactive material necessary and sufficient to permit antihormonal activities in vivo under specific circumstances.

BIOLOGICAL AND PHARMACOLOGICAL EFFECTS IN ANIMAL SYSTEMS

RU 486 is a Strong Antiprogesterone

RU 486 is a pure progesterone antagonist in all rodent systems as so far reported. It has no agonist activity but has the capacity to fully antagonize progesterone action (Table II). This is the case for: 1) estrogen-treated immature rabbit endometrium (Philibert et al., this volume); 2) the progesterone-induced increase in uteroglobin mRNA (Rauch et al., 1985; Chen et al., 1984); progesterone-induced giant mitochondria in rat endometrium (Secchi and Lecaque, 1984); 4) progesterone-supported

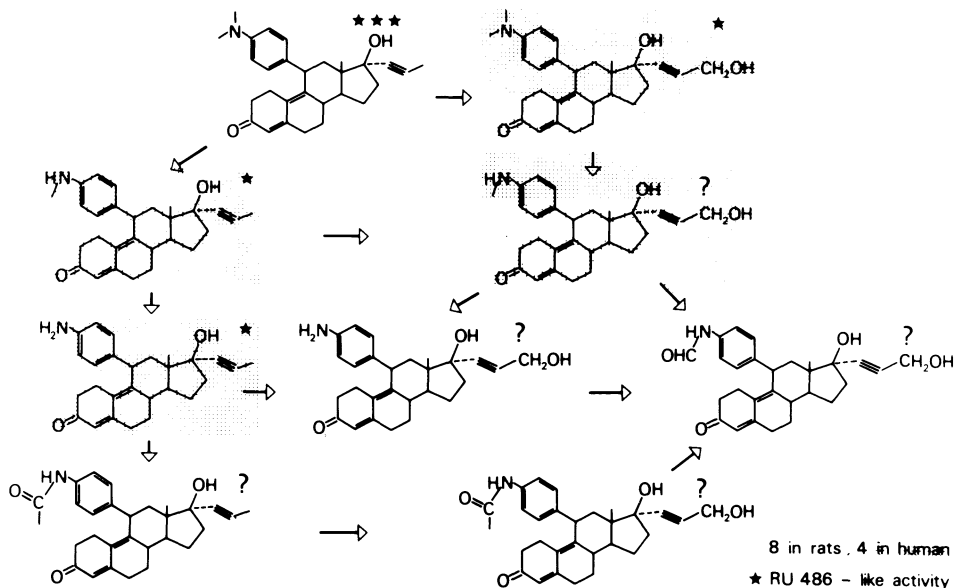


Fig. 4. Metabolites of RU 486. Arrows are logical metabolic pathways, but have not been formally demonstrated. Stars indicate RU 486-like biological activity; when it has not been tested, there is a question mark. See text and Deraedt et al., this volume.

deciduoma and pregnancy in castrated rats; 5) pseudo-pregnancy in the rat; 6) estrogen-primed rats after progesterone-induced LH increase; 7) cultured pituitary cells of estrogen-pretreated rats where LH, after treatment by GnRH and progesterone, is decreased *in vitro* by RU 486 (Philibert et al., this volume); and 8) progesterone-facilitated sex behavior of estradiol pretreated guinea pig is concerned (Brown and Blaustein, 1984). Active doses in these different systems depend on the experimental model, but all results confirm the great activity of the drug. The *in situ* experiment of Philibert et al. (this volume), introducing a few  $\mu$ g of RU 486 within the lumen of rat uterus horns, is remarkable in this respect, since it is compatible with the high affinity of the compound for the receptor, and also supports the concept that the antihormonal effect is exerted directly on the presumed target endometrial cells.

In cynomolgus monkeys, RU 486 causes uterine bleeding in estrogen-progesterone implanted castrated animals (artificial cycles) and as little as 1 mg or even 0.1 mg/kg are active after subcutaneous administration (Healy et al., 1983 a,b). It also decreases estrogen-progestin induced hyperprolactinemia (Healy et al., 1983 b).

There is no reason to believe that endometrial bleeding observed after administration of RU 486 during the luteal phase in cynomolgus and rhesus monkeys is not due to antiprogestone activity (Kreitmann-Gimbal et al., 1983; Nadler et al., this volume; B. Shortle, I. Dyrenfurth and M. Ferin, personal communication; Rojas et al., this volume). An effect at the hypothalamo-pituitary level is probably responsible for the increased length of the next cycle after a single high-dose injection (12 mg/kg i.m.) during the luteal phase (B. Shortle, I. Dyrenfurth and M. Ferin, personal communication). It seems probable that the spillover effect on the next cycle is related to drug persistence after the menses, and this may result

either from too large a dose or too slow release of the ethanol-injected compound. Preliminary results of Germain et al. (this volume) suggest that injection of RU 486 in oil makes it possible to obtain shorter and reproducible availability of injected RU 486 in cynomolgus monkeys.

Evidence obtained in vivo (Philibert et al., this volume) and from rat and cynomolgus monkey ovarian cells in vitro (D. Gospodorowitz, personal communication; Kreitmann-Gimbal et al., 1983) indicates that RU 486 does not inhibit progesterone biosynthesis (Schreiber et al., 1983). In rhesus monkeys, RU 486 given orally interrupted luteal phase extended by administration of hCG at doses that increased progesterone level (Rojas et al., this volume).

It is also likely that the antiprogesterone activity is responsible for pregnancy interruption in intact rats, mice and monkeys (Germain et al., this volume; G. Hodgen, personal communication). In rats, parturition can be readily synchronized (Bosc et al., this volume). In all studies where pregnancy was not interrupted, no fetal abnormality has ever been reported.

It has been verified that effects of RU 486 on decidualoma and pregnancy in rats occur even when animals are supplemented with dexamethasone, given in order to eliminate the possible interference of antiglucocorticosteroid activity with the effects ascribed to antiprogesterone action (Philibert et al., this volume). We report later in the text on antiglucocorticosteroid action of RU 486, that conversely is easily obliterated by concomitant administration of active glucocorticosteroid. To sum up, at this point, antihormonal activity appears to be mediated by the receptor of the corresponding endogenous agonist.

#### Does RU 486 Display Some Progesterone-Like Effects?

In human mammary cancer cells, RU 486 does not induce synthesis of specifically progesterone-inducible proteins, and actually strongly antagonizes progesterone action (Rochefort and Chabos, this volume). Bardon et al. (1985) and Horwitz et al., (1985) have found an antigrowth effect of RU 486 on human breast cancer cells in culture, that is mediated by progesterone receptor and is abolished by progesterone (H. Rochefort, personal communication). This is reminiscent of the effect of tamoxifen and analogs on MCF7 mammary cancer cells (Lippman et al., 1976) and mouse fibroblasts L cells (Jung-Testas and Baulieu, 1984). In this case, the antiestrogen decreases growth even in the absence of an estrogen agonist. Estrogen can overcome the suppressive effect. This interesting effect of RU 486, which apparently is not ascribable to the suppression of progesterone activity, may be of therapeutical importance.

In cynomolgus monkey endometrium, G. Hodgen and B. Kreitman (personal communication) have confirmed the observations of Gravanis et al. (1984) in estrogen-treated post menopausal women, showing that in absence of progesterone, RU 486 alone may provoke progesterone-like effects. The further decrease by RU 486 of gonadotropins already reduced by estrogens, was also observed in these post-menopausal women (who do not have circulating progesterone) (Gravanis et al., 1985). To date, these apparently paradoxical results have been observed only in primate systems.

#### RU 486 is Also a Potent Antiglucoctcosteroid

A very large number of biological results demonstrate the strong antiglucocorticosteroid activity of RU 486 and the absence of agonistic effects (Table III). Besides the results of DNA binding experiments

Table III. RU 486: Antigluccorticosteroid

Molecular

MMTV specific and non-specific DNA binding  
of glucocorticoid receptor

Cellular

growth: fibroblasts  
cytolysis: lymphoid cells  
liver enzymes (TAT)  
ACTH-induced decrease by glucocorticosteroid: pituitary cells

In Vivo

Anti-administered glucocorticosteroid

tyrosine aminotransferase, tryptophan oxygenase  
thymus weight  
cotton granuloma  
ACTH-induced decrease by glucocorticosteroid

Intact animals

brain/pituitary/adrenal system: activated  
very high doses: signs of hypocorticism

The Table enumerates the various experimental systems  
that have been studied. References are found in the text.

(mentioned above) that demonstrate differences between agonist and antagonist steroids at the molecular level (Bourgeois et al., 1984), RU 486 opposes active glucocorticosteroids in many cellular systems: 1) decreased growth of fibroblasts (Jung-Testas and Baulieu, 1983); 2) cytolysis of lymphoid cells (Bourgeois et al., 1984); 3) induction of enzymes such as tyrosine aminotransferase in liver cells (S. Chasserot-Golaz and G. Beck, personal communication; Gagne et al., 1984); 4) immunosuppression of in vitro antibody response in mice and autologous mixed lymphocyte reaction in human cells (Emilie et al., 1984); and 5) decrease of ACTH in cultured pituitary cells (Proulx-Ferland et al., 1982).

In vivo, pharmacological experiments in the rat (Philibert et al., 1981) have clearly indicated the antigluccorticosteroid effect of RU 486 on glycogen accumulation induced by administered corticosterone and on tyrosine aminotransferase and tryptophan oxygenase increase in liver, on thymus weight decrease, and on cotton pellet-induced granuloma. To obtain in vivo antigluccorticosteroid activity in intact animals has always been difficult for pharmacologists in the absence of very active compounds, since the physiological system is auto-regulated with negative feedback of glucocorticosteroids negatively regulating the production of ACTH (and other hormones of the pro-opiomelanocortin system, including lipotropin and  $\beta$ -endorphin). The increase of ACTH and of corticosterone (in rats Philibert et al., 1981) or of cortisol (in primates Healy et al., 1983 b) appears to compensate for the antigluccorticosteroid action of RU 486, and is more easily observed and quantified than antigluccorticosteroid effects on liver, thymus or granuloma, or on glucose or water metabolism. It is only if enough (and in fact much) of an active antigluccorticosteroid is given that the endogenous glucocorticosteroid-increase reaction can be overcome, and therefore that signs of hypocorticism may appear. This was the case, for instance, in cynomolgus monkeys that have been treated for tolerance and toxicity studies. The animals received for one month daily 100 mg/kg (Squires et al., 1982). This extraordinarily high dose led to an increase of adrenals concomitant with elevated cortisol levels, high ACTH levels, and of symptoms of hypocorticism (weakness, hypotension).

Among other changes related to the antiglucocorticosteroid activity, an interesting increase of arginine-vasopressin seems related to hypoglucocorticism (G. P. Chrousos, H. M. Schulte, P. W. Gold, G. D. Hodgen and D. L. Healy, personal communication). The secondary increase of aldosterone and plasma volume observed in some "responder" dogs treated for several days (and in whom water load test does not demonstrate peripheral hypo-glucocorticosteroid state), is not understood mechanistically (Wade et al., 1984).

#### RU 486 is a Weak Antiandrogen

Moderate but indisputable antiandrogen effects of RU 486 have been observed in vivo (Philibert et al., this volume) and in vitro (Jung-Testas and Baulieu, 1984).

#### RU 486 is not an Agonist of Steroid Hormones

With the exception of so-called progesterone-like effects indicated above, RU 486 demonstrates essentially no steroid agonistic action.<sup>5</sup> In particular, no estrogenic, androgenic or mineralocorticosteroid effects have been observed. (Large doses of RU 486 increase uterine weight and prolonged administration provokes estrus in the rat (Philibert et al., this volume).) The compound shows no anti-mineralocorticosteroid or anti-estrogenic activities (Philibert et al., this volume).

#### Species Differences and Dose-Related Effects

Dosage amounts are not transferable from one species to another; abortion of rat pregnancy is obtained only after more than 3 mg/kg, while in many cases 1 mg/kg is enough in the woman. The mouse is even less responsive than the rat. This is possibly due to differences among species in RU 486 metabolism. However, subcutaneous or intramuscular injections are more effective than oral administration for all species (antifertility in the rat, Chang et al., this volume). This is especially true in monkeys, for both antiprogesterone and antiglucocorticosteroid activities (Germain et al., 1984; Healy et al., 1983, Nadler et al., 1984). It also was observed that in the human, the antiprogesterone effect on the uterus is easier to obtain than that on LH release by the pituitary.

Finally, when comparing the two main antihormonal activities in humans and monkeys, the antiglucocorticosteroid effect (assessed by ACTH blood level) and the antiprogesterone property (provoking endometrium bleeding), it is clear that the latter is obtained with much less RU 486 than the former (Herrmann et al., 1982; Gaillard et al., 1984; Healy and Hodgen, this volume).

In conclusion, the difference among species, the mode of administration, the nature of the target organ and the type of antihormonal activity have to be considered before conclusions can be drawn in terms of active doses. More than receptor binding affinity, one should consider the concentration of steroid receptors and the distribution/metabolism status of hormones and RU 486 in each case.

#### CLINICAL APPLICATIONS

As indicated in various chapters of this volume, no side effects have been recorded in animal studies that could preclude the well-defined clinical use of the compound based on its endocrinological activities. The appropriate toxicological data (Squires et al., 1982; Deraedt et al. b, this volume) were submitted to the relevant authorities before clinical trials. We shall review some preliminary but important results obtained in humans.

Table IV. RU 486 in Non-Pregnant Women

	Endometrial bleeding	Luteolysis P+ BBT↓	LH+
<u>luteal phase</u>			
early: d. 18-21	a) + b) + (+)	+ -	+ +
late: d. 22-25	+	+	+
<u>preovulatory phase</u>			
: d. 10-13	0	delayed ovulation	

This Table is constructed essentially after the data of Herrmann et al. (1982 and this volume) and Schaison et al. (this volume). For the sake of simplicity, doses are not indicated (there is a dose-dependency in the 100 mg/day-25 mg/day range). Luteolysis stands for the decrease of both BBT and plasma progesterone. There were 2 approximately equal groups of patients who received RU 486 early during the luteal phase (they all bled and showed decreased of plasma LH). a) Those that underwent luteolysis and terminated prematurely their cycle. b) Those who did not undergo luteolysis and bled again at the expected time of normal menses. This second bleeding is indicated by the second cross in parentheses.

#### During the Menstrual Cycle

During the luteal phase in young women with normal cycles, the compound was first given for four days, 50 mg/day, starting on day 22 (Herrmann

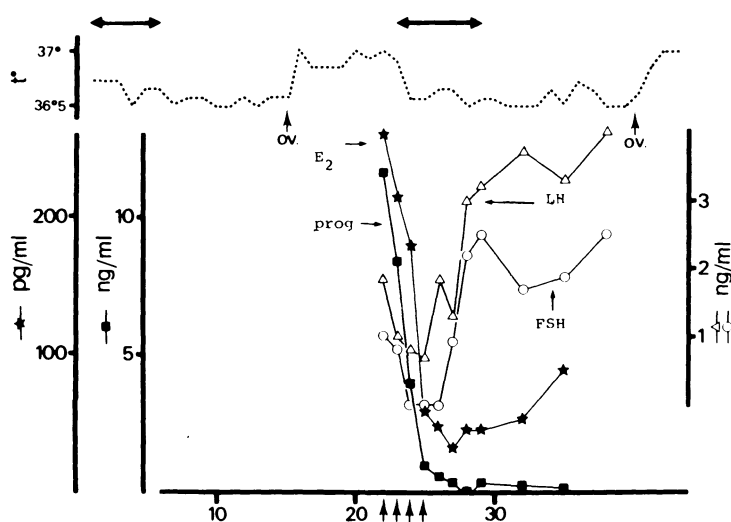


Fig. 5. Menstrual Cycle Interrupted on the 22nd Day by Administration of 50 mg RU 486 During 4 Days (↑). Horizontal line (arrows) = days of bleeding. Square, plasma progesterone; star, E<sub>2</sub>; circle, FSH; triangle, LH (original figure in Herrmann et al., 1982).

et al., 1982; Table IV). The cycle was interrupted, endometrium bleeding beginning on the second to third day of administration and simulating menses in its quantity (Fig. 5). There was irreversible luteolysis with rapid decrease of progesterone and estradiol in the plasma, and also decrease of LH and FSH. These results were interpreted in terms of antiprogestosterone activity: 1) at the endometrium level, resulting in hemorrhagia and 2) at the hypothalamus and/or pituitary level, the decrease of LH causing luteolysis. With the subsequent decrease in progesterone production, the shortening of the endometrial cycle is reinforced (Fig. 6a). Formal demonstration of this sequence of events was not possible. However, there was and still is no indication that RU 486 may inhibit progesterone synthesis in the corpus luteum and/or that it is directly luteolytic, while there is much evidence, to be presented below, that RU 486 can intervene at the hypothalamus/pituitary level. It is known that the corpus luteum in the mid and late periods of the luteal phase is dependent on LH, as confirmed by recent observations with GnRH agonists and antagonists (Vale et al., 1972; Schally, 1983; and S. C. C. Yen, personal communication). Finally, RU 486 induced menses in women whose luteal phase was extended by hCG administration at doses that simulate the hormonal profile of early pregnancy (Croxatto et al., 1984).

RU 486 was then given earlier in the cycle, for four days, beginning four days after the BBT shift, at three doses (100, 50 and 25 mg/day),

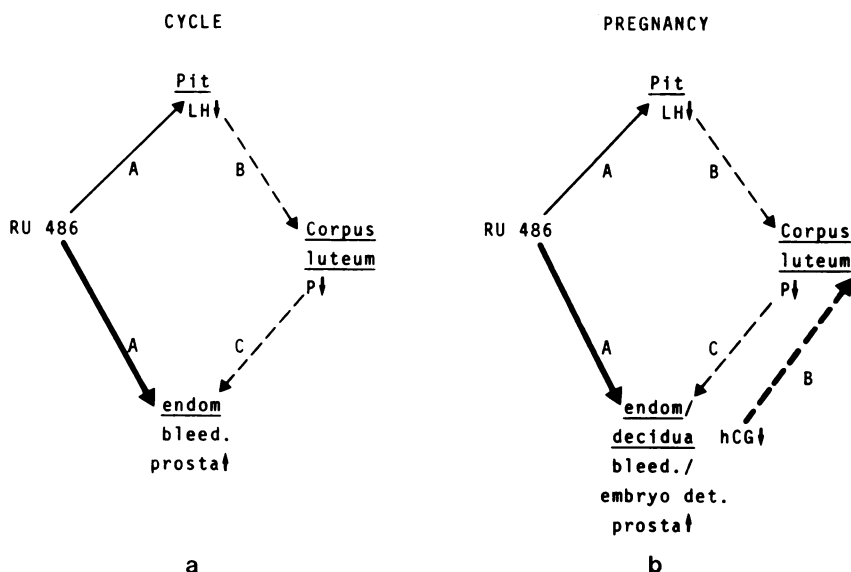


Fig. 6. Schemes of RU 486 "First Step" Effects in Non-Pregnant and Pregnant Women. These "first step" effects involve mostly hormones and endometrium/deciduoma (see text). a) RU 486 acts on endometrium or decidua, directly provoking bleeding. It also reaches the LH-making system at the level of the hypothalamus and pituitary cells. Endometrium or decidua alteration and bleeding, and LH decrease are primary responses. b) As a consequence of LH decrease, there is a secondary effect (though not always, see text) on the corpus luteum. This may decrease progesterone output. In pregnancy there is another secondary effect - the alteration/detachment of the trophoblast that reduces hCG and in turn affects the corpus luteum and decreases progesterone output. The decrease of progesterone, a tertiary effect in both cases, also may be deleterious to the endometrium/decidua.

(Schaizon et al., 1984 and this volume). It induced effects that confirmed previous conclusions and extended our knowledge of hormonal controls during the cycle (Table IV). Endometrium bleeding also occurred on day two or three of administration, constantly after 100 mg and in most cases after 50 or 25 mg, suggesting dose-dependent activity. However luteolysis, as indicated by the drop of body temperature and by decrease in progesterone and estradiol blood levels, did not occur regularly. On the contrary, only about 50% of treated cases experienced luteolysis in a dose-dependent manner (Table IV). It is of interest to note that endometrium bleeding occurred in many cases in absence of luteolysis or decrease of progesterone blood level, confirming the concept of direct endometrial antiprogesterone effect. There was also a dose-dependent decrease of LH output and a shortened period of its oscillations in plasma. Surprisingly, decrease in LH did not automatically lead to luteolysis. Perhaps the corpus luteum, during the early luteal phase, does not react to LH diminution because it is still under the influence of the mid-cycle LH surge. In cases where there was no luteolysis, a second bleeding occurred, approximately at the time of normally expected menses (Schaizon et al.; Herrmann et al., this volume). In all cases, after either premature or normally occurring luteolysis, the next ovulation took place, sometimes delayed by a few days. (See also Nieman et al., b, this volume.)

Fewer treatments have been done during the follicular phase of normal cycles. As expected, endometrium bleeding did not occur (Herrmann et al., this volume). Essentially, Herrmann observed reduction and delay of the preovulatory estradiol increase, and delay of the LH peak, which was often higher than normal. The role of estrogen in the effect of RU 486 was investigated by giving ethynylestradiol; ovulation was still delayed. Tests with GnRH indicated that, besides the slowing effect on follicle maturation, RU 486 causes a decrease of the pituitary response to the hypothalamic releasing factor. Preliminary experiments in monkeys (Collins et al., 1984) confirmed Herrmann's data, which themselves are compatible with the postulated action of progesterone in facilitating both follicular growth and positive feed-back exerted by estradiol at the pituitary level (Odell and Swerdloff, 1968).

These results suggest the possibility of using RU 486 as a once-a-month fertility-control agent, but indicate that much work is still necessary to achieve this goal. Effects on oocyte maturation should be studied, as well as the detailed hormonal pattern in women treated at various stages of the cycle. One also should consider whether RU 486 administered during one cycle will influence the next cycle. As already observed in monkeys, this may occur if the compound is administered persistently for a prolonged period. Further studies on this issue are required.

RU 486 already seems to be a very good candidate as a "late" post-coital<sup>6</sup> antifertility agent (Haspels, this volume), or "menses inducer," if given a few days before the expected date of menstruation in case of sexual exposure at mid-cycle. Trials are currently being conducted (for example, administration of 100 mg/day for 2-4 days, starting on the 24th day of a 28-day cycle). The compound may also be used as a "medical menstrual regulator" (an expression suggested by S. Segal); instead of undergoing a mechanical extraction, women with delayed menses and fearing pregnancy would immediately take RU 486. In all these cases, if the woman is not pregnant, this is a period of corpus luteum sensitivity, and the RU 486-induced decrease of LH will precipitate luteolysis. If there is very early pregnancy, this also is logically a good period to alter the decidua. Perhaps the decrease of LH may facilitate luteolysis. The occasional use of RU 486, either as a post-coital agent or as a menstrual regulator, seems imminent, even if further studies of different modes of administration, changing numbers of days of use and doses, are still necessary.

It is also possible that the use of another hormonal substance, such as an anti-GnRH or an estrogen in addition to RU 486, may help to develop an effective once-a-month fertility regulatory agent.

#### Other Antiprogesterone Uses in Non-Pregnant Women

We already have alluded to administration of RU 486 in post-menopausal women. The surprising results (Gravanis et al., 1985) were that RU 486 showed progesterone-like effects of its own in the endometrium of estrogen-treated women, while counteracting simultaneously the action of administered progesterone. We have also alluded to the LH decrease caused by RU 486 under these circumstances.

Based on the antiproliferative effect of RU 486 in mammary cancer cells in vitro (Bardon et al., 1985; Horwitz et al., 1985), we intend (with H. Rochefort) to use the compound in pilot trials in advanced breast cancers.

Moreover, since there is evidence of progesterone receptor in meningiomas (often with low estrogen receptor) and of an influence of progesterone on their evolution in women (Poisson et al., 1980; Blankenstein et al., 1983), the use of RU 486 in these (and perhaps other) brain tumors may be envisaged.

#### Medical Interruption of Pregnancy

RU 486 has been given under well defined conditions to more than 130 women asking for medical interruption of 4- to 10-week-old pregnancies. In most cases RU 486 was taken during four days at doses of 50, 100 or 200 mg/day (in one or two doses per day). The results may be divided schematically between pregnancies of 4-7 weeks and pregnancies of 8-10 weeks. The results were far better in cases of early pregnancy (seven weeks and less, that is up to 49 days after the beginning of the last menses, or about five weeks after fertilization, or about four weeks after implantation). For cases in this category of early pregnancy, the rates were: complete abortion, ca. 70%; incomplete abortion, ca. 20%; no clinical effect, ca. 10%. For 8-10-week pregnancies (between 50th and 70th days after last menses), the rates were: ca. 50%, ca. 35%, and ca. 15%, respectively (Herrmann et al., 1982; Elia, this volume; Haspels, this volume; Herrmann et al., this volume; Birgersson et al., this volume; Kovacs et al., 1984; Kovacs, this volume; Swahn et al., this volume). Interestingly, there was no dose-dependency in the percentages of successes and failures in the dose range of 50-200 mg/d for four days.

Whatever the outcome of pregnancy interruption, essentially no systemic side effects were recorded in the numerous clinical and laboratory tests performed. The pituitary-adrenal reaction measured by blood ACTH and increased cortisol levels, was observed mainly with the higher dosages used, and no clinical or biological sign of hyper- or hypo-corticosteroidism was registered. Pregnancy interruption itself included bleeding, usually starting not before the second day of drug treatment, expulsion occurring by day four or five but sometimes later. There was some tendency toward prolonged bleeding, and even the risk of heavy bleeding.

How should one interpret these results? It seems to me that the primary conclusion is that RU 486 has always worked exactly as an antiprogesterin should, even though the insufficient overall results were not medically acceptable.

This statement, that RU 486 has always worked, is based on the fact that all cases receiving 50-200 mg/day of RU 486 for four days demonstrated one or more of these following signs: 1) Bleeding (which occurred in more than

80% of cases); 2) diminution or cessation of the normal hCG daily increase that is seen at this period of pregnancy (see Fig. 7, however, statistics are not yet available); 3) effect on the cervix (in practically 100% of cases) facilitating secondary trans-cervical procedures, if necessary. Other effects of RU 486 were recorded also, such as prostaglandin increase, increase of myometrial contractility and, of course, signs of luteolysis (Herrmann et al., Swahn et al., this volume).

Figures 6b and 8 display diagrammatically my views on these primary effects of antiprogesterin RU 486 in early pregnancy and their immediate consequences. There is first an alteration of the uterine mucosa. The electron microscopy studies of Schindler et al. (1984) confirm that the drug's target is not the placenta, but elements of the decidua, in particular endothelial cells of capillaries. Three recognizable consequences result from interacting processes at the decidua-chorion level: 1) bleeding, which may vary in abundance; 2) increase of prostaglandin release, as observed in the rat (Philibert et al., this volume), in women (Herrmann et al., this volume), or in human endometrial cells *in vitro* (Kelly et al., this volume); 3) separation or alteration of the conceptus, initially the trophoblast, giving an explanation for the cessation of hCG increase and its eventual decrease. Finally, the effect on the uterine cervix is another primary effect of RU 486 consistent with current knowledge regarding progesterone action on this organ.

We propose to call the primary effects of antiprogesterin RU 486 and their above-mentioned consequences "first step" reactions. Even though one or several of these always take place, this does not lead to 100% complete abortion, as stated before. We are therefore contemplating the two following possibilities, not exclusive of one another, to explain failures.

The first proposes that RU 486 is not present at the target level in the proper amount and/or for a long enough period of time to effectively counteract progesterone. Supporting this possibility is the fact that the results are better with the earliest pregnancies and/or when hormone levels are low (low estrogen and hCG values are more predictive of the abortive action of RU 486 than is the progesterone level); in addition after about the 49th day, the chorion (placenta) cells locally produce high quantities of progesterone, which may be hard to antagonize. At this point in pregnancy, implantation is certainly more difficult to disrupt than it is

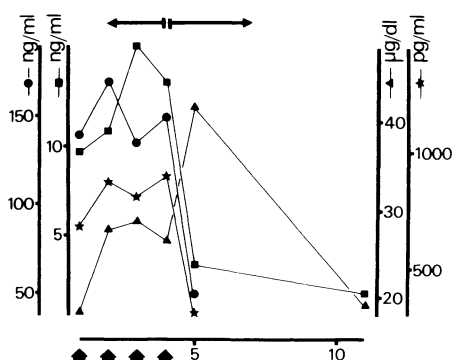
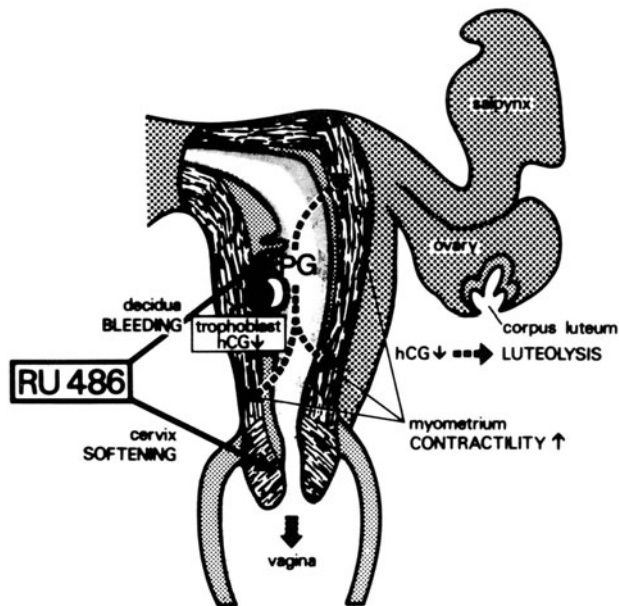


Fig. 7. Seven-Week Pregnancy. Administration of 4x 200 mg of RU 486(+). The horizontal line indicates length of bleeding, and the double vertical line signals the abortion itself. Square, progesterone; star, E<sub>2</sub> (estradiol); triangle, cortisol; and  $\beta$ -hCG in the plasma (original figure in Herrmann et al., 1982).



RU 486	Endometrium/decidua alteration	Bleeding.....	COMPLETE EVACUATION
		Prostaglandin↑ .....myometrium contractility↑	
		Alteration/detachment chorion.....hCG+ ..luteolysis (P+, E+)	
	Cervix.....	conceptus.....	
1st Step Reactions		2nd Step Consequences	

Fig. 8. A Representation of the Effects of RU 486 in Early Pregnancy. This Figure indicates the direct action on the cervix, in addition to the "first step" effects listed in Fig. 6b. The "second step" consequences on myometrium contractility (↑) are necessary for complete evacuation of uterus.

earlier. We realize that the lack of a dose-dependent effect above 50 mg/day of orally administered RU 486 may indicate that we already have obtained the best possible results with the antiprogesterin alone. Conversely, the result of Germain et al. (this volume) suggests that oral administration may not be satisfactory. Therefore other ways of administration will be tested (subcutaneous or intramuscular injections, vaginal route), before an approximately 70% success limit with an efficient antiprogesterin can be accepted. There is a precedent in the case of "spontaneous abortion" of chromosomal origin where complementary curettage is often necessary also.

The second possible explanation for failure is that the "second step" in RU 486 action does not work. As indicated in Figure 8, evacuation of the uterus, even when facilitated by the softening of the cervix, necessitates myometrial contractions. The mere decrease of progesterone action by RU 486 brings about greater contractility (Swahn et al., this volume), probably because there are both suppression of the "calming" effect of progesterone

and the increased release of excitatory prostaglandins. Insufficient endogenous prostaglandin production or availability may be the cause of no evacuation or incomplete evacuation of the uterus and of prolonged or excessive bleeding. Luteolysis, with the lowering of progesterone and estrogen output, is also related to the rather marked decrease of hCG and then is dependent on the alteration or expulsion of the trophoblast.<sup>7</sup>

We believe that the first hypothesis explains most so-called total failures, essentially characterized by no visible bleeding. This negative result is unacceptable on theoretical grounds, since it would mean that one of ten endometrium/decidua does not depend on progesterone during early pregnancy. The approximately 20% of so-called incomplete abortions, displaying bleeding but only partial evacuation of the uterus, are probably due to deficiency in the second-step reactions, either because the first step itself was already deficient, or because the prostaglandin-myometrium system itself did not work properly. It follows that considering the myotropism of prostaglandins, the results already obtained with them in abortion (see Bygdeman et al., 1983), and the tendency to long bleeding and even risk of heavy bleeding with RU 486 alone, the pharmacological use of prostaglandin appears very well suited for complementing anti-progesterone action. The aim is to reduce the rate of failure and possibly the extent of bleeding. It seems logical to give prostaglandin at the end of RU 486 administration. This is particularly acceptable in view of the change of the cervix obtained with RU 486 alone. The paper of Swahn et al. (this volume) is illuminating in this respect. It indicates that the amount of prostaglandin that is efficient after RU 486 administration does not interrupt pregnancy by itself, and also that it does not provoke the pain and gastro-intestinal disorders observed when the regular efficient dose of prostaglandin alone is given to interrupt pregnancy medically. The forthcoming trials will define doses, number of administrations of RU 486, nature of the prostaglandin used, as well as timing, dose and route of this complementary treatment.

Table V schematically summarizes the advantages and, pending problems of the use of RU 486, prostaglandin and aspiration in early pregnancy; and includes the results of Swahn et al. (this volume) obtained with 50 mg/day for 4 or 6 days, or 100 mg/day for 4 days of RU 486 given orally, plus one intramuscular injection of 0.25 mg of 16-phenoxo-tetranor-PGE<sub>2α</sub> methylsulfonylamide on the last day.

Other adjuncts than prostaglandin may be envisaged, such as oxytocin (E. Sakiz, personal communication) or anti-GnRH, on the basis of physiological and/or pharmacological considerations. One should avoid excessive potentiation with RU 486, and the aim will be to obtain the best self-administerable combination for complete, swift and safe effectiveness.

#### Other Aspects of the Clinical Use of RU 486

We already have mentioned the remarkable change of the uterine cervix obtained with RU 486. It may therefore be useful as an adjunct medication in late abortion and in parturition.

RU 486 has also been tested in extra-uterine pregnancies. As far as we know, there are at least two declared failures (Herrmann et al., this volume; Swahn et al., 1984). There is no indication of any real improvement of the prognosis of ectopic pregnancies by the use of RU 486 (Paris et al., 1984). Failure may be due to the difficulty of access by RU 486 to the fallopian-tube mucosa that, nonetheless contains progesterone receptor (Robertson et al., 1975). However, there is still the intriguing possibility that extra-uterine implantation of the blastocyst is not progesterone-dependent, contrary to uterine implantation. The hormonal

Table V. Methods for Early Abortion (Before 49th Day): a Comparison

	success rate (%)			med/ surg	trauma	pain	bleed	duration
	compl	incomp	fail					
RU 486	70	20	10	med	-	-	+	+
PROSTAGLANDIN	90			med	+	++	+	+
VACUUM ASPIRATION	> 95			surg	+	+	-	-
<hr/>								
RU 486 + PROSTAGLANDIN	100 <sup>1</sup>	0	0	med	-	-	+	-

The two medical methods, RU 486 and prostaglandin derivatives (med) are qualitatively compared to vacuum aspiration (surg). compl = complete, incomp = incomplete, and fail = failure. trauma = physical and psychological trauma. pain = uterine pain, nausea, vomiting and diarrhea. bleed = amount and length of bleeding. duration = duration of symptoms. <sup>1</sup>See the text of Swahn et al. (this volume) for further details and the afterward for a June 1985 update.

determinants in pregnancies implanted in the peritoneum or even under the kidney capsule have never been studied. Clearly, the potential use of RU 486 in ectopic pregnancies requires more study.

No clinical, metabolic or biochemical consequence of antiglucocorticosteroid activity has been recorded when using RU 486 in the context of its antiprogesterone activity (Herrmann et al., 1982; Bertagna et al., 1983), with the well understood exception of reset of the brain-pituitary-adrenal system. It remains that if any sign of hypocorticosteroidism should unfortunately appear, the remarkable reversal effect of dexamethasone (or of another active glucocorticosteroid) could be easily used (Gaillard et al., 1984; Bertagna et al., 1984). On the other hand, the use of RU 486 in a provocative test for the exploration of the brain-pituitary-adrenal system may be of great interest to analyze some aspects of the neuroendocrine functioning, as the part played by the adrenals in depression and other pathological states, for example hirsutism, hypertension, and various metabolic disorders. The compound has already been used successfully to counteract hypercortisolism in a Cushing syndrome of tumor origin, thus making surgical intervention possible (Nieman et al., a, this volume; see discussion in Gaillard et al., this volume). The tolerance of this patient to huge doses of RU 486 was remarkable (Nieman et al., a, this volume). The use of RU 486 as an antiglucocorticosteroid (Gaillard et al., 1984; Bertagna et al., 1984) is beyond the scope of this volume. It would be convenient to have an RU 486 analog devoid of antiprogesterone activity, making such an antiglucocorticosteroid more conveniently usable in women of fertile age. It is, however, remarkable that conversely, in practical terms, the antiglucocorticosteroid activity of RU 486 given in the 50-200 mg/day range, has not up to now been a drawback in the use of its antiprogesterone properties for contraception. Since the original position paper (Herrmann et al., 1982), well-publicized trials have been conducted. Public attention could be expected because of the extreme importance of the matter. All results have confirmed the original data and the principles and predictions set forth at that time have been substantial. There is increasing hope that appropriate forms of administration will soon be available to ensure wide use of RU 486 as a contraceptive.

## DEDICATION

This chapter is dedicated to the spirit that inspired Margaret Sanger, Katherine McCormick and Mary Lasker, and to the memory of Gregory Pincus.

## ACKNOWLEDGMENTS

This presentation would not have been possible but for the talents and devotion of many workers at Roussel-Uclaf and Inserm U 33. I hope I have appropriately cited their contributions. My special gratitude, respect and affection go to Dr. E. Sakiz and Professor W. Herrmann, whose moral and practical help have been invaluable.

## NOTES

<sup>1</sup>The specific suppression of progesterone biosynthesis in ovaries does not seem an appropriate goal, since progesterone is also synthesized in adrenal as a corticosteroid precursor, which should not be suppressed. In addition, the  $K_m$  of the related biosynthetic enzymes are relatively high, and the  $K_i$  of inhibitors also would probably necessitate large doses of drugs.

<sup>2</sup>Experimentally, antibodies to progesterone are very good contragestive agents (Wright et al., 1982). Their safe and convenient use in humans has not yet been perfected.

<sup>3</sup>Currently obtained data (unpublished) indicate that the agonist-receptor and the antagonist-receptor complexes soon will be distinguishable physicochemically.

<sup>4</sup>Teutsch and colleagues have synthesized estradiol derivatives with  $11\beta$ -substituents similar to that of RU 486. They behave as antiestrogenic tamoxifen derivatives (unpublished).

<sup>5</sup>We have described earlier specific hormonal conditions in the chick responsible for partial agonistic activity of antiestrogen (Catelli et al., 1980). The binding of any ligand to the receptor may, under appropriate conditions, induce/stabilize the receptor conformation able to trigger an agonistic response, and/or alternatively, specific conditions related to the state of differentiation, metabolic balance or functioning of target cells may modify receptor of chromatin (by alkylation, for example) in such a way that there will be an agonistic response.

<sup>6</sup>"Post-coital contraception" is almost always "contragestion." I prefer the latter terminology.

<sup>7</sup>The decrease of progesterone and estrogen subsequent to luteolysis may in turn influence myometrium contractility.

## REFERENCES

- Bardon S., Vignon, F., Chalbos, D., and Rochefort H., 1985, RU 486, a progestin and glucocorticoid antagonist inhibits the growth of breast cancer cells via the progesterone receptor, J. Clin. Endocr. Metab., in press.
- Baulieu, E. E., 1983, The progesterone receptor, in "Progestogens in Therapy," G. Benagiano, P. Zulli and E. Diczfaluzi, eds., Raven Press, New York, pp 27-38.

- Baulieu, E. E. and Raynaud, J. P., 1970, A "proportion graph" method for measuring binding systems, Eur. J. Biochem., 13:293-304.
- Baulieu, E. E. and Ulmann, A., 1985, RU 486: April 1985 update, this volume.
- Bergstrom, S., Diczfalussy, E., Borell, U., Karim, S., Samuelsson, B., Uvnas, B., Wikvist, N., and Bygdeman, M., 1972, Prostaglandins in fertility control, Science, 175:1280-1287.
- Bertagna, X., Bertagna, C., Girard, F., and Luton, J. P., 1983, Effet d'un antigluco corticoïde (RU 38486) sur l'axe hypophysio-surrénalien et l'élimination d'une charge en eau chez l'homme, 4ème Congrès Français d'Endocrinologie, Marseille (September 1983), Ann. Endocrinol., 44:191 (abstract no. 89).
- Bertagna, X., Bertagna, C., Luton, J. P., Husson, J. M., and Girard, F., 1984, The new steroid analog RU 486 inhibits glucocorticoid action in man, J. Clin. Endocrinol. Metab., 59:25-38.
- Bigerson, L., Odland, V., and Johansson, E., Clinical effects of RU 486, 50 mg x 2, for seven days in early pregnancy, this volume.
- Binart, N., Catelli, M. G., Geynet, C., Puri, V., Hannel, R., Mester, J., and Baulieu, E. E., 1979, Monohydroxytamoxifen: an antioestrogen with high affinity for the chick oviduct estrogen receptor, Biochem. Biophys. Res. Commun., 91:812-818.
- Blankenstein, M. A., Blaauw, G., Lambert, S. W. J., and Mulder, E., 1983, Presence of progesterone receptors and absence of estrogen receptors in human intracranial meningioma cytosols, Eur. J. Cancer Clin. Oncol, 19:365-370.
- Blondeau, J. P., and Baulieu, E. E., 1984, Progesterone receptor characterized by photoaffinity labelling in the plasma membrane of *Xenopus laevis* oocytes, Biochem. J., 219:785-792.
- Bosc, M. J., Germain, G., Nicolle, A., Philibert, D., and Baulieu, E. E., Control of birth in rats with the antiprogesterone compound RU 486, this volume.
- Bourgeois, S., Pfahl, M., and Baulieu, E. E., 1984, DNA binding properties of glucocorticosteroid receptors bound to the steroid antagonist RU 486, Embo J., 3:751-755.
- Brown, T. J., and Blaustein, J. D., 1984, Inhibition of sexual behavior in female guinea pigs by a progestin receptor antagonist, Brain Res., 301:343-350.
- Bygdeman, M., Christensen, N., Green, K., Zheng, S., and Lundstrom, V., 1983, Termination of early pregnancy - future development, Acta Obstet. Gynecol. Scand. suppl., 113:125-129.
- Catelli, M. G., Binart, N., Elkik, F., and Baulieu, E. E., 1980, Effect of tamoxifen on oestradiol and progesterone induced synthesis of ovalbumin and conalbumin in chick oviduct, Eur. J. Biochem., 107:165-172.
- Chang, C. C., Segal, S. J. and Bardin, C. W., In vivo assessment of anti-progesterone and anti-glucocorticoid activities of RU 486 in rats: efficacy in terminating early pregnancy in the rat, this volume.
- Chen, C. L. C., Chang, C. C., Bardin, C. W., and Janne, O. A., 1984, Inhibition of uteroglobin gene expression by an antiprogesterone, RU 38486, 7th International Congress of Endocrinology, Quebec, Canada (July 1984) Excerpta Medica, Amsterdam, abstracts nos. 519, 520.
- Collins, R. L., Kreitmann-Gimbal, B., Kreitmann, O. L., and Hodgen, G. D., 1984, Blockage of the spontaneous midcycle LH surge by a progesterone antagonist, 7th International Congress of Endocrinology, Quebec, Canada (July 1984) Excerpta Medica, Amsterdam, abstract no. 375.
- Croxatto, H. B., Slavatierra, A. M., and Spitz, I., 1984, Induction of menses by RU 38486 during extension of the luteal phase by exogenous hCG, 7th International Congress of Endocrinology, Quebec, Canada (July 1984) Excerpta Medica, Amsterdam, abstract no. 520.

- Csapo, A. I., 1979, Antiprogesterone in fertility control, in: "Pregnancy Termination: Procedures, Safety, and New Developments," G. I. Zatuchni, J. J. Sciarra, and J. J. Speidel, eds., Harper & Row, Hagerstown, pp 16-34.
- Deraedt, R., Bonnat, C., Busigny, M., Chatelet, P., Cousty, C., Mouren, M., Philibert, D., Pottier, J., and Salmon, J., Pharmacokinetics of RU 486, this volume, a.
- Deraedt, R., Vannier, B., and Fournex, R., Toxicological studies on RU 486, this volume, b.
- Djerassi, C., 1970, Birth control after 1984, Science, 169:941-951.
- Elia, D., Clinical study on RU 486 in early pregnancy, this volume.
- Emilie, D., Galanaud, P., Baulieu, E. E., and Dormont, J., 1984, Inhibition of in vitro immunosuppressive effects of glucocorticosteroids by a competitive antagonist RU 486, Immunol. Letters, 8:183-186.
- Gagne, T., Pons, M., and Philibert, D., 1984, RU 486 a potent antiglucocorticoid in vivo in vitro, J. Ster. Biochem., submitted.
- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., 1984, RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day, Proc. Natl. Acad. Sci. USA, 81:3879-3882.
- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., RU 486: studies of its antiglucocorticosteroid activity in man, this volume.
- Gasc, J. M., Ennis, B. W., Baulieu, E. E., and Stumpf, W. E., 1983, Recepteur de la progestérone dans l'oviducte de poulet: double revelation par immunohistochimie avec des anticorps antirecepteur et par autoradiographie a l'aide d'un progestagene tritié, C. R. Acad. Sci. Paris, 297:477-482.
- Germain, G., Philibert, D., Pottier, J., Mouren, M., Baulieu, E. E., and Sureau, C., Effects of an antiprogesterone agent (RU 486) on the time-course of the natural cycle and gestation in intact cynomolgus monkeys (*macaca fascicularis*), this volume.
- Geynet, C., Shyamala, G., and Baulieu, E. E., 1983, Similarities and differences of the binding of estradiol and 4-hydroxytamoxifen (an antiestrogen) in the chick oviduct cytosol, Biochim. Biophys. Acta, 756:439-353.
- Gravanis, A., Schaison, G., George, M., De Brux, J., Satyaswaroop, P. G., Baulieu, E. E., and Robel, P., 1984, Endometrial and pituitary responses to the steroidal anti-progestin RU 486 in post-menopausal women, J. Clin. Endocrinol. Metab., 60:156-63.
- Groyer, A., Radanyi, C., Joab, I., Lebouc, Y., Renoir, J. M., Robel, P., and Baulieu, E. E., 1982, Cross-reactivity of anti-chick oviduct progesterone receptor antibodies with glucocorticosteroid receptor, Sixth International Congress on Hormonal Steroids, Jerusalem (September 1982), J. Ster. Biochem. 17:abstract no. 131.
- Haspels, A. A., Interruption of early pregnancy by an antiprogestational compound - RU 486, this volume.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: sites of action, dose-response relationships, and hormonal effects, Fertil. Steril., 40:253-257.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen, G. D., 1983, Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863-865.
- Healy, D. L., and Hodgen, G. D., Non-human primate studies with RU 486, this volume.
- Herrmann, W., Wyss, R., Riondel A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effet d'un steroide anti-progesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut, C.R. Acad. Sci. Paris, 294:933-938.

- Hospital, M., Busetta, B., Bucourt, R., Weintraub, H., and Baulieu, E. E., 1972, X-ray crystallography of estrogens and their binding to receptor sites, Mol. Pharmacol., 8:438-445.
- Joab, I., Radanyi, C., Renoir, J. M., Buchou, T., Catelli, M. G., Binart, N., Mester, J., and Baulieu, E. E., 1984, Immunological evidence for a common non hormone-binding component in "non-transformed" chick oviduct receptors of four steroid hormones, Nature, 308:850-853.
- Jung-Testas, I., and Baulieu, E. E., 1983, Inhibition of glucocorticosteroid action in cultured L-929 mouse fibroblasts by RU 486, a new anti-glucocorticosteroid of high affinity for the glucocorticosteroid receptor, Exp. Cell. Res., 147:177-182.
- Jung-Testas, I., and Baulieu, E. E., 1984, Effects of steroid hormones and antihormones in cultured cells, Exp. Clin. Endocrinol., in press.
- Kelly, R. W., Healy, D. L., Cameron, M. J., Cameron, I. T., and Baird, D. T., RU 486 stimulates PGF<sub>2α</sub> production in isolated endometrial cells in short-term culture, this volume.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486, an antiprogesterone compound, Contraception, 29:399-410.
- Kovacs, L., Termination of very early pregnancy with different doses of RU 486 - a phase I controlled clinical trial, this volume.
- Kreitmann-Gimbal, B., Kreitmann, O. L., Sopelak, V. M., Kurman R. J., Baulieu, E. E., and Hodgen, G. D., 1983, Menstrual induction in the primate fertile and non-fertile cycle: anti-progesterone RU 486 binds to endometrial progesterone receptors without affecting luteal cells, J. Ster. Biochem., suppl. 19:112S, abstract no. 336.
- Levy, C., Robel, P., Gautray, J. P., De Brux, J., Verma, U., Descomps, B., Baulieu, E. E., and Eychemme, B., 1980, Estradiol and progesterone receptors in human endometrium: normal and abnormal menstrual cycles and early pregnancy, Am. J. Obstet. Gynecol., 136:646-651.
- Lippman, M., Bolan, G., and Huff, K., 1976, The effects of estrogens and antiestrogens on hormone-responsive human breast cancer in long-term tissue culture, Cancer Res., 36:4595-4601.
- Milgrom, E., and Baulieu, E. E., 1968, Liaison spécifique de la progesterone a une proteine dans l'uterus, C. R. Acad. Sci. Paris, 267:2005-2007.
- Milgrom, E., Atger, M., and Baulieu, E. E., 1970, Progesterone in uterus and plasma. IV. Progesterone receptor(s) in guinea pig uterus cytosol, Steroids, 16:741-54.
- Mogulewsky, M., and Philibert, D., 1984, RU 38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation, J. Ster. Biochem., 20:271-276.
- Nadler, R. D., Roth-Meyer, C., and Baulieu, E. E., Behavioral and endocrine consequences of long-term antiprogesterone-RU 486 administration in cynomolgus monkeys: preliminary results, this volume.
- Nieman, L. K., Chrousos, G. P., Kellner, C., Spitz, I. M., Nisula, B. C., Cutler, G. B., Jr., Merriam, G. R., Bardin, C. W., and Loriaux, D. L., Use of the glucocorticoid antagonist RU 486 in the treatment of Cushing's syndrome, this volume, a.
- Nieman, L. K., Healy, D. L., Spitz, I. M., Merriam, G. R., Bardin, C. W., Loriaux, D. L., and Chrousos, G. P., Use of single doses of the antiprogesterone steroid RU 486 for induction of menstruation in normal women, this volume b
- Odell, W. D., and Swerdloff, R. S., 1968, Progestogen-induced luteinizing and follicle-stimulating hormone surge in postmenopausal women: a simulated ovulatory peak, Proc. Natl. Acad. Sci. USA, 61:529-36.
- Paris, F. X., Henry-Suchet, J., Tesquier, L., Loysel, T., Loffredo, V., and Pez, J. P., 1984, Le traitement médical des grossesses extra-utérines par le RU 486, La Presse Médicale, 13:1219
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 438486 - a potent antiglucocorticoid in vivo, 8th International Congress of Pharmacology, Tokyo (1981), abstract no. 1463.

- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, RU 486 a new lead for steroidal anti-hormones, 64th Annual Meeting of the Endocrine Society (June 1982), abstract no. 668.
- Philibert, D., Moguilewsky, M., Mary, I., Lecaque, D., Tournemine, C., Secchi, J., and Deraedt, R., Pharmacological profile of RU 486 in animals, this volume.
- Pincus, G., 1965, "The Control of Fertility," Academic Press, New York.
- Poisson, M., Magdalenat, H., Foncin, J. F., Bleibel, J. M., Philippon, J., Pertuiset, B., and Buge, A., 1980, Récepteurs d'oestrogènes et de progesterone dans les méninges, Rev. Neurol., 136:193-203.
- Proulx-Ferland, L., Cote, J., Philibert, D., and Deraedt, R., 1982, Potent antiglucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat, 6th International Congress on Hormonal Steroids, Jerusalem (September 1982), J. Ster. Biochem., 17:xvii, abstract no. 80.
- Rauch, M., Loosfelt, H., Philibert, D., and Milgrom, E., 1985, Mechanism of action of an antiprogestin in the rabbit endometrium. Effects of RU 486 on the PR and on the expression of the uteroglobin gene, Eur. J. Biochem., in press.
- Robertson, D. M., Landgren, B. M., Guerrero, R., 1975, Oestradiol receptor levels in the human fallopian tube during the menstrual cycle, Acta Endocrinol., 80:705-718.
- Rocheffort, H., and Chabos, D., RU 486 is a full progestin antagonist in human breast cancer cell lines, this volume.
- Rocheffort, H., and Borgna, J. L., 1981, Differences between estrogen receptor activation by estrogen and antioestrogen, Nature, 292:257-59.
- Rojas, F. J., O'Conner, J. L., and Asch, R. H., Studies on the antireproductive mechanisms of action of RU 486, this volume.
- Sadler, S.E., Bower, M. A., and Maller, J. L., 1983, Studies of a plasma membrane steroid receptor in *xenopus laevis* oocytes, J. Cell. Biol., part 2, 97:21a, abstract no. 76.
- Sakiz, E., Euvrard, C., and Baulieu, E. E., 1984, The antiprogestin activity of RU 486, a contragestive agent in the human, 7th International Congress of Endocrinology, Quebec, Canada (July 1984), Elsevier, Amsterdam, Symposium Paper S40, in press.
- Schaison, G., George, M., Lestrat, N., Lagoguey, M., and Baulieu, E.E., Inhibitory effects of the antiprogestin steroid RU 486 on gonadotropin secretion in women, 7th International Congress of Endocrinology, Quebec, Canada (July 1984) Excerpta Medica, Amsterdam, abstract no. 2278.
- Schaison, G. George, M., Lestrat, N., and Baulieu, E. E., RU 486 in women with normal or anovulatory cycles, this volume.
- Schally, A. V., 1983, Current status of antagonistic analogs of LH-RH as a contraceptive method in the female, Res. Front. Fert. Regul., 2:5.
- Schindler, A. M., Zanon, P., Obradovic, D., Wyss, R., Graff, P., and Herrmann, W. L., 1984, Interruption of early pregnancy with RU 486, Gynecol. Obstet. Invest. (submitted).
- Schreiber, J. R., Hsueh, A. J. W., and Baulieu, E. E., 1983, Binding of the antiprogestin RU 486 to rat ovary steroid receptors, Contraception, 28:77-85.
- Segal, S. J., and Atkinson, L. E., 1973, Systemic Contragestational Agents, in: "The Abortion Experience," H. J. Osofsky and J. D. Osofsky, eds., Harper & Row, Hagerstown, pp 400-14.
- Segal, S. J., and Teitze, C., 1971, Contraceptive technology: current and prospective methods, Reports on Population/Family Planning, 1:1-24.
- Secchi, J., and Lecaque, D., 1984, Effect of progestins and antiprogestins on mitochondria in uterine glandular cells in the rat. A quantitative investigation, Cell Tiss. Res., in press.
- Smith, H. E., Smith R. G., Toft, D. O., Neergard, J. R., Burrows, E. P., and O'Malley, B. W., 1974, Binding of steroids to progesterone receptor proteins in chick oviduct and human uterus, J. Biol. Chem., 249:5924-5932.

- Squires, P. F., Allen, D. G., Heywood, R., Buist, D. P., Street, A. E., Read, R. M., Cherry, C. P., Prentice, D. E., 1982, Oral toxicology study in cynomolgus monkeys, Huntingdon Research Centre, Cambridgeshire.
- Swahn, M. L., Bygdeman, M., and Lundstrom, B., 1984, Termination of very early pregnancy with an antiprogestational compound, International Symposium on Future Aspects in Contraception, Heidelberg (September 1984), abstract no. 76.
- Swahn, M. L., Cekan, S., Wang, B., Lundstrom, V., and Bygdeman, M., Pharmacokinetic and clinical studies on RU 486 for regulation of fertility, this volume.
- Teutsch, G., Analogues of RU 486 for the mapping of the progestin receptor synthetic and structural aspects, this volume.
- Vale, W., Rivier, G. G., Monahan, M., Amoss, M., Blackwell, R., Gurgus, R., and Guillemin, R., 1972, Synthetic polypeptide antagonists of the hypothalamic luteinizing hormone releasing factor, Science, 176:933-934.
- Wade, C. E., Spitz, I. M., and Krieger, D., 1984, The effects of the antiglucocorticoid and antiprogesterone RU 38486, on adrenal function in dogs, 7th International Congress of Endocrinology, Quebec, Canada (July 1984) Excerpta Medica, Amsterdam, abstract no. 2485.
- Wilks, J. W., 1983, Pregnancy interception with a combination of prostaglandins: studies in monkeys, Science, 221:1407-1409.
- Wright, L. J., Feinstein, A., Heap, R. B., Saunders, J. C., Bennett, R. C., and Wang, M. Y., 1982, Progesterone monoclonal antibody blocks pregnancy in mice, Nature, 295:415-417.

## ANALOGUES OF RU 486 FOR THE MAPPING

### OF THE PROGESTIN RECEPTOR: SYNTHETIC AND STRUCTURAL ASPECTS

Georges Teutsch

Centre de Recherche Roussel Uclaf  
93230 Romainville, France

The synthesis of 11 $\beta$ -substituted 19-norsteroids, including RU 486 and analogues, is briefly discussed. Relative binding affinities for the rabbit progestin receptor of a series of these compounds, varying either by the 11 $\beta$ -substituent or D-ring substitution, are reported, leading to preliminary structure-affinity relationships. It was found that rather large substituents, both in position 11- $\beta$  and 17- $\alpha$  of the steroid nucleus, can be accommodated by the receptor, suggesting the existence of two unusually large hydrophobic pockets in the receptor protein in addition to the main hydrophobic site that binds the steroid framework. A computer-drawn mapping of these hydrophobic pockets has been attempted, based on crystal structures and calculated molecular conformations. The results obtained so far suggest that anti-hormones of the RU 486 type interfere with the activation of the steroid-receptor complex.

## INTRODUCTION

The discovery of the fascinating anti-hormonal activities of RU 486 and its analogues is rightly considered a major breakthrough in steroid endocrinology, especially in view of its expected impact on human fertility control. However, beyond the short-term use of these compounds as contragestive agents and their potential long-term use as medications for glucocorticoid related diseases, they constitute unique tools, at present, for the investigation of hormone action. This review, which also will include some up to now unpublished results, will discuss our chemical and biochemical itinerary through the 11- $\beta$ -substituted 19-norsteroid series and will draw preliminary conclusions on the shape and function of the progesterone receptor site. Although our original interest in this particular chemical series was not motivated by an oriented search for antiprogestins but rather by a more general structure-affinity study, it rapidly appeared that 11 $\beta$ -substitution could lead to compounds with unexpected biochemical profiles (Teutsch et al., 1978; Belanger et al., 1981). The first evidence of antihormonal activity by some of these steroids was provided by the *in vitro* antiglucocorticoid activity of RU 25 055 on TAT induction in HTC cells (Giesen et al., 1981; Giesen and Beck, 1982), a result that encouraged us to seek even more potent compounds by trying to further increase the relative binding affinity (RBA) for the glucocorticoid receptor. It turned out that this approach was correct in spite of its evident disagreement with existing theories. Indeed, RU 486 and most of its analogues show interaction kinetics with the receptors that

had been suggested to be characteristic of agonists rather than of antagonists (Raynaud et al., 1980); this applies for both the glucocorticoid receptor (GR) and the progestin receptor (PR) (D. Philibert, this volume).

Nevertheless, it is now well known that these compounds behave as pure antagonists. We had succeeded in materializing the dream of many a researcher in reproductive biology, summarized quite accurately only a few years ago by Laumas: "A good antagonist would be a compound which would bind with high affinity to the uterine progesterone receptors to exclude progesterone from occupying binding sites and which would have such a structure that its interaction with the receptor molecule does not lead to any hormonal agonistic effect" (Verma and Laumas, 1981). In fact, early attempts to achieve this goal by introducing bulky substituents at positions 1- $\alpha$ , 7- $\alpha$  and 17- $\alpha$  of active progestins failed (Beyer et al., 1976; Beyer et al., 1980). It was concluded that the progesterone receptor does not possess hydrophobic sites large enough to accommodate these substituents. These results were in partial agreement with independent QSAR studies that pointed to the existence of small hydrophobic pockets with the maximum size of a methyl group only in the vicinity of positions 6 $\alpha$  and 11 $\beta$ . Somewhat larger pockets would exist in the 16- $\alpha$ , 17- $\alpha$  region (Lee et al., 1977).

Our results therefore came as a double surprise - the existence of a much larger pocket than expected above C-11 of the bound steroid and the fact that its occupancy led to an antagonistic response, rather than an agonistic one. This point is of particular importance for understanding the mechanism of action and will be discussed in more detail in the last section of this review. The other sections will include a brief summary of the chemistry involved, a discussion of the structural features of 11 $\beta$ -substituted 19-norsteroids, and selected examples of structure-affinity and structure-activity relationships that allowed us to gain additional information on progesterone receptor topography and the mechanism of anti-hormonal action.

## CHEMISTRY

Chemistry has unquestionably played a determining role in the development of the novel anti-progestins. Prior to the 1975 discovery of our original synthetic scheme, general access to 11 $\beta$ -substituted 19-norsteroids was not available. Existing methods proved cumbersome, generally ineffective and limited to primary and secondary alkyl substituents (for a review see Teutsch, 1984). The finding that unsaturated epoxides of type 1 (Nedelec, 1970) react with organocuprates or copper-catalyzed Grignard reagents to produce high yields of the 11 $\beta$ -substituted steroids 2 via conjugate addition opened the road to practically limitless variations (Teutsch and Belanger, 1979). Thus, almost any type of organic group can be introduced, provided that the corresponding organometallic reagent (Grignard reagent, lithium derivative or copper reagent) is accessible. Substituents as diverse as alkyl, alkenyl, aryl and heteroaromatic groups have been introduced without difficulty. Though steric hindrance is generally not a problem as evidenced by the quantitative introduction of a tertiary-butyl group, the strong buttressing effect present in 2,6-disubstituted aromatics does not let the reaction proceed (Fig. 1).

The adopted methodology thus far has remained unchallenged, and no comparable results could be obtained by changing either the organometallic reagent or the catalyst. The use of aluminum derivatives predictably led to 10 $\beta$ -substitution, whereas lithium dimethylaurate gave a poor yield of the 11 $\beta$ -substituted compound. This result could not be extended to alkyl groups other than methyl (Teutsch, 1982). Among a variety of catalysts that were

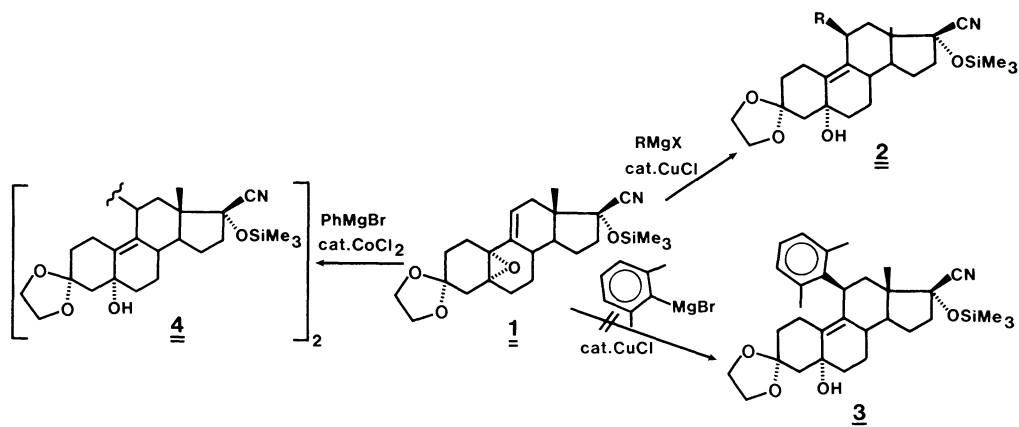


Fig. 1. Synthesis of 11 $\beta$ -substituted 19-norsteroids via conjugate epoxide opening.

tried, only cobaltous chloride in the presence of phenylmagnesium bromide gave a remarkable result: it led to the steroid dimer 4 (G. Teutsch, unpublished results), the structure of which has been confirmed by X ray diffraction studies (J. P. Mornon, personal communication).

The intermediates of general structure 5, when submitted to hydrolytic ketal cleavage, undergo simultaneous dehydration to the dienones 6, which can be further aromatized to 7 (Fig. 2).

This scheme was applied to our initial synthesis of a selection of 11 $\beta$ -substituted 19-norsteroids, in order to explore their biological potential (Belanger et al, 1981) and ultimately led to the design of RU 486 (Teutsch, 1984). Consistent with the nature of D-ring substitution, there is an adequate degree of flexibility in the synthetic scheme; the main reagent (Grignard reagent in the presence of cuprous salt) is compatible with a number of functional groups such as cyano, unconjugated ketones, esters or hydroxyls (in which case one has to use a twofold excess).

Figure 3 exemplifies two routes of access to RU 486, while Figure 4 illustrates the usefulness of the particular 17 $\beta$ -cyano, 17- $\alpha$  trimethylsilyloxy substitution pattern (Gasc and Nedelec, 1971) as a precursor to various D-ring substituents (Belanger et al, 1981; Teutsch et al., 1982a). This convenient chemical flexibility has been exploited in the preparation of the two hundred odd compounds designed to assess the

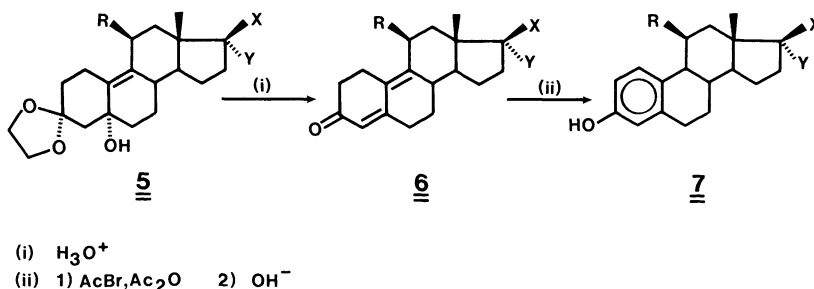


Fig. 2. Synthesis of 11 $\beta$ -substituted-4,9-estradien-3-ones and 1,3,5(10)-estratrienes.

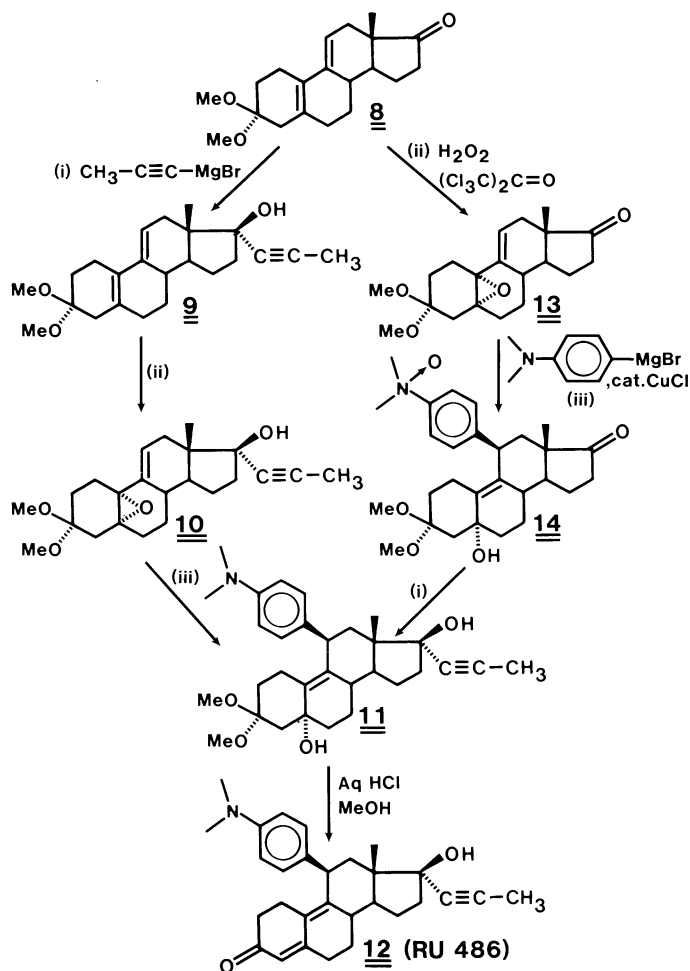


Fig. 3. Two synthetic pathways to RU 486.

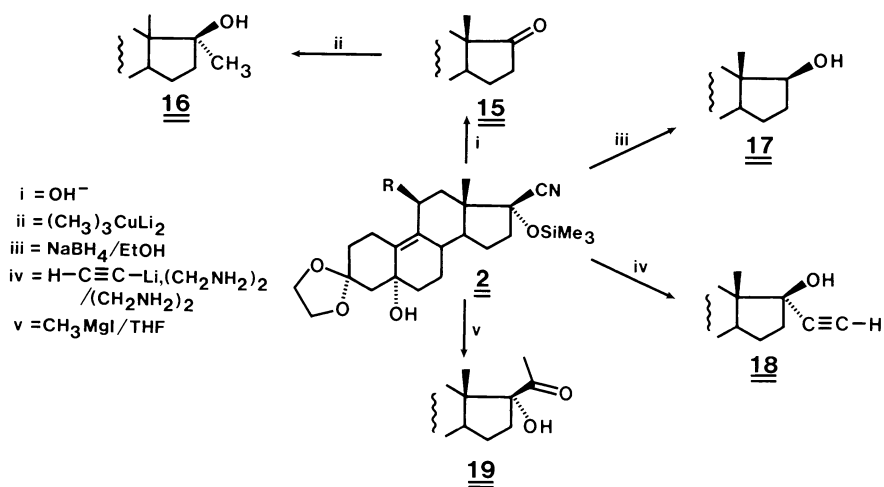


Fig. 4. Chemical modifications of the O-trimethylsilyl-cyanohydrin moiety.

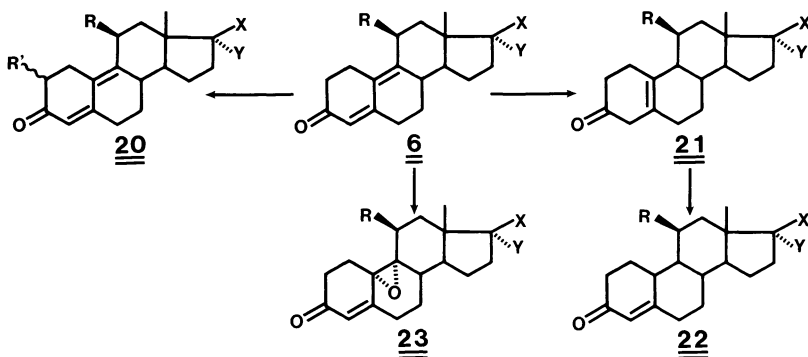


Fig. 5. Synthetic transformations in rings A and B of the 4,9-estradien-3-one system.

influence of 11 $\beta$ -substitution, as well as D-ring substitution on relative binding affinity (RBA) for the steroid hormone receptors and/or biological activity.

Of course the 11 $\beta$ -substituted dienones themselves can be subjected to various transformations such as reduction to the enones (Neef et al., 1983; Teutsch, 1984), alkylation at C-2 (Teutsch et al., 1983) or epoxidation of the delta-9 double bond as shown in Figure 5 (Teutsch et al., 1982b). It should be noted, however, that the transformation 21 to 22 (Fig. 5) has not been achieved when R represents an aromatic ring. The hydroxyketal precursors, on the other hand, can be useful in the functionalization of the 11 $\beta$ -substituent such as the hydroboration of a vinyl group (Fig. 6) (Teutsch, unpublished results) or in backbone modifications such as the photochemical C-13 epimerization described in Figure 7 (Neef et al., 1984). These few examples illustrate the remarkable versatility of the synthetic scheme, opening access to literally hundreds of thousands of potentially active steroids. Thus one can make a reasonable prediction that this single class of compounds can yield all types of sexual and adrenal hormone agonists and antagonists. As a conclusion to this section it should be pointed out that the strict regio- and stereo-selectivity of this scheme provide some clues about the mechanism of the epoxide opening reaction (Teutsch, 1982).

## STRUCTURAL FEATURES

The assignment of beta versus alpha configuration of the C-11 substituents based on  $^1\text{H-NMR}$  data has been discussed in a previous review (Teutsch, 1984) and will not be developed any further in this chapter. However, complementary information is provided by X ray crystallographic

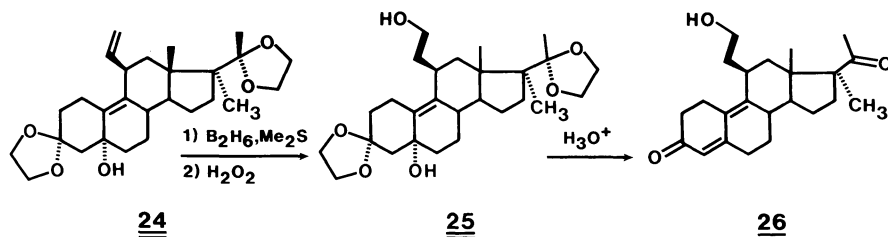


Fig. 6. Hydroboration of an 11 $\beta$ -vinyl group.

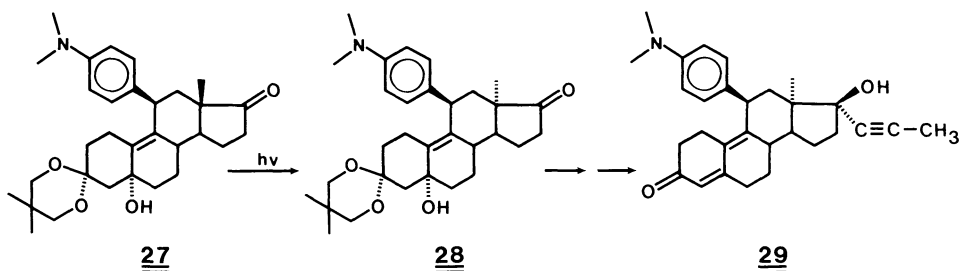


Fig. 7. Photochemical epimerisation of the C-13 methyl group.

analysis of a few selected compounds, represented in Figures 8-12 (J. P. Mornon, personal communication). These can be compared to the computer graphic representations (Figs. 13-17) generated by the conformational energy minimizing SCRIPT program (Cohen et al., 1981).

The represented compounds are all 4,9-estradien-3-ones substituted in the 11 $\beta$  position respectively by: vinyl (Figs. 8 and 13), p-MeO-C<sub>6</sub>H<sub>4</sub> (Figs. 9 and 14), o-MeO-C<sub>6</sub>H<sub>4</sub> (Figs. 10 and 15), 2-thienyl (Figs. 11 and 16) and t-butyl (Figs. 12 and 17). All of them possess a 17 $\beta$ -hydroxy-17 $\alpha$ -ethynyl substitution except for the 11 $\beta$ -t-butyl derivative, which contains the 17 $\alpha$ -propynyl side chain (Figs. 12 and 17). The X ray structure (Fig. 12) of this compound is drawn only with the carbon atoms, as is the vinyl derivative (Fig. 8). All other compounds are shown with the hydrogen as well as carbon atoms. Though the two sets of compounds are not shown from identical perspectives, it is evident that, for any one compound, the overall conformation calculated by SCRIPT is consistent with the X-ray structures if one excludes the 11 $\beta$ -o-MeO-C<sub>6</sub>H<sub>4</sub> derivative (Fig. 10 vs. Fig. 15) and, to a lesser extent, the 11 $\beta$ -t-butyl-derivative (Fig. 12 vs. Fig. 17). In these cases there is a discrepancy in the general curvature of the steroid framework, resulting from different A-ring conformations. On the other hand, the distortion of the C-ring, apparent in Figure 12, agrees with our NMR data in solution that show an unusual doublet for the 11- $\alpha$ -proton, suggesting a flattening of the C<sub>8</sub>C<sub>9</sub>C<sub>11</sub>C<sub>12</sub> dihedral angle.

Also of interest is the observation that the plane of 11 $\beta$ -unsaturated substituents (vinyl, p-MeO-C<sub>6</sub>H<sub>4</sub> and thienyl) closely

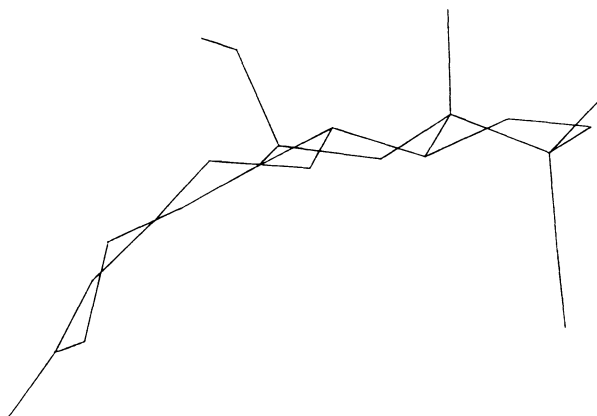


Fig. 8. Crystal structure of 17 $\beta$ -hydroxy-11 $\beta$ -(4-methoxy-phenyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

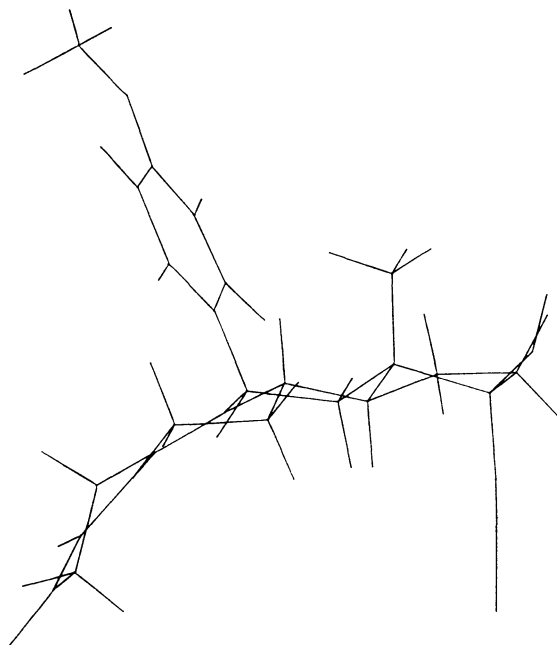


Fig. 9. Crystal structure of 17 $\beta$ -hydroxy-11 $\beta$ -(4-methoxy-phenyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

eclipses the C<sub>9</sub>-C<sub>11</sub> single bond, both in the crystal structures and in the calculated conformers. However, in the vinyl substituted compound, SCRIPT generated a second rotamer with equal energy in which the vinyl group is in staggered position (not shown).

Our overall conclusion from this comparison is that the molecular energy minimization program SCRIPT can predict quite accurately the conformation of 11 $\beta$ -substituted 19-norsteroids, especially when the substituent is of the p-X-C<sub>6</sub>H<sub>4</sub> type. Thus it is useful for our attempt at receptor mapping.

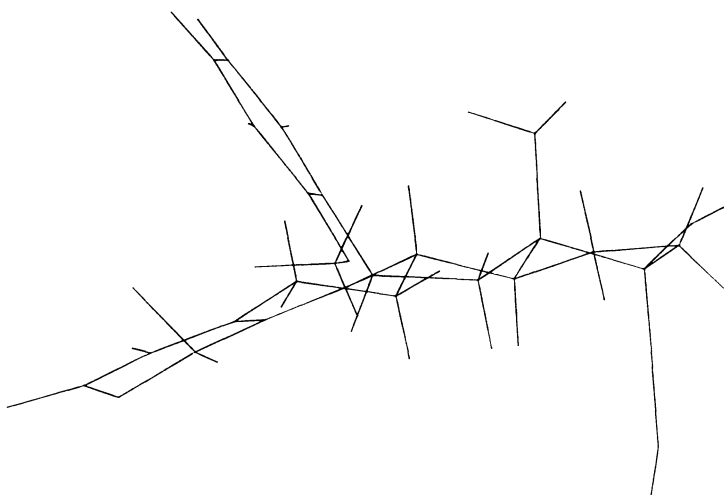


Fig. 10. Crystal structure of 17 $\beta$ -hydroxy-11 $\beta$ -(2-methoxy-phenyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

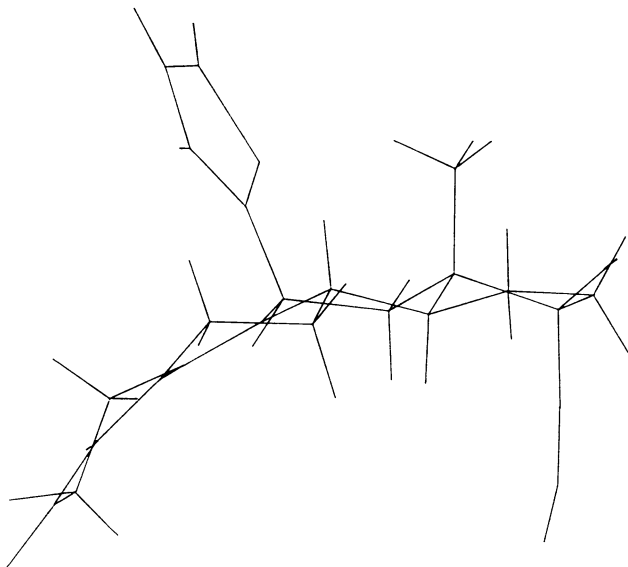


Fig. 11. Crystal structure of 17 $\beta$ -hydroxy-11 $\beta$ -(2-thienyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

#### STRUCTURE-ACTIVITY RELATIONSHIPS

As Teutsch points out (1984), most 11 $\beta$ -substituted 19-norsteroids of the 4,9-dien-3-one series bind both to the progestin and the glucocorticoid cytosolic receptors. A prerequisite for good antihormonal activity is, in most instances, a high affinity for the relevant receptor (i.e. the PR for anti-progestagenic activity). Our strategy, therefore, was to design compounds with as high an affinity as possible for the PR and subsequently to check by *in vivo* experiments (McPhail test in rabbits, abortive test in rats) whether the compounds were agonists or antagonists.

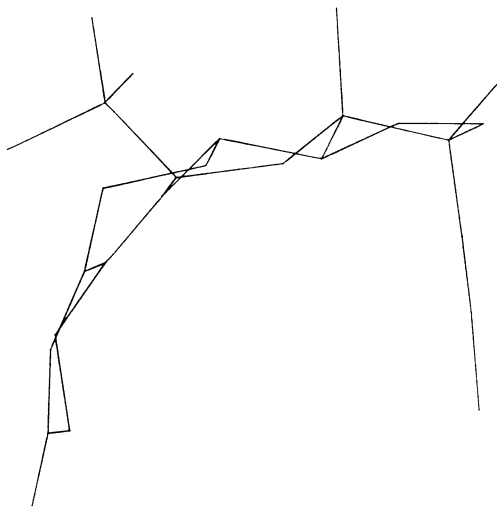


Fig. 12. Crystal structure of 11 $\beta$ -(2,2-dimethyl-ethyl)-17 $\beta$ -hydroxy-17 $\alpha$ -(prop-1-ynyl)-estra-4,9-dien-3-one.

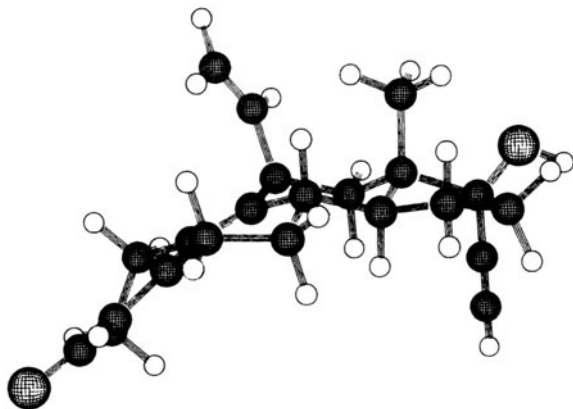


Fig. 13. SCRIPT-determined conformation of 17 $\beta$ -hydroxy-11 $\beta$ -vinyl-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

This section will deal with some of the parameters that affect PR binding, starting with the nature of the 17-substituent. The importance of this factor for the progestational activity of steroids was suspected quite early by a number of investigators. Their patent literature claims good activities for a variety of 17-hydroxy-17 $\alpha$ -substituted compounds, both in the androstene and in the estrene series. Only a few of these examples have appeared in the form of scientific papers and have dealt mainly with substituted ethynyl and vinyl side chains, including propynyl, butynyl, pentynyl, decynyl (Barton et al., 1959), chloroethynyl (Fried et al., 1961; Burgess et al., 1962), trifluoropropynyl, trifluorovinyl, trifluoropropenyl, etc. (Fried et al., 1961), as well as such exotic stick-like substituents as

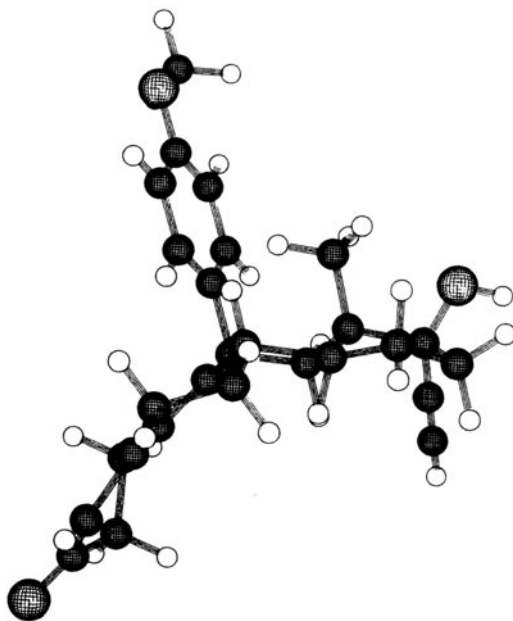


Fig. 14. SCRIPT-determined conformation of 17 $\beta$ -hydroxy-11 $\beta$ -(4-methoxyphenyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

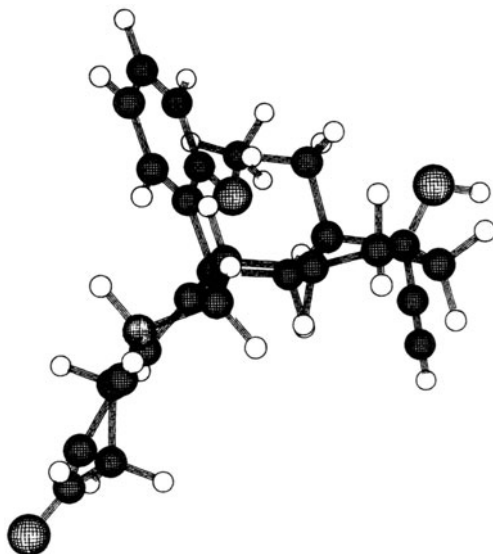


Fig. 15. SCRIPT-determined conformation of 17 $\beta$ -hydroxy-11 $\beta$ -(2-methoxyphenyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

butadiynyl (Burgess et al., 1965). Though some of the chloroethynyl derivatives showed improved progestational activities (Fried et al., 1961), as compared to their well-known and clinically used ethynyl analogues, only the propynyl bearing dimethisterone was developed for transient commercialization (for a review see Petrow, 1970). It was also found that hydrophilic 17 $\alpha$ -substituents lacked progestational activity, as in the case of the 3'-hydroxy-propynyl ethisterone analogue synthesized by Petrow's group (Barton et al., 1957) and the well-known 17 $\alpha$ -hydroxy progesterone (Pfiffner and North, 1941). Although these results did not make much sense at that time, we may nowadays interpret them in terms of steroid-receptor

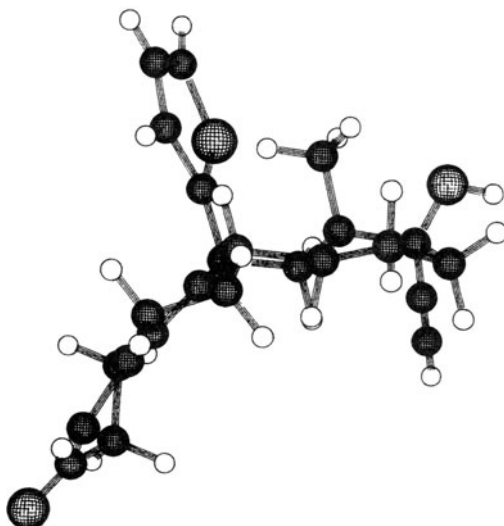


Fig. 16. SCRIPT-determined conformation of 17 $\beta$ -hydroxy-11 $\beta$ -(2-thienyl)-19-nor-17 $\alpha$ -pregna-4,9-dien-20-yn-3-one.

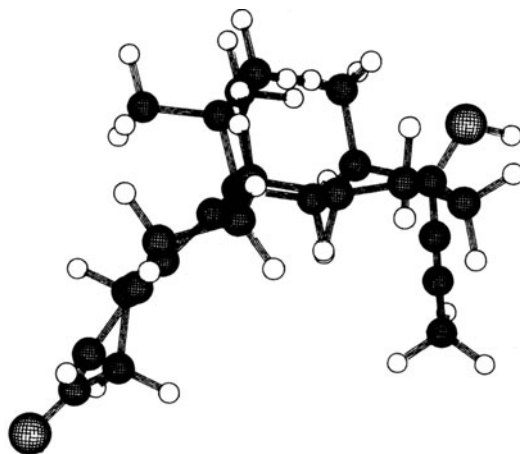


Fig. 17. SCRIPT-determined conformation of 11 $\beta$ -(2,2-dimethyl-ethyl)-17 $\beta$ -hydroxy-17 $\alpha$ -(prop-1-ynyl)-estra-4,9-dien-3-one.

interaction, that is to say that steroids with a hydrophilic 17 $\alpha$ -substituent have a poor affinity for the progestin receptor. This view is confirmed by a number of PR binding data (see for example Kontula et al., 1975 and Raynaud et al., 1979) and has led to the proposal that a hydrophobic pocket exists in the receptor protein, in the close vicinity of the 17 $\alpha$ -substituent (Lee et al., 1977; Zeelen, 1983).

In our particular chemical series, the same principles apply as can be inferred from the results shown in Table I. Hydrophilic 17 $\alpha$ -substituents or no substituent (entries b through d and o) are clearly detrimental to binding, whereas hydrophobic groups tend to increase considerably the RBA values. Of course, excessive steric hindrance will set a limit to the achievable hydrophobicity, as illustrated by the extremely lipophilic but very bulky trimethylsilylethynyl substituent (entry j). The propynyl substituent of RU 486 (entry f) and the chloroethynyl group (entry g) appear to provide a good compromise between steric hindrance and hydrophobicity, suggesting a potential interest in other substituents such as bromoethynyl or iodoethynyl, studied by Petrow's group (Burgess et al., 1967). In view of their incipient reactivity, however, these latter substituents do not seem to be well suited to compounds designed for human use. They could nevertheless be tested as possible affinity labels.

The variety of the 17 $\alpha$ -side chains we synthesized thus gives us quite a good idea about the size of the hydrophobic pocket that exists in the vicinity of the D-ring of the bound steroid. This idea will be developed further in the section dealing with receptor mapping. But what about biological activity? For obvious reason, it is too early to draw a complete structure-activity relationship, but it may be of significance to notice that the compounds that elicit an *in vivo* antiprogestational activity superior to that of RU 486 all possess hydrophobic 17 $\alpha$ -substituents. Two of them are represented in Table I (entries i and l). Much more important than the D-ring substitution, however, is the nature of C-ring substitution for binding to the PR. In fact, it has been observed earlier that in the 19-nor series, introduction of a 11 $\beta$ -methyl (Baran et al., 1970) or a 11 $\beta$ -chloro substituent (Gilbert et al., 1974) considerably increased progestational activity. A subsequent RBA determination of 11 $\beta$ -chloro-17 $\alpha$ -ethynyl-17 $\beta$ -hydroxy-4-estren-3-one confirmed the high affinity of this compound for the progestin receptor (Kontula et al., 1975). However, the belief remained that the size of a hydrophobic pocket around the 11 $\beta$

Table I. Relative Binding Affinities of 11 $\beta$ -R-17 $\alpha$ -X-17 $\beta$ -Y-4,9-estradien-3-ones\* for the Rabbit Progesterin Receptor\*\*

Entry	R	X	Y	RBA-PR (24hrs)
a	m-OMeC <sub>6</sub> H <sub>4</sub>	C $\equiv$ C-CH <sub>3</sub>	OH	14
b	"	C $\equiv$ C-CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	OH	0.8
c	"	OH	COCH <sub>3</sub>	0.6
d	p-NMe <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	OH	C $\equiv$ C-H	4.3
e	"	C $\equiv$ C-H	OH	350
f	"	C $\equiv$ C-CH <sub>3</sub>	OH	530
g	"	C $\equiv$ C-Cl	OH	460
h	"	C $\equiv$ C-C <sub>6</sub> H <sub>5</sub>	OH	250
i	"	C $\equiv$ C-(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	OH	420
j	"	C $\equiv$ C-SiMe <sub>3</sub>	OH	40
k	"	-CH=CH <sub>2</sub>	OH	290
l	"	-CH <sub>2</sub> -CH <sub>3</sub>	OH	260
m	"	-CH <sub>2</sub> -CN	OH	100
n	"	-C <sub>6</sub> H <sub>5</sub>	OH	160
o	"	-H	OH	35

\* General formula 6 in Fig. 2

\*\* Rabbit uterus, 0°C, 24 hrs (Progesterone = 100)

Table II. Relative Binding Affinities (RBA) of 11 $\beta$ -R-17 $\beta$ -hydroxy-17 $\alpha$ -(prop-1-ynyl)-4,9-estradien-2-ones for the PR and the GR

	R	PR*	GR**
a	H	16	2
b	Methyl	234	62
c	Ethyl	22	60
d	Vinyl	390	340
e	Cyclopentyl	3.6	150
f	2-Thienyl	438	300
g	2-Furyl	170	280

\* Rabbit uterus progesterin receptor, 0°C, 24hrs (Progesterone = 100)

\*\* Rat thymus glucocorticoid receptor, 0°C, 24hrs (Dexamethasone = 100)

Table III. RBA of 11 $\beta$ -(p-X-C<sub>6</sub>H<sub>4</sub>)-17 $\beta$ -hydroxy-17 $\alpha$ -(prop-1-ynyl)-4,9-estradien-3-ones for the Rabbit Progesterin Receptor

	X	RBA*	$\eta$ **	$\sigma$ **
a	H	90	0	0
b	CH <sub>3</sub>	295	0.56	- 0.17
c	F	85	0.14	0.06
d	CF <sub>3</sub>	55	0.88	0.54
e	OMe	500	- 0.02	- 0.27
f	SMe	600	0.61	0
g	NMe <sub>2</sub>	530	0.18	- 0.83
h	OH	60	- 0.67	- 0.37
i	CH <sub>2</sub> NMe <sub>2</sub>	50	- 0.15	0.01
j	Ph	280	1.96	- 0.01

\*.Rabbit uterus, 0°C, 24 hrs (Progesterone = 100)

\*\* Values taken from Hansch et al., 1973

region would not exceed the dimensions of a methyl substituent (Lee et al., 1977). Zeelen's group had found a marked drop in progestational activity when changing from 11 $\beta$ -methyl to 11 $\beta$ -ethyl and even more so for propyl or butyl. Their tentative explanation was, "groups that stick out too far at the 11 $\beta$ -position interfere with receptor binding" (v. d. Broek et al., 1977). Our results in the 17 $\alpha$ -ethynyl substituted estradiene series confirmed this finding, but we showed that it was true for saturated alkyl substituents, not for vinylic or aromatic substituents demonstrating the existence of a deep cleft-like lipophilic pocket. The binding seemed to involve a very specific interaction between the unsaturated 11 $\beta$ -substituent and the receptor protein. For para-substituted aromatic rings in particular, we noticed a direct relation between the electrodonating power of the para substituent and the RBA for the progesterin receptor (Belanger et al., 1981). These results have been confirmed for a larger set of substituents in the 17 $\alpha$ -propynyl estradiene series, including RU 486. Some of them are summarized in Tables II and III. If we take the Hammett  $\sigma$  (sigma) constant as a measure of electron donation, it can be easily seen that for compounds which have similar lipophilicities (Hansch  $\pi$  values) and different sigmas (entry a vs. entry e and entry c vs. entry g), the one with the most negative  $\sigma$  value has the highest RBA (Table III). Conversely, of two compounds that bear para-substituents with similar  $\sigma$  values, the one with the higher lipophilicity will show the higher RBA (entries f and i). Unfortunately, our experience in this series has shown that, although high affinity for the progesterin receptor is needed for in vivo anti-progestational activity, it is not a sufficient condition; and animal experiments cannot be circumvented. One interesting case concerns RU 39 973 (compound 30 in Fig. 18), which is somewhat superior to RU 486 as an antiprogestational and interceptive agent in animals despite its low lipophilicity (the compound is water soluble) and consequently its low affinity (ca 8%) for the rabbit progesterin receptor. It is not yet known if the good activity is mediated by in vivo reduction of the N-oxide or simply by a more favorable tissue distribution.

Table. IV. RBAs of the Compounds Used for the Mapping of the Progesterin Receptor in the 11 $\beta$ -Region

	R <sup>1</sup>	R <sup>2</sup>	RBA*
<u>32</u>			
a	Me	Me	234
b	p-tBuC <sub>6</sub> H <sub>4</sub>	Me	48
c	p-NMe <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	Et	430
d	p-(4-Methyl-piperazinyl)-C <sub>6</sub> H <sub>4</sub> )	Me	25
<u>33</u>			270**

\* Rabbit uterus progesterin receptor, 0°C, 24 hrs (Progesterone = 100)

\*\* From Pitt et al., 1979; the original value (136 for the racemic compound) has been corrected

Based on the necessity of the 3-keto-4-ene system for receptor binding (see for example Duax et al., 1982), we also confirmed our earlier observation that the presence of an 11 $\beta$ -vinyl or aryl group on 1,3,5(10)-estratrienes conferred to these compounds a remarkable affinity for the progesterin receptor (Belanger et al., 1981). For instance the aromatic (compound 31 in Fig. 18) exhibited a strong affinity for both the glucocorticoid and the progesterin receptors (2 times dexamethasone and 1.3 times progesterone respectively), while being only poorly bound to the estrogen receptor. This result suggests that the strong favorable hydrophobic interaction of the 11 $\beta$ -substituent with the receptor can compensate for loss of part of the hydrogen bond interaction involving the 3-ketone, roughly estimated at 3 kcal/mole (Kontula et al., 1975). New opportunities for the design of further hormone agonists and antagonists are thus created.

#### TOWARDS THE MAPPING OF THE PROGESTIN RECEPTOR

In addition to the immediate and foreseeable benefits resulting from the discovery of potent anti-progestins, there is a more fundamental aspect that deserves further development. It concerns our increased information about the progesterin receptor. Using the well known technique of receptor mapping (Raynaud et al., 1981a; Raynaud and Ojasoo, 1983), we have used the 11 $\beta$ -substituted 19-norsteroids to elaborate a crude picture of the hydrophobic steroid binding site in the GR and the PR. Our objective was to design compounds that would bind selectively to one or the other receptor (Teutsch, 1984).

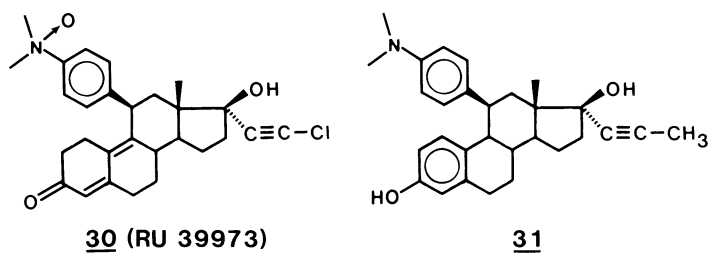


Fig. 18.

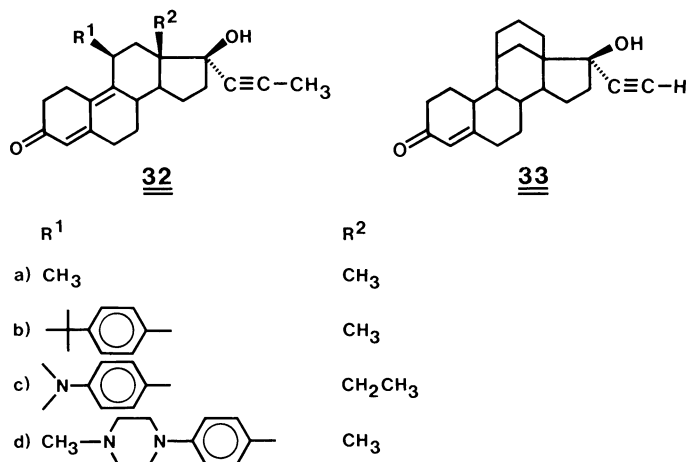


Fig. 19. Compounds used for the mapping of the progesterin receptor in the "11β-region."

It was found that there appears to exist, in both receptors, a large hydrophobic pocket able to accept 11β-substituents up to 12 angstroms (1.2 nm) in length; the general shape is different from one receptor to the other. Using the same technique, that is, determining the Van der Waals envelope of a selection of substituents accepted by the PR, we tried to improve this first outline. As previously, conformations were determined by the energy minimizing SCRIPT program (Cohen et al., 1981). For the mapping of the "11β-region" of the PR, we used the phenylpiperazine substituent in addition to the methyl and p-terbutylphenyl groups exploited earlier (entry d in Table IV and Fig. 19). The two possible conformations of the piperazinyl cycle were taken into account, creating an envelope with axial symmetry. We expected from our previous work that the angular methyl group (C-18) would fit into the hydrophobic pocket, and the good RBA found for the 11β-13β-propano-bridged compounds (Pitt et al., 1979) is good evidence for it. It is included in the drawing, as is the 13-ethyl analogue 32c of RU 486 (Fig. 9). To the contrary, hydrophilic substituents at the C-13 position in the pregnene series have been shown to be devoid of progestational or antiprogestational activity (Auel et al., 1978). Similar results have been obtained recently in the estrene series (Pillai et al., 1984).

We mapped the 17α-region using 11β-(4-dimethylaminophenyl) substituted compounds varying only by 17α-substitution (entries h, j and n in Table I).

The result is shown in Figures 20 and 21, which represent respectively the front side (A ring to the left) and rear side (A ring to the right) views of the substituted steroid models. The white Van der Waals spheres represent the steroid framework, whereas the others represent the superimposition of the acceptable substituents. Though the figures do now show a continuity between the 11β-pocket and the 17α-pocket, it is very likely that it does exist. In this case, the steroid binding site in the PR would appear to be formed by two hydrophobic clefts: a smaller one containing the steroid backbone, about 11 angstroms in length, limited at either end by a hydrogen bonding zone (with O-3 and O-17 or O-20), and a larger one, at least 20 angstroms in length, extending above and below the plane of the steroid. The planes of the two grooves are roughly perpendicular, forming an X-shaped hydrophobic site that can tightly lock the steroid in place. It is this tight locking that might be responsible

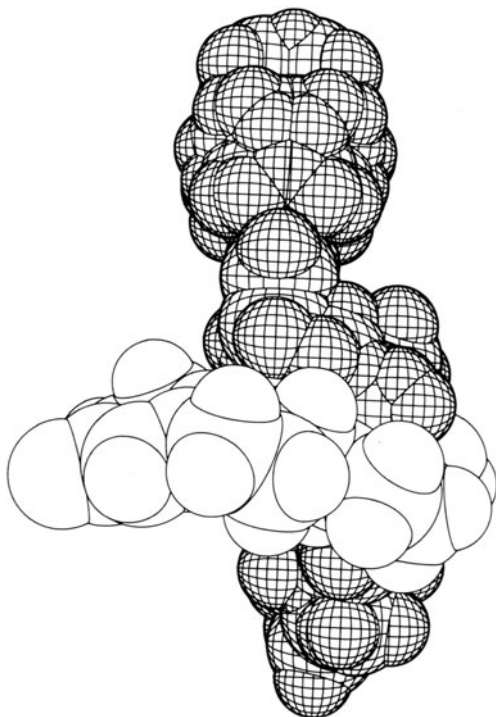


Fig. 20. Computer-drawn mapping of the progestin receptor showing the lipophilic pockets above and below the plane of the steroid. Front view (A ring to the left).

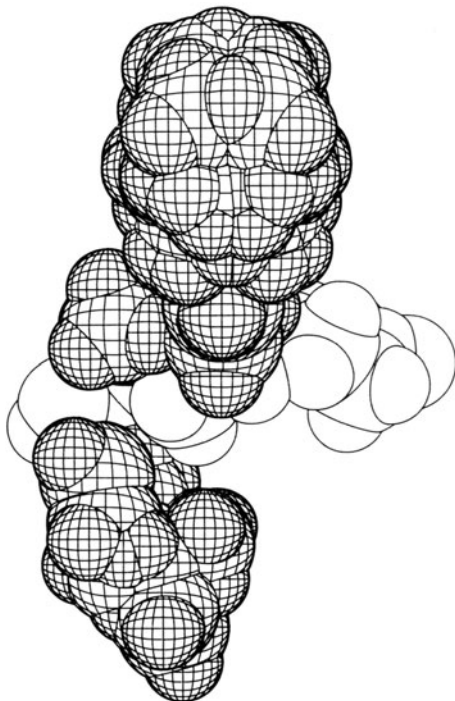


Fig. 21. Computer-drawn mapping of the progestin receptor. Rear view (A ring to the right).

Table V. Qualitative Evaluation of Agonistic Versus Antagonistic Progestational Activities of Selected 11 $\beta$ -substituted-19-norsteroids\*

Entry	RU code	RBA**	Agonist <sup>†</sup>	Antagonist <sup>††</sup>
<u>34</u>	RU 25 253	178 - 530	++	-
<u>35</u>	RU 42 764	140 - 390	++	-
<u>36</u>	RU 25 055	70 - 85	+	nd
	RU(38)486	78 - 530	-	++
<u>37</u>	RU 39 009	5 - 11	nd	+

\* The structures of the compounds appeared in Fig. 22

\*\* Rabbit uterus progestin receptor, 0°C, 2hrs and 24hrs (Progesterone=100)

<sup>†</sup> Progestational activity as determined by the McPhail test :

+ = potency < Progesterone; ++ = potency > Progesterone

<sup>††</sup> Antiprogestational activity against progesterone (0.2 mg/Kg, s.c) on the endometrial proliferation in rabbit uterus : + = active at 10 mg/Kg orally; ++ = active at 3 mg/Kg orally; nd = not determined

for the anti-hormonal activities of 11 $\beta$ -substituted 19-norsteroids. We have shown that it is possible to design compounds in this series that are either pure agonists or pure antagonists with all the intermediated stages (Teutsch, 1984).

One example, RU 25 253 (compound 34 in Fig. 22), an 11 $\beta$ -vinyl derivative, proved to be a powerful progestin (Teutsch et al., 1982a). Another, the 17 $\alpha$ -propynyl analogue 35, differs from RU 486 only by the 11 $\beta$ -vinyl group, whereas RU 25 055 (36) an 11-thienyl derivative had only modest progestational activity (Raynaud et al., 1981 b). From the results shown in Table V, it appears that agonistic and antagonistic activities are independent of both affinity and affinity-time courses.

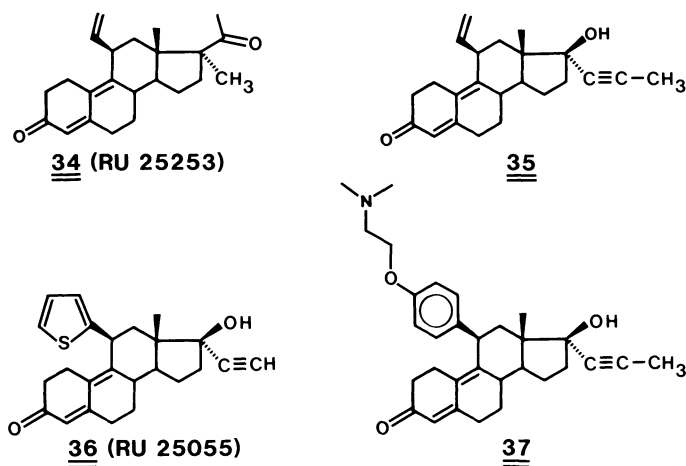


Fig. 22.

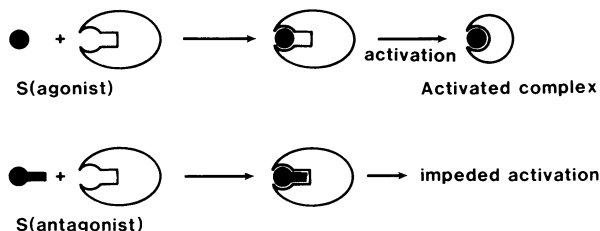


Fig. 23. Schematic representation of the postulated mechanism of action of progesterone agonists and antagonists.

A method based on this latter criterion has been suggested to distinguish between agonists and antagonists (Raunaud et al., 1980). It clearly does not apply in our series, where antagonism seems to be brought about by substituents that protrude deep enough into the 11 $\beta$ -pocket. This realization led to the hypothesis that large 11 $\beta$ -substituents could interfere with the activation step of the hormone receptor complex (Teutsch, 1984). During this process, a deep conformational change of the receptor protein is thought to occur, and the large, generally unoccupied hydrophobic pocket just might provide the protein with the degree of freedom it needs to undergo conformational change. If, however, the steroid is locked in the totality of the X-shaped site, this process would be compromised; and the expected biological response will not occur. A simplified representation of this concept is shown in Figure 23.

## CONCLUSION

The development of a convenient route of access to 11 $\beta$ -substituted-19-norsteroids and the study of their structure-activity relationship has culminated in the discovery of the first true anti-progestins. A large hydrophobic pocket has been localized in the progestin receptor above the C-ring of the bound steroid. This pocket is likely to be involved in the mechanisms of hormone action. The occupation of the lower part by a small lipophilic substituent like chloro, methyl or vinyl leads to potent agonists, whereas occupation of the upper part by larger substituents leads to powerful antagonists. These results suggest that the anti-progestational properties of RU 486 and its analogues could be the consequence of impeded activation of the hormone-receptor complex. We hope they will provide further impetus for the quest of even better and more dissociated antihormones and that the hypotheses evolved from this work will help to solve the riddle of hormone action at the molecular level.

## ACKNOWLEDGMENTS

I wish to thank all my friends and collaborators who participated at one time or another in this work:

1. Chemistry: A. Belanger (Universite Laval, Quebec), G. Cahiez (Universite Pierre et Marie Curie, Paris), L. Carbonaro (ENSC Mulhouse), G. Costerousse, S. Didierlaurent, F. Goubet, A. M. Guerin, G. Millot and V. Torelli (Roussel-Uclaf)
2. Biology: M. Moguilewsky, D. Philibert and C. Tournemine (Roussel Uclaf)
3. Computing with the SCRIPT Program: P. Broto and G. Lemoine (Roussel Uclaf)

4. X ray studies: J. P. Mornon, I. Morize and E. Surcouf (Universite Pierre et Marie Curie, Paris)
5. Typing of the original manuscript: D. Salaun (Roussel Uclaf)

#### REFERENCES

- Auel, R. A. M., Freerksen, R. W., and Watt, D. S., 1978, Synthesis of various 18-substituted progesterones, Steroids, 31:367.
- Baran, J. S., Lennon, H. D., Mares, S. E., and Nutting, E. F., 1970, 11 $\beta$ -methyl-19-norsteroids: novel progestational hormones, Experientia, 26:762.
- Barton, S. P., Burn, D., Cooley, G., Ellis, B., and Petrow, V., 1959, Modified steroid hormones, Part XI, Some ethisterone homologues, J. Chem. Soc., 1959:1957.
- Belanger, A., Philibert, D., and Teutsch, G., 1981, Regio and stereospecific synthesis of 11 $\beta$ -substituted 19-norsteroids. Influence of 11 $\beta$ -substitution on progesterone receptor affinity, Steroids, 37:361.
- Beyer, B., Terenius, L., Brueggemeier, R. W., Ranade, V. V., and Counsell, R. E., 1976, Synthesis of potential antiprogestins, Steroids, 27:123.
- Beyer, B., Terenius, L., and Counsell, R. E., 1980, Synthesis of potential antiprogestins, Steroids, 35:481.
- Burgess, C., Burn, D., Ducker, J. W., Ellis, B., Feather, P., Hiscock, A. K., Leftwick, A. P., Mills, J. S., and Petrow, V., 1962, Modified steroid hormones, Part XXIX, Some 17 $\alpha$ -chloroethynyl-17 $\beta$ -hydroxy-derivatives, J. Chem. Soc., 1962:4995
- Burgess, C., Burn, D., Feather, P., Howarth, M., and Petrow, V., 1965, Modified steroid hormones - XXXVII. Some 17 $\alpha$ -butadiynyl-17 $\beta$ -hydroxy- and 17 $\alpha$ -(2'-thienyl)-17 $\beta$ -hydroxy-derivatives, Tetrahedron, 21:1197.
- Burgess, C., Cooley, G., Feather, P., and Petrow, V., 1967, Modified steroid hormones: a new route to 17 $\alpha$ -bromoethynyl and 17 $\alpha$ -iodoethynyl-17 $\beta$ -hydroxy steroids, Tetrahedron, 23:4111.
- Cohen, N. C., Colin, P., and Lemoine, G., 1981, Script: interactive molecular geometrical treatments on the basis of computer-drawn chemical formula, Tetrahedron, 37:1711.
- Duax, W. L., Griffin, J. F., Rohrer, D. C., and Weeks, C. M., 1982, Steroid agonists and antagonists: molecular conformation, receptor binding and activity, in: "Hormone Antagonists," M. K. Agarwal, ed., Walter de Gruyter & Co, Berlin, New York.
- Fried, J. H., Bry, T. S., Oberster, A. E., Beyler, R. E., Windholz, T. B., Hannah, J., Sarett, L. H., and Steelman, S. L., 1961, Novel gonadotrophin inhibitors in the 19-norsteroid series, J. Am. Chem. Soc., 83:4663.
- Gasc, J. C., and Nedelec, L., 1971, A new approach to corticoid total synthesis, Tetrahedron Lett., 1971:2005.
- Giesen, E. M., Bollack, C., and Beck, G., 1981, Relation between steroid-cell contact, steroid binding and induction of tyrosine aminotransferase, Molec. Cell. Endocr., 22:153.
- Giesen, E. M., and Beck, G., 1982, Hormonal deinduction of tyrosine aminotransferase, Horm. Metab. Res., 14:252.
- Gilbert, H. G., Philipps, G. H., English, A. F., Stephenson, L., Woollett, E. A., Newall, C. E., and Child, K. J., 1974, The progestational and anti-estrogenic activities of some novel 11 $\beta$ -substituted steroids, Steroids, 23:585.
- Hansch, C., Leo, A., Unger, S. H., Kim, K. H., Nikatani, D., and Lien, E., 1973, "Aromatic" substituent constants for structure-activity correlations, J. Med. Chem., 16:1207
- Kontula, K., Janne, O., Vikho, R., de Jager, E., de Visser, J., and Zeelen, F., 1975, Progesterone binding proteins: in vitro binding and biological activity of different steroidal ligands, Acta Endocr., 78:574.

- Lee, D. L., Kollman, P. A., Marsh, F. J., and Wolff, M. E., 1977, Quantitative relationships between steroid structure and binding to putative progesterone receptors, J. Med. Chem., 20:1139.
- Nedelec, L., 1970, Sur l'époxydation des estradiènes-5(10),9(11), Bull. Soc. Chim. France, 1970:2548.
- Neef, G., Sauer, G., and Wiechert, R., 1983, Influence of bulky 11 $\beta$ -substituents on reactivity of estrene derivatives, Tetrahedron Lett., 24:5205.
- Neef, G., Sauer, G., Seeger, A., and Wiechert, R., 1984, Synthetic variations of the progesterone antagonist RU 38 486, Tetrahedron Lett., 25:3425.
- Petrow, V., 1970, The contraceptive progestagens, Chem. Rev., 70:713.
- Pfiffner, J. J., and North, H. B., 1941, Isolation of 17-hydroxyprogesterone from adrenal gland, J. Biol. Chem., 139:855.
- Pillai, K. M. R., Murray, W. V., Shooshani, I., Williams, D. L., Gordon, D., Wang, S. Y., and Johnson, F., 1984, Synthesis of C-18 functionalized steroids via the Smith-Hughes route, J. Med. Chem., 27:1131.
- Pitt, C. G., Rector, D. H., Cook, C. E., and Wani, M. C., 1979, Synthesis of 11 $\beta$ , 13 $\beta$ - and 13 $\beta$ , 16 $\beta$ -propano steroids: probes of hormonal activity, J. Med. Chem., 22:966.
- Raynaud, J. P., Ojasoo, T., Bouton, M. M., and Philibert, D., 1979, Receptor binding as a tool in the development of new bioactive steroids, in: "Drug Design," volume 8, A. J. Ariens, ed., Academic Press, Inc.
- Raynaud, J. P., Bouton, M. M., and Ojasoo, T., 1980, The use of interaction kinetics to distinguish potential antagonists from agonists, TIPS, 1980:324.
- Raynaud, J. P., Delettre, J., Ojasoo, T., Lepicard, G., and Mornon, J. P., 1981a, Steps towards mapping of steroid hormone receptors, in: "Physiopathology of Endocrine Diseases and Mechanism of Hormone Action," R. J. Soto, A. Denicola and J. Blaquier, eds., Alan R. Liss, Inc., New York.
- Raynaud, J. P., Ojasoo, T., and Labrie, F., 1981b, Steroid hormones - agonists and antagonists, in: "Mechanisms of Steroid Action," G. P. Lewis and M. Ginsburg, eds., MacMillan Publishers Ltd., England.
- Raynaud, J. P., and Ojasoo, T., 1983, The relevance of structure-affinity relationship in the study of steroid hormone action, in: "Steroid Hormone Receptors: Structure and Function," H. Eriksson and J. A. Gustafsson, eds., Elsevier Science Publishers, B. V.
- Teutsch, G., Belanger, A., Philibert, D., 1978, Regio and stereospecific synthesis of 11 $\beta$ -substituted 19-norsteroids, J. Steroid Biochem., 9:814, abstract no. 9.
- Teutsch, G., and Belanger, A., 1979, Regio and stereospecific synthesis of 11 $\beta$ -substituted 19-norsteroids, Tetrahedron Lett., 1979:2051.
- Teutsch, G., 1982, Concerning the opening of 1,3-diene monoepoxides by organometallic reagents: a general synthesis of 9 $\beta$ -substituted 19-norsteroids, Tetrahedron Lett., 23:4697.
- Teutsch, G., Belanger, A., Philibert, D., and Tournemine, C., 1982a, Synthesis of 11 $\beta$ -vinyl-19-norsteroids as potent progestins, Steroids, 39:607.
- Teutsch, G., Costerousse, G., Philibert, D., and Deraedt, R., 1982b, Nouveaux dérivés stéroïdes substitués en 11  $\beta$ , procede et intermédiaires de préparation, leur application comme médicaments et les composition les renfermant, European Patent, 0 057 115 A2.
- Teutsch, G., Torelli, V., Deraedt, R., and Philibert, D., 1983, Nouveaux 19-norstéroïdes substitués en 11 et éventuellement en 2, leur préparation, leur application comme médicaments, les compositions les renfermant et les nouveaux intermédiaires obtenus, European Patent, 0 097 572 A1.
- Teutsch, G., 1984, 11- $\beta$  substituted 19-norsteroids: at the crossroads between hormone agonists and antagonists, in: "Adrenocorticoid Antagonists," M. K. Agarwal, ed., Walter de Gruyter, Berlin, New York.

- V. D. Broek, A. J., Broess, A. I. A., v. d. Heuvel, M. J., de Jongh, H. P., Leemhuis, J., Schonemann, K. H., Smits, J., de Visser, J., van Vliet, N. P., and Zeelen, F. J., 1977, Strategy in drug research. Synthesis and study of the progestational and ovulation inhibiting activity of a series of 11 $\beta$ -substituted-17 $\alpha$ -ethynyl-4-estren-17 $\beta$ -ols, Steroids, 30:481.
- Verma, U., and Laumas, K. R., 1981, Screening of anti-progestins using in vitro human uterine progesterone receptor assay system, J. Steroid Biochem., 14:733.
- Zeelen, F., 1983, Receptor models: learning from the progesterone receptor, TIPS, 1983:520.

## PHARMACOLOGICAL PROFILE OF RU 486 IN ANIMALS

D. Philibert, M. Moguilewsky, I. Mary, D. Lecaque,  
C. Tournemine, J. Secchi and R. Deraedt

Centre de Recherches Roussel Uclaf  
93230 Romainville, France

### SUMMARY

RU 486 is an original multifaceted antihormone. It appears to be a potent progestin and glucocorticoid antagonist while exhibiting no agonistic effect, even at very high doses. Thus, according to the bioassay used, RU 486 administered orally at doses between 3 and 20 mg/kg completely inhibits the effect of exogenous progesterone on the endometrial proliferation in rabbits, on the volume density of uterine gland cell mitochondria, on the decidual formation and on the maintenance of pregnancy in ovariectomized rats. Furthermore, it proves to be antinidatory and abortive in rats and mice. In cycling monkeys it induces menstruation when administered during the mid-luteal phase.

Acting as an antiglucocorticoid component, RU 486 effectively antagonizes, at  $10^{-6}$  M, dexamethasone's effect on uridine incorporation into rat thymocytes RNA and on ACTH secretion from rat pituitary cells. In vivo (at a dose of 10 mg/kg to adrenalectomized rats) it fully prevents the thymolytic effect of corticosterone and dexamethasone; it also completely blocks urinary volume increase and potassium excretion induced by dexamethasone. In this latter test, its antiglucocorticoid activity is rapidly and fully reversed by increasing doses of dexamethasone. Using a perfusion technique, we observe that RU 486 does not inhibit corticosterone biosynthesis in rat adrenal cells stimulated by ACTH. The compound also possesses a moderate antiandrogenic activity on seminal vesicles and prostate weights, about 20 times weaker than its two other antagonist properties (evaluated in rats). In various species (rats, rabbits and mice) it exhibits a slight uterotrophic activity, approximately 10,000 times weaker than that of estradiol; but, unlike estrogens, it fails to induce estrus in ovariectomized rats given up to 300 mg/kg. When RU 486 is administered chronically for 15 days in adult female rats, it displays no antiovarian activity; on the contrary, it provokes dose-dependent increases in serum LH and progesterone levels and ovarian weight.

### INTRODUCTION

RU 486 displays much higher relative binding affinities for both progestin and glucocorticoid receptors than those of the corresponding natural hormones. It also possesses a moderate affinity for the androgen receptor (Moguilewsky and Philibert, this volume). These biochemical data led us to study in detail the pharmacological properties of RU 486 in vitro

and in vivo, using animal models specific for these three hormonal activities. The aim of this paper is to present the results of these intensive investigations and thus to establish the endocrinological profile of RU 486 as completely as possible.

## RESULTS

Unless otherwise stated, Sprague-Dawley rats, Swiss mice, New Zealand rabbits and cynomolgus monkeys (*Macaca fascicularis*) were used. In all experiments RU 486 was administered orally, suspended in aqueous solution containing 0.25% carboxymethyl-cellulose and 0.2% Polysorbate 80 (CMCP). Estradiol, progesterone, R 5020 and testosterone propionate were injected by subcutaneous route in sesame oil containing 5% benzyl alcohol. All results are presented as the mean  $\pm$  SEM. Statistical comparisons between groups were made using Dunnett's test (1955); the significance of the values is indicated by means of an asterisk \* $p$  < 0.05; \*\* $p$  < 0.01.

### Antiprogesterin Activity of RU 486

The effect of RU 486 has been studied on various biological responses mediated by progesterone. The bioassays employed took into account the role of progesterone in various physiological processes such as: endometrial transformation during the luteal phase of the menstrual cycle, the decidual

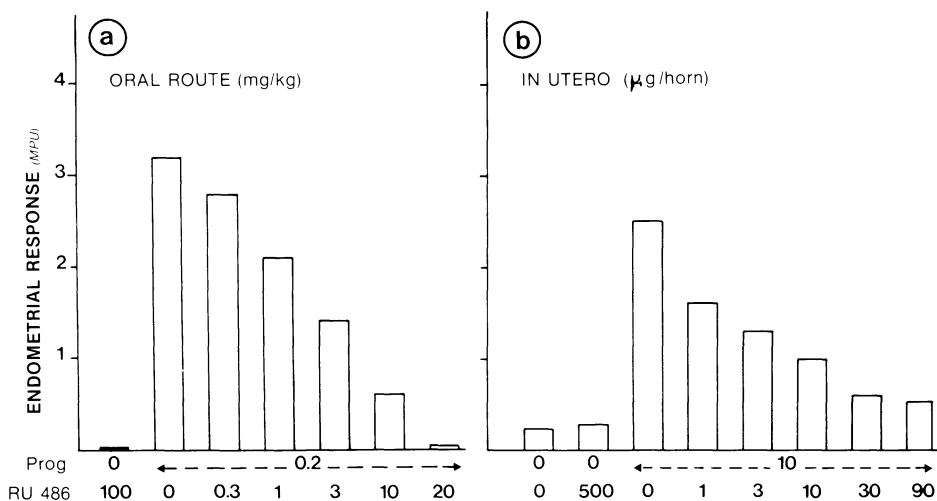


Fig. 1. Effect of RU 486 on progesterone-induced endometrial proliferation in rabbits. a) Groups of immature rabbits weighing about 1 kg received s.c. injections of 5 µg/kg estradiol from day 1 to 5. As shown on the abscissa, the animals were treated daily with progesterone (s.c.) and RU 486 (p.o.) from day 7 to day 10 and were killed on day 11. b) On day 1, the animals received a deposit of 25 µg estradiol, dissolved in ethanol, on the dorsal skin. On day 4, the test compounds dissolved in sesame oil- 5% benzyl alcohol were introduced in utero within 2 ligatures. The rabbits were killed on day 6. The uteri were removed, fixed in Bouin fluid, and their transformations were graded histologically according to McPhail's scale (MPU = McPhail's unit).

Table I - Effect of RU 486 on Deciduomata Formation  
Induced by Progesterone in Rats

RU 486 p. o. mg/kg	PROGESTERONE s. c. mg/kg	RATS WITH DECIDUOMATA %
3	0	0
10	0	0
0	10	100
0.3	10	75
1	10	20
3	10	0
10	10	0

Groups of 5 adult female rats were ovariectomized in estrus (day 1) and treated from day 1 to 10 by oral route with RU 486 alone or in combination with progesterone injected subcutaneously, a thread being passed through one uterine horn on day 5. The animals were sacrificed on day 11, the traumatized horn was removed and fixed in Bouin solution for evaluation of decidual response by histological analysis.

reaction occurring during ovum implantation, and the maintenance of pregnancy in ovariectomized animals.

Endometrial proliferation in rabbits. As illustrated in Figure 1a, 0.2 mg/kg of progesterone induces, in estradiol primed immature rabbits, a strong endometrial transformation graded 3.2 MPU, according to the standard scale of McPhail (1934). RU 486 alone (up to 100 mg/kg) is completely devoid of progestogen-mimetic activity. When administered in combination with progesterone, however, it inhibits the action of the natural hormone in a dose-dependent manner. The ED50 of this inhibitory effect is about 3 mg/kg, and at 20 mg/kg the inhibition is complete. When the two steroids are introduced in utero (McGinty et al., 1939), RU 486 exhibits a more potent antagonist activity (Fig. 1b), the ED50 being about 1/3 the dose of progesterone used (10 µg/horn). This result is in agreement with the relative binding affinities of these two compounds for the rabbit uterine progestin receptor (Moguilewsky and Philibert, this volume).

Deciduomata formation in rats. In pseudopregnant animals and in animals ovariectomized during estrus or ovariectomized and pretreated with estrogen, progesterone induces the apparition of deciduomata in uteri traumatized mechanically or by injection in situ of various chemical agents (Chambon, 1952; Tachi and Tachi, 1974). Thus, in rats (Table I) progesterone at a dose of 10 mg/kg provoked a decidual response in all animals. This effect was completely prevented by simultaneous administration of 3 mg/kg RU 486, whereas this compound alone at a dose of 10 mg/kg was not accompanied by deciduogenic activity.

Table II - Antiprogestin Activity of RU 486 on  
the Maintenance of Pregnancy in Rats

RU 486 p. o. mg/kg	PROGESTERONE s. c. mg/kg	FETUS ON DAY 12/DAY 8 %
75	0	0
0	75	86
1	75	81
5	75	0
20	75	0

RU 486	RU 5020	%
0	2.5	75
1	2.5	75
5	2.5	0

Groups of 5 pregnant rats were ovariectomized, and the number of implantations was determined after laparotomy on day 8. Treatment was initiated immediately after operation and continued to day 11. The animals received RU 486 daily by oral route either alone or in combination with progesterone or R 5020 injected subcutaneously. The animals were killed on day 12, and the number of implantations was evaluated. Results were expressed as the ratio of the number of implantations on day 12/number of implantations on day 8.

Maintenance of pregnancy in rats. As shown in Table II, progesterone at a dose of 75 mg/kg maintained, until day twelve, 86% of the implantation sites determined in pregnant rats ovariectomized on day eight. Under the same experimental conditions, R 5020, a potent progestin (Raynaud, 1977), exhibits similar effects on 75% of implantation sites at a dose of 2.5 mg/kg. When 5 mg/kg of RU 486 were administered in combination with these progestational compounds, no conceptuses were seen at autopsy (day twelve). Given alone, at the high dose of 75 mg/kg, it did not maintain pregnancy.

Volume density of uterine gland cells mitochondria. It has been shown by Ljungkvis (1971), Nilsson (1975), and Secchi and Lecaque (1984) that, in ovariectomized rats, progesterone provokes an increase in the volume density of mitochondria localized in uterine epithelial-gland cells. This response is particularly interesting because, unlike many of progesterone's other biological effects, it does not necessitate estrogen priming. As illustrated in Figure 2, progesterone at 20 mg/kg induces a three times increase in the volume density of mitochondria compared to the control, whereas RU 486 alone up to 30 mg/kg has no effect. When 10 mg/kg RU 486 is administered simultaneously with progesterone, it completely antagonizes the action of the natural hormone, the ED50 being about 3 mg/kg.

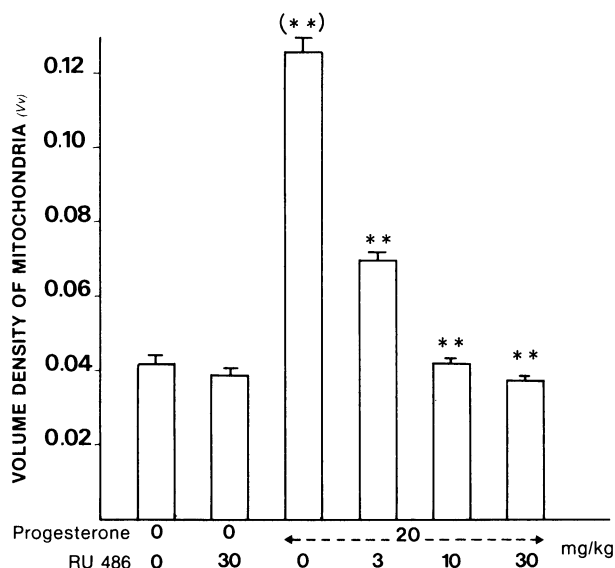


Fig. 2. Effect of RU 486 on the volume density of uterine gland-cell mitochondria in ovariectomized rats. A group of 5 3-week ovx rats (250 g) were treated for 3 days with progesterone (s.c.) and RU 486 (orally). The animals were killed on day 4, the uterus removed and ultrathin sections from each uterus were examined at a magnification of 30,000. The volume density of glandular cell mitochondria (vv) was determined as described by Secchi and Lecaue (1984). (\*\*) Compared to control group. \*\* Compared to progesterone-treated group.

All the bioassays described so far demonstrate the antagonistic effect of RU 486 against exogenous progesterone in ovariectomized or immature animals, that is to say in animals practically devoid of any sexual hormone. These results suggest that RU 486 acts directly at the target organ level.

In the following tests, we have studied the efficacy of this compound against endogenous progesterone in intact animals under the influence of the pituitary-ovarian axis. Rats were studied during pregnancy and monkeys during the luteal phase of the menstrual cycle.

Effect of RU 486 in pregnant rodents. Figure 3 shows the effect of single oral administrations, in increasing doses, of RU 486 to rats in any stage of pregnancy from day one to eighteen inclusive. We observed that, at 2 mg/kg, the product exhibits only a partial activity during days eight to twelve. At 10 mg/kg it is not antinidatory and exercises total abortive activity from days three to eighteen with the exception of day fifteen. At 30 mg/kg, RU 486 totally prevents implantation but was still inactive on day 15. At 100 mg/kg, only a partial abortion is noticed.

It therefore appears that (with the exception of day fifteen) the preimplantation period is less sensitive to the product than mid-pregnancy. The same observation has been made by Kendle (1982), using inhibitors of progesterone biosynthesis. As far as the relative lack of activity of RU 486 on day fifteen is concerned, experiments are under progress to confirm the results reported here.

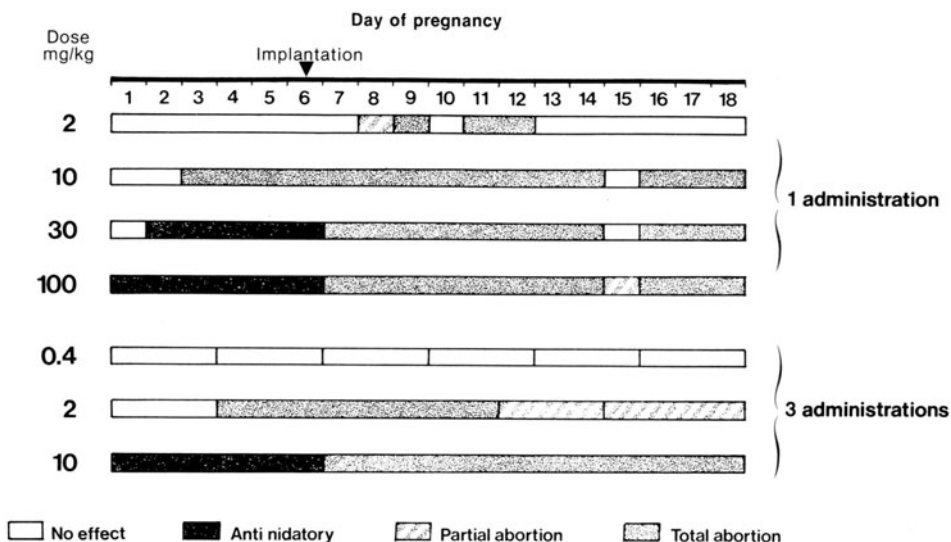


Fig. 3. Antinidatory and abortive activities of RU 486 in rats. Following the determination of the first day of pregnancy in mated female rats by detection of spermatozoa in the vaginal smears, RU 486 was administered orally either in a single dose on any day between days 1 and 18 inclusive or for 3 consecutive days during the same period. The animals were sacrificed from 48 hours to 7 days after the treatment, and the number of implantations was determined.

Using pregnant mice under the same experimental conditions (results not shown), we have obtained the same spectrum of activity at doses at least three times higher. We have not observed the day-fifteen insensitivity to the treatment in mice.

Figure 3 also shows the effect of oral administration of RU 486 for 3 consecutive days (days 1, 2, 3; days 4, 5, 6; etc.). We noticed that at 10 mg/kg it displays both antinidatory and abortive activities in all animals.

Effect of RU 486 in cycling monkeys. Ten cynomolgus monkeys received RU 486 by oral route during the mid-luteal phase, as indicated in Figure 4. No more than three administrations/animal were performed with a dose range from 25 to 75 mg (from about 8 mg/kg to 25 mg/kg). We observed that RU 486 was inactive only in the animal receiving the lowest dose of 25 mg. All other monkeys menstruated within 48 hours after the last administration. Menstruation appeared before the 24th day of the cycle, and no significant modification on the length of the following cycle was seen. These results differ from those obtained by Healy et al. (1983), who found that RU 486 was active at a dose of 0.1 mg/kg (compared to about 16 mg/kg in our experiments) injected by i.m. route in artificially cycled ovx monkeys. The possibility exists that these divergences can be explained by the fact that RU 486 administered orally undergoes a high presystemic effect (Deraedt et al., this volume) and that, in intact animals, the pituitary-ovarian axis might exert a compensatory activity.

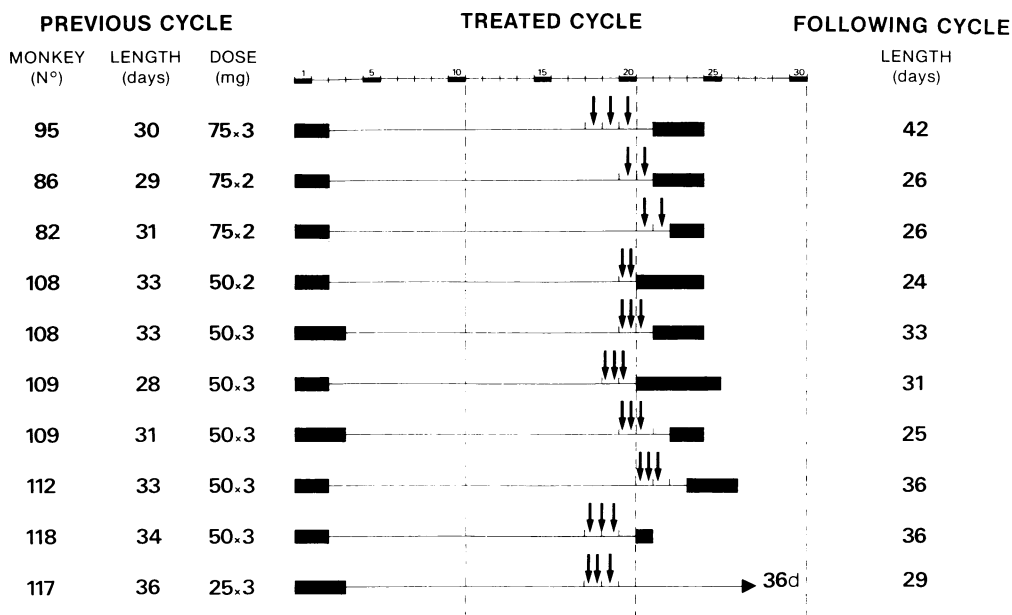


Fig. 4. Effect of RU 486 on the menstrual cycle in monkeys. Adult female monkeys (*Macaca fascicularis*) weighing between 3 and 4 kg were treated orally during the mid luteal phase with different doses of RU 486. The arrows indicate days of administration and the solid bars days of menstruation.

#### Antiglucocorticoid Activity of RU 486 in Rats

It is well known that glucocorticoids trigger biological responses in many target tissues (see Baxter and Rousseau, 1979). Therefore, the properties of RU 486 have been studied in vitro as well as in vivo, using various classical tests in rats such as uridine incorporation into RNA in thymocytes, ACTH secretion from pituitary cells, thymus weight, and diuresis (Philibert et al., 1981).

Thymocytes. In rat thymocytes,  $5.10^{-8}M$  of dexamethasone provoked a nearly maximum inhibition of 38% (relative to the control) of uridine incorporation into RNA. This value arbitrarily was made to equal 100% (Fig. 5). When RU 486 was incubated with  $5.10^{-8}M$  of dexamethasone, it antagonized, in a concentration-dependent manner, the effect of the synthetic glucocorticoid. Its inhibitory effect was total at  $5.10^{-6}M$  whereas its  $EC_{50}$  was similar to the concentration of dexamethasone used. These results agree with the relative binding affinities of these two compounds for the thymus glucocorticoid receptors (Moguilewsky and Philibert, this volume). RU 486 alone was devoid of any glucocorticoid activity in concentrations of up to  $10^{-6}M$ .

Pituitary cells. As shown in Figure 6, dexamethasone at  $10^{-8}M$  totally inhibited the secretion of ACTH from rat pituitary cells stimulated by  $Br^8cAMP$ . This effect was reversed in a concentration-dependent manner by RU 486, which at  $10^{-6}M$  displayed a full antiglucocorticoid effect. It was also observed that up to  $10^{-6}M$  of RU 486 alone was devoid of any agonist activity. These two compounds incubated separately did not modify the release of ACTH in non- $Br^8cAMP$ -stimulated cells (results not shown).

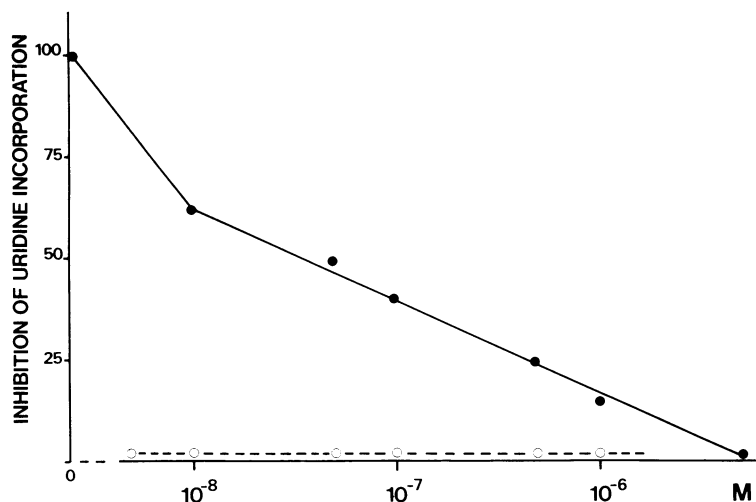


Fig. 5. Antigluco-corticoid activity of RU 486 on uridine incorporation in rat thymocytes. Thymocytes were prepared by mincing pooled thymi of adx rats in Hank's buffer (Dausse et al., 1977). They were washed and suspended at a final concentration of  $10^7$  cells/ml in MEM (GIBCO). Aliquots of 300  $\mu$ l were incubated under  $O_2$  95% -  $CO_2$  5% for 3 h at  $37^\circ C$  with the RU 486 alone 0---0 or with  $5 \times 10^{-8} M$  of dexamethasone in the presence of increasing concentrations of RU 486 0 0. 1  $\mu$ Ci of  $^3H$ -uridine was added, and incubation was continued for 1 h. Radioactivity incorporated into trichloroacetic precipitable material was determined.

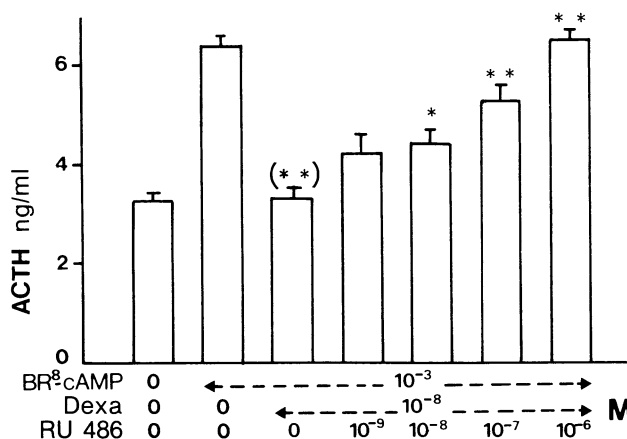


Fig. 6. Effect of RU 486 on BR<sup>8</sup>cAMP-induced ACTH release from rat pituitary cells. The anterior pituitary was removed from 2-week adrenalectomized 200g male rats and dispersed by trypsinization (Rotsztein et al., 1981). Cell concentration was adjusted at  $2 \times 10^5$  cells/ml in 0.2% glucose Krebs-Ringer bicarbonate buffer, 0.1% BSA and 0.1% lima bean trypsin inhibitor. It was incubated under  $O_2$  95% -  $CO_2$  5% for 4 h at  $37^\circ C$  (test compounds indicated on the abscissa). BR<sup>8</sup>cAMP was added to each tube and incubation continued for 1 h. Nutrient medium ACTH was evaluated, after acidification with HCl, by radioimmunoassay. (\*\*) - relative to control (BR<sup>8</sup>cAMP). \*\* - relative to dexamethasone treated cells.

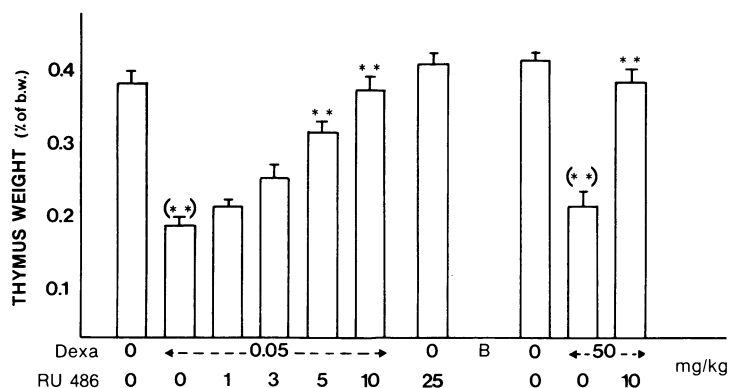


Fig. 7. Antigluco-corticoid activity of RU 486 on thymus weight in intact rats. Groups of 5 intact rats weighing about 100g received an oral administration of the compounds daily for 4 days, as indicated on the abscissa (B = corticosterone). 24 h after the last administration, the animals were killed, and the thymus was removed and weighed.

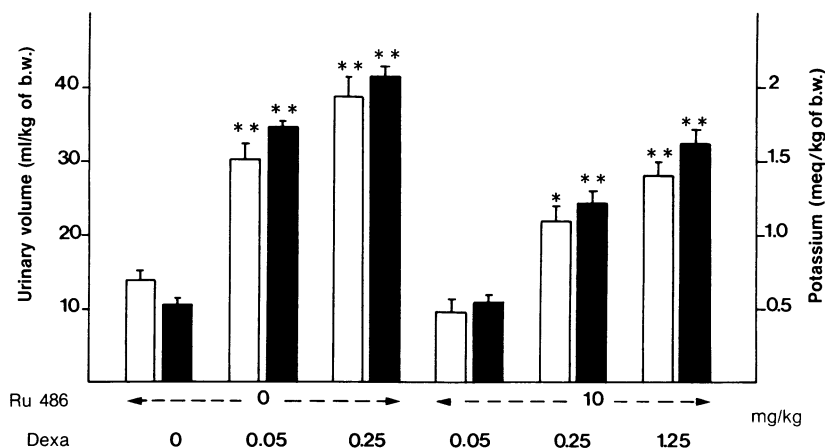


Fig. 8. Reversibility of the antigluco-corticoid activity of RU 486 by dexamethasone on diuresis in rats. Adx male rats (160-180g) were divided into 2 groups. One received a single oral dose of RU 486 and the other the vehicle. One h later the animals received a single s.c. injection of (0-1.25 mg/kg) dexamethasone in normal saline containing 1% ethanol and simultaneously a surcharge of 5 ml/100g of body weight of normal saline intraperitoneally. The animals were immediately placed in diuresis cages. Urine was collected for 4 hours. The volume was measured (white bars) and the urinary potassium (black) was determined by flame photometry (6 rats/groups).

Similar antiglucocorticoid properties have been described on gamma-glutamyl-transferase and tyrosine amino-transferase in HTC cells (Chobert et al., 1983; Gagne et al.; Chasserot-Golaz and Beck), on the viability of murine T-lymphoid cells line W7TB (Bourgeois et al., 1984), on L-929 mouse fibroblasts proliferation (Jung-Testas and Baulieu, 1984), on uridine incorporation in mouse thymocytes (Duval et al., 1984), and on ACTH release from rat perfused pituitary gland (Sakly et al., 1984).

Anti-thymolytic activity in rats. Thymic involution is one of the biological responses most used to detect glucocorticoid compounds *in vivo*. Thus, in normal rats (Fig. 7), dexamethasone and corticosterone (B) given orally for four consecutive days at doses of 0.05 and 50 mg/kg respectively, induce the same significant decrease of thymus weight. When RU 486 is administered in combination with these glucocorticoids, it exhibits a full antagonistic effect at a dose of 10 mg/kg (ED50 about 4 mg/kg). Administered alone, however (up to 25 mg/kg), it does not modify the thymus weight. Using the same bioassay, we have obtained similar data in adrenalectomized animals (not shown). RU 486 also has been proved fully effective in adx rats on hepatic tyrosine aminotransferase and tryptophan pyrrolase (Philibert, 1984) and on liver glycogen (Chrousos et al.).

Diuresis. It was also important to know if the antiglucocorticoid effect of RU 486 could be reversed by dexamethasone. We used a modified version of the test described previously by Johnson-Bia et al. (1982), showing that acute administration of a glucocorticoid increases the urinary excretion of potassium in adrenalectomized rats. In our experimental conditions, a single subcutaneous injection of dexamethasone in increasing doses (0-0.25 mg/kg) significantly enhanced both urinary volume and potassium excretion in adx male rats (Fig. 8). When animals were pretreated with a single oral dose of 10 mg/kg RU 486 one hour before the injection of dexamethasone, we noticed that RU 486 completely blocked the effect at 0.05 mg/kg of dexamethasone. This inhibitory effect was reversed by higher doses of dexamethasone. In these cases, 1.25 mg/kg of dexamethasone produced the same effect as 0.05 mg/kg of dexamethasone injected alone. Thus, 25 times more glucocorticoid was needed to induce the same response in rats pretreated with RU 486 as compared to non-pretreated animals.

Under the same experimental conditions (results not shown), aldosterone was injected at a dose of 1 µg/kg in place of dexamethasone (Kagawa 1960). We observed a classical mineralocorticoid effect characterized by a significant decrease in urinary sodium excretion and a slight increase of potassium excretion. Up to 30 mg/kg of RU 486 administered orally one hour before aldosterone did not display any antimineralocorticoid activity. Given alone, at 30 mg/kg, it exhibited no mineralocorticoid or glucocorticoid activities on the urinary ionic balance.

Effect of RU 486 on corticosterone biosynthesis in rat adrenal cells. All bioassays used so far show that RU 486 exerts an antiglucocorticoid effect at the target-tissue level. We wanted to determine whether this compound possesses another type of antagonism by inhibiting the corticosterone biosynthesis. As shown in Figure 9, using a perfusion system, ACTH introduced in the nutrient medium for fifteen minutes stimulated, in a dose-related manner, the secretion of corticosterone from adrenal cells. When RU 486 was added to the medium at  $10^{-5}$ M, it did not modify the stimulatory effect of 3 ng of ACTH. However,  $10^{-5}$ M of trislostane, a well-known inhibitor of glucocorticoid biosynthesis (Potts et al., 1978), totally prevented the corticosterone biosynthesis.

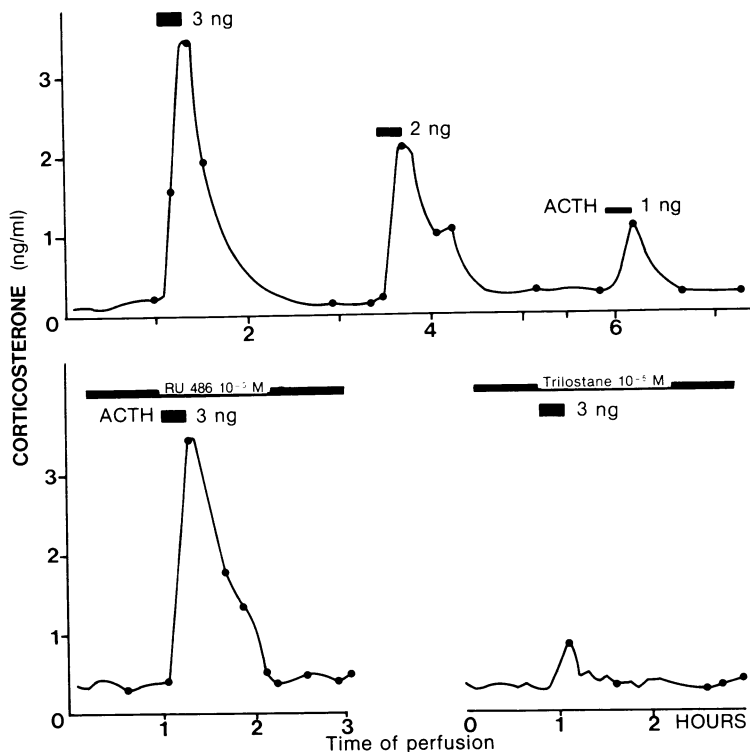


Fig. 9. Study of the effect of RU 486 in ACTH-induced corticosterone biosynthesis from rat adrenal cells using a perfusion system. In each experiment, 6 adult male rats (200-250g) were killed, and the adrenal glands were removed, dissected free of fat and minced. Isolated cells were prepared by trypsinization (Lowry et al., 1973). The cells were suspended in MEMS (Flow Laboratories) buffer supplemented with 10% fetal calf serum and whole egg ultrafiltrate and introduced into a small glass column (0.9 x 15 cm) containing Biogel P<sub>2</sub> (Biorad) (Leboulanger et al., 1978). The perfusion medium, gassed with O<sub>2</sub> 95%-CO<sub>2</sub> 5% and containing ACTH + test compounds, was pumped through the perfusion chamber at a constant flow rate of 600 ul/min. The temperature was maintained at 37°C by water circulating in a jacket. The effluent was collected at 4-minute intervals, and corticosterone concentrations measured by radioimmunoassay.

#### Antiprogesterone Activity of RU 486 Administered in Combination with Dexamethasone in Rats

The following experiments were performed to determine whether the antiglucocorticoid component of RU 486 intervenes in the expression of its antiprogesterone activity. As shown in Table III, progestogen-mimetic (deciduomata formation) and glucocorticoid (thymus and cotton-induced granuloma weights) responses were evaluated in the same ovx rats. 10 mg/kg of progesterone induced deciduogenic activity in all treated rats. The administration of 0.6 mg/kg of dexamethasone in combination with progesterone did not modify the deciduogenic response but significantly decreased both thymus and granuloma weights. When oral doses of RU 486 were given simultaneously with both progesterone and dexamethasone (6 mg and 20 mg/kg), it fully antagonized the effect of progesterone on deciduomata formation and was devoid of antiglucocorticoid activity.

Table III - Antiprogesterone Activity of RU 486 on Deciduomata Formation and Abortion in Rats Simultaneously Treated with High Doses of Dexamethasone.

A)

TREATMENT	mg/kg	RATS WITH DECIDUOMATA	THYMUS mg	DRY GRANULOMA mg
PROGESTERONE (s.c.)	10	6/6	537 $\pm$ 85	25.0 $\pm$ 3
+ DEXA (s.c.)	0.6	5/5	109 $\pm$ 13**	4.3 $\pm$ 0.5**
+ DEXA + RU 486 (p.o.)	6	0/6	93 $\pm$ 4**	4.7 $\pm$ 0.7**
+ DEXA + RU 486 (p.o.)	20	0/7	111 $\pm$ 11**	5.9 $\pm$ 0.8**

B)

TREATMENT Day 9+10+11	mg/kg	NUMBER OF ABORTIONS	THYMUS mg
CONTROL	0	0/5	314 $\pm$ 25
DEXA (s.c.)	1	0/5	54 $\pm$ 5**
RU 486 (p.o.)	10	5/5	302 $\pm$ 27
DEXA + RU 486	-	5/5	103 $\pm$ 14**

A) Female Wistar rats were ovariectomized on day 1 and treated with 5  $\mu$ g of estradiol by s.c. route from day 1-4. From day 5-14, the animals received the test compounds at doses indicated on the table. On day 9, a thread was passed through one uterine horn, and two pellets of cotton were introduced under the dorsal skin. The animals were killed, and the decidual response was evaluated as described in Table I. The thymus and granuloma were removed and weighed.

B) Pregnant rats were treated with the test compounds from day 9-11 and killed 24 hours after the last treatment. The abortive effect was evaluated, and the thymus was removed and weighed.

Furthermore, RU 486 administered orally to adx pregnant rats on day nine for three consecutive days exerted a total abortive effect without affecting the thymus weight (Table III). When this compound was given simultaneously with 1 mg/kg dexamethasone, it was also fully abortive, although the thymus-weight index indicated that its antiglucocorticoid component was blocked by dexamethasone.

#### Anti-Androgenic Activity in Rats

In castrated rats, testosterone propionate at a dose of 0.5 mg/kg caused a sharp increase of both seminal vesicles and prostate weights (Fig. 10). RU 486, given orally, inhibited in a dose-dependent fashion, the effect of testosterone. Its antiandrogenic activity was more important on the seminal vesicles than on the prostate, 85% and 55% inhibition respectively. At 100 mg/kg, RU 486 alone had no androgenic activity.

#### Estrogenic Activity of RU 486

We have studied the effect of RU 486 on two classical biological responses mediated by estrogens: the induction of estrus in ovariectomized rats characterized by the presence of keratinized cells in vaginal smears (Allen and Doisy, 1923) and the increase of the uterine weight in various species (Rubin et al., 1951).

Induction of estrus in ovx rats. Groups of six female rats weighing about 200 g were ovariectomized. Fifteen days later, vaginal smears were examined for signs of permanent diestrus (presence of numerous polymorphonuclear leucocytes) in order to confirm that the animals were truly ovariectomized. The maintenance of the sensitivity of the vaginal mucosa was then ensured by a single subcutaneous injection of 5 µg/kg estradiol benzoate. Three weeks after the priming injection, the rats received a single oral administration of RU 486, in increasing doses of 3-300 mg/kg. We observed that none of the RU 486 doses provoked the appearance of an estrus within the week following its administration, whereas estradiol at 5 µg/kg induced the keratinization of vaginal cells within 48 hours. RU 486 also was devoid of any estrogenic activity in this test when administered for five days at 10 and 50 mg/kg.

Uterine weight increase in various species. As shown in Figure 11, 10-300 mg/kg of RU 486, administered orally to immature rabbits, mice and rats, exhibited a weak uterotrophic activity that plateaued, according to the species, at a level 4-10 times lower than that observed with estradiol. In this test, RU 486 was at least 10,000 times less active than estradiol. We have also studied the kinetics of RU 486's uterotrophic effect in normal, ovariectomized, and ovariectomized and adrenalectomized rats. We noticed (Fig. 12) that RU 486 at a dose of 250 mg/kg induced similar uterine weight increases in both intact and operated rats. These results suggest that RU 486 at this high dose may act by direct interaction with the uterine estrogen receptor (Moguilewsky and Philibert, this volume).

#### Effect of Chronic Administration of RU 486 on Estrus Cycle in Rats

RU 486 was administered orally for 15 days to rats. As illustrated in Figure 13, RU 486, administered orally to hysterectomized and intact rats, induced a dose-related increase in the ovarian weight, the number of colorless lutea (Bennett et al., 1967) and serum progesterone. Serum LH, measured only in intact rats, was enhanced from 20 ng/ml in the control animals to 60 ng/ml in animals treated with 10 mg/kg of RU 486. Daily examinations of vaginal smears from days 12-16 (day of sacrifice) indicated that the amount of estrus observed over a period of five days per animal increased in a dose-dependent fashion. At doses of 10 mg/kg and above, all

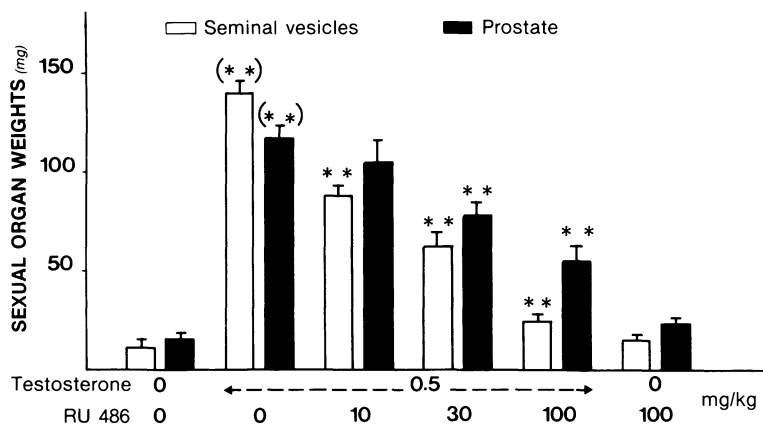


Fig. 10. Effect of RU 486 on seminal vesicles and prostate weights in castrated rats. Groups of 5 castrated male rats (about 100 g) simultaneously received RU 486 for 8 days by oral route and testosterone propionate by s.c. route. The animals were killed 24 h after the last treatment. The seminal vesicles and prostates were removed, fixed for 24 h in a 10% solution of formaldehyde in physiological saline, dissected and weighed.

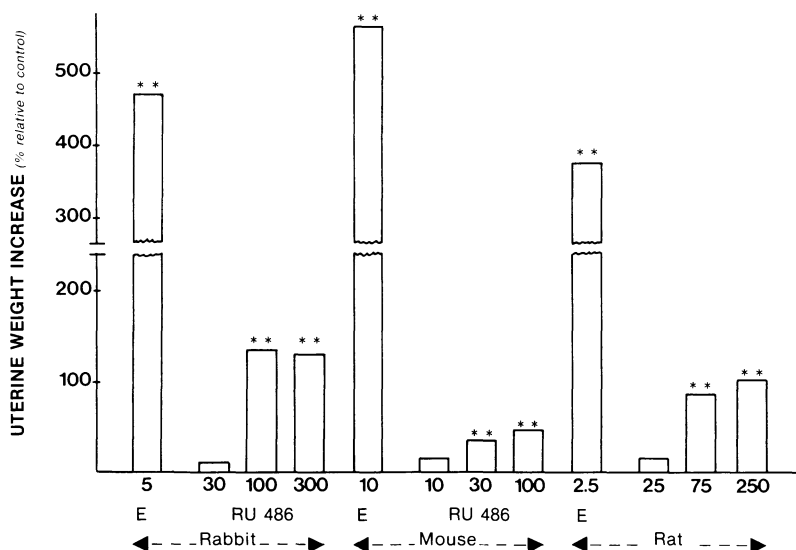


Fig. 11. Uterotrophic activity of RU 486 in various species. Groups of 3 immature rabbits (about 1 kg), 5 immature rats (40 g) and 5 mice (10 g) received either RU 486 (mg/kg) orally or estradiol (ug/kg) subcutaneously for 3 days. Twenty four hours after the last administration, the animals were killed, and the uteri were excised and weighed. The uterine-weight increase was equal to  $100 \times (\text{weight of treated animals} - \text{weight of control}) / \text{weight of control}$ .

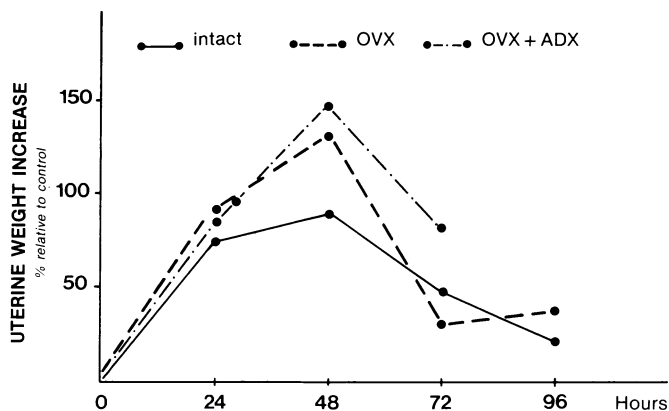
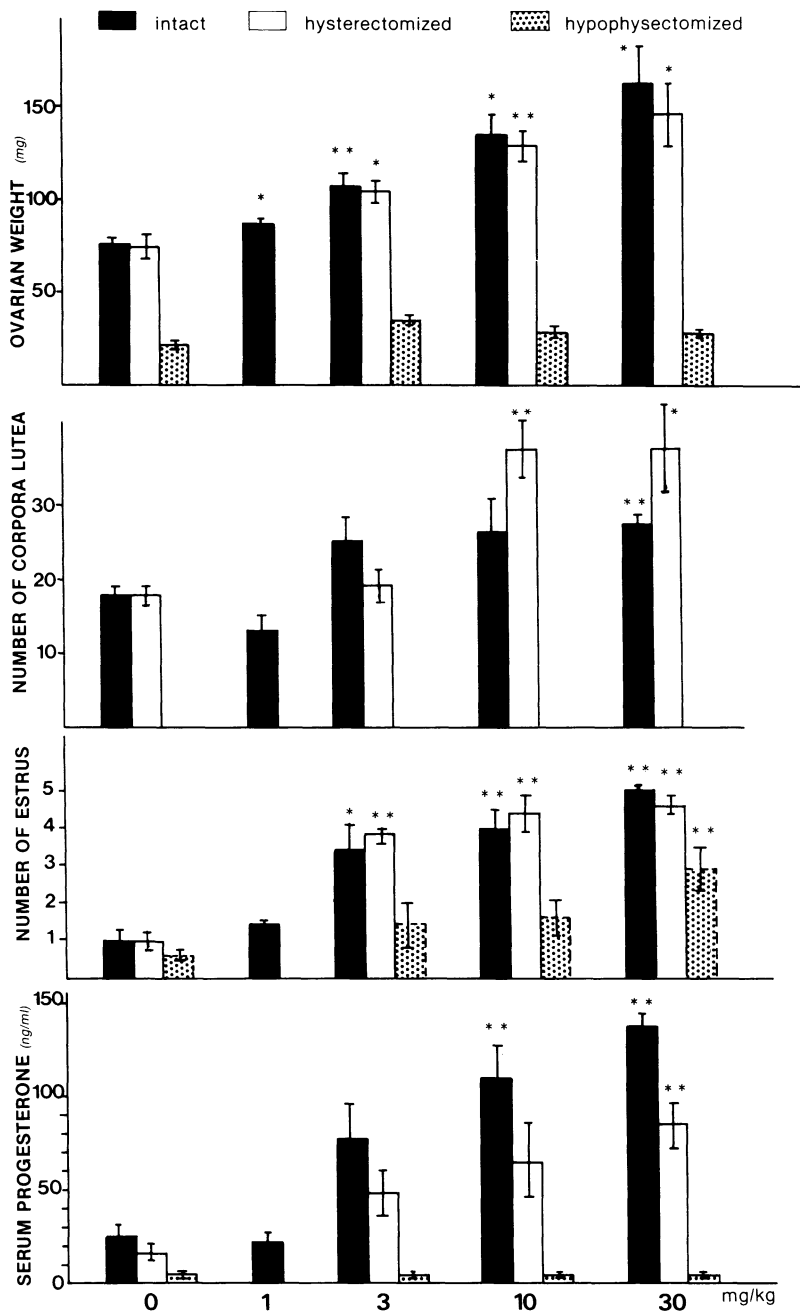


Fig. 12. Kinetics of the RU 486 uterotrophic activity in rats. A single oral dose of RU 486 (250 mg/kg) was administered to groups of 5 intact, ovx or ovx + adx rats. The animals were sacrificed at different times after the treatment, and the uteri were removed and weighed. The uterine-weight increase was expressed as indicated in the legend of Figure 11.

animals displayed permanent estrus. In hypophysectomized animals we observed no estrus. In these cases the vaginal smears were characterized by the presence of leucocytes with a few keratinized cells (dotted bars in the figure) that could correspond to an intermediary state between diestrus and proestrus. Thus RU 486 does not exhibit any antiovarulatory activity. The induction of estrus in intact and hysterectomized animals might be explained by its antiprogesterone activity at the vaginal level, which should in turn indirectly amplify the action of endogenous estrogens.

## CONCLUSION

RU 486 appears to be the most potent known antiprogesterone and antiglucocorticoid observed both in vitro and in vivo. At oral doses ranging from 3-20 mg/kg, it prevents progestin and glucocorticoid action at the level of their own receptors. No agonist effect is exhibited; the effect is seen in all species and with all bioassays used. In the rat, the species studied the most, the strengths of these two antagonist profiles are quite similar. In castrated male rats, RU 486 also displays antiandrogenic activity on prostate and seminal vesicle weights that is 10-20 times weaker than its two other antagonist properties. Unlike estrogens, it does not induce estrus at doses up to 300 mg/kg in ovariectomized rats. In contrast, it provokes permanent estrus after chronic administration to intact rats at doses of 10 mg/kg and above. Under the same experimental conditions, the compound does not display antiovarulatory activity but does seem to stimulate the pituitary ovarian axis. In various species, RU 486 also exhibited slight uterotrophic activity (30-300 mg/kg). This always occurred, even in ovariectomized and adrenalectomized rats. These results suggest that this uterine weight increase was mediated by a transient interaction between RU 486 and the uterine estrogen receptor, although the histological aspect of the uteri was



different than that obtained with estradiol (Secchi et al., this volume). As expected, RU 486's negligible binding affinity for the rat kidney mineralocorticoid receptor makes it completely devoid of mineralocorticoid and antialdosterone activities in adrenalectomized rats (doses up to 30 mg/kg). It was also observed that the antiglucocorticoid action of RU 486 on diuresis was rapidly and completely reversed by increasing doses of dexamethasone. However, RU 486 seems devoid of any inhibitory effect on the biosynthesis of corticosterone and progesterone. Kendle (1979, 1982), who has recently listed all known antiprogestins, indicated that only one, R 2323 (Sakiz and Azadian-Boulanger, 1971; Azadian-Boulanger et al., 1971), seems to act directly at the receptor level. However, this compound exhibits only partial antagonism. In addition, it displays estrogenic and androgenic activities. Other drugs, such as RMI 12936, seem to be effective in interfering with the biosynthesis of progesterone.

Fig. 13. Effect of chronic administration of RU 486 on the estrous cycle in rats. RU 486 was administered orally for 15 days to adult female rats. From day 5-8 inclusive, Dianil Blue 2R was injected by i.p. route; all corpora lutea formed during this time or already present thus were stained blue. The vaginal smears were examined daily from day 12-16; the animals were killed on day 16. Ovaries were removed and weighed. Serum LH and progesterone were measured by RIA. The number of colorless corpora lutea/rat, indicating that ovulation had occurred between day 9 and 16, was determined. The number of estrus/rat from day 12-16 was evaluated.

Chrousos et al. (1982a, 1983) have recently reviewed the effective antiglucocorticoid compounds such as cortexolone (Kaiser et al., 1972; Cutler et al., 1979), steroidal 17 $\beta$  carboxamides (Rousseau et al., 1979), delta<sup>1,9(11)</sup>-11-deoxycortisol (Chrousos et al., 1980), 21-mesylate derivatives of cortisol and dexamethasone (Simons et al., 1980; Simons and Thompson, 1981), and delta<sup>1</sup>-11 oxa-11 deoxycortisol (Chrousos et al., 1982b). All of these compounds exhibit a much weaker antagonistic effect in vitro than RU 486. In vivo, they are effective at very high doses (only for certain tests) and, unlike RU 486, exhibit partial agonist activity in most cases.

RU 486 is the first example of a true antagonist in the areas of progestins and glucocorticoids. In addition to its wide clinical applications, particularly for fertility control and treatment of diseases due to hypercorticism, RU 486 represents a useful tool to elucidate the physiological processes mediated by progesterone and glucocorticoids.

## REFERENCES

- Allen, E., and Doisy, E., 1923, An ovarian hormone. Preliminary report on its localization. Extraction and partial purification and action in test animals, J. Am. Med. Ass., 81:819
- Azadian-Boulanger, G., Secchi, J., and Sakiz, E., 1971, Biological study of the antiprogesterone effect of R 2323, Excerpta Med. Inst. Congr. Ser., 278:129
- Baxter, J. D., and Rousseau, G. G., eds., 1979, in: "Glucocorticoid Hormones Action," Springer-Verlag, New York.
- Bennett, J. P., Vallance, D. K., and Vickery, B. H., 1967, A method for the direct observation of ovulation in the mature rat, J. Reprod. Fert., 13:567.
- Bourgeois, S., Pfahl, M., and Baulieu, E. E., 1984, DNA binding properties of glucocorticosteroid receptors bound to the steroid antagonist RU 486, The EMBO Journal, 3:751.
- Chambon, Y., 1952, Sur le conditionnement endocrinien du déciduome traumatique chez la rate castrée, C. R. Soc. Biol., 146:1095.
- Chasserot-Golaz, S., and Beck, G., An approach to the mechanism of the potent antiglucocorticoid: 17 $\beta$ -hydroxy-11 $\beta$  4-dimethylamino-phenyl-17 $\alpha$ -propynyl-estra-4, 9-dien-3-one, submitted to J. Ster. Biochem.
- Chobert, M. N., Barouki, R., Finidor, J., Aggerbeck, M., Hanoune, J., Philibert, D., and Deraedt, R., 1983, Antiglucoctcoid properties of RU 38 486 in a differentiated hepatoma cell line, Biochem. Pharmacol., 32:3481.
- Chrousos, G. P., Barnes, K. M., Sauer, M. A., Loriaux, D. L., and Cutler, G. B., Jr., 1980, delta<sup>1,9(11)</sup>-11-deoxycortisol, An improved glucocorticoid antagonist, Endocrinology, 107:472.
- Chrousos, G. P., Cutler, G. B., Jr., Simons, S. S., Jr., Pons, M., Loriaux, D. L., John, L. S., and Moriarty, R. M., 1982a, Development of antiglucoctcoids with potential clinical usefulness in: "Progress in Research and Clinical Applications of Corticosteroids," H. J. Lee and T. J. Fitzgerald, eds., Heyden and Son Co., Philadelphia.
- Chrousos, G. P., Sauer, M. A., Cutler, G. B., Jr., and Loriaux, D. L., 1982b, delta<sup>1</sup>-11-oxa-11-Deoxycortisol: A new antiglucoctcoid with activity in vivo, Steroids, 40:425.
- Chrousos, G. P., Cutler, G. B., Jr., Sauer, M., Simons, S. S., Jr., Loriaux, D. L., 1983, Development of glucocorticoid antagonists, Pharmac. Ther., 20:263.
- Chrousos, G. P., Nieman, L., Healy, D., Spitz, I., Hodgen, G., Bardin, C. W., Cutler, G. B. Jr., Schulte, H. M., Merriam, G. R., Brandon, D. D., and Loriaux, D. L., Antiglucoctcoids: General aspects and clinical implications.
- Cutler, G. B., Jr., Barnes, K. M., Sauer, M. A., and Loriaux, D. L., 1979, 11-Deoxycortisol: A glucocorticoid antagonist in vivo, Endocrinology, 104:1839.
- Dausse, J. P., Duval, D., Meyer, P., Gagnault, J. C., Marchandeau, C., and Raynaud, J. P., 1977, The relationship between glucocorticoid structure and effects upon thymocytes, Mol. Pharmacol., 13:948.
- Dunnett, C. W., 1955, A multiple comparison procedure for comparing several treatments with a control, Am. Stat. Assoc. J., 50:1096.
- Duval, D., Durant, S., and Homo-Delarche, F., 1984, Effect of antiglucoctcoids on dexamethasone-induced inhibition of uridine incorporation and cell lysis in isolated mouse thymocytes, J. Steroid Biochem., 20:283.
- Gagne, D., Pons, M., and Philibert, D., RU 486, a potent antiglucoctcoid in vitro and in vivo, submitted to J. Steroid Biochem.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationship, and hormonal effects, Fertility and Sterility, 4:253.

- Johnson Bia, M., Tyler, K., and De Fronzo, R. A., 1982, The effect of dexamethasone on renal electrolyte excretion in the adrenalectomized rat, Endocrinology, 111:882.
- Jung-Testas, I., and Baulieu, E. E., 1984, Anti-steroid action in cultured L-929 mouse fibroblasts, J. Steroid Biochem., 20:301.
- Kagawa, C., 1960, Blocking the renal electrolyte effects of mineralocorticoids with an orally active steroidal spironolactone, Endocrinology, 67:125.
- Kaiser, N., Milholland, R. J., Turnell, R. W., and Rosen, F., 1972, Cortexolone: binding to glucocorticoid receptors in rat thymocytes and mechanism of its antiglucocorticoid action, Biochem. Biophys. Res. Comm., 49:516.
- Kendle, K. E., 1979, Current investigations of antiprogestational steroids, in: "Antihormones," M. K. Agarwal, ed., Elsevier/North Holland Biomedical Press, New York.
- Kendle, K. E., 1982, Advances in the study of antiprogestational agents, in: "Hormone Antagonists," M. K. Agarwal, ed., Walter de Gruyter, Berlin and New York.
- Leboulanger, F., Delarue, C., Tonon, M. C., Jegou, S., and Vaudry, H., 1978, In vitro study of frog (*Rana redebunda* Pallas) interrenal function by use of a simplified perfusion system, Gen. Comp. Endocrinol., 36:327.
- Ljunkvist, I., 1971, Attachment reaction of rat uterine luminal epithelium. II. The effect of progesterone on the morphology of the uterine glands and the luminal epithelium on the spayed virgin rat, Acta Soc. Med. Uppsalien, 76:110.
- Lowry, P. J., McMartin, C., and Peteras, J., 1973, Properties of a simplified bioassay for adrenocorticotrophic activity using the steroidogenic response of isolated cells, J. Endocr., 59:43.
- McGinty, D. A., Anderson, L. P., and McCullough, N. B., 1939, Effect of local application of progesterone on the rabbit uterus, Endocrinology, 24:829.
- McPhail, M. K., 1934, The assay of progestin, J. Physiol., Lond., 83:145.
- Nilsson, O., 1975, Influence of progesterone on the mitochondrial size in the uterine glands of the rat, mouse, hamster and guinea-pig, Acta Endocr., 78:349.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 38486: a potent antiglucocorticoid in vivo, VIII Int. Congr. of Pharmacology, Tokyo, abstract no. 1463.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, A new lead for steroidal antihormones, Endocrine Society, 64th annual meeting, San Francisco, abstract no. 668.
- Philibert, D., 1984, RU 38 486: an original multifaceted antihormone in vivo, in: "Adrenal Steroid Antagonism," M. K. Agarwal, ed., Walter de Gruyter and Co., Berlin and New York.
- Potts, G. O., Greange, J. E., Harding, H. R., and Schane, H. P., 1978, Trilostane: an orally active inhibitor of steroid biosynthesis, Steroids, 32:257.
- Raynaud, J. P., 1977, R5020, a tag for the progestin receptor, in: "Progesterone Receptors in Normal and Neoplastic Tissues," W. L. McGuire, J. P. Raynaud and E. E. Baulieu, eds., Raven Press, New York.
- Rotsztejn, W. H., Dusallant, M., Nobou, S., and Rosselin, G., 1981, Rapid glucocorticoid inhibition of vasoactive peptide (VIP)-induced cyclic AMP accumulation and prolactin release on rat anterior pituitary cells in culture, Proc. Natl. Acad. Sci. USA, 78:7584.
- Rousseau, G. G., Kirchhoff, J., Formstecher, P., and Lustenberger, P., 1979, 17- $\beta$  carboxamides steroids are a new class of glucocorticoid antagonists, Nature, 279:158.
- Rubin, B. L., Dorfman, A. S., Black, L., and Dorfman, R. I., 1951, Bioassay of estrogens using the mouse uterine response, Endocrinology, 49:429.
- Sakiz, E., and Azadian-Boulanger, G., 1971, R 2323, An original contraceptive compound, Excerpta Med. Int. Congr. Ser., 219:865.

- Sakly, M., Philibert, D., Lutz-Bucher, B., and Koch, B., 1984, Paradoxical involvement of glucocorticoid receptors in the aldosterone-induced impairment of ACTH secretion from perifused pituitary glands, J. Steroid Biochem, 20:1101
- Secchi, J., and Lecaque, D., 1984, Effects of progestins and antiprogestins on mitochondria in uterine glandular cells in the rat: a quantitative investigation, Cell and Tissue Res., 238:247.
- Simons, S. S., Jr., Thompson, E. B., and Johnson, D. F., 1980, Unique long active antiglucocorticoid in whole and broken cell systems, Proc. Natl. Acad. Sci. USA, 77:5167
- Simons, S. S., Jr., and Thompson, E. B., 1981, Dexamethasone-21-mesylate: An affinity label of glucocorticoid receptors from rat hepatoma tissue culture cells, Proc. Natl. Acad. Sci. USA, 78:3541.
- Tachi, C., and Tachi, S., 1974, Cellular aspects of ovum implantation and decidualization in the rat, in: "Physiology and the Genetics of Reproduction," Part B, E. M. Coutinho and F. Fuchs, eds., Plenum Press, New York and London.

THE USE OF THE ANTIPROGESTERONE COMPOUND RU 486

TO CONTROL TIMING OF PARTURITION IN RATS

M. J. Bosc,<sup>1</sup> G. Germain,<sup>2-5</sup>  
A. Nicolle,<sup>1</sup> D. Philibert,<sup>3</sup> and E. E. Baulieu<sup>4</sup>

<sup>1</sup>Station de Physiologie de la Reproduction  
I.N.R.A. Nouzilly  
37380 Monnaie, France

<sup>2</sup>Clinique Universitaire Baudelocque  
I.N.S.E.R.M. U. 262  
Paris Cedex, 14

<sup>3</sup>Laboratoires Roussel-Uclaf  
93230 Romainville, France

<sup>4</sup>Faculte de Medecine Paris-Sud  
Lab. Hormones I.N.S.E.R.M. U. 33  
94270 Bicetre, France

<sup>5</sup>Station Centrale de Physiologie Animale  
C.N.R.Z. de l'I.N.R.A.  
78350 Jouy en Josas, France

The end of progesterone inhibition is a prerequisite for parturition in rats (Deansley, 1956). In this species, birth is preceded by a drop in uterine progesterone (Davies and Ryan, 1973) and by low blood-progesterone concentrations during the last 25-26 hours (Sherwood et al., 1983). Therefore, an antiprogesterone compound might be used to control the time of birth. To test this possibility, a single dose of RU 486 (10 mg/rat in 0.2 ml of ethanol) was given subcutaneously to rats late in pregnancy. Ninety nine pregnant rats, isolated at mating (day 1 of pregnancy) and fed ad libitum, were submitted to eight hours daily of light (lights on from 12 noon to 8 p.m.) from day eight of gestation. RU 486 was given to five groups as follows: at 8 a.m. on day 21 (group A), at 12 noon on day 21 (group B), at 7 p.m. on day 21 (group C), at 8 a.m. on day 22 (group D) and at 12 noon on day 22 (group E). In the sixth group (T), the solvent was given once at each of the preceding times. As previously observed (Bosc and Nicolle, 1980), group T births were distributed in two periods with 2/3 of the rats giving birth in the late light phase of day 22 (mean time 6 p.m.) and 1/3 after lights on on day 23 (mean time 4 p.m.). The rats treated on day 22 (groups D and E) had a similar distribution.

All rats in groups A, B, and C treated on day 21 gave birth at single periods on the 22nd day, 8.5 hours ( $p < 0.01$ ), 3.8 hours ( $p < 0.001$ ) and 1.3 hours earlier, respectively, than the controls (T). Thus, the births

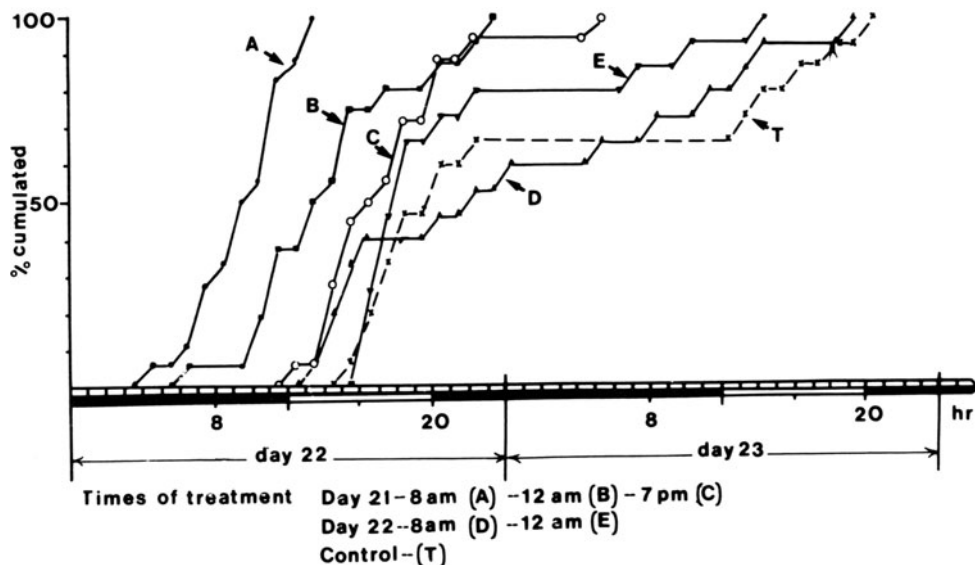


Fig. 1. Birth Distributions of Rats Treated with RU 486

occurred 25.4 h (+ 2.5), 26.1 h (+ 2.8) and 21.6 h (+2.8) ( $p < 0.01$ ) respectively, after treatment. The number of pups dead after day one was affected by the length of gestation ( $p < 0.01$ ) (extremes: 50.6% in group A; 3% in group E), as was live pup weight ( $p < 0.01$ ) (extremes: 5.25 g in group A; 6.10 g in group T). However, postnatal mortality and weight at weaning were not different from normal. These results show that an antiprogesterone molecule may provide an efficient tool for the control of birth timing.

#### ACKNOWLEDGMENTS

We wish to thank Ms. A. Daifuku for editing the English manuscript.

#### REFERENCES

- Bosc, M. J., Nicolle, A., 1980, Influence of photoperiod on the time of parturition in the rat. I-Effect of the length of day illumination on normal or adrenalectomized animals, *Reprod. Nutr. Develop.*, 20:735.
- Davies I. J., Ryan, R. J., 1973, The modulation of progesterone concentration in the myometrium of the pregnant rat by changes in cytoplasmic "receptor" protein activity, *Endocrinology*, 92:394.
- Deansley, R., 1956, The endocrinology of pregnant and fetal life in: "Marshall's Physiology of Reproduction," A. S. Parkes, ed., Longmans, Green and Co., London.
- Sherwood, O. D., Downing S. J., Golost, G., Gordon W. L., and Tarbell, M. K., 1983, Influence of light-dark cycle on antepartum serum relaxin and progesterone immunoactivity levels and birth in the rat, *Endocrinology*, 113: 997.

IN VIVO ASSESSMENT OF ANTI-PROGESTERONE AND ANTI-GLUCOCORTICOID ACTIVITIES  
OF RU 486 IN RATS: EFFICACY IN TERMINATING EARLY PREGNANCY IN THE RAT

C. C. Chang,<sup>1</sup> Sheldon J. Segal,<sup>2</sup> and C. Wayne Bardin<sup>1</sup>

<sup>1</sup>Center for Biomedical Research, The Population Council and  
<sup>2</sup>Population Sciences Division, The Rockefeller Foundation  
New York, NY

INTRODUCTION

The present study was undertaken (1) to assess the efficacy of the anti-progesterone and anti-glucocorticoid activities of RU 486 in the same animal; (2) to determine whether different routes of administration affect the ratio of anti-glucocorticoid and anti-progestational activity of the compound and (3) to evaluate the efficacy of RU 486 in terminating early pregnancy by different routes of administration.

Ovariectomized and adrenalectomized pseudopregnant rats were used for the test system. The anti-progesterone activity of RU 486 was determined by the deciduoma formation assay (Miyake, 1962), and the anti-glucocorticoid activity of the compound was evaluated by a thymolytic assay (Dorfman, 1962) and an anti-inflammatory assay (Meyer, et al., 1953, Dubin, 1955).

MATERIALS AND METHODS

Nulliparous female rats of Sprague-Dawley strain, weighing 160-180 gm and obtained from the Charles River Breeding laboratories (Waltham, MA), were used in this study. The animals were housed in a temperature- (24.5-26.5°C) and illumination- (14 hr. light and 10 hr. dark) controlled room and maintained on Purina laboratory chow and tap water ad libitum.

Pseudopregnancy was induced by mechanical stimulation of the cervix with a glass rod during the afternoon of estrus. The first morning that vaginal smear cells were predominantly leukocytes was considered day one of pseudopregnancy. On day four of pseudopregnancy, only those animals that remained in diestrus were selected for the experiment. Animals in proestrus in the morning were caged overnight with males of proven fertility. Positive matings were verified in the presence of either spermatozoa or copulation plugs the following morning. If one or the other, or both, were present, this day was designated as day one of pregnancy.

Experiment 1: Anti-Progesterone and Anti-Glucocorticoid Activities of RU 486 in Ovariectomized and Adrenalectomized Pseudopregnant Rats

Bilateral ovariectomy and adrenalectomy were performed by dorso-lateral incision on day four of pseudopregnancy. For the deciduoma formation assay

of progesterone, the endometrium of one uterine horn was traumatized by longitudinal, antimesometrial scratching with a burred-tip hypodermic needle (De Feo, 1963). The contralateral horn that was not traumatized served as the control. For systemic anti-inflammatory assay of cortisol, the cotton-pellet test was employed, according to the methods described by Meyer et al. (1953) and Dubin (1955) and modified in our laboratory. It consisted essentially of implanting two non-sterile cotton pellets (No. 2), weighing 5-7 mg, into the dorso-lateral subcutaneous connective tissue of the neck region, one on each side (two per rat) through a small incision in the skin which was closed with a wound clip. The implanting of pellets was done at the time of adrenalectomy while the animals were under ether anesthesia.

The animals were treated either with daily subcutaneous injections of 2 mg of progesterone in 0.2 ml of corn oil or concomitantly with 0.375 mg of cortisol in 0.2 ml of vehicle consisting of 1 part 100% ethanol and 9 parts 50% aqueous propylene glycol. RU 486 dissolved in 100% ethanol and suspended in corn oil was administered either orally by means of a gavage needle inserted into the stomach, subcutaneously, or intravaginally, to progesterone- and cortisol-treated animals from day four through day seven of pseudopregnancy. For the intravaginal route of administration, RU 486 suspension was placed on a cotton pellet (No. 0) with the desired volume according to the method reported by Yamazaki (1982) and modified in our laboratory. This cotton pellet was inserted into the vagina and then covered with a rubber disc (about 10 mm in diameter) and a second cotton pellet without the compound was placed on top of the rubber disc. These cotton pellets and the rubber disc were replaced with new ones every 24 hours during the treatment period. The first cotton pellet of the set dipped with corn oil served as the control. The animals were allowed free access to drinking water containing 1% NaCl and 5% glucose throughout the experiment.

All animals were sacrificed on day eight of pseudopregnancy. At autopsy, the body weight was recorded and the uteri and thymus removed, trimmed of fat, and weighed to the nearest 0.1 mg on a torsion balance. The cotton pellets together with the accumulated granuloma were recovered, dried at 60°C for 72 hr, and weighed. The net granuloma dry weight was obtained by subtracting the original cotton weight of the cotton pellet from the total dry weight.

#### Experiment 2: Termination of Early Pregnancy with RU 486 by Different Routes of Administration in the Rat

RU 486 in oil suspension was administered either orally, subcutaneously, intramuscularly, or intravaginally, as described in Experiment 1 on days 6, 7 and 8 of pregnancy, or by a single dose on day six of pregnancy. All animals were sacrificed on day nine of pregnancy, and the number of implantation sites was counted.

The results of these experiments were expressed as mean plus or minus standard error of the mean. The data were analyzed statistically using Student's t-test. A probability of less than 0.05 was considered statistically significant.

## RESULTS

#### Experiment 1: Anti-Progesterone and Anti-Glucocorticoid Activities of RU 486 in Ovariectomized and Adrenalectomized Pseudopregnant Rats

The inhibitory effects of RU 486 on progesterone and cortisol action by different routes of administration are summarized in Table I. Daily subcutaneous injection of 2 mg of progesterone to ovariectomized and

**Table I. Anti-Progesterone and Anti-Glucocorticoid Activities of RU 486 in Ovariectomized and Adrenalectomized Pseudopregnant Rats**

Treatment	Daily dose* (No. of rats)	Wt of uterine horn, mg (mean $\pm$ SEM)		Thymus wt. (mg/100 gm B.W. $\pm$ SEM)	Granuloma dry wt. mg (mean $\pm$ SEM)
		traumatized	control		
Control	mg				
Progesterone (P)	2.0 (6)	687.2 $\pm$ 57.9	108.9 $\pm$ 9.3	295.9 $\pm$ 30.1 <sup>b</sup>	10.5 $\pm$ 0.9 <sup>a</sup>
P + Cortisol (C)	0.375 (8)	566.9 $\pm$ 70.5	101.9 $\pm$ 5.3	206.5 $\pm$ 13.4 <sup>b</sup>	5.7 $\pm$ 0.3 <sup>a</sup>
RU 486 + P + C	mg/kg				
Oral	4.0 (5)	102.5 $\pm$ 5.8 <sup>c</sup>	95.3 $\pm$ 7.5	252.1 $\pm$ 20.7	8.2 $\pm$ 0.6
	2.0 (10)	145.5 $\pm$ 10.4 <sup>c</sup>	111.5 $\pm$ 4.5	233.7 $\pm$ 16.4	7.2 $\pm$ 0.7
	1.0 (5)	299.0 $\pm$ 104.3 <sup>f</sup>	112.8 $\pm$ 9.5	227.9 $\pm$ 29.1	6.8 $\pm$ 0.7
Subcutaneous	1.0 (8)	104.4 $\pm$ 5.4 <sup>c</sup>	87.1 $\pm$ 4.9	211.3 $\pm$ 12.1	6.5 $\pm$ 0.5
	0.4 (5)	184.3 $\pm$ 26.3 <sup>c</sup>	109.2 $\pm$ 7.1	200.0 $\pm$ 9.7	5.2 $\pm$ 0.4
	0.2 (6)	218.4 $\pm$ 32.0 <sup>d</sup>	110.8 $\pm$ 5.2	197.1 $\pm$ 18.6	5.1 $\pm$ 0.5
	0.1 (5)	526.0 $\pm$ 64.3	100.6 $\pm$ 7.4	167.2 $\pm$ 10.7	5.6 $\pm$ 0.5
Intravaginal	2.0 (5)	149.2 $\pm$ 12.2 <sup>c</sup>	127.7 $\pm$ 6.3	199.8 $\pm$ 18.5	4.4 $\pm$ 0.3
	1.0 (5)	174.4 $\pm$ 27.5 <sup>c</sup>	114.0 $\pm$ 9.1	205.5 $\pm$ 5.9	4.4 $\pm$ 0.9
	0.4 (5)	225.8 $\pm$ 108.6 <sup>e</sup>	114.6 $\pm$ 4.1	196.4 $\pm$ 15.8	4.9 $\pm$ 1.0
	0.2 (8)	561.2 $\pm$ 62.2	111.3 $\pm$ 5.2	170.0 $\pm$ 9.2	5.1 $\pm$ 0.7

\* RU 486 was administered from day 4 through day 7 of pseudopregnancy.

<sup>a,b</sup> Significant difference vs. P control: <sup>a</sup><sub>p</sub> 0.001; <sup>b</sup><sub>p</sub> 0.02.

<sup>c-f</sup> Significant difference vs. P + C control: <sup>c</sup><sub>p</sub> 0.001; <sup>d</sup><sub>p</sub> 0.002; <sup>e</sup><sub>p</sub> 0.02; <sup>f</sup><sub>p</sub> 0.05

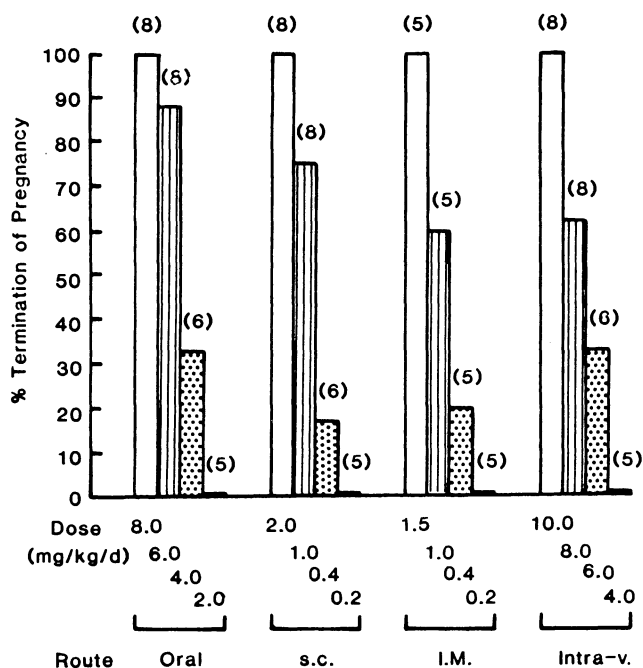
adrenalectomized pseudopregnant rats maintained pseudopregnancy and supported deciduoma formation. A daily dose of 0.375 mg of cortisol given concomitantly with progesterone produced involution of the thymus gland (about 30%,  $p < 0.02$ ) and inhibited granuloma formation around the cotton pellet (about 45%,  $p < 0.001$ ). The decidual response measured by the weight of the traumatized uterine horn in animals treated with progesterone and cortisol was slightly reduced (17.5%), but it was not significantly different from that of the progesterone control. An oral dose of 4 mg/kg/day of RU 486 exerted both anti-progesterone and anti-cortisol activities, as evidenced by inhibition of decidual response by blockade of cortisol-induced reduction of thymus weight and by prevention of cortisol-induced reduction of granuloma formation. It is of interest that a lower dose of RU 486 was required by the subcutaneous or intravaginal routes to produce inhibition of progesterone action (e.g. 1.0 mg/kg/day by subcutaneous injection or 2.0 mg/kg/day by intravaginal application). Unexpectedly, these doses did not interfere with cortisol action, as evidenced by the thymus weight and granuloma around the cotton pellet, which did not differ from the progesterone and cortisol control.

The same data on the effects of RU 486 on inhibition of decidual response, thymus involution, and anti-inflammatory activity, when administered orally, subcutaneously, and intravaginally are presented in Table II. The compound at an oral dose of 4 mg/kg/day inhibited cortisol action by 51% by thymolytic assay and 52% by anti-inflammatory assay, whereas a subcutaneous dose of 1 mg/kg/day or an intravaginal dose of 2 mg/kg/day had only about 5% or none of anti-cortisol activity, respectively.

The relative anti-progesterone and anti-cortisol potencies of RU 486 administered by different routes of administration are presented in Table III. The efficacy for the compound at the most successful efficacious dose in inhibiting decidual response was found to be 400% by subcutaneous injection and 200% by intravaginal application, while the oral route of administration was assigned a value of 100%. Considering the anti-cortisol activity of the compound, subcutaneous injection and intravaginal application were 7.7% and none as active as oral administration.

Table II. Inhibition of Decidual Response, Thymus Involution, and Anti-Inflammatory Activity with RU 486 by Oral, Subcutaneous and Intravaginal Routes

Route	Daily dose (mg/kg)	No. of rats	Inhibition of		
			Decidual response	Thymic involution	Anti-inflammatory activity
Oral	4.0	5	99	51	52
	2.0	10	93	30	31
	1.0	5	60	24	23
Subcutaneous	1.0	8	96	5	4
	0.4	5	84	0	0
	0.2	8	77	0	0
	0.1	5	9	0	0
Intravaginal	2.0	5	99	0	0
	1.0	5	87	0	0
	0.4	5	75	0	0
	0.2	8	3	0	0



Treatment: D<sub>6,7,8</sub> of Pregnancy

Fig. 1. Termination of early pregnancy with RU 486 by different routes of administration in the rat. The compound was given on days 6, 7 and 8 of pregnancy. Autopsy was done on day 9 and the number of implantation sites was counted. Data were expressed as a percentage of termination of pregnancy. The number of animals used is indicated in parentheses.

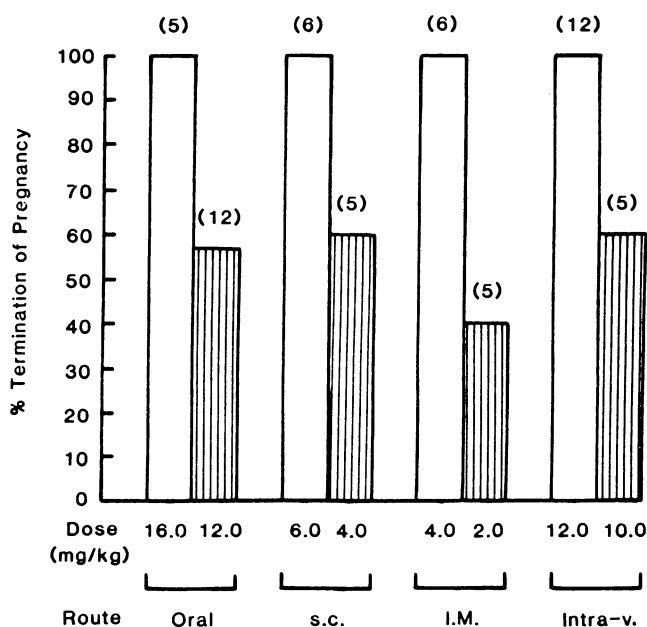
Table III. The Relative Anti-Progesterone and Anti-Cortisol Potencies of RU 486 Administered by Different Routes to Pseudopregnant Rats

Route	Relative anti-progesterone activity (%)*	Relative anti-cortisol activity (%)*
Oral	100	100
Subcutaneous	400	7.7
Intravaginal	200	0

\* The relative potencies of RU 486 by different routes were expressed as the percentage of the activity of RU 486 administered orally.

#### Experiment 2: Termination of Early Pregnancy with RU 486 by Different Routes of Administration in the Rat

The effects of RU 486 on early pregnancy when administered by oral, subcutaneous, intramuscular, and intravaginal routes are illustrated in Figure 1. Implantation was completely inhibited by the compound given on days 6, 7, and 8 of pregnancy by either oral (8 mg/kg), subcutaneous (2mg/kg), intramuscular (1.5 mg/kg), or intravaginal (10 mg/kg) routes. The



Treatment: D<sub>6</sub> of Pregnancy

Fig. 2. Pregnancy terminating effect of RU 486 by a single dose in the rat. The compound was administered on day 6 of pregnancy. Autopsy was done on day 9 and the number of implantation sites counted. Data were expressed as a percentage of termination of pregnancy. The number of animals used is indicated in parentheses.

Table IV. The Relative Potencies of RU 486 in Terminating Early Pregnancy by Different Routes of Administration in the Rat

Route	Relative potency (oral route = 1)	
	Multiple doses*	Single dose**
Oral	1	1
Subcutaneous	4	2.7
Intramuscular	5.3	4
Intravaginal	0.8	1.3

\* RU 486 was administered on days 6, 7 and 8 of pregnancy.

\*\* RU 486 was administered on day 6 of pregnancy.

numbers of rats with implantation sites increased with decreasing doses. The numbers of implantation sites per animal with implantations did not differ significantly from those of control animals.

Figure 2 shows the results of the pregnancy terminating effects of RU 486 given in a single dose on day 6 of pregnancy. Implantation was completely inhibited when the compound was given orally, subcutaneously, intramuscularly, and intravaginally on day 6 of pregnancy at 16 mg/kg, 6 mg/kg, 4 mg/kg, and 12 mg/kg, respectively. Lower doses did not completely suppress implantation; the number of implantation sites in these animals was not reduced.

Table IV summarizes the relative potencies of RU 486 in terminating early pregnancy by different routes of administration. The pregnancy terminating activity of the compound by intramuscular or subcutaneous injection was significantly higher than that by oral administration, whether the compound was given in multiple doses or in a single dose. The relative activities of the compound given orally or intravaginally did not differ significantly in terminating pregnancy. The efficacy ratio for the compound at the most effective dose in terminating early pregnancy for intramuscular injection is 4, for subcutaneous injection 2.7, and for intravaginal application is 1.3, whereas the oral administration is assigned a value of 1

## DISCUSSION

Deciduoma formation tests for progestational activity in the ovariectomized pseudopregnant rat (Miyake, 1962) and thymolytic or anti-inflammatory activity measures glucocorticoid activities in the adrenalectomized immature male rat (Dorfman, 1962). In order to assess, in the same animal at the same time, the in vivo anti-progesterone and anti-glucocorticoid activities of RU 486, we have developed a test system in which a combination of a deciduoma formation assay, thymolytic assay, and an anti-inflammatory assay is employed in the ovariectomized and adrenalectomized pseudopregnant rat. With regard to deciduoma formation for progesterone activity, a dose of 2 mg/kg/day of progesterone is capable of inducing and supporting decidual cell reaction in adult ovariectomized pseudopregnant rats (unpublished data). Administration of cortisol (0.375 mg/kg/day) concomitant with progesterone in our test system produced less decidual response as judged by the weight of traumatized uterine horn. However, the weight of the traumatized uterine horn of the animal treated with progesterone and cortisol did not significantly differ from that of the

animal treated with progesterone alone. Regarding the combined thymolytic assay and anti-inflammatory assay for cortisol activity, the results obtained in the present test system demonstrated that a daily dose of 0.375 mg of cortisol given concomitantly with 2 mg of progesterone produces about a 30% involution of the thymus gland and an inhibition of about 45% in the granuloma formation around implanted cotton pellets. These values agree with those obtained in adrenalectomized immature male rats treated with cortisol alone (Dorfman et al., 1961; Dubin, 1955). The data clearly demonstrated that, under the present experimental conditions, cortisol does not interfere with progesterone action in the deciduoma formation assay, and progesterone does not antagonize cortisol action in the combined thymolytic and anti-inflammatory assay for cortisol action.

The present study demonstrates that the anti-cortisol but not the anti-progesterone activity of RU 486 is dependent upon the route of administration. The anti-cortisol activity of the compound can be differentiated from its anti-progesterone activity by subcutaneous injection or intravaginal application. RU 486 given orally inhibits both progesterone and cortisol action. At a dose of 4 mg/kg of the compound, for example, it exerts a 99% inhibition of decidual response. Concurrently, it produces about a 50% block of cortisol-induced thymus involution and about a 50% prevention of cortisol-induced anti-inflammatory activity. When the compound is given subcutaneously or intravaginally (at the most effective dose inhibiting progesterone action) it exerts little or no interference with cortisol action. The efficacy of RU 486 in inhibiting progesterone action is also dependent upon the route of administration. It is apparent that the compound exerts its highest efficacy as an antiprogesterone agent by subcutaneous injection or intravaginal application.

Early pregnancy in the rat can be terminated by RU 486 at either multiple doses or a single dose regimen by different routes of administration. Our findings in the rat confirm the previous reports that the compound given orally to pregnant rat terminates early pregnancy (Philibert et al., 1982). Moreover, the data obtained in the present experiments clearly demonstrate that RU 486 exerts its most potent antifertility action following intramuscular or subcutaneous injection by either multiple doses or a single dose.

RU 486 has been shown to have no overt estrogenic, androgenic, glucocorticoid or progestomimetic activity in rats (Philibert et al., 1981) and in monkeys (Healy, et al., 1983). The present study demonstrates that its efficacy in terminating early pregnancy can be increased by using a systemic route of administration.

#### SUMMARY

RU 486, a 19-norsteroid with substituted radicals in C<sub>17</sub> and C<sub>11</sub>, was assessed in vivo for its effects on progesterone and cortisol action under different routes of administration in the rat. A test system was developed in which a combination of deciduoma formation assay, thymolytic assay, and anti-inflammatory assay was employed in the ovariectomized and adrenalectomized pseudopregnant rat. RU 486 was given either orally, subcutaneously or intravaginally to progesterone- and cortisol-treated rats. A dose of 4 mg/kg/day of RU 486 given orally exerted both anti-progesterone and anti-cortisol activities. A lower dose of RU 486 was required by the subcutaneous or intravaginal routes to produce inhibition of progesterone action (e.g. 1.0 mg/kg/day by subcutaneous or 2.0 mg/kg/day by intravaginal route). Unexpectedly, these doses did not interfere with cortisol action.

In the rat, implantation was completely inhibited by multiple doses of RU 486 given on days 6, 7, and 8 of pregnancy either by oral (8 mg/kg/day), subcutaneous (2 mg/kg/day), intramuscular (1.5 mg/kg/day) or intravaginal (10 mg/kg/day) route. A single dose of RU 486 given on day six of pregnancy either by oral (16 mg/kg), subcutaneous (6 mg/kg), intramuscular (4 mg/kg) or intravaginal (12 mg/kg) route was also effective in terminating early pregnancy. The data indicate that, in rats, the anti-cortisol, but not the anti-progesterone, activity of RU 486 is dependent upon the route of administration and that RU 486 exerts its most potent antifertility effect following intramuscular or subcutaneous injection.

#### REFERENCES

- De Feo, V. J., 1963, Determination of the sensitive period for the induction of deciduoma in the rat by different inducing procedures, Endocrinology, 73:488.
- Dorfman, R. I., 1962, Corticoids, in: "Methods in Hormone Research, Volume II, Bioassay," R. I. Dorfman, ed., Academic Press, New York.
- Dorfman, R. I., Dorfman, A. S., Agnello, E. J., Figdor, S. K., and Laubach, G. D., 1961, Relative thymolytic activities of cortisol and prednisolone derivatives in the adrenalectomized rat, Acta Endocrinol. (Kbh), 37:343.
- Dubin, W. E., 1955, Anti-inflammatory activity of delta'-9alpha-fluorohydrocortisone acetate, Proc. Soc. Exp. Biol. Med., 90:115.
- Gravanis, A., Schaison, G., de Brux, J., George, M., Satyaswaroop, P. G., Baulieu, E. E., and Robel, P., 1984, Endometrial responses to the anti-progesterone steroid RU 486 in post-menopausal women, 7th International Congress of Endocrinology, Quebec City, Abstract 1183.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an anti-progesterone steroid (RU 486) in primate: Site of action, dose-response relationships, and hormonal effects, Fert. Steril., 40:253.
- Meyer, R. K., Stucki, J. C., and Aulsebrook, K. A., 1953, Effect of pregnancy and lactation on granuloma tissue formation and joint permeability in rats, Proc. Soc. Exp. Biol. Med., 84:624.
- Miyake, T., 1962, Progestational substances, in: "Methods in Hormone Research, Volume II, Bioassay," R. I. Dorfman, ed., Academic Press, New York.
- Philibert, D., Deraedt, R., and Teutsch, C., 1981, RU 486--A potent antiglucocorticoid in vivo, 8th International Congress of Pharmacology, Tokyo, abstract 1463.
- Philibert, D., Deraedt, R., Teutsch, C., and Tournemine, C., 1982, RU 486--A new lead for steroidal anti-hormones, 64th Annual Meeting of the Endocrine Society, San Francisco, Abstract 668.
- Yamazaki, I., 1982, Pregnancy terminating effect of a highly active LHRH agonist by vaginal application in rats, Endocrinology Japonica, 29:197.

## HISTOPHARMACOLOGY OF RU 486

J. Secchi, D. Lecaue, C. Tournemine and D. Philibert

Roussel Uclaf, 102-111  
Route de Noisy  
93230 Romainville, France

### SUMMARY

Histological studies were done by light and electron microscopy to improve biological tests used to evaluate the pharmacological properties of RU 486. The compound prevented the progesterone-induced appearance of giant mitochondria in uterine glandular cells in ovariectomized rats and did not show any progestogen-mimetic effect. Its antiprogestational activity was demonstrated by abortion in rats where RU 486-induced changes in the decidual cells could be observed, even prior to the changes caused by ovariectomy. The hypertrophic effect of RU 486 on the uterus of immature animals was distinguished from the hyperplastic effect of estradiol.

### INTRODUCTION

As previously described, pharmacological studies of RU 486 were carried out by means of different tests based on gross morphological changes induced by this compound on the target organs. Under these experimental conditions, observations were made about the organs; tissue-specific responses were not distinguished. In order to improve these pharmacological tests, histological techniques involving light and electron microscopy were used, and the early effects of the drug at cellular and subcellular levels were analyzed.

### ANTIPROGESTATIONAL ACTIVITY

The antiprogestational activity of RU 486 in the rat was established in two ways: RU 486 prevents the appearance of giant mitochondria in the uterine glandular cells of animals treated with progesterone. It also induces cellular disorders of decidual cells and abortion at the ninth day of gestation.

#### Giant Mitochondria

It has been shown that progesterone administered to ovariectomized rats induces the appearance of giant mitochondria in glandular uterus cells (Ljunkvist, 1971; Nilsson, 1975) (Figs. 1a, b). This hormonal effect was obtained without estrogen priming. Under the same experimental conditions

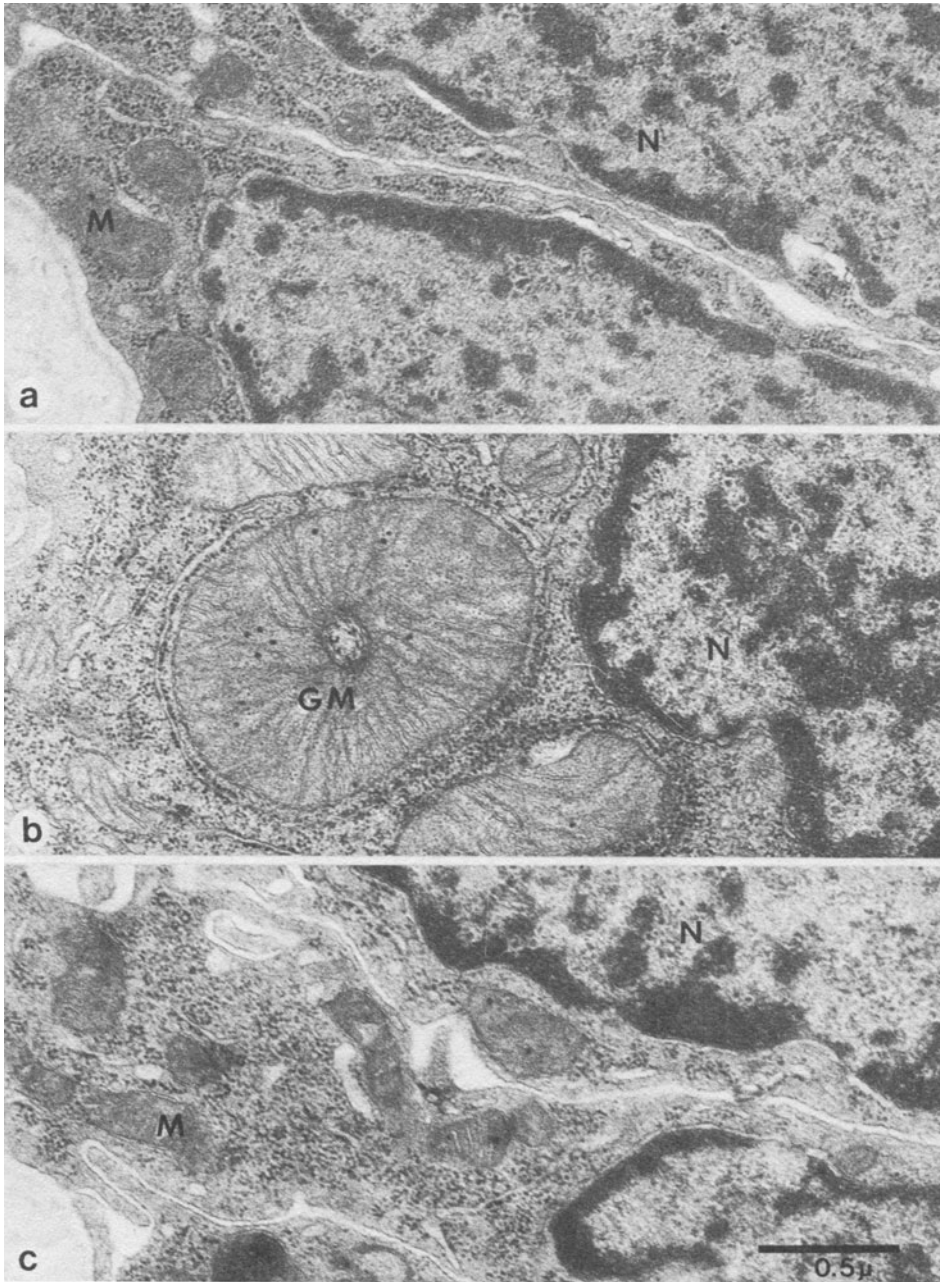


Fig. 1. Electron micrographs of uterine glandular cells in rats ovariectomized for 3 weeks. a) Control. m = mitochondria. b) Progesterone given at 20 mg/kg/day for 3 days induces the appearance of giant mitochondria (g.m.). c) RU 486 (10 mg/kg/day) administered simultaneously with progesterone prevents the appearance of giant mitochondria.

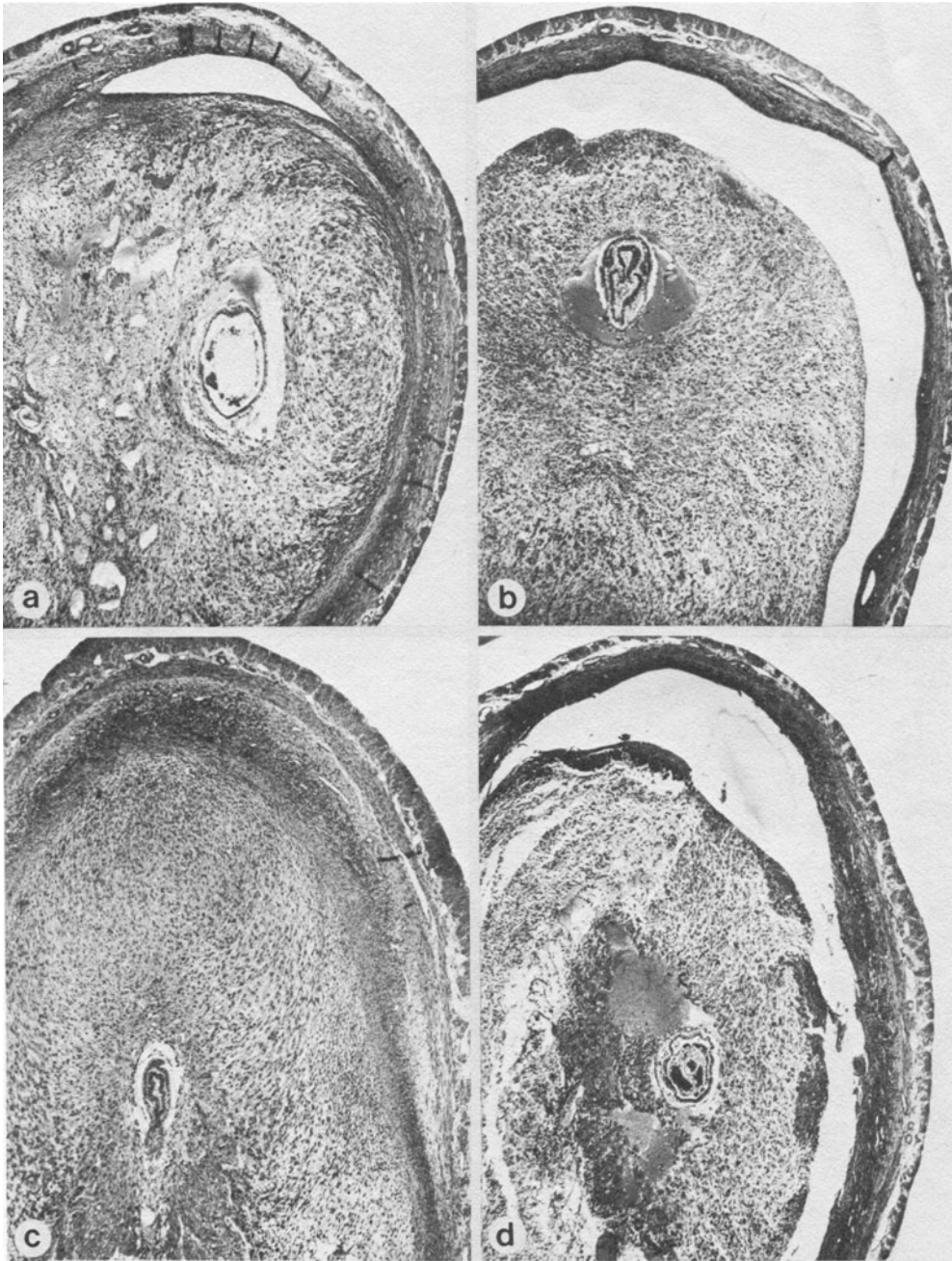


Fig. 2. Histological study of abortion in the rat. Pregnant rats were treated at day 9 of gestation with RU 486 (50 mg/kg/p.o.) and killed at the 6th, 8th, 10th and 16th hours. In comparison, a group of 9-day pregnant rats were ovariectomized and sacrificed at the same time. a) Hour 10 and b) hour 16 after RU 486. Note the necrotic aspect of the deciduoma as early as hour 10. c) Hour 10 and d) hour 16 after ovariectomy. At hour 10 any signs of necrosis are visible (G x 50).

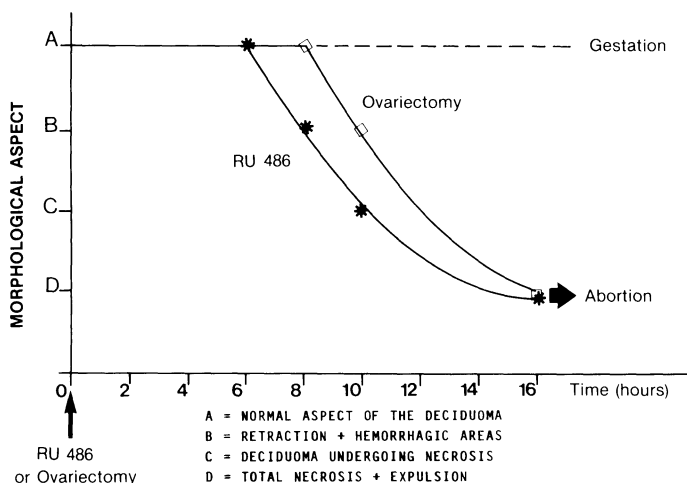


Fig. 3. Histological study of abortion in the rat: a graphic representation.

and by use of quantitative analysis on electron micrographs, we have demonstrated that there is an increase in the volume density of the entire mitochondrial population (Secchi et al., 1984; Secchi and Lecaque, 1984). This test, which is useful for evaluating the hormonal effect at the subcellular level, allows one to appreciate the antiprogestational activity of RU 486.

The administration of RU 486 and progesterone (0.3 and 3 mg/kg/day, respectively) for three days to 3-week-old ovariectomized rats prevented both volume density increase in the mitochondrial population and development of giant mitochondria (Fig. 1c). Moreover, under these experimental conditions, the morphological aspect of the glandular cells was similar to that of the control. RU 486 never showed a progestogen-mimetic effect when it was administered in place of progesterone.

#### Abortion in Rats

Progestogens are absolutely essential for the maintenance of pregnancy in all mammals. In some species (monkey, human), the placenta is the primary source of progesterone; but, in the rat and mouse, ovarian progestagens are required during the greater part of pregnancy. Ovariectomy induces abortion in these rodents until the 14th day of pregnancy. Thus, in our experiments, the antiprogestational activity of RU 486 was tested on pregnant rats and compared to the early effects of ovariectomy (for procedures, see Fig. 2 legend).

There were no observed microscopic changes in the treated and ovariectomized animals occurring before six hours. In the animals given RU 486, a retraction of the whole deciduoma from the uterine wall was visible at the eighth hour. At higher magnification, numerous decidual cells showing pycnotic nuclei were visible in the same animals.

Small hemorrhagic areas were observed around the embryo. At the tenth hour, the deciduoma in the RU 486-treated animals were undergoing necrosis and were filled with polymorphonuclear infiltrates and hemorrhagic areas (Figs. 2a, 3). The structure of the embryos seemed partially disorganized. In the ovariectomized rats, the decidual cells were elongated in shape with occasionally pycnotic nuclei, but no sign of necrosis was visible in the

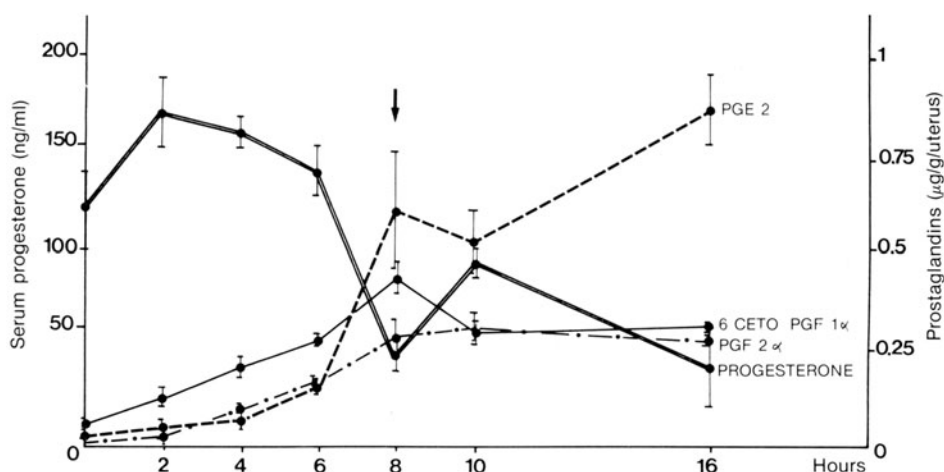


Fig. 4. Serum progesterone and uterine prostaglandin levels.

deciduoma (Figs. 2c, 3). At the 16th hour, the deciduoma in both RU 486-treated and ovariectomized rats showed necrotic aspects and were almost separated from the uterine walls (Figs. 2b, d, 3). In both groups, embryogenesis was disrupted, and the embryos were floating in large hemorrhagic areas.

These observations show that the effects of RU 486 on the deciduoma occur earlier than those induced by ovariectomy and that later embryonic lesions may be due to deciduomal changes rather than the compound's direct effect. Measurements of serum progesterone levels were made by RIA, and uterine-prostaglandin levels were determined according to the method described by Takekuchi et al. (1971).

At the eighth hour, when the first cellular disorders were observed, we noted a striking decrease in the serum progesterone level (Fig. 4). This low progesterone level was maintained until the 16th hour. Uterine prostaglandins showed a regular increase as early as the second hour; this increase was particularly marked for PGE<sub>2</sub>. We cannot conclude that the abortifacient effect of RU 486 is dependent on a decrease in serum progesterone levels or that the modified levels of prostaglandins are responsible for the abortion. The severe cellular lesions observed by light microscopy at the eighth hour lead one to hypothesize that earlier subcellular alterations appear in the deciduoma cells. If this is so, prostaglandins cannot be directly involved in the mechanism of abortion, and the increase in these prostaglandins would have to be the result of the cellular changes.

#### UTEROTROPHIC ACTIVITY

The evaluation of the uterine weight increase of immature rats and rabbits served as a convenient bioassay to test the uterotrophic activity of RU 486 as compared to estradiol. In this study, the tissular components involved in the uterine hypertrophy remained unknown. A semi-quantitative analysis of paraffin uterine sections allowed us to dissociate the activities of both compounds.

#### Experiments Using Rats

Uterine surfaces and luminal epithelial layer thicknesses were measured, and the number of cells undergoing mitosis in different areas of the uterus

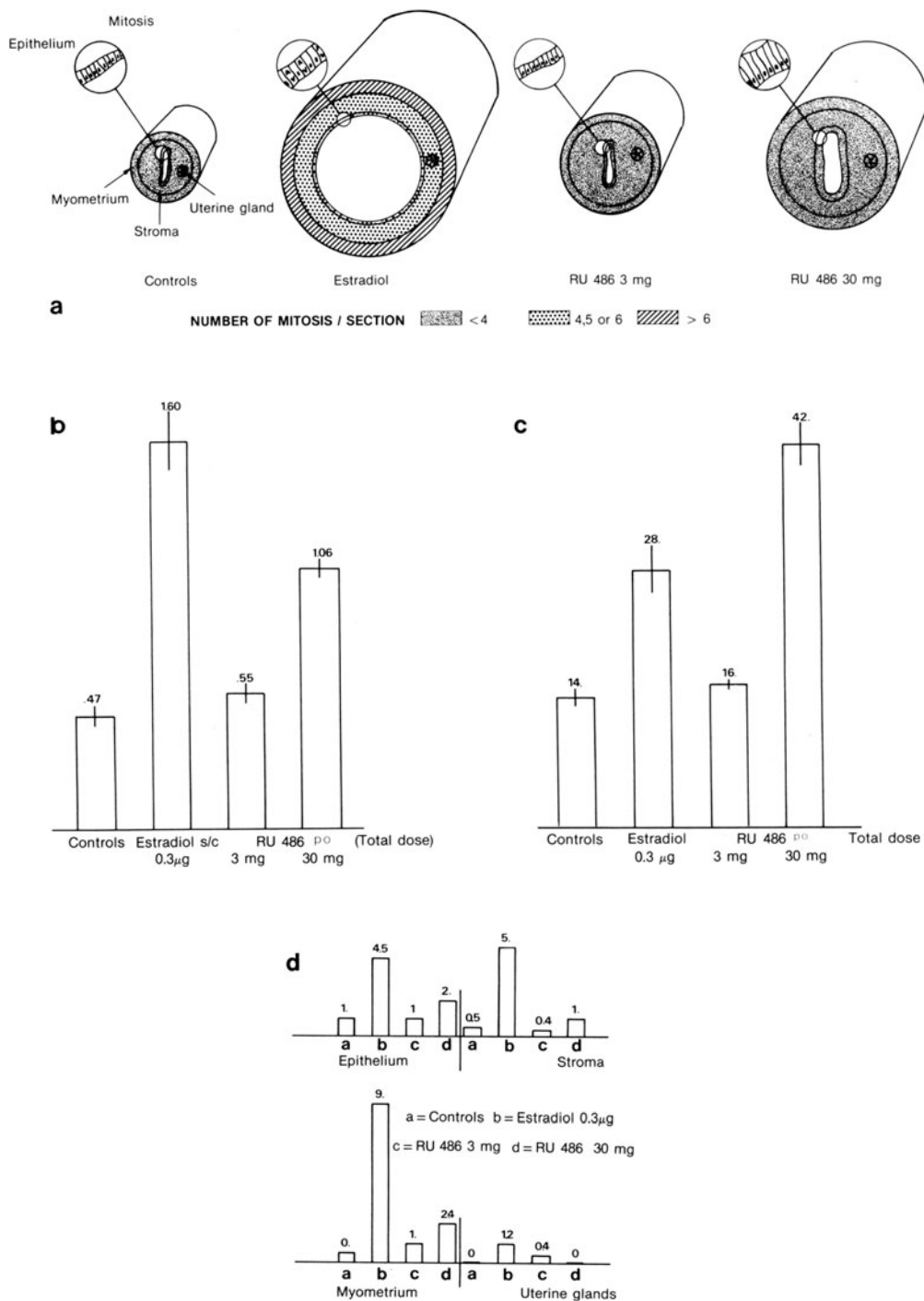


Fig. 5. Histological study of uterotrophy in the rat. a) Drawings of uterine horn sections in immature rats treated with estradiol (0.3 µg/kg/day s.c.), and RU 486 (3-30 mg/kg/day p.o). b) Measurements of transverse sections of the uterine horn (mm<sup>2</sup>). c) Measurement of the thickness of the luminal epithelium (µm). d) Number of mitosis/section.

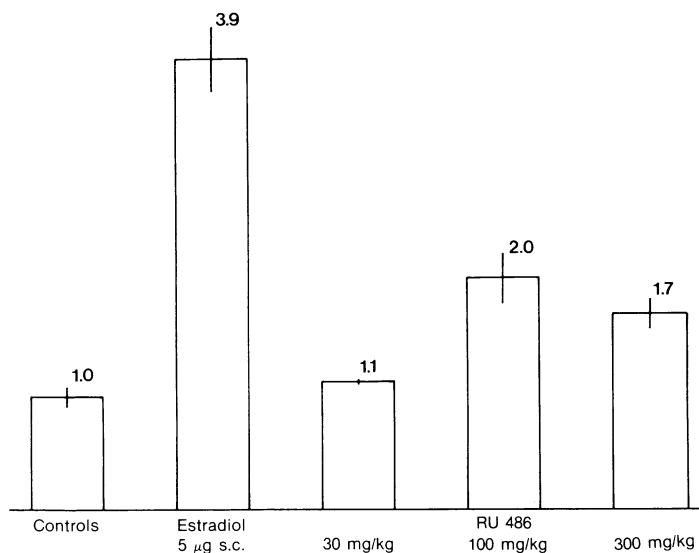


Fig. 6. Histological study of uterotrophy in the rabbit. The measurements of the uterine surface (mm<sup>2</sup>) on paraffin sections were made in immature rabbits treated with estradiol (5 µg/kg/day s.c.) or RU 486 (30-100-300 mg/kg/day p.o.) for 3 days.

was counted. An important size increase with estradiol was observed consecutively with hypertrophy of the stroma and myometrium, (Fig. 5a). The luminal epithelium was high and the lumen enlarged, giving a dilated appearance to the uterine horns. After RU 486 treatment, hypertrophy was dose-related and affected all tissue layers (Figs. 5a, b). At higher RU 486 doses, the uterine size increase was less than with estradiol. At 30 mg/kg, we observed a striking increase in the thickness of the epithelial layer surrounding the lumen (Fig. 5c). Unlike the estradiol-treated horns, epithelial cells did not show many cells undergoing mitosis (Fig. 5d). These observations indicate that the hypertrophic changes induced by estradiol and RU 486 seem to be quite different from one another. Estradiol's hyperplastic effect, clearly demonstrated by the high rate of mitosis in all uterine layers, can be distinguished from the hypertrophic activity of RU 486, which principally affects the luminal epithelium.

#### Experiments using Rabbits

The measurement of uterine horn surface sections, using the Rubin test, allowed us to appreciate in a quantitative manner the significant increase in uterine size (Fig. 6). The estradiol-treated rabbits showed uterine surface increases about four times greater than those of the controls. In RU 486-treated animals, the increase plateaued at a dose of 100 mg/kg, with the increase only two times greater than those of the controls. Other changes observed with estradiol treatment (thickened luminal epithelium, increased number of mobile cells in the stroma and mitosis in the myometrium) are not visible in animals receiving RU 486.

#### CONCLUSION

These histological studies give further information about the biological activities of RU 486. At the subcellular level, the compound's anti-progestational effect is clearly demonstrated, affecting the mitochondria of

the rat uterine glandular cells without revealing progestogen-mimetic activity. Administration of RU 486 is effective in inducing rat abortions earlier in pregnancy than progesterone deprivation by ovariectomy. RU 486-induced hypertrophy of the uterus is less severe than estradiol-induced hypertrophy and, in particular, acts on the epithelial layer. Unlike estradiol, RU 486 does not increase the mitotic rate of uterine cells.

#### REFERENCES

- Ljunkvist, I., 1971, Attachment reaction of rat uterine luminal epithelium. II. The effect of progesterone on the morphology of the uterine glands and the luminal epithelium on the spayed, virgin rat, Acta Soc. Med. Uppsalien, 76:110.
- Nilsson, O., 1975, Influence of progesterone on the mitochondrial size in the uterine glands of the rat, mouse, hamster and guinea pig, Acta Endocr., 78:349.
- Secchi, J., Lecaue, D., and Philibert, D., 1984, In vivo effects of progesterone on mitochondria in the glandular uterine cells: a quantitative analysis, Biology of the Cell, 51:18a.
- Secchi, J., and Lecaue, D., 1984, Effects of progestins and antiprogestins on mitochondria in uterine glandular cells in the rat. A quantitative investigation, Cell and Tissue Res., 238:247.
- Tagekuchi, C., Kuhno, E., and Sih, G. J., 1971, Mechanism of prostaglandin biosynthesis. I. Characterization and assay of bovine prostaglandin synthetase, Biochemistry, 10:2372.

## BIOCHEMICAL PROFILE OF RU 486

M. Moguilewsky and D. Philibert

Centre de Recherches Roussel Uclaf  
93230 Romainville, France

### ABSTRACT

The binding characteristics of RU 486 with the glucocorticoid receptor (GR) and the progesterin receptor (PR) were studied in order to explain the potent antiglucocorticoid and antiprogesterin activities of the compound. In vitro, ( $^3\text{H}$ )RU 486 bound to the same cytosol receptors as ( $^3\text{H}$ )dexamethasone (GR) and ( $^3\text{H}$ )R 5020 (PR); the sedimentation coefficients, number of binding sites and specificity were similar. However, the affinity of ( $^3\text{H}$ )RU 486 for these receptors was higher than that of the potent agonists, as indicated by the high affinity constant determined from Scatchard plots and by the slow dissociation rates from the GR and PR. With the cytosol receptor under heat activation, the agonists were able to give rise to more stable complexes, but ( $^3\text{H}$ )RU 486 dissociated faster from the activated than from the non-activated GR. This impeded activation of the ( $^3\text{H}$ )RU 486-GR complex was confirmed by observations of its lower affinity than that of ( $^3\text{H}$ )dexamethasone-GR complex for DNA-cellulose and by a low retention in the nucleus that may be related to RU 486's lack of glucocorticoid activity. Conversely, the uterine ( $^3\text{H}$ )RU 486-PR complex did not undergo an acceleration of dissociation rate under heat activation, and its affinity for DNA-cellulose was similar to that of the activated ( $^3\text{H}$ )R 5020-PR complex. This led to a high level of nuclear retention unrelated to the lack of progesterin activity of RU 486. In contrast to in vitro interaction, high in vivo doses of RU 486 were needed to interact with the cytosol receptors in the rat; this cannot be explained by a binding to rat plasma protein.

### INTRODUCTION

RU 486 has been proven to fully antagonize the action of glucocorticoids and progestins (Philibert, 1984; Philibert et al., this volume) in different in vitro and in vivo models. The compound exhibits no agonistic activity in most of the systems tested. These potent antiglucocorticoid and antiprogesterin effects are related to the inhibition by low concentrations of RU 486 of the binding of ( $^3\text{H}$ )dexamethasone and ( $^3\text{H}$ )R 5020 to their specific cytosol receptors, respectively the GR and the PR. However, in spite of this strong interaction with cytosol receptors, the lack (or weakness) of agonistic activity by RU 486 remained unexplained.

The availability of radioactive RU 486, tritiated with a high specific activity, allowed us to study its binding characteristics with cytosol

receptors, as compared to glucocorticoid or progestin agonists. We wanted to ascertain whether this interaction involved RU 486's binding directly to the GR and PR. In addition we wanted to investigate its ability to promote the subsequent steps leading to the biological response, i.e. "activation" of the receptor and "nuclear translocation" in tissues where RU 486 has been shown to exert true antihormonal effects.

In order to explain the discrepancies between the in vivo and in vitro ratios of RU 486:agonist concentrations needed to antagonize the hormonal effects (Philibert et al., this volume), we studied both the interaction of RU 486 with the cytosol steroid binding sites after in vivo administration of the compound, as well as plasma binding.

## RESULTS

### In Vitro Interaction of RU 486 with Steroid Hormone Receptors

Relative binding affinities for cytosol receptors (Table I). RU 486 displayed a very high relative binding affinity (RBA) for the rat thymus GR and the rabbit uterus PR. After 24 hours of incubation at 0°C, its RBA was much higher than that of the natural hormones corticosterone (31 for the GR) and progesterone (100 for the PR). RU 486's RBA was at least as high as that of potent agonists such as dexamethasone (GR) and R 5020 (PR), 100 and 530 respectively. The RBA of RU 486 for the androgen receptor (AR) in the rat prostate was about 1/4 that of testosterone; no interaction with the rat mineralocorticoid and the mouse estrogen receptor was detectable.

Binding characteristics of (<sup>3</sup>H)RU 486 with the rat cytosol GR and PR. Sedimentation coefficients (Fig. 1): Following ultracentrifugation, on a sucrose gradient, of rat thymus cytosol incubated either with (<sup>3</sup>H)RU 486 or (<sup>3</sup>H)dexamethasone (Fig. 1a), a similar marked radioactivity peak was observed in the 9S region. Addition of 100 fold-excess of radioinert dexamethasone nearly abolished the (<sup>3</sup>H)RU 486 peak. Similar studies performed with uterine cytosol (Fig. 1b) showed that (<sup>3</sup>H)RU 486 formed a complex with the PR that sedimented in the same 8S zone as (<sup>3</sup>H)R 5020 and that cold R 5020 displaced the (<sup>3</sup>H)RU 486 peak.

Table I. Relative Binding Affinities (RBAs) of RU 486 for the Cytosol Steroid Receptors

Receptor (tissue)	GR (rat thymus)	PR (rabbit uterus)	AR (rat prostate)	MR (rat kidney)	ER (mouse uterus)
	24 h 0°C	24 h 0°C	24 h 0°C	24 h 0°C	24h 0°C
Radioligand	<sup>3</sup> H dexamethasone	<sup>3</sup> H R 5020	<sup>3</sup> H R 1881	<sup>3</sup> H aldosterone	<sup>3</sup> H estradiol
RBA of RU 486	300	530	23	< 0.1	< 0.1

The RBAs for glucocorticoid (GR), progestin (PR), androgen (AR), mineralocorticoid (MR) and estrogen (ER) receptors, were measured in a routine screening assay, as previously described (Raynaud et al., 1975; Ojasso and Raynaud, 1978). The RBAs of dexamethasone, progesterone, testosterone, aldosterone and estradiol for GR, PR, AR, MR and ER, respectively, were taken to be equal to 100. Each value is the mean of 3 determinations.

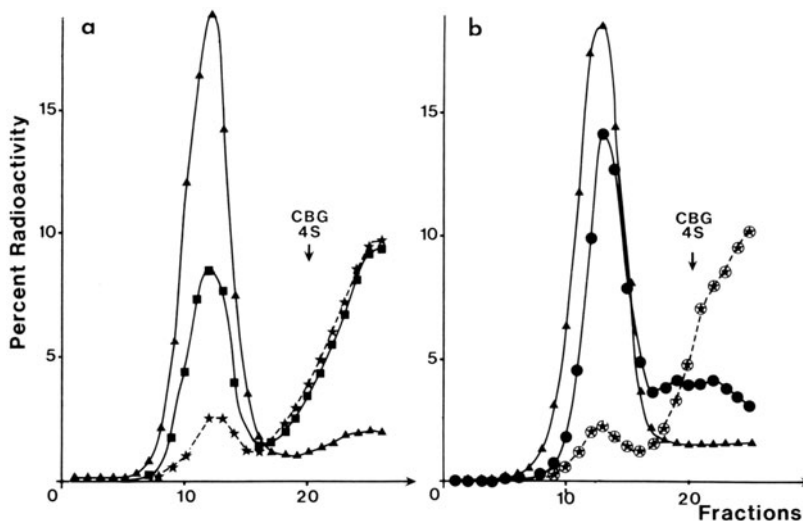


Fig. 1. Sucrose gradient analysis of ( $^3\text{H}$ )RU 486 binding to rat thymus (a) and uterus (b) cytosols. Thymus from adrenalectomized (ADX) male rats (a) or uterus from estrogen-primed (10  $\mu\text{g}$  estradiol s.c. 40 h before sacrifice) ovariectomized (OVX) and ADX female rats (b) were homogenized in 10 volumes (W/V) of buffer (Tris-HCl 10 mM, EDTA 1.5 mM, mercaptoethanol 1.5 mM, sodium molybdate 10 mM). Cytosols (105,000 g 1h supernatants) were prepared (for uterus, 100 nM dexamethasone was added during centrifugation in order to mask glucocorticoid binding sites) and incubated for 2 h at  $0^\circ\text{C}$  with ( $^3\text{H}$ )RU 486 (triangles), ( $^3\text{H}$ )dexamethasone (squares), ( $^3\text{H}$ )R 5020 (circles), or ( $^3\text{H}$ )RU 486 in the presence of 100 nM of dexamethasone (left) or R 5020 (right). Two hundred  $\mu\text{l}$  aliquots were deposited on a linear 5-20% sucrose gradient, prepared in the homogenization buffer, and ultracentrifuged in a VTI 65 rotor for 1 h at 65,000 rpm. The radioactivity of 2-drop fractions collected from the bottom of the tubes was counted.

Binding parameters (Fig. 2): Scatchard plot analysis of ( $^3\text{H}$ )RU 486 binding to the thymus cytosol receptor (Fig. 2a) indicated that ( $^3\text{H}$ )RU 486 bound to the same number of glucocorticoid binding sites as ( $^3\text{H}$ )-dexamethasone, whereas its association constant ( $K_a = 4 \times 10^8 \text{M}^{-1}$ ) was about 3 times higher than that of dexamethasone ( $K_a = 1.4 \times 10^8 \text{M}^{-1}$ ). The association constant of ( $^3\text{H}$ )RU 486 for the rat uterus progesterin receptor ( $K_a = 1.8 \times 10^9 \text{M}^{-1}$ ; Fig. 2b) was 2 times higher than that of the potent agonist ( $^3\text{H}$ )R 5020 ( $K_a = 0.9 \times 10^9 \text{M}^{-1}$ ; Fig. 2b).

Dissociation rate from the GR and PR (Fig. 3): The rate of dissociation of ( $^3\text{H}$ )RU 486 from the rat thymus cytosol GR at  $0^\circ\text{C}$  (Fig. 3a) was much slower than that of the potent agonist ( $^3\text{H}$ )dexamethasone and of the natural hormone ( $^3\text{H}$ )corticosterone, since the half-lives ( $t_{1/2}$ ) of these 3 ( $^3\text{H}$ )steroid-GR complexes were more than 100 hours, 16 hours and 150 min, respectively. Similarly, the dissociation rate of ( $^3\text{H}$ )RU 486 from the rat uterine PR ( $t_{1/2} = 16\text{h}$ ) was much slower than that of ( $^3\text{H}$ )RU 5020 ( $t_{1/2} = 70\text{min}$ ) and progesterone ( $t_{1/2} = 10\text{min}$ ; Fig. 3b).

These biochemical binding characteristics of ( $^3\text{H}$ )RU 486 with the thymic or uterine cytosol appear to suggest that ( $^3\text{H}$ )RU 486 binds to the same receptors as ( $^3\text{H}$ )dexamethasone (GR) and ( $^3\text{H}$ )R 5020 (PR).

Table II. Effect of Molybdate on the Dissociation Rate of Preheated Complexes Formed with Rat Thymus GR or Rabbit Uterine PR

Receptor	Steroid	Temperature of dissociation	Half-lives of the complex	
			Non activated (+ molybdate)	Activated (- molybdate)
Rat thymus GR	Dexamethasone	0°C	16 h	24 h
		25°C	49 min	70 min
	Cortisol	0°C	50 min	144 min
	Corticosterone	0°C	150 min	310 min
	Progesterone	0°C	70 min	70 min
	RU 486	25°C	150 min	70 min
Rabbit uterus R 5020 PR		0°C	15 h	22 h
		22°C	20 min	47 min
	Progesterone	0°C	100 min	160 min
	RU 486	22°C	95 min	85 min

Thymuses from ADX male rats or uteri from estrogen-primed female immature rabbits (25 ug estradiol s.c. 96 h before sacrifice) were homogenized in 10 (thymus) or 25 (uterus) volumes (w/v) of buffer (Tris-HCl 10 mM, sucrose 0.25 M, pH = 7.4; dithiothreitol 2 mM for thymus only). Cytosols were prepared and incubated with 20 nM (<sup>3</sup>H) steroid overnight at 0°C. The "activation" process was performed by heating the labeled cytosol for 30 min at 25°C. Unbound radioactive steroid was then removed by vortexing the labeled cytosol at 4°C with 10% (v/v) of 6.25% charcoal (Norit A) and 3.125% Dextran in buffer, and centrifuging it at 1500 g for 10 min. The "activated" complex was heated in the absence of molybdate, while the "native" or "non-activated" complex was heated in the presence of molybdate. The dissociation of (<sup>3</sup>H)steroid at the specified temperature was then studied as described in Fig. 3.

In order to evaluate whether this strong interaction with the cytosol receptor was able to promote the essential biochemical steps leading to the biological response, we then measured the ability of RU 486 to "activate" the cytosol receptor and to translocate it into the nucleus. These studies were performed on rabbit uterine PR, since the dissociation rate of the PR complex is slower than that in the rat and since endometrial cells are easier to prepare in this species. The GR was studied in the rat.

#### Comparison of Effects of RU 486 and Agonists on the Activation Process of Cytosol GR and PR.

Effect of heat activation on the dissociation rates of RU 486 and agonists from GR and PR: we compared the rates of dissociation of "heat-activated" and "non-activated" complexes formed with GR or PR and (<sup>3</sup>H)RU 486 or different glucocorticoid or progestin agonists (Table II).

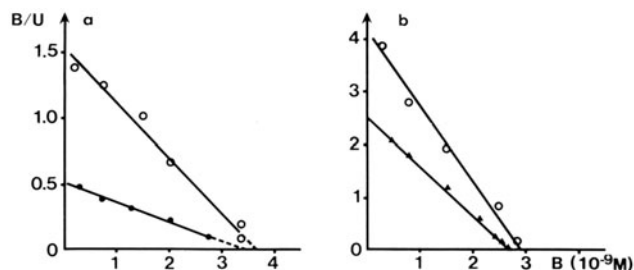


Fig. 2. Scatchard plot analysis of ( $^3\text{H}$ )RU 486 binding to rat thymus (a) and uterus (b) cytosols. Cytosols were prepared as described in Fig. 1, except buffer (Tris-HCl 10 mM, sucrose 0.25 M, pH = 7.4; dithiothreitol 2 mM was added only for thymus) and incubated for 4 h at  $0^\circ\text{C}$  with increasing concentrations of ( $^3\text{H}$ )RU 486 (O-O), ( $^3\text{H}$ )dexamethasone (circles) or ( $^3\text{H}$ )R 5020 (triangles) in the absence or presence of 500 fold excess of the corresponding radioinert steroid. Bound (B) and unbound (U) steroid were measured by the Dextran Coated Charcoal Adsorption (DCCA) technique (Raynaud et al., 1975; Ojasoo and Raynaud, 1978).

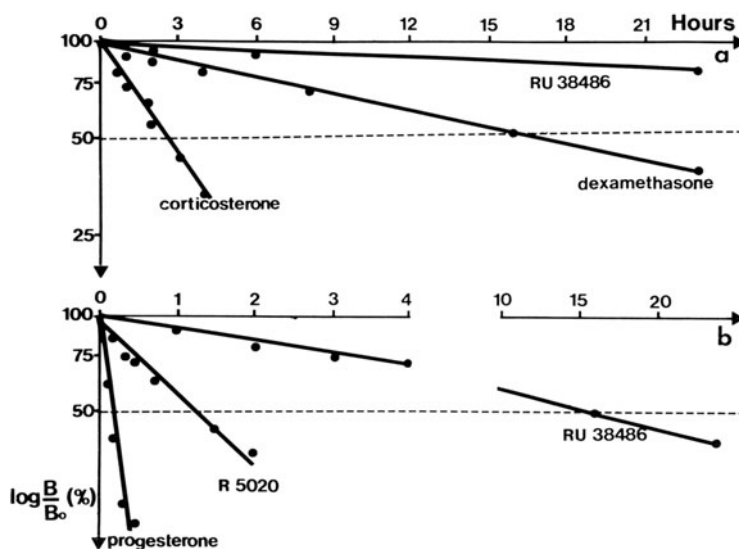


Fig. 3. Dissociation rate of ( $^3\text{H}$ )RU 486 from rat thymic GR (a) and rat uterine PR (b). Cytosols were prepared as in Fig. 2 and incubated with 10 nM ( $^3\text{H}$ ) steroid for 2 h at  $0^\circ\text{C}$ . The dissociation of the ( $^3\text{H}$ )steroid receptor complex was initiated by addition of 1000 fold excess of the corresponding radioinert steroid (time 0) at  $0^\circ\text{C}$ . After different times, bound radioactivity (B) of 0.1 ml samples was measured by the DCCA technique. ( $B_0$  = bound radioactivity at time 0.) Non-specific binding was estimated by performing parallel incubations of cytosols with 10 nM ( $^3\text{H}$ ) steroid and 1  $\mu\text{M}$  unlabeled steroid.

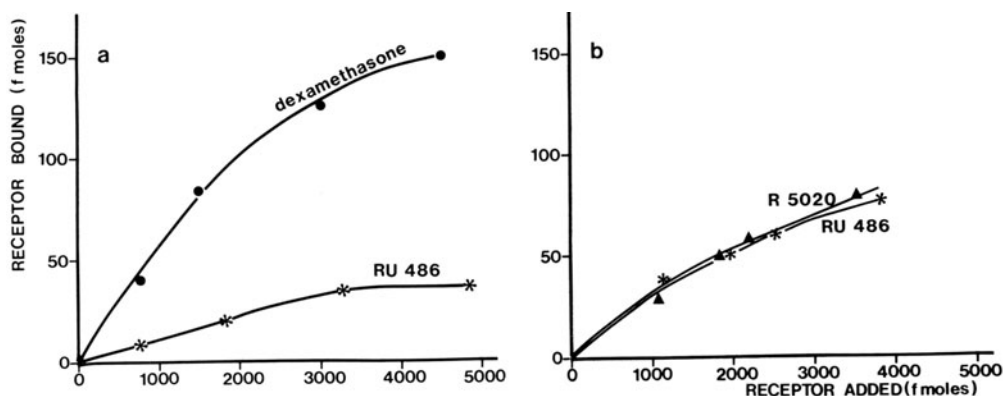


Fig. 4. Binding of the heat-transformed ( $^3\text{H}$ )RU 486-GR (a) or -PR (b) complex to DNA-cellulose. Cytosols from rat thymus (a) or rabbit uterus (b) were prepared in the absence of molybdate, incubated and "activated" by heating as described in Table II. Two hundred  $\mu\text{l}$  of cytosol (containing increasing amounts of activated ( $^3\text{H}$ )steroid receptor) were then incubated with 200  $\mu\text{l}$  of DNA-cellulose (8  $\mu\text{g}$  DNA) for 45 min at  $0^\circ\text{C}$  with gentle shaking. At the end of the incubation, 2 ml of ice-cold buffer (Tris-HCl 10 mM, EDTA 1 mM, pH = 7.4) were added, mixed and centrifuged for 5 min at 600 g. The washing procedure was repeated 3 times, then the final DNA-cellulose pellet was dissolved in 15 ml scintillation fluid for  $^3\text{H}$  measurements. Controls were taken using plain cellulose to correct for the small amount of binding of complex to cellulose, and with cytosol incubated in the presence of a 100 fold excess of non-radioactive steroid to determine the non-specific binding to DNA-cellulose.

Although the half-lives of the complexes formed by ( $^3\text{H}$ )dexamethasone, ( $^3\text{H}$ )corticosterone or ( $^3\text{H}$ )cortisol with GR were very different, all three glucocorticoid agonists dissociated much more slowly from the activated than from the non-activated complex. In contrast, RU 486 dissociated much more rapidly from the activated than from the non-activated GR, indicating that the heat-transformed RU 486-GR complex was less stable than the non-activated complex. ( $^3\text{H}$ )Progesterone, a partial agonist/antagonist, dissociated at the same rate from both the activated and non-activated GR.

Similarly, progestin agonists, i.e. ( $^3\text{H}$ )R 5020 and ( $^3\text{H}$ )progesterone, formed a complex with the progestin receptor, dissociating at a slower rate after heat activation than in the presence of molybdate (Table II). Conversely, ( $^3\text{H}$ )RU 486 dissociated at least as fast from the activated as from the non-activated receptor.

Therefore, in contrast to agonists that dissociated more or less rapidly from the GR or PR while forming by activation a complex much more stable, RU 486 dissociated very slowly from the non-activated GR or PR, but the complex was transformed by heat in a less stable form.

Since binding to polyanions has been taken as a test for receptor activation (Kalimi et al., 1975; Schmidt and Litwack, 1982), we then studied the binding of the heat-activated RU 486-PR or -GR complexes to DNA-cellulose.

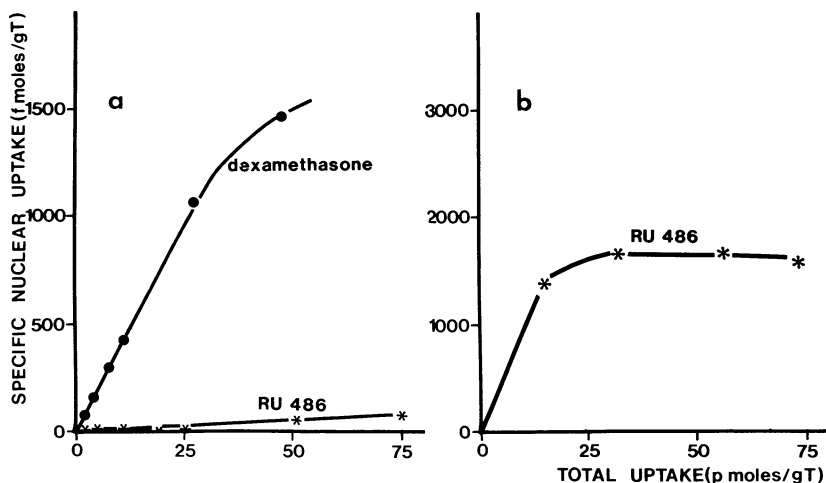


Fig. 5. In vitro specific nuclear uptake of  $(^3\text{H})$ RU 486 in rat thymus (a) or rabbit uterus (b). Rat thymuses were pooled and finely minced with scissors. Rabbit uteri were scraped with a razor blade to prepare endometrial cells. Aliquots of minced thymus or endometrial cells were incubated in 2 ml Krebs' solution containing increasing concentrations of  $(^3\text{H})$  steroid, in the presence or absence of a 100-fold excess of the corresponding radioinert steroid for 2 h at  $25^\circ\text{C}$ . After incubation, minces or cells were washed twice with 2 ml ice cold Krebs and homogenized in 2 ml of 0.32 M sucrose, K phosphate (pH = 6.5),  $\text{MgCl}_2$  3 mM, and 0.25% Triton X 100 (v/v). Purified nuclei were isolated by the McEwen and Zigmond procedure (1972). Radioactivity was extracted from aliquots of the homogenate (total uptake) and from the final nuclear pellet (nuclear uptake) by 15 ml of toluene containing 0.4% omnifluor (w/v). Specific nuclear uptake was the difference between the radioactivity measured in the absence and in the presence of an excess of radioinert steroid. The purity of nuclear preparation was assessed by contrast phase microscopy.

Binding of heat "transformed" complexes to DNA-cellulose (Fig. 4): in the presence of molybdate, neither the  $(^3\text{H})$ RU 486-GR or -PR complexes, the  $(^3\text{H})$ DM-GR nor the  $(^3\text{H})$ R 5020-PR complexes bound to DNA-cellulose. This was not affected by preheating. When heat transformation was promoted in the absence of molybdate, the  $(^3\text{H})$ RU 486-GR and -PR complexes were able to bind to DNA-cellulose. The affinity of the  $(^3\text{H})$ RU 486-GR complex was much lower than that of the  $(^3\text{H})$ DM-GR complex (Fig. 4a), whereas the affinity of the complex formed by  $(^3\text{H})$ RU 486 and the PR was similar to that of the activated  $(^3\text{H})$ R 5020-PR complex (Fig. 4b).

Nuclear uptake of  $(^3\text{H})$ RU 486 in thymus and uterus in vitro (Fig. 5). When thymic minces were incubated at  $25^\circ\text{C}$  with increasing concentrations of  $(^3\text{H})$ RU 486, a very low level of specific nuclear uptake was measured in purified nuclei after two hours (Fig. 5a) in comparison with that of  $(^3\text{H})$ dexamethasone measured in the same conditions. However, the total tissue incorporation of  $(^3\text{H})$ RU 486 was higher than that of  $(^3\text{H})$ dexamethasone. Similar observations were made at  $37^\circ\text{C}$  or at different times of incubation at  $25^\circ\text{C}$  (Moguilewsky and Philibert 1984).

In contrast, when endometrial cells of the rabbit uterus were incubated with ( $^3\text{H}$ )RU 486, a large amount of specific radioactivity uptake was recovered in purified nuclei (Fig. 5b).

#### IN VIVO INTERACTION OF RU 486 WITH STEROID HORMONE RECEPTORS

As predicted from *in vitro* studies, RU 486 was able to interact with the GR, PR and AR after oral administration to rats (Fig. 6), but, surprisingly, doses as high as 10 mg/kg were needed to completely occupy (or block the access to) glucocorticoid, progestin and androgen binding sites. Higher doses were necessary to interact with the GR in the hippocampus, while even at the higher dose tested (100mg/kg), RU 486 did not modify the concentration of mineralocorticoid binding sites (as predicted from the RBA value). This *in vivo* interaction of RU 486 with cytosol receptors is relatively weak compared to that of compounds such as dexamethasone, R 5020 or even testosterone, which occupy their specific receptors at doses lower than 50 ug/kg in spite of *in vitro* RBA values of the same order of magnitude as that of RU 486. This explains why much higher doses of RU 486 are needed to counteract dexamethasone or R 5020 *in vivo* than *in vitro* (Philibert, this volume).

RU 486 was able to interact with the uterine estrogen receptor (ER) at high doses in the rat, while no interaction with the mouse uterine ER was detectable *in vitro*.

#### PLASMA BINDING OF RU 486

In order to explain RU 486's loss of potency after *in vivo* administration (as compared to its *in vitro* activity), we investigated the

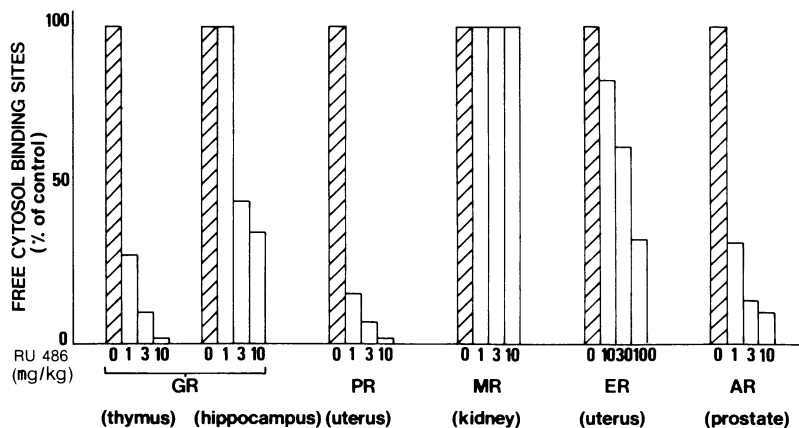


Fig. 6. Interaction of RU 486 with steroid hormone receptors after *in vivo* administration to rats. Estrogen-primed OVX and ADX female rats (10 ug estradiol s.c. 40 h before treatment) (for GR, PR, MR and ER), or castrated (CX) and ADX male rats (for AR), received an oral administration of increasing doses of RU 486. Rats were sacrificed 2 h later, and target organs were removed, homogenized in buffer (Tris-HCl 10 mM, sucrose 0.25 M (pH = 7.4), dithiothreitol 2 mM), and the cytosols (105,000 g supernatants) were incubated for short periods of incubation with specific radioligands (see Table I). The steroid binding sites remaining free in the cytosols were measured by the DCCA technique.

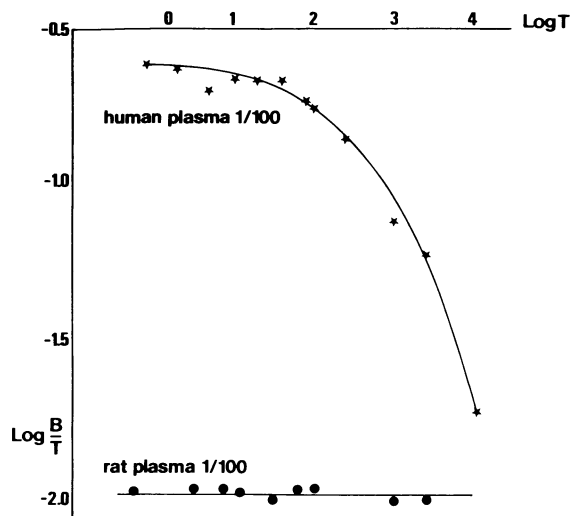


Fig. 7. Binding to rat and human plasma. Rat or human plasma diluted in 100 volumes of buffer (Tris-HCl 10 mM, sucrose 0.25 M (pH = 7.4)) was incubated for 4 h at 0°C with increasing concentrations of radioinert RU 486, in the absence or presence of increasing concentrations of radioinert RU 486. Bound radioactivity (B) was measured by the dextran-coated charcoal adsorption technique (DCCA), and the number of binding sites and the affinity constant were evaluated by the method of proportion graph (Baulieu and Raynaud, 1970).

binding of ( $^3\text{H}$ )RU 486 to the plasma of different species. No specific binding of ( $^3\text{H}$ )RU 486 was detected in rat, rabbit, guinea pig or monkey (*Maccaca fascicularis*) plasma (Fig. 7).

In contrast, specific binding was measured in human plasma (Fig. 7) with a high number of sites ( $N = 7 \text{ } \mu\text{mol/l}$ ) and an association constant ( $K_a$ ) of about  $4 \times 10^6 \text{ M}^{-1}$ . Both parameters were increased when the binding was measured by dialysis technique. This binding protein was neither transcortin nor Sex Binding Protein (SBP), because even at very high concentrations, radioinert RU 486 was unable to compete with ( $^3\text{H}$ )cortisol for transcortin or with ( $^3\text{H}$ )testosterone for SBP. Preliminary results indicate that it might be  $\alpha_1$  globulin. The role of this binding to human plasma on the subsequent activity of RU 486 is currently under investigation.

## DISCUSSION

The potent antagonistic activity of RU 486 to glucocorticoid and progestin hormones is related to the strong inhibition of the binding of these hormones to the GR and PR. This inhibition seems to be in direct competition with the GR and PR, since ( $^3\text{H}$ )RU 486 was shown to bind in rat thymic and uterine cytosols with the same number of sites that ( $^3\text{H}$ )dexamethasone or ( $^3\text{H}$ )R 5020 formed with the receptors. ( $^3\text{H}$ )RU 486 sedimented on a sucrose gradient in the same zone as these ( $^3\text{H}$ )agonists. The affinity of ( $^3\text{H}$ )RU 486 for these receptors was very high at 0°C and related to a very slow dissociation rate.

Such strong interaction with the cytosol receptors at 0°C is more characteristic of agonist compounds than antagonists (Raynaud et al., 1980). However, it was not followed by a heat "activation" to a more stable complex, as observed for glucocorticoid and progestin agonists in this study and by others (Wolfson et al., 1980; Seeley and Castas, 1983) and as previously shown for estrogens (Weichmann and Notides, 1977; Shyamala and Leonard, 1980). This stabilization of the receptor by agonists leads to an increased affinity for chromatin, whether the initial interaction with the native receptor takes place in the nucleus of the cell or in the cytoplasm (King and Greene, 1984; Welshons et al., 1984; Schrader, 1984). In contrast, the (<sup>3</sup>H)RU 486-GR complex was destabilized by heat activation, and this "transformed" or "poorly-activated" complex (as confirmed by the lower affinity for DNA-cellulose than the activated dexamethasone-GR complex) was weakly retained by the chromatin, as shown by the low level of specific incorporation of (<sup>3</sup>H)RU 486 into purified nuclei of thymus after in vitro incubation. The weak nuclear uptake of (<sup>3</sup>H)RU 486 in comparison to (<sup>3</sup>H)dexamethasone, also observed in HTC cells (Chasserot-Golaz and Beck et al., 1984) might be related to a lack of nuclear translocation of the transformed receptor or to a rapid dissociation of the nuclear complex during nuclear preparation; by using a different technique of nuclear isolation, some authors (Jung-Testas and Baulieu, 1984) have found higher concentrations of (<sup>3</sup>H)RU 486. This impaired activation, which can be related to the lack of glucocorticoid agonist properties of RU 486 in most systems tested, was also observed by other authors (Bourgeois et al., 1984).

Conversely, the uterine (<sup>3</sup>H)RU 486-PR complex did not undergo an acceleration of dissociation rate under heat activation but rather an absence of stabilization compared to progestin agonists. This effect was similar to that reported for triphenylethylene antiestrogens (Rochefort and Borgna, 1981) and like these latter, the heated (<sup>3</sup>H)RU 486-PR complex was able to bind to DNA-cellulose and to be retained in the nuclei of the rabbit uterus. However, contrary to what was observed in human endometrium (Gravanis et al., 1984), no progestomimetic activity of RU 486 could be detected in this organ (Philibert et al., this volume). Therefore, the reason for the inability of the RU 486-PR complex to trigger agonistic progestin effects might be localized in a further step in the mechanism of hormonal action, i.e. at the genomic level.

The discrepancy between the doses of RU 486 necessary to counteract the glucocorticoid and progestin activities in vivo and in vitro (2 to 10 times the concentration of dexamethasone or R 5020 in vitro against about 100 times in vivo) was related to an interaction of RU 486 with the cytosol receptors of target tissues after in vivo administration to rats, much lower than that expected from the affinity measured in vitro. High doses of RU 486 were needed to interact with the receptor in vivo and thus to impede the access of agonists and their biological effects. The need of such high doses cannot be explained by a binding to plasma protein in the rat, contrary to human, but rather by pharmacokinetics or metabolism of the compound (Deraedt et al., this volume).

#### REFERENCES

- Baulieu, E. E., and Raynaud, J. P., 1970, a "proportion graph" method for measuring binding systems, Europ. J. Biochem., 13:293.
- Bourgeois, S., Pfahl, M., and Baulieu, E. E., 1984, DNA binding properties of glucocorticosteroid receptors bound to the steroid antagonist RU 486, EMBO Journal, 3:751.
- Chasserot-Golaz, S., and Beck, G., 1984, an approach to the mechanism of the potent antiglucocorticoid: 17 $\beta$ -hydroxy-11 $\beta$ -4-dimethyl-aminophenyl-17 $\beta$ -propynyl-estra-4, 9-dien-3-one, submitted to J. Steroid Biochem.

- Deraedt, R., Bonnat, C., Busigny, M., Chatelet, P., Cousty, C., Mouren, M., Philibert, D., Pottier, J., and Salmon, J., Pharmacokinetics of RU 486, this volume.
- Gravanis, A., Schaison, G., George, M., Satyaswaroop, P. G., Baulieu, E. E., and Robel, P., 1985, Endometrial and pituitary responses to the anti-progesterone steroid RU 486 in post-menopausal women, J. Clinical Endocrinol. Metab., 60:156.
- Jung-Testas, I., and Baulieu, E. E., 1984, Anti-steroid action in cultured L-929 mouse fibroblasts, J. Steroid Biochem., 20:301.
- Kalimi, M., Colman, P., and Feigelson, P., 1975, the "activated" hepatic glucocorticoid receptor complex. Its generation and properties, J. Biol. Chem., 10:1080.
- King, W. J., and Greene, G. L., 1984, Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells, Nature, 307:745.
- McEwen, B. S., and Zigmond, R. E., 1972, Isolation of brain cell-nuclei, in: "Method in Neurochemistry," N. Marks and R. Rodnight, eds., Plenum Press, New York, pp 140-61.
- Moguilewsky, M., and Philibert, D., 1984, RU 38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation, J. Steroid Biochem., 20:271.
- Ojasso, T., and Raynaud, J. P., 1978, Unique congeners for receptor studies, Cancer Res., 38:4186.
- Philibert, D., 1984, RU 38486: an original multifaceted antihormone in vivo, in: "Adrenal Steroid Antagonism," K. W. Agarwal, ed., Walter de Gruyter & Co., Berlin and New York, pp 77-101.
- Philibert, D., Moguilewsky, M., Mary, I., Lecaque, D., Tournemine, C., Secchi, J., and Deraedt, R., Pharmacological profile of RU 486, this volume.
- Raynaud, J. P., Bonne, C., Bouton, M. M., Moguilewsky, M., Philibert, D., and Azadian-Boulanger, G., 1975, Screening for anti-hormones by receptor studies, J. Steroid Biochem., 6:615.
- Raynaud, J. P., Bouton, M. M., and Ojasoo, T., 1980, The use of interaction kinetics to distinguish potential antagonists from agonists, TIPS: 324.
- Rochefort, H., and Borgna, J. L., 1981, Differences between oestrogen receptor activation by oestrogen and antioestrogen, Nature, 292:257.
- Schrader, W. T., 1984, New Model for steroid hormone receptors, Nature, 308:17.
- Schmidt, T. J., and Litwack, G., 1982, Activation of the glucocorticoid receptor complex, Physiol. Rev., 62:1131.
- Seeley, D. H., and Costas, P. D., 1983, Transformation of a rabbit progesterone receptor from an 8S form to 5.5S and 4S forms, Mol. Cell. Endocr., 30:161.
- Shyamala, G., and Leonard, L., 1980, Inhibition of uterine estrogen receptor transformation by sodium molybdate, J. Biol. Chem., 255:6028.
- Weichman, B. M., and Notides, A. C., 1977, Estradiol-binding kinetics of the activated and nonactivated estrogen receptor, J. Biol. Chem., 252:8856.
- Wolfson, A., Mester, J., Chang-Ren, Y., and Baulieu, E. E., 1980, "Non-activated" form of the progesterone receptor from chick oviduct: characterization, Biochem. Biophys. Res. Commun., 95:1577.
- Welshons, W. V., Lieuerman, M. E., and Gorski, J., 1984, Nuclear localization of unoccupied oestrogen receptors, Nature, 307:747.

## RADIOIMMUNOASSAY OF RU 486

J. Salmon and M. Mouren

Centre de Recherches Roussel-Uclaf  
93230 Romainville, France

### SUMMARY

A rapid and sensitive radioimmunoassay for RU 486 has been developed. The straightforward assay procedure is described in detail.

An antigen was prepared by coupling bovine serum albumin with the 3-carboxymethyloxime of RU 486, and an anti RU 486 antiserum was produced in rabbits. Its specificity for 50% inhibition of maximum binding, is reported in Table I.

Sensitivity is about 10 pg/assay tube, and 100 pg of RU 486 reduce maximum binding by half its value. Non specific binding, measured with excess unlabelled RU 486 (100 ng), represents 4% of the added radioactivity. Using labelled RU 486, the recovery of radioactivity after diethyl ether extraction is  $89.3 \pm 1.3\%$  ( $n=9$ ).

### REAGENTS

A phosphate buffer containing 0.1% gelatin was prepared by dissolving 9 g NaCl, 1 g sodium azide and 1 g gelatin in 1 liter of 0.1M phosphate buffer, pH 6.9. It was stored at +4°C.

The anti-RU 486-3carboxymethyloxime-BSA antiserum was raised in New Zealand rabbits using methods previously described by Raynaud et al. (1974). It had been stable for several months at 4°C when diluted 1/100 in phosphate-gelatin buffer.

A stock solution of RU 486 at 0.1 mg/ml ethanol was stored at 4°C. For the standard curve, solutions of RU 486 were prepared just before use by dilution in the phosphate-gelatin buffer supplemented with 0.025% Triton X 100. They contain, respectively, 0, 3.91, 7.81, 15.63, 32.25, 62.5, 125, 250, 500, 1000 and 2000 pg/0.1 ml.

Tritiated RU 486 (specific activity = 37 Ci/mmol), stored at 4°C in ethanol, was diluted at the time of use in the phosphate-gelatin-Triton X 100 buffer at a concentration of 25,000 cpm per ml.

The charcoal suspension was composed of 250 mg charcoal (Norit A) and 25 mg dextran T70 (Pharmacia) for 100 ml of phosphate buffer without gelatin. Scintillation fluid was Dynagel (Baker).

Table I - Percentage of Cross-Reaction of Various Steroids  
with the Anti-RU 486 Antiserum (in the RIA Conditions)

RU 486	100
N-Didemethyl RU 486	84
N-Monodemethyl RU 486	60
Propargyl Alcohol RU 486	0.80
Progesterone	< 0.01
Testosterone	< 0.01
Cortisol	< 0.01
Desoxycorticosterone	< 0.01
17 $\beta$ -Estradiol	< 0.01
Estrone	< 0.01
Estriol	< 0.01
Dexamethasone	< 0.01

#### ASSAY PROCEDURE

The standard curve ranged from 0.04 to 20 ng/ml (Fig. 1), and plasma samples had to be diluted 1/200 to 1/4000 when 100 mg of RU 486 were administered to humans. Plasmas were diluted in phosphate-gelatin buffer supplemented with 0.025% Triton X 100. 0.1 ml of these dilutions or 0.1 ml of standard solutions were added, in triplicate, to 0.4 ml water. The RU 486 was extracted with 3.0 ml diethyl ether from a freshly opened bottle, in 80 x 13 mm glass hemolysis tubes, using a Multivortexer.

After four minutes of agitation, the aqueous phases were frozen in a methanol-dry ice bath. The ether phases were decanted in 65 x 13 mm glass hemolysis tubes and evaporated in a 40°C water bath. The residues were taken up in 0.2 ml of the tritiated RU 486 solution (5,000 cpm), vortexed and allowed to stand 30 minutes at room temperature before addition of 0.8 ml of antiserum (batch 655) diluted 1/100,000 in the phosphate-gelatin buffer. After an overnight incubation at 4°C, 0.75 ml of ice cold dextran-coated charcoal suspension was added to each tube. After ten minutes of incubation, the charcoal was pelleted at 3,000 rpm for ten

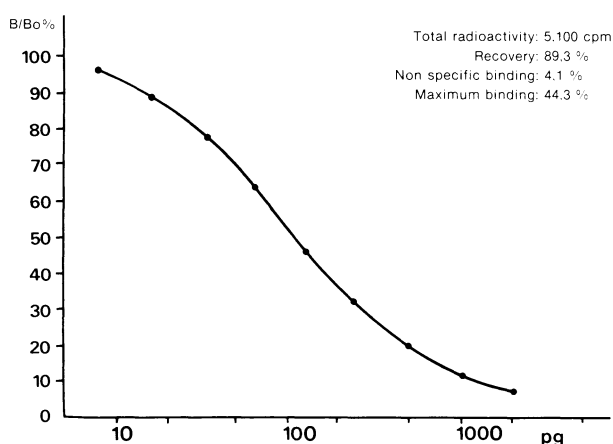


Fig. 1. RU 486 radioimmunoassay standard curve. RU 486 was diethyl ether extracted, the final antiserum dilution was 1/125,000 and separation of the unbound was obtained by dextran-coated charcoal.

minutes in a cooling centrifuge. Finally, supernatants were transferred to polyethylene counting vials and mixed with 10 ml of scintillation fluid for two minutes before they were counted.

#### REFERENCE

Raynaud, J. P., Azadian-Boulanger, G., and Bucourt, R., 1974, Anticorps spécifiques de l'estradiol, J. Pharmacol.(Paris), 5:27.

## PHARMACOKINETICS OF RU 486

Roger Deraedt, Claude Bonnat, Monique Busigny, Pierre Chatelet, Christian Cousty, Michel Mouren, Daniel Philibert, Jacques Pottier and Jean Salmon

Centre de Recherches Roussel-Uclaf  
93230 Romainville, France

### SUMMARY

The pharmacokinetics of RU 486 have been studied in man, rats and cynomolgus monkeys. Single pharmacological doses were given (from 1.3 mg/kg<sup>-1</sup> in man (oral) to 3-5 mg/kg<sup>-1</sup> in animal species), using a tritiated compound. Radiometric assay and, in some cases, RIA were used. Absorption was satisfactory in all species, but the compound underwent a presystemic effect that reduced its bioavailability to 40% in man and rats and 15% in monkeys. Extravascular diffusion was much greater in animal species than in man; the apparent initial volume of distribution was equivalent to the body weight in rats and twice the body weight in monkeys. However, it accounted for only 10% of the body weight in man.

Similarly, plasma clearance was much higher in rats and monkeys (3 and 1.5 l/h<sup>-1</sup> per kg body weight) than in man (23 ml/h<sup>-1</sup> per kg body weight). These striking differences result from the presence of a certain binding protein found only in human plasma. Studies are in progress to identify this protein and to evaluate its role in the pharmacological activity of RU 486. The study of rat tissue distribution, by radiometry, confirmed the large extravascular diffusion seen in this species and showed that the compound and its metabolites were rapidly cleared from all tissues except erythrocytes. We found, from autoradiographs of female rats, that selective areas of labelling appeared in the brain (hippocampus, cortex, pineal gland and hypophysis) as well as in the cortical adrenal, ovaries, uterus, epithelium and clitoris gland. The major route of radioactivity excretion in all species was via the fecal route; less than 1/10 of the dose was recovered in urine. Seven metabolites, identified in rat bile or plasma, resulted from two routes, either alone or combined: 1) mono, then di-N-demethylation followed by N-acetylation; 2) oxidation of the methyl of the 17- $\alpha$  propynyl chain to alcohol. Moreover, an N-formyl derivative of the alcohol metabolite was also characterized. Four of these metabolites were synthesized, and their activities were assessed in vitro and in vivo.

### GENERAL CONDITIONS

The pharmacokinetics of RU 486 have been studied in man and animal species using a tritiated compound labelled in 6 and 7 positions (Fig. 1). From nuclear magnetic resonance (NMR) spectrum, 2/3 of the isotope is in the

7- $\beta$  position. In vivo, less than 1% of the administered radioactivity appeared as tritiated water; and, to rule out this interference, the radioactivity was determined in most samples after desiccation and combustion.

The assay of unchanged compound has been carried out after extraction and separation by thin layer chromatography (TLC). The excellent agreement of the results obtained with two TLC systems strongly supports the specificity of this assay. In humans, the compound was also determined with a radioimmunoassay (RIA) fully detailed further.

The metabolic pathway was established from rat bile, collected in anesthetized animals after catheterization of the common bile duct. Metabolites were extracted, isolated by high pressure liquid chromatography (HPLC), and identified from physical methods (U.V., NMR and mass spectra). In one case (metabolite IX) the characterization was obtained from rat plasma by reverse isotope dilution using the synthesized radio-inert compound.

#### PHARMACOKINETICS IN THE RAT

Fasted male or female Sprague Dawley rats (200 g) were used. The compound was intravenously or orally administered at a dose of 5 mg/kg<sup>-1</sup> dissolved in methylacetamide.

#### Plasma Kinetics After Intravenous and Oral Administration

After intravenous dosing, the plasma kinetics of radioactivity and of unchanged compound (Fig. 2) showed that extravascular diffusion was great, and elimination was fast. From an open, three-compartment model, the apparent initial volume of distribution (AIVD) of RU 486 accounted for 135% of body weight, its clearance was 3 l/h<sup>-1</sup>/kg of body weight, and its apparent terminal half life was about one hour.

Following oral treatment (Fig. 2), absorption proceeded rapidly, and peaks of both radioactivity and unchanged compound were reached as early as 15 minutes after administration (2.2 and 0.6 ug.ml<sup>-1</sup> respectively, expressed as the equivalent of RU 486). Subsequent kinetics were close to those observed after injection.

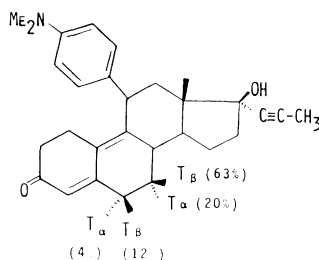


Fig. 1. Tritiated RU 486.

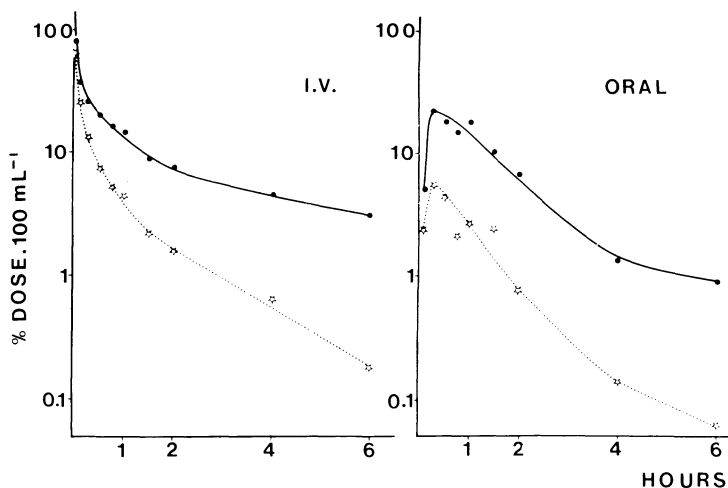


Fig. 2. Plasma kinetics of radioactivity (circles) and of unchanged compound (stars) in the rat after intravenous or oral administration of 5 mg.kg<sup>-1</sup> of <sup>3</sup>HRU 486 (n = 5).

From comparison of oral and intravenous data, we calculate that absorption accounted for 3/4 of the ingested dose, but the bioavailability was 39%, showing that a moderate presystemic effect occurs in the rat.

#### Tissue Distribution of Radioactivity

The tissue distribution of radioactivity (sum of the compound and its metabolites) has been studied, using radiometry and autoradiography. The radiometric study was carried out in rats of both sexes 1/2 and 24 hours after oral dosing.

The male tissue, pictured 1/2 hour after ingestion (Fig. 3), confirms great extravascular diffusion, since most tissue concentrations are higher than that of plasma. Highest levels were found in the liver and digestive tracts.

Twenty four hours after treatment (Fig. 4), the tissue state confirms the fast elimination of RU 486 and its metabolites. Compared to previous results, concentrations strongly decreased in all tissues (from 15 to 140 times), except in erythrocytes (two times). In particular, the disappearance rate in the brain, testis, seminal vesicles, prostate, thymus and adrenal was close to that observed in plasma.

A similar picture is seen in genital tissues from female rats (Fig. 5). Again, the decrease of radioactivity was slow only in erythrocytes. This slow decrease is due to covalent binding to the protein part of hemoglobin. No radioactivity was associated with hemin after precipitation and crystallization, but radioactivity persisted in globin even after sequential treatments with various solvents (Kappus et al., 1973; Peter and Bolt, 1981). For the total erythrocytes, this binding accounted for less than 1 µg of equivalent RU 486 after ingestion of 1 mg.

An autoradiographic study was carried out in female rats 1/4, one and six hours after intravenous dosing. Regularity of cycle had been previously established, and the animals were treated in the metestrus phase.

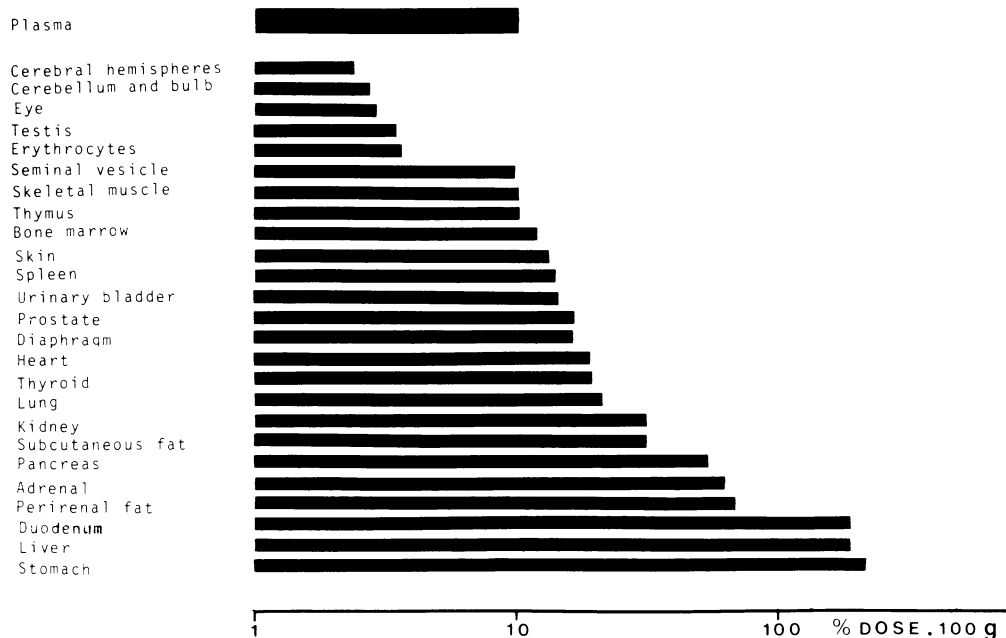


Fig. 3. Radioactivity distribution in male rat tissue 1/2 hour after oral administration of 5 mg.kg<sup>-1</sup> of <sup>3</sup>H-RU 486 (n = 5).

The corresponding autoradiographs (Figs. 6, 7 and 8) illustrate the high rate of extravascular diffusion by the contrast between blood radioactivity in the cardiac cavity and heart radioactivity. Selective areas of labelling appeared in the brain (hippocampus, cortex, pineal gland and hypophysis). Adrenal radioactivity measured by radiometry was mainly cortical. In the reproductive tract, the ovaries, uterus epithelium and clitoris gland were selectively labelled.

#### Excretion of Radioactivity After Intravenous and Oral Administration

The excretion of radioactivity was complete within four days (Fig. 9) and was almost entirely fecal; the urinary route accounted for only about 3% of the dose.

#### PHARMACOKINETICS IN THE MONKEY

Fasted female cynomolgus monkeys (3 kg) were used. The compound was administered at a dose of 3 mg/kg<sup>-1</sup>, either intravenously or orally, dissolved in PEG 300; or intramuscularly, in aqueous suspension or oily solution.

#### Plasma Kinetics After Intravenous and Oral Administrations

Following intravenous dosing, the plasma kinetics of radioactivity and of unchanged compound (Fig. 10) showed a high rate of extravascular diffusion, but the disappearance was slower than in rats. From an open three-compartment model the AIVD of RU 486 was twice the body weight, its plasma clearance 1.5 l/h<sup>-1</sup>/kg of body weight, and its apparent terminal half life about 15 hours.

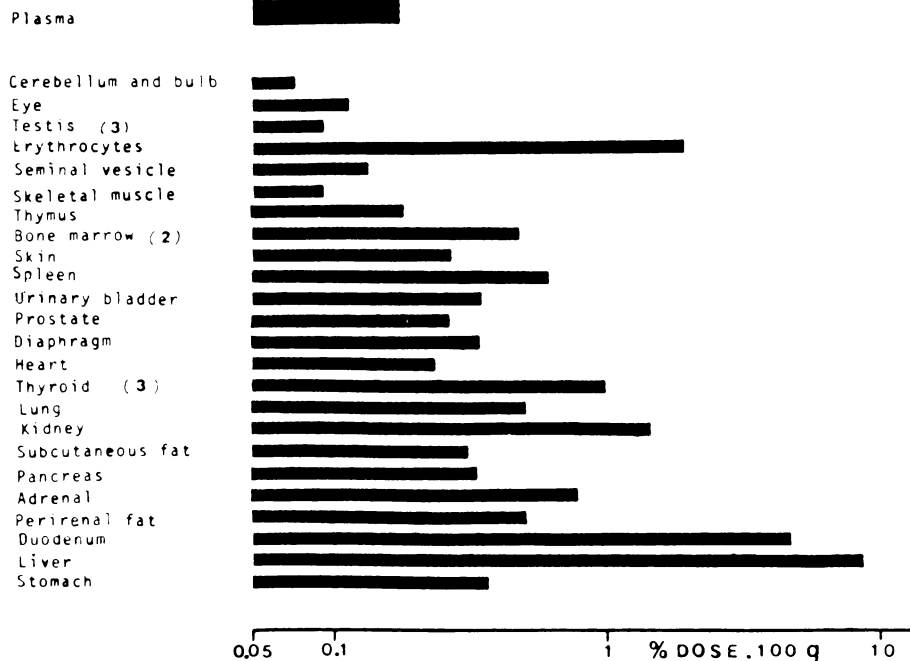


Fig. 4. Radioactivity distribution in male rat tissue 24 hours after oral administration of 5 mg/kg<sup>-1</sup> of <sup>3</sup>H-RU 486 (n = 5)

The absorption began early after oral treatment (Fig. 10) but proceeded slowly and irregularly. The peak of radioactivity was reached three hours after ingestion (420 ng/ml<sup>-1</sup> mean). In contrast, the curve of unchanged compound plateaued from 1/4 to three hours after dosage at a mean level of about 30 ng/ml<sup>-1</sup>.

From a comparison of oral and intravenous data, we found that absorption accounted for 3/4 of the ingested dose but bioavailability for only 15%. A strong presystemic effect occurs in the monkey.

#### Plasma Kinetics After Intramuscular Administration

Two formulations were assayed by intramuscular route in three out of the four animals previously used. Figure 11 shows the kinetics of unchanged compound observed after the intravenous, oral and two intramuscular treatments.

The aqueous suspension led to a very slow release of RU 486, completed only after 1.5 month. Plasma concentrations of RU 486 plateaued at a low level, about 12 ng/ml<sup>-1</sup>, during the first six hours, then decreased slowly to 1 ng/ml<sup>-1</sup> 38 days after injection. In contrast, the absorption of RU 486 injected in oily solution was fast and led to sustained plasma concentrations from 1 to 6 hours (90-130 ng/ml<sup>-1</sup>).

From comparison with intravenous data, we found that absorption from the injection site was complete within about one day for the oily solution,

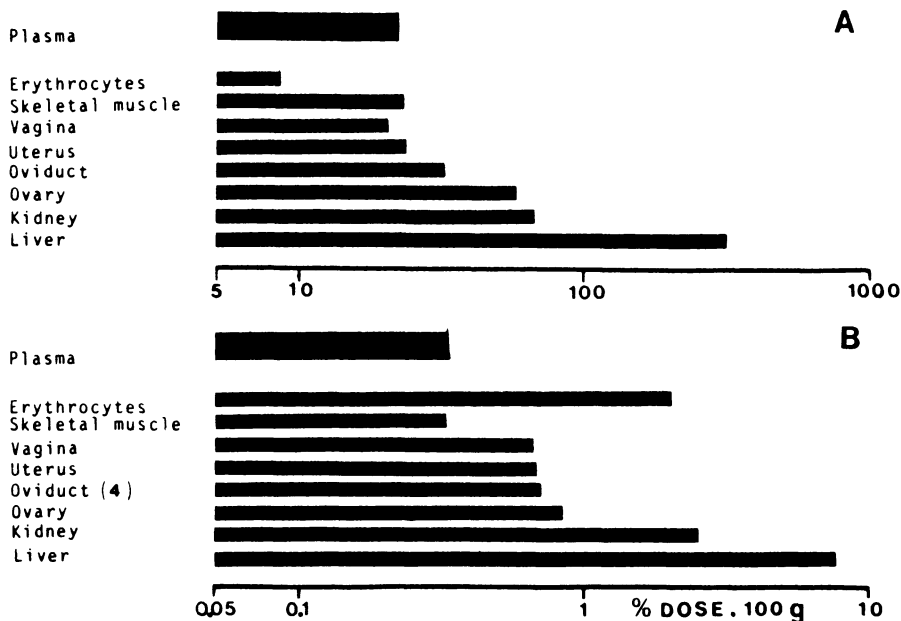


Fig. 5. Radioactivity distribution in female rat tissue 1/2 (A) and 24 Hours (B) after oral administration of 5 mg/kg<sup>-1</sup> of <sup>3</sup>H-RU 486 (n = 5).

whereas it accounted for only about 1/10 of the dose for the aqueous suspension during the same period. Moreover, the intramuscular route rules out a presystemic effect, and concentrations of RU 486 obtained up to six hours after administration of the oily solution were 3 to 6 times higher than after ingestion.

#### Excretion of Radioactivity After Intravenous Administration

The sum of fecal and urinary radioactivity within one week accounted for 85.1 ± 1.1% of the dose (n = 3). Excretion is mainly fecal, since only 6.8 ± 0.4% of the dose is recovered in the urine.

#### Interspecies Comparison

Figure 12, which represents the kinetics of RU 486 in both species after intravenous or oral treatment, summarizes the differences. The results, expressed as % dose per kg per 100 ml, can be compared directly.

Intravenous kinetics show that the compound was largely distributed in tissues of both species but that it disappeared faster in rats. Oral kinetics differed markedly: in monkeys, the slower absorption rate and the more important presystemic effect led to a plateau, in contrast to the peak-shaped curve observed in rats.

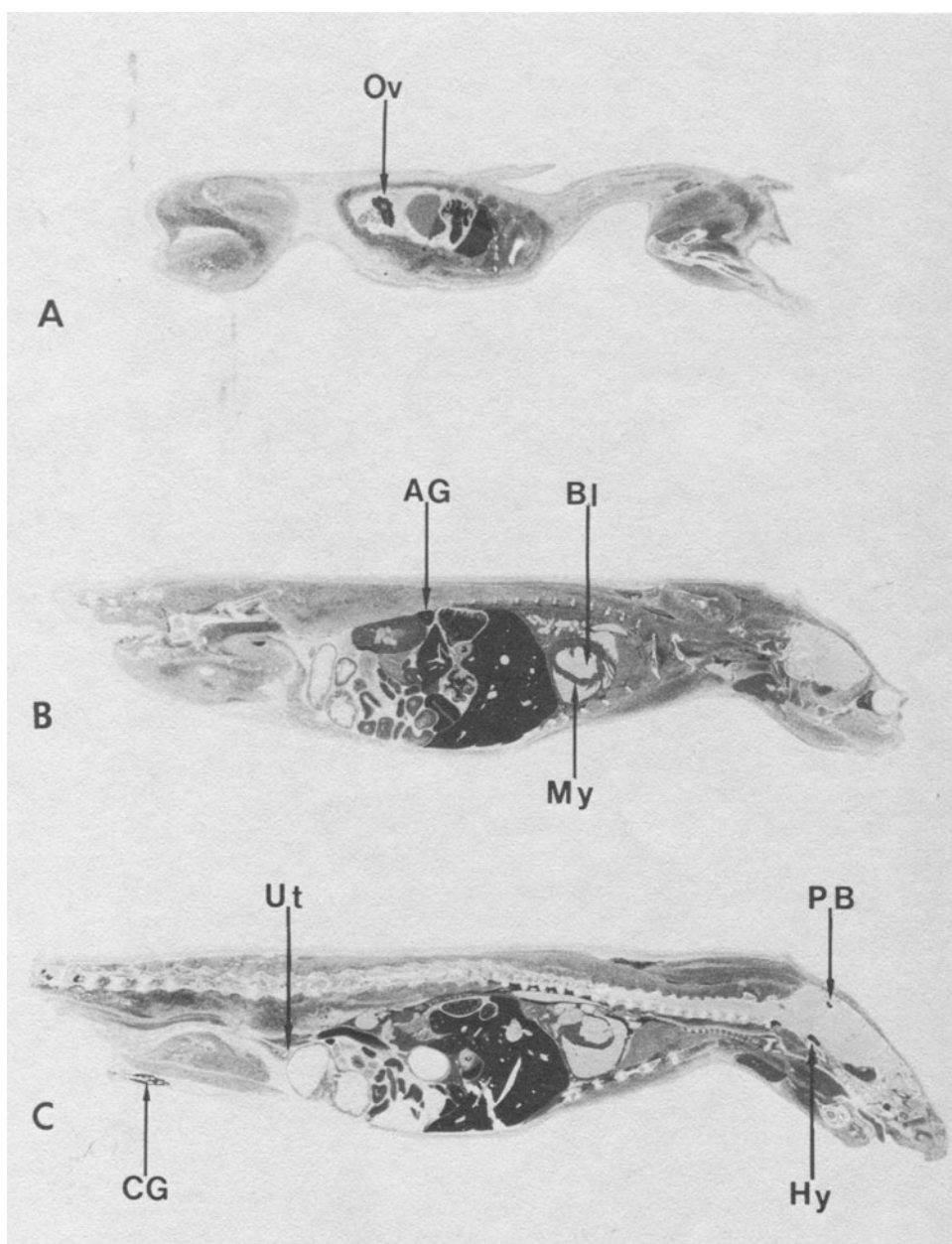


Fig. 6. Autoradiographs of a female rat 1/4 hour after intravenous administration of  $5 \text{ mg/kg}^{-1}$  of  $^3\text{H}$ -RU 486. AG=adrenal gland, Bl=blood, CG=clitoris gland, Hy=hypophysis, My=Myocardium, Ov=Ovary, PB=Pineal Body, Ut=Uterus.

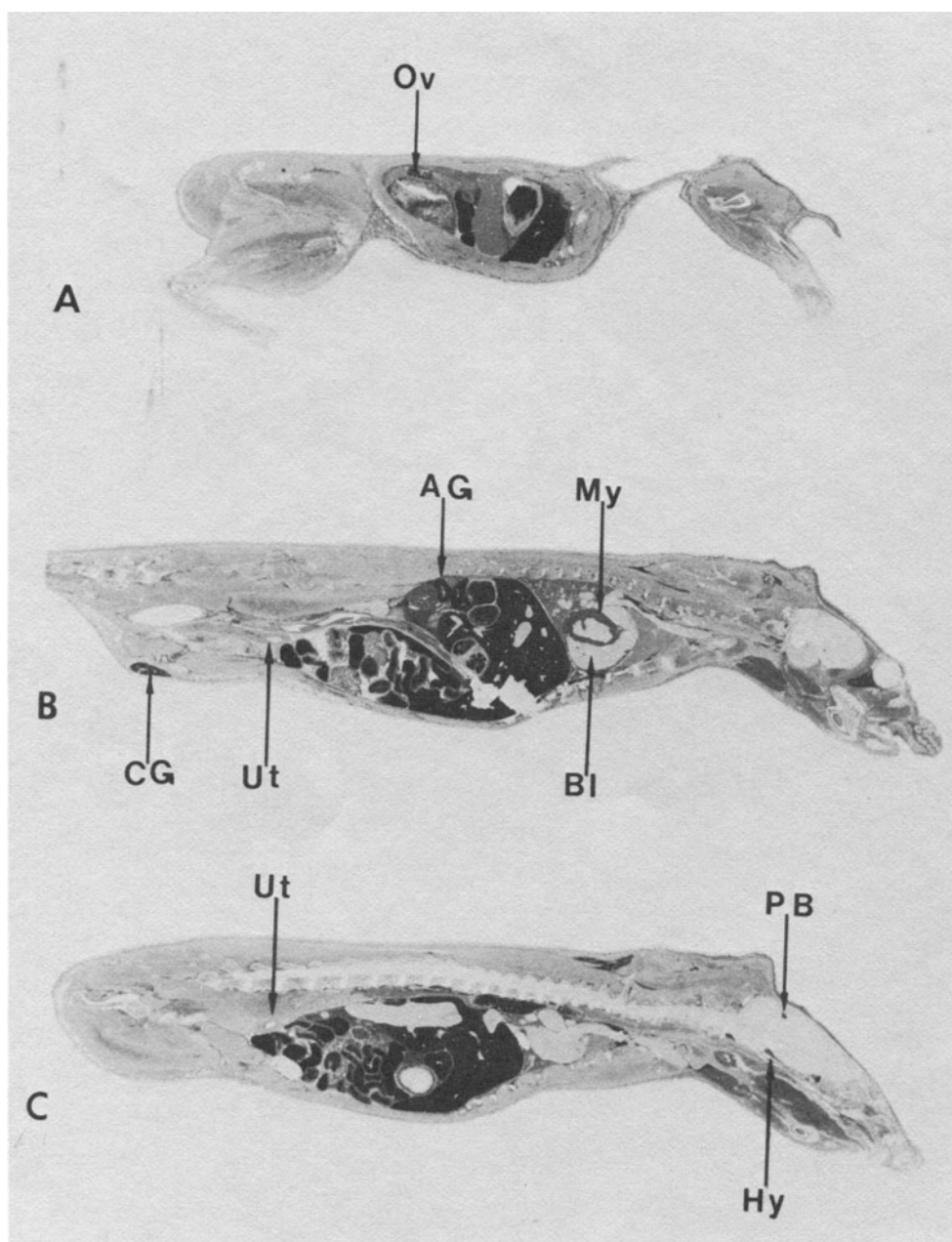


Fig. 7. Autoradiographs of a female rat 1 hour after intravenous administration of  $5 \text{ mg/kg}^{-1}$  of  $^3\text{H}$ -RU 486. For abbreviations see caption of Figure 6.

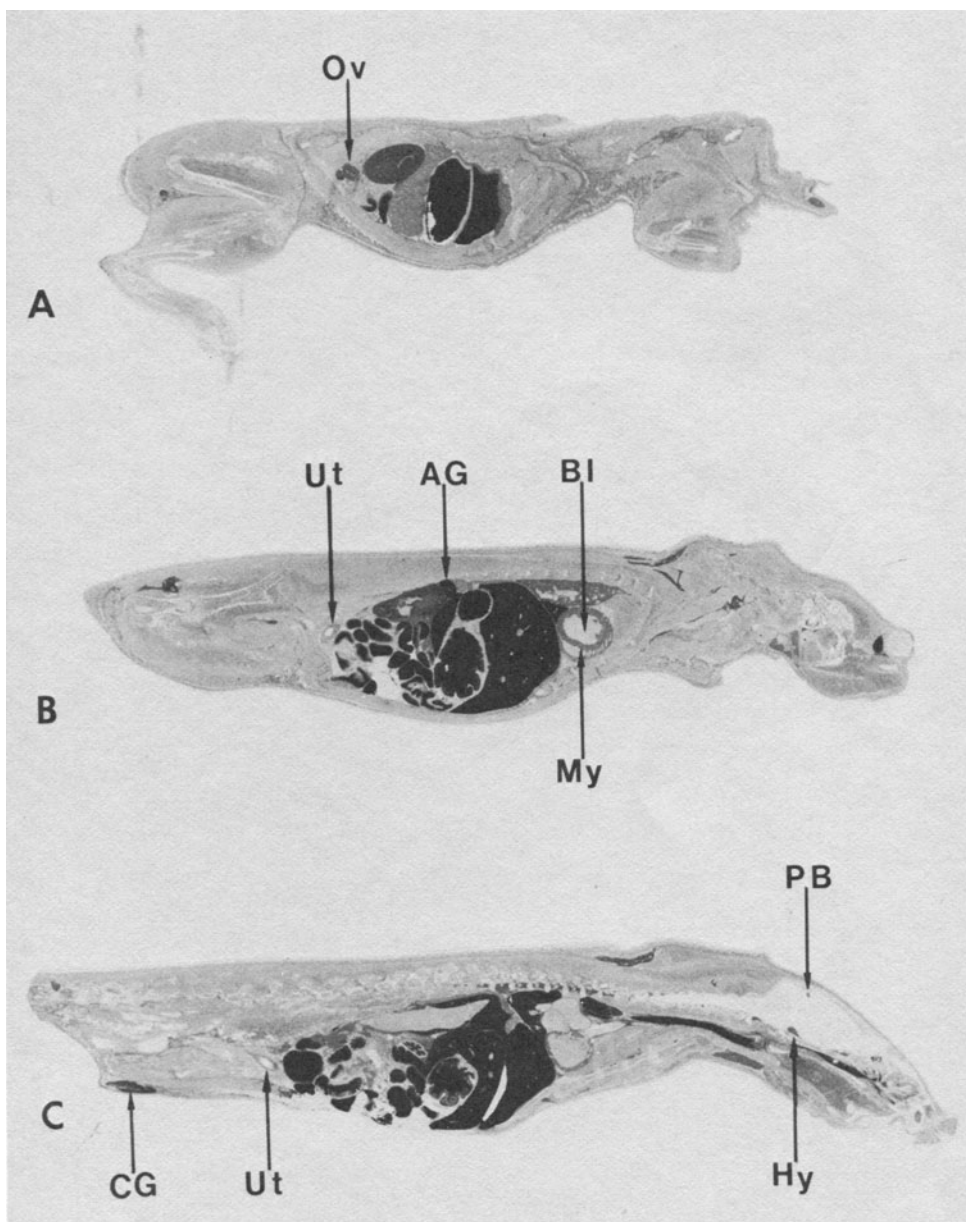


Fig. 8. Autoradiographs of a female rat 6 hours after intravenous administration of  $5 \text{ mg/kg}^{-1}$  of  $^3\text{H}$ -RU 486. For abbreviations see caption of Figure 6.

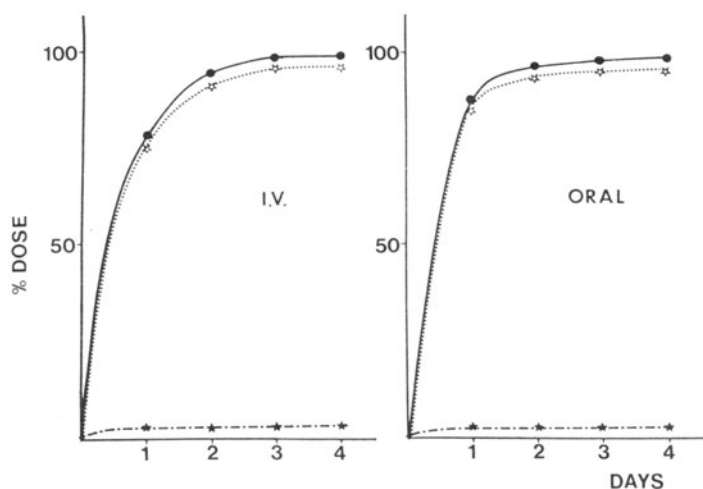


Fig. 9. Urinary (closed stars), fecal (open stars) and total (circles) excretions of radioactivity in the rat after intravenous or oral administration of 5 mg/kg<sup>-1</sup> of <sup>3</sup>H-RU 486 (n = 5).

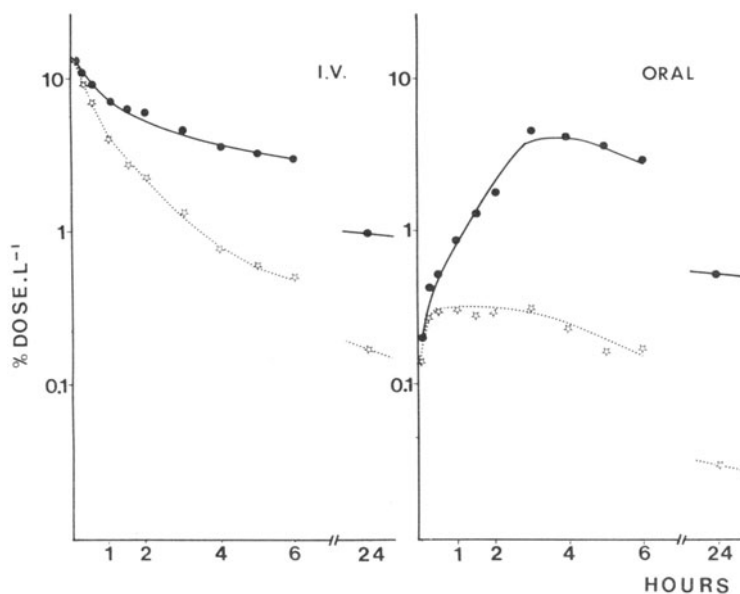


Fig. 10. Plasma kinetics of radioactivity (circles) and of unchanged compound (stars) in the monkey after intravenous or oral administration of 3 mg/kg<sup>-1</sup> of <sup>3</sup>H-RU 486 (n = 4).

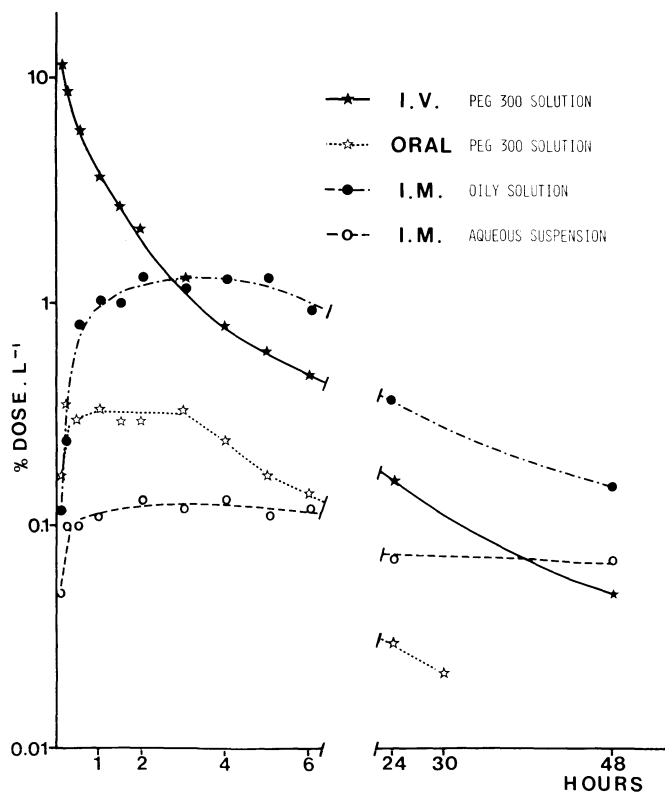


Fig. 11. Mean plasma kinetics of unchanged compound after administration of  $3 \text{ mg/kg}^{-1}$  of  $^3\text{H}$ -RU 486 to the same monkeys ( $n = 3$ ) (i.v. - oral - i.m., two formations).

#### METABOLIC PATHWAY OF RU 486

The structures depicted in Figure 13 have been identified in the bile or plasma (metabolite IX) of rats intravenously treated with 50 or  $5 \text{ mg/kg}^{-1}$  respectively. All retained the 3-oxodien structure and resulted from two routes: 1) mono, then N-didemethylation, followed by N-acetylation leading to compounds II, III and IV. 2) oxidation of the methyl of the 17- $\alpha$  propynyl chain leading to the alcoholic metabolite IX, found only in plasma. The combination of the two pathways led to the metabolites V, VI and VII. Lastly, an N-formyl derivative of VI was also characterized.

The dotted arrows in the scheme indicate that V, for example, can arise from N-demethylation of IX and/or from oxidation of the propynyl chain of II. The N-formyl metabolite VIII could be intermediary from V to VI, or, alternatively, could arise from formylation of VI.

N-Demethylation and further acetylation of aromatic amines, as well as hydroxylation of alkyl chains, are common pathways. In contrast, few examples of N-formylation have been described. In addition to N-acetyl metabolites, 2-amino anthraquinone and aminogluthethimide lead to N-formyl derivatives in the rat for the former (Gothoskar et al., 1979) and in man for the latter (Foster et al., 1984). 4-Formyl antipyrine was excreted in the urine of man and of various animal species treated with aminopyrine

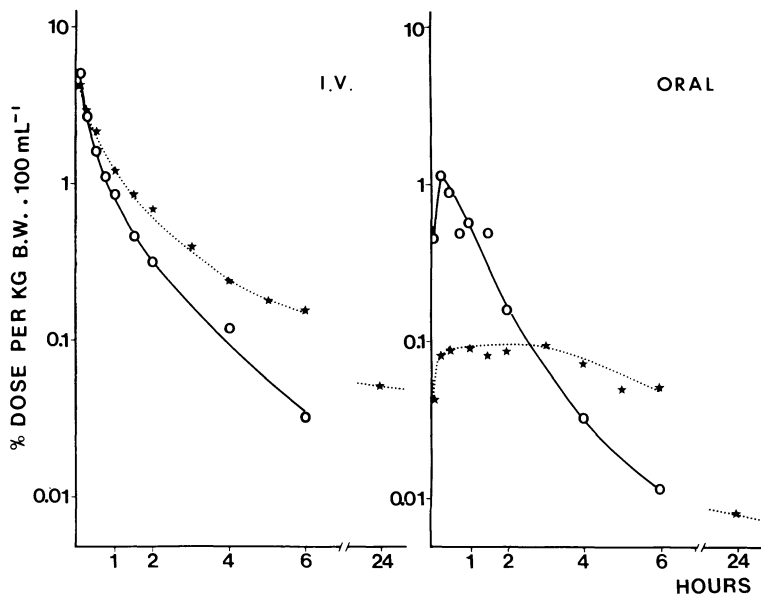


Fig. 12. Plasma kinetics of unchanged compound after administration of  $^3\text{H}$ -RU 486 to the rat ( $\circ$  - 5  $\text{mg/kg}^{-1}$ ) and the monkey ( $\star$  - 3  $\text{mg/kg}^{-1}$ ).

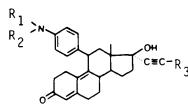
(4-dimethylamino antipyrine) but not after treatment with 4-amino antipyrine. The formation of the N-formyl metabolite was attributed to the oxidative removal of one of the methyl groups (Noda et al., 1976).

The six-hour bile radioactivity accounted for 1/4 of the injected dose. This relatively low excretion contrasts with the high rate of clearance shown before. High doses ( $50 \text{ mg/kg}^{-1}$ ) used to enable structural identification delayed the biliary excretion of radioactivity: corresponding figures for 6 hour-tertiary excretion of radioactivity were 81% (5  $\text{ug/kg}^{-1}$ ), 70% ( $0.5 \text{ mg/kg}^{-1}$ ), and 65% ( $5 \text{ mg/kg}^{-1}$ ). Half of the radioactivity was extractable. The main metabolites were the N-mono and N-didemethylated compounds (II and III) and the N-acetylated alcohol compound VII (from 6 to 10% of the excreted radioactivity). The N-didemethylated alcohol VI and its N-formylated derivative VIII accounted for 3-4%. Metabolites IV and V were minor products (1-2%). RU 486 was excreted in minute amounts ( $<0.5\%$ ), and though alcohol IX was not detected, it represented 15% of the plasma radioactivity one hour after injection of  $5 \text{ mg/kg}^{-1}$  of RU 486. Thus, the three metabolites resulting only from modification of the dimethylamino group accounted for 17.5% of the biliary excretion, and the four compounds containing an alcoholic function accounted for 15.5%.

#### IN VITRO AND IN VIVO ACTIVITIES OF SOME METABOLITES

Four of these metabolites have been synthesized: the N-mono and N-didemethylated compounds II and III; the N-acetylated derivative of the latter, IV, all found in bile; and the alcohol IX identified in plasma. Their activities have been compared to those of the parent compound.

Table I. Comparative Activities of RU 486 and Some Metabolites


$$R_1 = R_2 = R_3 = \text{CH}_3$$
$$R_1 = H, R_2 = R_3 = CH_3$$
$$R_1 = R_2 = H, R_3 = CH_3$$
$$R_1 = R_2 = CH_3, R_3 = CH_2OH$$

RU 486

II (RU 42 633)

III (RU 42 848)

IX (RU 42 698)

- RBA TO RECEPTORS

## PROGESTERONE

2 HR

78

95

44

44

24 HR

397

40

47

GLUCOCORTICO

4 HR

280

211

164

166

24 HR

294

118

127

- ABORTIVE EFFECT ED 100\*

3

10

IN 10

10

#### - INHIBITION OF THYMOLYTIC

EFFECT OF DEXAMETHASONE

APPROXIMATIVE ED 50\*

4

12

30

8

\* DOSES IN  $\text{MG} \cdot \text{KG}^{-1}$  ORAL

IN = INACTIVE

Relative binding affinities (RBA) for cytosolic receptors were determined as described by Moguilewsky and Philibert. The abortive effect was evaluated after a single oral dose of test compound on day 9 of pregnancy. The inhibition of dexamethasone's thymolytic effect was evaluated after a 4-day oral treatment with dexamethasone (0.05 mg/kg<sup>-1</sup>) and test compounds (see Philibert et al., this volume)

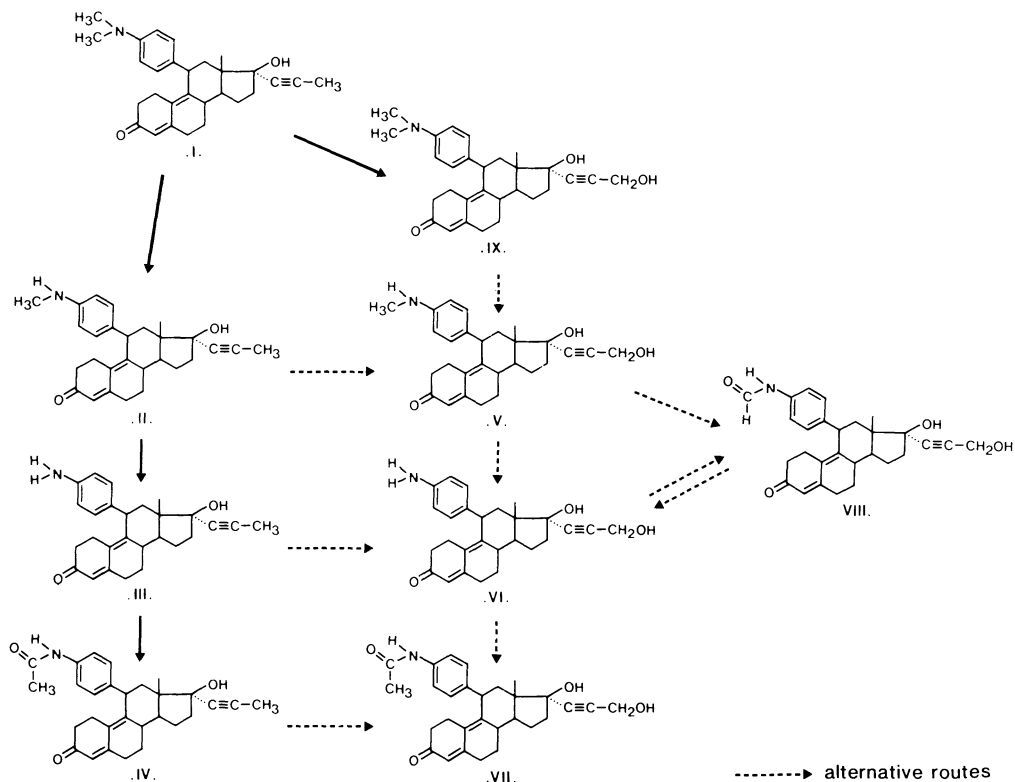


Fig. 13. Metabolic pathway of RU 486 (compound I) in the rat.

Preliminary results showed a clear decrease of the activity of the N-acetylated compound IV in vitro (data not shown), but it does retain some potency. Although phase II metabolites (conjugates) are usually considered to be inactive, the beta-blocker acebutolol is metabolized by a similar pathway - N-desalkylation and then acetylation of the free amino group to a active metabolite (Zaman et al., 1984).

As shown in Table I, compound II has similar affinities to RU 486 for the progesterone and glucocorticoid receptors. The affinities of compounds III and IX, however, are lower, particularly for the progesterone receptor, after a 24 hour incubation.

In vivo, compound IX exhibited the same abortive effect as compound II and a similar anti-glucocorticoid effect (activities about 3 times weaker than that of RU 486), while compound III was clearly less potent.

## PHARMACOKINETICS IN MAN

### Plasma Kinetics After Intravenous Administration

A tracer dose of tritiated RU 486 (280 ng-22 uCi) was injected intravenously into three fasted young male volunteers. Eighteen blood samples were obtained over 72 hours.

The plasma kinetics of radioactivity, of unchanged compound and of its N-monodemethylated derivative (RU 42 633) were determined (Fig. 14). The fitted curves correspond to an open two-compartment model for RU 486 and to an open one-compartment model for RU 42 633. The area under the plasma level curve (AUC), maximum concentration ( $C_{Max}$ ), time of maximum concentration ( $t_{Max}$ ), and half life of elimination ( $t_{1/2}$ ) are reported in Table II.

The AIVD of RU 486 was very low,  $8.0 \pm 0.8$ . The volume of distribution at steady state (VDSS),  $25.7 \pm 2.3$  was also relatively low, showing that an important part of the dose remained in the central or plasma compartment.

### Plasma Kinetics After Oral Administration

Two tablets, each containing 50 mg-25 uCi of tritiated RU 486, were administered to three fasting young male volunteers. Two of them had received the compound by injection two months before. Seventeen blood samples were obtained over 120 hours. The plasma kinetics of radioactivity RU 486 and RU 42 633 are shown in Figure 15. For RU 486 and RU 42 633, the fitted curves correspond to an open two-compartment model. Calculated or observed pharmacokinetic parameters are listed in Table II.

The absorption was fast, the time-to-peak level of RU 486 being observed  $0.7 \pm 0.1$  hour after ingestion of the tablets, and mean levels were  $1750 \text{ ng/ml}^{-1}$ . However, at this time, the plasma concentration of RU 486, expressed as % of the dose, was only one third of that observed after intravenous administration.

Plasma levels were also determined by RIA in one subject. The disappearance curve obtained by this method has been compared to those determined previously (Fig. 16). This method overestimates the RU 486 concentrations by an amount that roughly corresponds to the sum RU 486 + RU 42 633; but, since it is not time consuming, this may be of interest if man samples have to be measured.

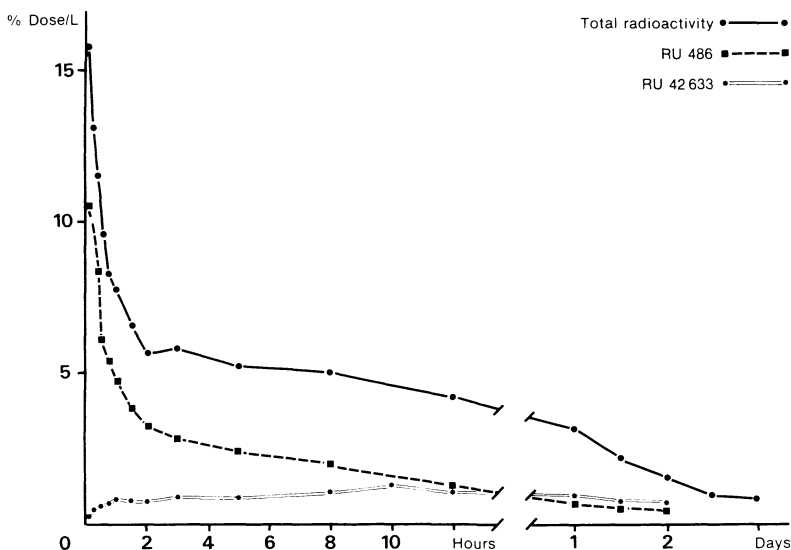


Fig. 14. Mean plasma kinetics after I.V. administration of 280 ng-22 uCi of  $^3\text{H}$ -RU 486 to 3 male volunteers.

#### Excretion of Radioactivity After Intravenous and Oral Administration

Urinary and fecal excretion of radioactivity reached completion in 6-7 days with 84% of the intravenous dose and 92% of the oral dose recovered. Whatever the route of administration, only 8.8% of the dose was excreted in urine, suggesting an important elimination via the bile.

#### Bioavailability

Two subjects received RU 486 intravenously and, two months later, orally. It was then possible to determine the absolute bioavailability.

From the ratio of areas under the plasma level curve (AUCs), we found that the absolute bioavailability was 56% for one subject and 30% for the other presystemic effect. In the urine, RU 486 was not excreted unchanged; but, if a similar profile of metabolites is assumed after oral and intravenous administrations, the absorption calculated from the ratio of urinary radioactivity is very good: 103% for one subject and 89% for the other (Table III).

Harris and Riegelman (1969) have proposed a method to differentiate between incomplete absorption and a presystemic effect. They compare the ratios of areas under the curve upon oral and intravenous administrations for parent drug and metabolite(s). This evaluation appears in Table III. For the two subjects, the ratios of AUCs of the metabolite RU 42 633 were higher than the ratios of AUCs of the parent product RU 486. In one case the AUCs of the metabolite were even higher after oral than after intravenous administration. Moreover, the time it took to reach maximum concentration of the metabolite was eight hours after intravenous administration and only 1 1/2 and two hours after oral administration.

Table II. Pharmacokinetic Parameters of RU 486 and  
RU 42 663 After I.V. (280 mg) or Oral (100 mg)  
Administration of RU 486 to Three Male Volunteers

		RU 486	RU 42 633
- AUC (% DOSE L <sup>-1</sup> .HR)	I.V.	60.6 ± 3.7	54.9 ± 8.1
	ORAL	23.5 ± 6.7	44.0 ± 11.9
- C MAX (% DOSE L <sup>-1</sup> )	I.V.	12.7 ± 1.3	1.2 ± 0.1
	ORAL	1.8 ± 0.4	1.7 ± 0.2
- T MAX (HR)	I.V.		9.3 ± 1.3
	ORAL	0.7 ± 0.1	2.2 ± 0.3
- T 1/2 (HR)	I.V.	11.8 ± 1.7	23.7 ± 5.8
	ORAL	23.7 ± 3.0	23.4 ± 1.5

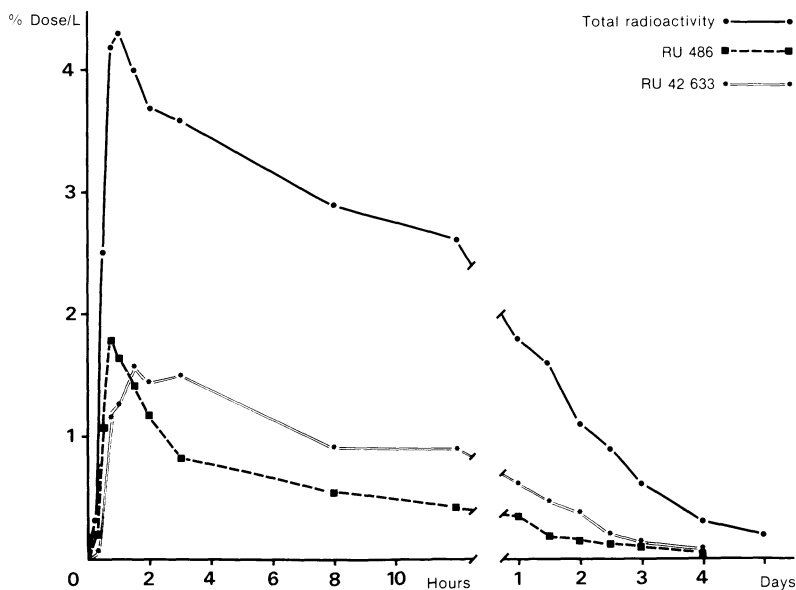


Fig. 15. Mean plasma kinetics after oral administration of  
100 mg-50 uCi of <sup>3</sup>H-RU 486 to 3 male volunteers.

Incomplete absorption cannot be ruled out. It clearly appears, however, that presystemic effect is mainly, if not completely, responsible for the incomplete bioavailability.

## DISCUSSION

Table IV summarizes kinetics data in man and two other animal species. In all species, the absorption was satisfactory; and a presystemic effect, more marked in monkeys, reduced the bioavailability to about 40% in man and rat and only 15% in the monkey.

In contrast to these roughly comparable results, a striking difference appears between man and the other animal species with respect to distribution volume and plasma clearance. In both animal species, the AIVD was either equivalent to or twice the body weight. In man it accounted for 1/10 of the body weight. Clearances were 3 and 1.5 l/h<sup>-1</sup>/kg body weight in the rat and the monkey, respectively, and only 23 ml/h<sup>-1</sup>/kg in man. This difference does not result from injection of only trace amounts in man, a 10<sup>-6</sup> ratio compared to animals (3.5 ng vs. 3-5 mg/kg body weight), since a similar difference was observed after oral treatment with comparable doses (1.3 mg vs 3-5 mg/kg body weight). Corrected for a 5 mg/kg<sup>-1</sup> dose, the maximum levels reached after oral administration were 7400 ng/ml<sup>-1</sup> in man, 600 ng in rats, and 50 ng in monkeys.

RU 486 binding to undiluted serum (dialysis four hours, 37°C) is high in the three species, accounting for 98-99% in rats and man and 97% in the monkey, in the range from 10 to over 6000 ng/ml<sup>-1</sup>. These results do not seem to justify the observed differences. The presence in human plasma, but not in numerous other animal species plasma investigated to date, of a protein that strongly binds RU 486 (Moguilewsky and Philibert, this volume) probably explains these kinetics differences. The identification of this protein, which has not yet been clearly distinguished from albumin, is

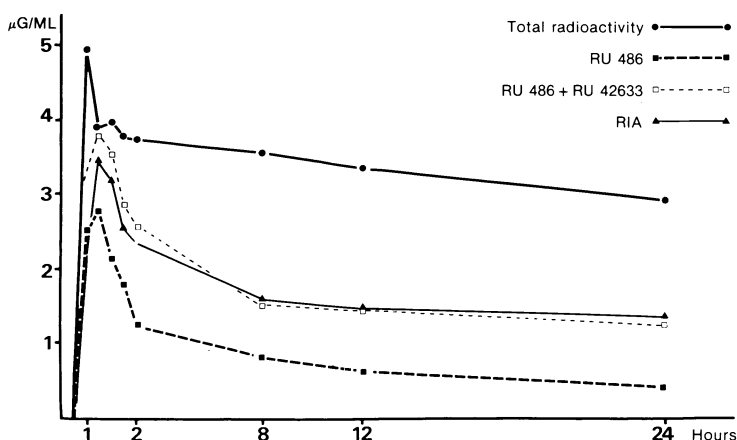


Fig. 16. Plasma kinetics after oral administration of 100 mg of <sup>3</sup>H-RU 486 to 1 male volunteer; comparison of radioactive and RIA methods.

Table III. Estimation of Absorption and Absolute Bioavailability of RU 486 After Oral (100 mg) or I.V. 280 ng) to 2 Volunteers

		SUBJECT 1	SUBJECT 2
- AUC RU 486 (% DOSE L <sup>-1</sup> .HR)	I.V. ORAL	65.2 36.7	63.9 19.1
B IN %		56	30
- URINARY EXCRETION OF TOTAL RADIOACTIVITY (% DOSE)	I.V. ORAL	10.3 10.6	9.8 8.7
A IN %		103	89
- AUC RU 42 633 (% DOSE L <sup>-1</sup> .HR)	I.V. ORAL	43.6 64.8	70.5 43.6
B IN %		149	61
- T MAX RU 42 633 (HR)	I.V. ORAL	8.0 1.5	8.0 2.3

A = ABSORPTION (ORAL/I.V.)

B = ABSOLUTE BIOAVAILABILITY (ORAL/I.V.)

Table IV. Comparison of Kinetic Parameters in Man and Animal Species

	RAT (5 MG·KG <sup>-1</sup> ) (I.V. - ORAL)	MONKEY (3 MG·KG <sup>-1</sup> ) (I.V. - ORAL)	MAN (3.5 MG·KG <sup>-1</sup> I.V.) (1.3 MG·KG <sup>-1</sup> ORAL)
AIVD* (% B.W.)	135	200	10
CLEARANCE (L·HR <sup>-1</sup> PER KG B.W.)	2.95	1.45	0.02
C MAX (MG·ML <sup>-1</sup> ) (CORRECTED FOR 5 MG·KG <sup>-1</sup> )	600	50	7400
T MAX (HR)	0.25	0.25 TO 3	0.75
ABSORPTION (% DOSE)	71	75	89 - 103
BIOAVAILABILITY (% DOSE)	39	15	30 - 56
PRESYSTEMIC EFFECT	MODERATE	STRONG	MODERATE

\* APPARENT INITIAL VOLUME OF DISTRIBUTION

underway. Due to the high binding capacity of albumin, the lack of this saturable binding in animal species cannot be demonstrated in undiluted serum.

In the same way, the AIVD and clearance of progesterone differ in the virgin and pregnant guinea pig, due to a high increase of progesterone-binding globulin in the course of pregnancy (Raynaud et al., 1982).

Classically, only free amounts of drug that can diffuse from plasma to tissue receptor are active, and the drug's binding to plasma proteins influences the pharmacological response. For example, the increase in corticosteroid binding globulin in pregnant women decreases progesterone elimination and leads to the formation of a hormone reservoir that ensures an adequate supply in the target tissue over time. However, the activity of the natural hormone, estradiol, that binds to estrogen binding protein (EBP), is reduced in immature rats compared to that of synthetic moxestrol, which does not bind to EBP (Raynaud et al., 1980). In the same way, increased binding of disopyramide in rabbits pretreated with human  $\alpha_1$ -glycoprotein is associated with decreased cardiac response (Huang and Oie, 1982).

Studies are currently underway to demonstrate the eventual influence of this particular binding upon the pharmacological activity of RU 486. We have observed that co-administration of human serum and the compound to rats leads to a dramatic decrease of AIVD. This effect was transient, however, and was only obtained at subpharmacological doses. Isolation of the protein in sufficient quantities would allow us to solve this question.

#### ACKNOWLEDGMENTS

The authors thank R. C. Gaillard (Hopital Cantonal de Geneve) for the clinical part of this study and V. Delaroff, N. Dupuy, D. Jovanovic and R. Smolik (Roussel-Uclaf) for the physical analysis.

#### REFERENCES

- Foster, A. B., Griggs, L. J., Howe, I., Jarman, M., Leung, C. S., Manson, D., and Rowlands, M. G., 1984, Metabolism of aminoglutethimide in humans. Identification of four new metabolites, Drug Metab. Dispos., 12:511.
- Gothoskar, S. V., Benjamin, T., Roller, P. P., and Weissburger, E. K., 1979, N-Formylation of an aromatic amine as a metabolic pathway, Xenobiotica, 9:533.
- Harris, P. A., and Riegelman, S., 1969, Influence of the route of administration on the area under the plasma concentration-time curve, J. Pharm-Sci., 58:71.
- Huang, J. D., and Oie, S., 1982, Effect of altered disopyramide binding on its pharmacologic response in rabbits, J. Pharmacol. Exp. Ther., 223:469.
- Kappus, H., Bolt, H. M., and Remmer, H., 1973, Irreversible protein binding of metabolites of ethynylestradiol in vivo and in vitro, Steroids, 22:203.
- Noda, A., Goromaru, T., Tsubone, N., Matsuyama, K., and Iguchi, S., 1976, In vivo formation of 4-formylamino-antipyrine as a new metabolite of aminopyrine, Chem. Pharm. Bull., 24:1502.
- Peter, H., and Bolt, H. M., 1981, Irreversible protein binding of acrylonitrile, Xenobiotica 11:51.

- Raynaud, J. P., Moguilewsky, M., and Vannier, B., 1980, Influence of rat estradiol binding plasma protein (EBP) on estrogen binding to its receptor and on induced biological responses, in: "Advances in the Biosciences, Volume 25, Development of Responsiveness to Steroid Hormone," A. M. Kaye and M. Kaye, eds., Pergamon Press, Oxford.
- Raynaud, J. P., Ojasoo, T., Pottier, J., and Salmon, J., 1982, Chemical substitution of steroid hormones: effect on receptor binding and pharmacokinetics, in: "Biochemical Actions of Hormones, Volume IX," G. Litwack, ed., Academic Press, New York.
- Zaman, R., Wilkins, M. R., Kendall, M. J., and Jack, D. B., 1984, The effect of food and alcohol on the pharmacokinetics of acebutolol and its metabolite, diacetolol, Biopharm. Drug Dispos., 5:91.

## TOXICOLOGICAL STUDY ON RU 486

Roger Deraedt, Bernard Vannier and Robert Fournex

Centre de Recherches  
Roussel-Uclaf  
93230 Romainville, France

### SUMMARY

RU 486 showed no toxic effects after acute oral administration to mice and rats. Six-month toxicological studies were performed, using rats (15, 25, and 125 mg/kg) and cynomolgus monkeys (5, 15, and 45 mg/kg). Most of the modifications induced by the high doses are due to antihormonal properties of the compound such as: antiprogesterone activity in females (frequent estrus and mammary-gland development in rats, suppression of menstruation and a decrease in serum progesterone in monkeys); antiglucocorticoid activity (increase in kidney and adrenal weights, and, in monkeys, increase in serum ACTH and cortisol); and antiandrogenic activity in male rats (decrease in prostate and seminal vesicle weights). RU 486 showed no mutagenic effect either in vitro or in vivo and provoked no teratogenic effect at usable doses, that is to say non-abortion doses (0.5 mg/kg in rats and 1 mg/kg in rabbits). Studies were performed on albino mice (Swiss - SPF) and albino rats (Sprague-Dawley - SPF), on cynomolgus monkeys (*Macaca fascicularis*) and on rabbits (HY). RU 486 was administered orally suspended in water containing 0.25 to 1% of carboxymethylcellulose with or without polysorbate 80 (0.20%).

### RESULTS

#### Acute Toxicity

RU 486 was well tolerated at doses of 1 g/kg by mice (10 males and 10 females) observed for 21 days after treatment. In rats observed for 14 days, the same dose provoked one death in 20 animals. Thus the compound is not toxic to the mouse, even at very high doses, and its LD50 should be superior to 1 g/kg in the rat.

#### Chronic Toxicity (Table I)

In previous studies involving oral administration once a day for 30 days, RU 486 was not toxic to the rat, even at 200 mg/kg. After oral administration of 100 mg/kg to the monkey, three animals out of six were

Table I. RU 486: Oral Chronic Toxicity

RAT	1 month: 8 - 40 - 200 mg/kg
	6 months: 5 - 25 - 125 mg/kg
CYNOMOLGUS MONKEY	1 month: 4 - 20 - 100 mg/kg
	6 months: 5 - 15 - 45 mg/kg

sacrificed after 12 to 17 days of treatment because of their physical conditions, the main symptoms being vomiting, diarrhea, loss of appetite and body weight, and increased blood urea. The antiglucocorticoid properties of the compound may be the major cause of these symptoms.

In rats, however, hormonal effects appeared even at 40 mg/kg, resulting from a disturbance of the estradiol-progesterone equilibrium in the female (estrus and mammary secretion) and from antiandrogenic properties of the molecule in the male. Similarly, in the monkeys an increase in serum cortisol was observed at doses of 20 mg/kg.

Six-month toxicological studies. In rats orally treated once a day at 5, 25, or 125 mg/kg (groups of 20 males and 20 females), RU 486 induced no deaths after six months. In the female it provoked a dose-related increase in food and water consumption and an increase in diuresis at 25 and 125 mg/kg. In the male, a decrease in body weight gain was noted at the highest dose.

A dose-related increase in serum proteins was observed in both sexes, and an increase in serum cholesterol was noticed in the female, notable at doses of 125 mg/kg.

An increase in liver weight was noted at 25 and 125 mg/kg, as well as an increase in male thyroid weight at all doses; female thyroid weight increased only at 125 mg/kg; an increased follicular epithelium height was noted on histological examination. An increase in kidney weight at 25 and 125 mg/kg and in adrenal weight at 125 mg/kg was observed in the female.

The hormonal activities of RU 486 provoked, at all doses, more frequent estrus than in female controls, a decrease in uterine weight, and an increase in female pituitary weight (due to hyperplasia of the pars anterior). In addition, at the two higher doses, we noticed distension of acini and mammary gland-ducts and a decrease in serum ACTH.

In the male, at all doses, the compound's antiandrogenic activity led to a dose-related reduction of prostate and seminal vesicle weights and, at 125 mg/kg, a reduction of testicle weight.

In monkeys treated orally once a day at 5, 15, or 45 mg/kg (groups of five males and five females), RU 486 provoked no deaths at six months; but a decrease in food consumption and a loss of body weight was observed in animals receiving the highest dose during the first five weeks.

The biochemical investigations revealed a decrease in Cl and K urinary excretion at all doses and, at 45 mg/kg, a decrease in Na excretion. At this latter dose, a decrease in serum cholesterol was observed.

An increase in kidney and adrenal weights was observed at all doses and in liver weight at 15 and 45 mg/kg. A decrease in pancreas weight was observed at the highest dose.

Histological examination revealed important modifications in the adrenals, such as increased eosinophilia of the zona fasciculata with loss of distinction between this zona and the zona reticularis or increased width of the zona reticularis. In the hormonal field, the treatment increased serum ACTH and cortisol at 45 mg/kg and decreased serum progesterone in all females. Menstruation was suppressed, and dilation of the fallopian tubes occurred frequently. Histological changes were noted in these latter organs, as in the uterus and cervix.

In conclusion, repeated high doses of orally administrated RU 486 to the rat and the monkey produced modifications in biochemistry, organ weights and histopathology. Most of these effects can be explained by the potent antiprogestosterone, antigluco-corticoid and antiandrogenic properties of the compound.

#### Embryotoxicity

RU 486 was administered orally, from day six to day 18 of pregnancy, to rats (22 to 23 animals per group) and rabbits (12 to 14 animals per group) at 0.25, 0.5, and 1 mg/kg. Animals were sacrificed on the day before the expected delivery day.

At the highest dose, rat abortions occurred in six out of 22 animals. A preliminary experiment has shown that in rabbits, 2 mg/kg of RU 486 have been found to be abortifacient.

Except for the group of rats having received 1 mg/kg, the treatment did not increase fetal loss. This was determined by totalling dead fetuses and resorptions.

RU 486 did not modify the mean fetal weight and induced no anomalies or malformations in the rat (including the surviving fetuses at 1 mg/kg) or the rabbit. Thus the compound showed no teratogenic effect in the two species studied.

#### Genetic toxicity

RU 486 produced no mutagenic effect in the Ames test (up to 5 mg per dish on five strains of S. typhimurium), neither with nor without metabolic activation (Ames et al., 1975).

In the micronucleus test (Heddle and Salamone, 1981) RU 486 did not change the incidence of micronucleated polychromatic erythrocytes in the bone marrow of mice, either 24, 48, or 72 hours after oral treatment with 1 g/kg of RU 486.

#### DISCUSSION

RU 486 appears to be a non-toxic drug upon acute oral administration. Most of the modifications induced by repeated administration of high doses are due to the potent antihormonal properties of the compound, particularly the antiprogestosterone activity in the female and the antiandrogenic activity in the male. The product showed no teratogenic effect in the rat or rabbit at non-abortive doses. The lowest dose administered in the studies was 5 mg/kg, which corresponds to the mean total dose ingested in a day by women desiring contraception or interruption of early pregnancy. This dose was

well-tolerated by rats and monkeys treated every day for six months. It must be emphasized that when this drug is used as an antiprogesterin, treatment will be of short duration, one to four days per month.

#### REFERENCES

- Ames B. N., Mc Cann J. and Yamasaki E., 1975, Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutation Res., 31:347.
- Heddle, J. A. and Salamone, M. F., 1981, The micronucleus assay I-In vivo, in: "Short-Term Tests for Chemical Carcinogens," H. F. Stich and R. H. C. San, eds., Springer Verlag, New York.

## NON-HUMAN PRIMATE STUDIES WITH RU 486

David L. Healy<sup>1</sup> and Gary D. Hodgen<sup>2</sup>

<sup>1</sup>Medical Research Centre  
Prince Henry's Hospital  
St. Kilda Road  
Melbourne, Australia

<sup>2</sup>The Howard & Georgeanna Jones Institute  
for Reproductive Medicine  
Department of Obstetrics & Gynecology  
Eastern Virginia Medical School  
Norfolk, Virginia, U.S.A.

### ABSTRACT

Non-human primate studies with RU 486 have established that this steroid can reliably induce menstruation by direct action upon the endometrium. When injected daily for four days, the minimal effective dose is approximately 0.1 mg/kg. RU 486 also acts acutely upon hypothalamic pituitary function to increase circulating ACTH, cortisol and AVP concentrations. However, it decreases plasma PRL levels in hyperprolactinemic monkeys induced by an estrogen-progesterone synergy,. In contrast to its menstrual action, doses of 1.0, 5.0 and 10.0 mg/kg of RU 486 are necessary to significantly elevate serum ACTH, cortisol and AVP concentrations, respectively.

### INTRODUCTION

Our challenge in this manuscript is to review our experience with RU 486 through physiological studies in non-human primates. Complete experimental details can be found by reference to our published papers (Healy et al., 1983a; Healy et al., 1983b; Healy et al., 1985) and abstracts (Kreitmann-Gimbal et al., 1983; Collins et al., 1984). Our studies have addressed the following areas: 1) whether RU 486 administration to primates can induce menstruation by direct endometrial action, 2) the acute effects of RU 486 administration upon pituitary hormone secretion, 3) the action of RU 486 when administered daily from days 10-12 of the menstrual cycle and 4) the dose-response relationships between RU 486 and its endometrial and pituitary effects.

### MENSTRUAL INDUCTION STUDIES: CASTRATES

#### Experimental Model

In this initial investigation, adult female cynomolgus monkeys (Macaca fascicularis, n=17) were castrated at least two months prior to use. Each

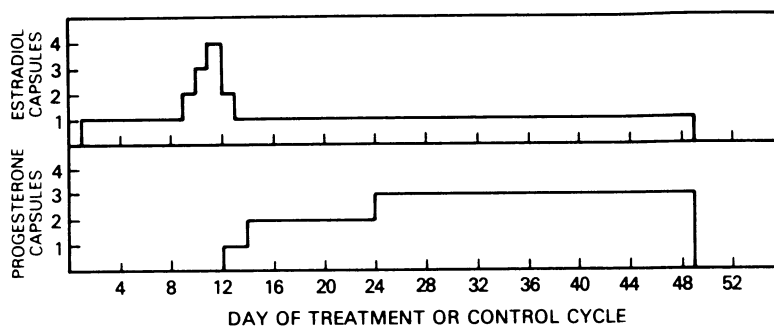


Fig. 1. Schema for implantation of subcutaneous E<sub>2</sub> and P capsules. All capsules were removed on day 49 of each experimental cycle.

animal received a schedule of silastic capsules (Medical grade tubing, 0.132 in internal diameter; 0.183 in external diameter, Dow Corning Midland, MI) over a 49-day course, as described in Figure 1, and had previously been shown capable of menstruating by a test removal of all capsules. Each capsule was implanted subcutaneously in the interscapular region. The estradiol (E<sub>2</sub>) capsules contained crystalline E<sub>2</sub> and were 3 cm long, and the progesterone (P) capsules were 2.25 cm long and packed with crystalline P. This regimen previously has been shown to mimic the circulating steroidal milieu of the normal ovarian/menstrual cycle and early pregnancy in monkeys (Kreitmann-Gimbal et al., 1979).

#### Experimental Design

Each monkey received the placebo or RU 486 on days 31-34 of each 49-day treatment cycle in a randomized controlled trial. These days were chosen to correspond with the time in women at which RU 486 might be considered for clinical use, that is, when an expected menstruation had not occurred, perhaps due to early pregnancy. Treatment cycles were separated by at least six weeks.

RU 486 was supplied as a powder by Roussel-Uclaf and administered intramuscularly to nine monkeys at a dose of 1.0 to 3.0 mg/kg in 90% ethanol from days 31 to 34 of the experimental protocol. Dose-response relationships were investigated by subsequent studies. Five monkeys received 0.1 mg/kg RU 486, whereas a separate group of three animals were injected with 10 mg/kg.

#### Circulating Hormone Levels

In both placebo and test cycles, daily femoral blood samples (3.5 ml) were taken from days one to 49 for radioimmunoassay of serum E<sub>2</sub>, P, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL) by established radioimmunoassays (RIA). On days 31 to 34, the injection of the placebo (vehicle only) or RU 486 was given after the morning blood sample had been taken.

#### Assessment of Menstruation

Daily menstrual records of all placebo and RU 486 cycles were carefully taken from days one to 56 of each treatment protocol. Both the duration and quality of menstruation were recorded. Menses were graded as: heavy (:), when blood was seen at the base of the monkey's cage; moderate (.), when blood was observed only about the vulva; and light (.), when blood was detected only by vaginal swab.

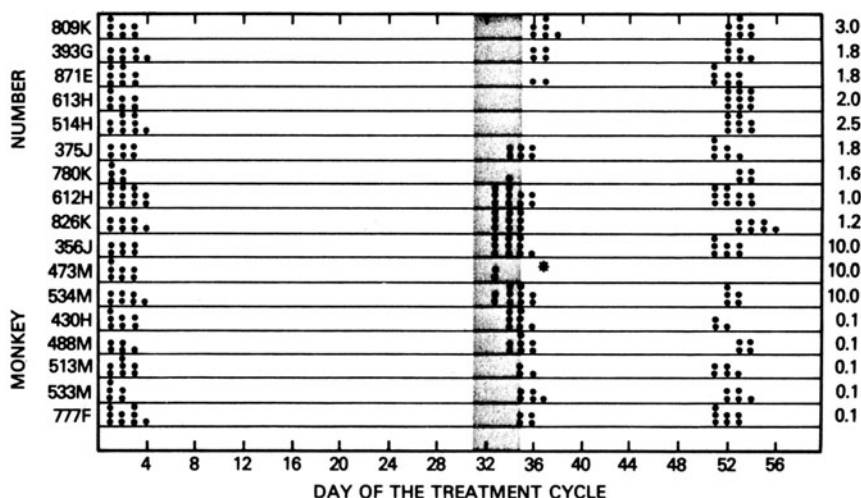


Fig. 2. Composite patterns of menstrual bleeding before, during and after RU 486 administration (Shaded Area) in 17 monkeys. The daily dose of steroid injected from days 31 to 34 of the treatment cycle is indicated in mg/kg on the right of the figure. Note that the top five monkeys of the pilot study received only partly dissolved RU 486: all animals subsequently injected with soluble RU 486 menstruated. Bleeding patterns from days 1-5 reflect a test removal of all capsules. Menses were graded as defined in the text. \*Monkey 473M died of iatrogenic trauma on day 33.

#### Results: Menstruation

Fifteen of 17 monkeys that received RU 486 menstruated within five days after initiation of the drug (Fig. 2). Note that the two monkeys that failed to menstruate were administered incompletely dissolved steroid in our first experimental series. Menses occurred in all primates injected with fully solubilized RU 486, regardless of whether the dose was 0.1, 1.0-3.0, or 10.0 mg/kg. In nine animals, menstruation began within 72 hours of the first injection. Bleeding persisted for at least three days in eight animals and was heavy in seven monkeys.

No menstruation was observed in any of the primates injected with ethanol alone (control) from days 31-34 of the cycle. Randomization of treatment and control cycles did not affect the bleeding patterns observed. Furthermore, all monkeys that menstruated during or after RU 486 administration also bled within 72 hours after removal of exogenous  $E_2$  and P on day 49 of the treatment cycle. No side effects of RU 486 exposure were noted in these primates.

#### Results: Gonadotrophin and Steroid Hormone Levels

Figure 3 depicts mean serum FSH, LH, PRL,  $E_2$ , and P values during the treatment cycles of four monkeys (375J, 780K, 612H, 826K) receiving moderate doses of RU 486 (1.0-1.8 mg/kg).

As expected, serum FSH and LH initially fell within 24 hours after  $E_2$  administration. Note the retention of responsiveness of the pituitary gland

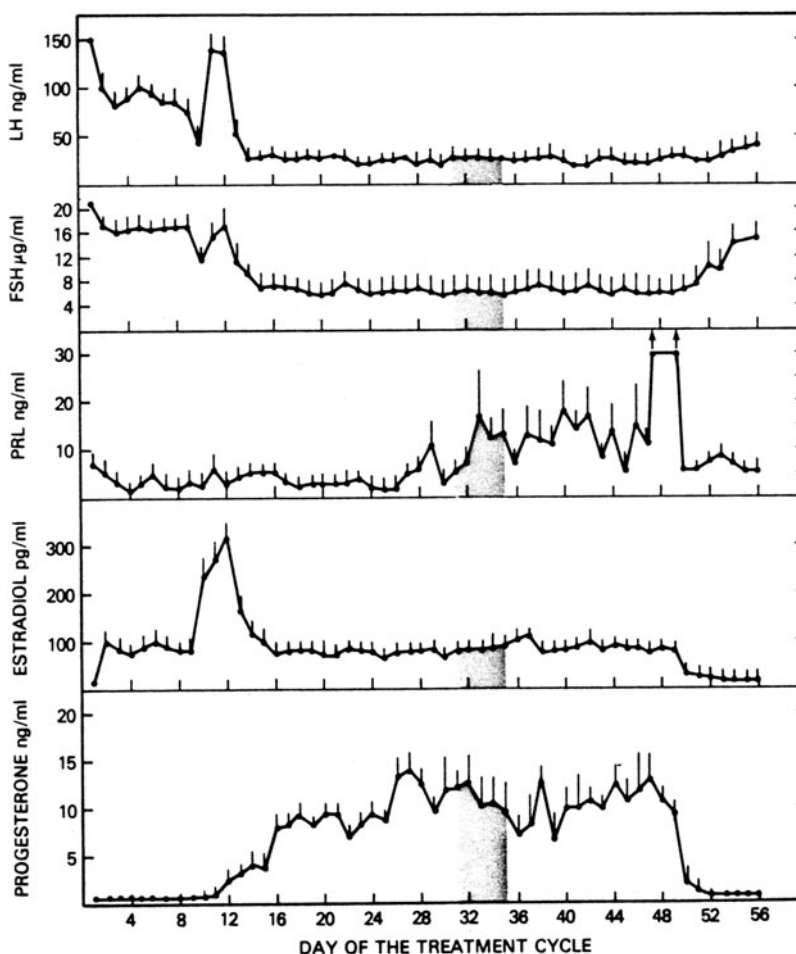


Fig. 3. Composite patterns of FSH, LH, PRL, E<sub>2</sub> and P from the serum of four monkeys injected with RU 486 1.0 to 1.8 mg/kg daily from days 31-34, as indicated by the shaded area. The data points represent the mean  $\pm$  1 standard error.

in these primates to E<sub>2</sub>-positive feedback from days 9-12, with the surge release of both FSH and LH. There was no significant change in mean serum PRL values, despite circulating levels of E<sub>2</sub> near 200 pg/ml from days 10-13 of the treatment cycles. However, serum PRL did increase significantly from days 29-49 ( $F_{48,392} = 2.40$ ;  $P < 0.01$ ); this coincided with the subcutaneous insertion of three P capsules and concurrent elevations of serum P ( $> 12$  ng/ml). RU 486 administration from days 31-34 did not alter the daily mean serum gonadotrophin profiles.

#### MENSTRUAL INDUCTION STUDIES: INTACT PRIMATES

We also tested to see if RU 486 could induce menses in cycling, pseudopregnant or pregnant cynomolgus monkeys (Kreitmann-Gimbal et al., 1983). A dose of RU 486, 5 mg/day i.m., was administered from day 24-26 of ovulatory menstrual cycles ( $n=4$ ), during pseudopregnancy in intact monkeys ( $n=4$ ), when hCG was given daily from day 21 in doubling i.m. injections from 10 I.U. per day (to mimic pregnancy), and to two pregnant monkeys. Menses

were induced within 48 hours after the first administration of RU 486 in these three groups of animals.

After the second dose of RU 486 to cycling animals, concentrations of steroid receptors were measured in the endometrium and in dispersed corpus luteal cells. Tritiated RU 486 binding to cytosol and nuclear fractions of endometrium was greater than for tritiated progesterone (cytosol: RU 486-2500  $\pm$  745 fmole/mg DNA  $\frac{1}{2}$ mean  $\pm$  SE $_{\frac{1}{2}}$ ; P-2112  $\pm$  530. nuclei: RU 486-1550  $\pm$  750; P-410  $\pm$  100). In contrast to this binding to the endometrium RU 486 did not appear to bind to corpus luteal cells and did not alter in vitro P production from dispersed luteal cells.

#### ACUTE PITUITARY RESPONSES TO RU 486: TREATED CASTRATES

##### Experimental Model

To study the effects of acute administration of RU 486 upon pituitary hormone secretion in primates, we studied an additional group of eight cynomolgus castrates, treated previously for 30 days with exogenous E<sub>2</sub> and P silastic capsules. This treatment is known to induce hyperprolactinemia. Two groups of four monkeys were fitted with a vest and mobile tether assembly that allowed chronic cannulation of the inferior vena cava via the femoral vein (Sopelak et al., 1983). This tether system permits repetitive blood sampling from freely-moving, unanesthetized primates with the investigator operating the cannula from an adjacent room.

Two days after cannulation, the duration of action of RU 486 was evaluated by taking 5 ml blood samples -30, -15, 0, +15, +30, +60, +90, +120, +180, +240, +360 and +480 min after administration of RU 486 or vehicle only at 0700h. Erythrocytes were returned hourly during each experiment. Test or placebo treatments were randomized and separated by two days.

In the first group of four monkeys, plasma was harvested for RIA measurement of PRL, FSH, LH, GH and TSH. Assay sensitivity was 3.0 uU/ml for FSH, 15 ng/ml for LH, 0.8 ng/ml for GH and 0.8 uU/ml for TSH.

In the second group, blood for determination of plasma ACTH,  $\beta$ -endorphin ( $\beta$ -END), AVP and cortisol by RIA was collected in prechilled vacutainers containing EDTA, placed on ice and immediately spun in a refrigerated centrifuge at 2000 g for 20 min at 4°C. Plasma was stored at -60°C until assayed. Plasma cortisol was assayed by RIA. Plasma for measurement of ACTH,  $\beta$ -END and AVP was extracted and concentrated on ODS cartridges (Waters Assoc., Milford, Mass.), lyophilized and reconstituted in RIA buffer. The antibody used for ACTH RIA was ACTH 1 (IgG Corporation; Nashville, TN). Detection limits of these RIAs were 5 pg/ml for ACTH, 10 pg/ml for  $\beta$ -END and 0.05 pg/ml for AVP.

RU 486 was synthesized by Roussel-Uclaf (Paris). The crystalline powder was dissolved in 70 percent ethanol in water (v:v). RU 486 was injected intramuscularly at a dose of 10 mg per kg. Results were expressed as the mean  $\pm$  SE. Duncan's multiple range t test was used to analyze hormonal changes following RU 486 administration and to compare acute responses to RU 486 versus responses to vehicle alone.

##### Results

Figure 4 illustrates the time courses and magnitude of the acute pituitary and adrenal responses to RU 486 administration. Plasma PRL had

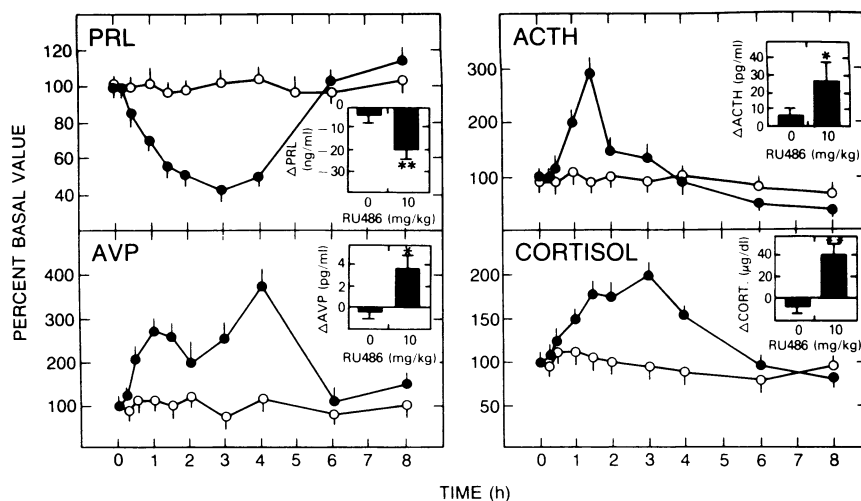


Fig. 4. Patterns of circulating levels of PRL, ACTH, AVP and cortisol after 10 mg/kg RU 486 (i.m.) (closed circle) or vehicle (open circle) administration. Drug or vehicle was administered at 0700 h to groups of 4 ovariectomized monkeys receiving exogenous estradiol and progesterone. Hormone concentrations are expressed as the percent of the transverse mean of 12 basal values: PRL ( $40.5 \pm 3.1$  ng/ml), ACTH ( $12.9 \pm 2.1$  pg/ml), AVP ( $1.3 \pm 0.2$  pg/ml) and cortisol ( $38.8 \pm 8.8$  ug/dl). Vertical bars indicate 1 SE. Inserts: A comparison of the greatest change from mean basal level ( $\Delta$ ) after administration of RU 486 (10 mg/kg) or vehicle. \*  $P < 0.05$ ; \*\*  $P < 0.025$ .

fallen significantly by 1 h after RU 486 injection ( $F_{3,48} = 26.70$ ;  $P < 0.05$ ). The nadir of circulating PRL occurred at 3 hours and was nearly 60% below the initial PRL concentration ( $17.8 \pm 4.5$  versus  $40.5 \pm 3.1$  ng/ml;  $P < 0.025$ ). PRL secretion remained suppressed 4 h after RU 486 was given, but by 6 h plasma PRL levels had returned to the pretreatment range. No significant change in plasma PRL followed injection of the vehicle alone.

In contrast to the inhibition of PRL secretion, plasma ACTH, cortisol and AVP increased after RU 486 administration. ACTH was significantly elevated from 1-2 h, with peak ACTH levels 280% above the basal value at the 90 min interval ( $F_{3,48} = 16.34$ ;  $P < 0.025$ ). Plasma ACTH values declined to the pretreatment range within 4 h after RU 486 was given. Note that cortisol secretion rose steadily to a peak mean value at 3 h (205% basal;  $= 39.1 \pm 8.1$  ug/dl;  $P < 0.025$ ) and had returned to the basal range 6 hours following RU 486 injection. Cortisol was significantly elevated from 90 min through 4 hours after RU 486 exposure ( $F_{3,48} = 21.26$ ;  $P < 0.01$ ). AVP also rose in a step-wise fashion: the releasable AVP peaked 4 hours after RU 486 administration ( $\Delta = 3.7 \pm 1.1$  pg/ml;  $P < 0.05$ ).

Although mean concentrations of  $\beta$ -END increased steadily from a basal state at  $42.5 \pm 16.1$  pg/ml to  $73.2 \pm 28.2$  pg/ml at 90 min, thus mimicking the time course of the ACTH rise, these evaluations were not statistically significant. No discernible changes in plasma FSH, LH, GH or TSH followed this regimen of RU 486 treatment.

Introduction

It is clearly established that the pituitary gonadotrophins FSH, LH and PRL are secreted in a pulsatile mode throughout the menstrual cycle. We have recently demonstrated that P is also secreted in a pulsatile or episodic fashion by the primate corpus luteum in the midluteal phase of the menstrual cycle (Healy et al., 1984a). Pulsatile P secretion appeared dependent upon LH secretion as every LH pulse was associated with a concomitant P pulse within 15 to 30 minutes. Moreover, there was an 80% concordance between episodes of LH and PRL secretion. This relationship was especially striking at the onset of darkness. A pulse of LH was identified simultaneously in the LH bioassay and RIA at this stage of the menstrual cycle.

The exact role of P at midcycle is disputed. Earlier data suggests that P can both block and facilitate gonadotrophin release (Dierschke et al., 1973; Helmond et al., 1980). Recent studies in normal cycling women demonstrate that the preovulatory P rise begins as much as twelve hours prior to the initiation of the midcycle LH surge and that P can slow LH, hence presumably LHRH-pulse frequency (Soules et al., 1984). To investigate this putative role of P, RU 486 was administered to four normal adult cynomolgus monkeys.

Experimental Method

RU 486 was given (5 mg/day, i.m.) on days 10-12 of the menstrual cycle. Femoral blood for RIA of FSH, LH, PRL,  $E_2$ , P was obtained daily under ketamine anesthesia from D8 of treatment cycle until D2 of subsequent menses. Daily menstrual records were kept.

Results

One monkey menstruated within 48 hours after receiving RU 486. She had had a spontaneous LH surge prior to RU 486 treatment, as evidenced by rising plasma P and corpus luteum formation. Three preovulatory monkeys failed to manifest an LH surge, despite familiar elevations of serum estradiol ( $<200$  pg/ml), suggesting an emergence of a dominant follicle; plasma P remained  $<0.4$  ng/ml. An attenuated LH rise (2-fold above basal) occurred  $11.7 \pm 0.8$  day after RU 486 treatment, without an intervening increase in plasma P. The intermenstrual interval ( $75.4 \pm 9.7$  days) was significantly lengthened ( $p < 0.05$ ) when compared to pretreatment control cycles ( $31.33 \pm 1.5$  days).

## RU 486 DOSE-RESPONSE RELATIONSHIPS IN PRIMATES

Endometrial Dose-Response

Figure 2 summarizes our experience that the menstrual induction  $ED_{50}$  for i.m. RU 486 in primates is at or below 0.1 mg/kg. Menstrual loss was assessed as heavy in 7/15 monkeys. Note that both the duration and amount of menstrual blood loss did not appear to be dose-related to the amount of RU 486 injected.

Pituitary Dose-Response

To further examine the action of RU 486 upon plasma ACTH, cortisol and AVP concentrations, we injected RU 486 i.m. at concentrations of 1.0, 5.0 and 10.0 mg/kg.

## Experimental Method

Two days after chronic femoral vein cannulation, RU 486 or the vehicle was administered at 0700 hours, and 5 ml blood samples were collected -60, -30, 0, +30, +60, +120, +180, +240 and +360 min for determination of plasma ACTH,  $\beta$ -endorphin ( $\beta$ -END), cortisol and AVP by RIA. Erythrocytes were returned hourly during each experiment. Test or placebo (vehicle) treatments were randomized, and each monkey received RU 486 only once.

All blood samples were immediately placed into prechilled vacutainer tubes containing EDTA and allowed to sit in ice and separated within 20 min by centrifugation at 2000 x g at 4°C for 20 min. Plasma samples were frozen at -60°C until assay.

To examine the glucocorticoid dependency of the RU 486-induced increases in plasma AVP concentrations, additional monkeys (n=6) received 1.0 or 0.1 mg of dexamethasone intramuscularly, 2 hours before 10 mg/kg RU 486 was given, also i.m. Blood samples for AVP measurements were collected -120, -60, 0, +30, +60, +90, +120, +180, +240 and +360 min. Other protocol procedures were followed as described above. All samples from an individual animal were analyzed at the same time.

## Results

RU 486 produced a dose-dependent increase in plasma ACTH, cortisol and AVP concentrations (Fig. 5). For ACTH, the peak elevation occurred at 1-2 hours for each dose level. Even at 1.0 mg/kg RU 486, the ACTH elevation was significant at 1 hour ( $F_{3,48} = 15.92$ ;  $p < 0.05$ ). Each peak elevation in ACTH was 190%, 270% and 310% above baseline after 1.0, 5.0 and 10.0 mg/kg RU 486 respectively, and these values were statistically comparable among the three doses. Note that the duration, but not the magnitude, of elevated plasma ACTH concentrations increased with higher dosages of RU 486. Plasma ACTH values returned to within the basal range after 2 hours following the 1.0 mg/kg dose and after 4 hours following 5.0 and 10.0 mg/kg injections of RU 486.

Peak cortisol concentrations occurred between 3-4 hours after RU 486 administration. The maximum cortisol level after 1.0 mg/kg was only 130% above the basal value of that time ( $p < 0.05$ ); it was 180% above basal after 5.0 mg/kg and 190% above basal after 10 mg/kg. AVP concentrations rose after 10 mg/kg RU 486 to a maximum at 4 hours 310% above the basal value ( $X = 3.2 \pm 0.9$  pg/ml;  $F_{3,48} = 18.32$ ;  $p < 0.05$ ). At 5 mg/kg RU 486, AVP concentrations increased to 260% at 1 hour and 150% at 4 hours, respectively, but these elevations were not significant. No change in plasma AVP followed administration of 1.0 mg/kg RU 486.  $\beta$ -END concentrations rose from a basal mean value of  $45.6 \pm 17.1$  (SE) pg/ml to  $76.8 \pm 31.3$  pg/ml 2 h after 10 mg/kg RU 486, but this increase was not significant.

## Cumulative Responses to Various Dosages of RU 486

Analysis of the areas of response of plasma ACTH, cortisol and AVP to RU 486 is shown in Table 1. Released ACTH increased significantly between 1.0 and 5.0 mg/kg RU 486, but there was no further increase at 10 mg/kg dosage. Cortisol release did not rise appreciably until 5 mg/kg, and there was no further increase in the area of response following the 10 mg/kg injection. Cumulative AVP secretion became significant only after administration of 10 mg/kg RU 486.

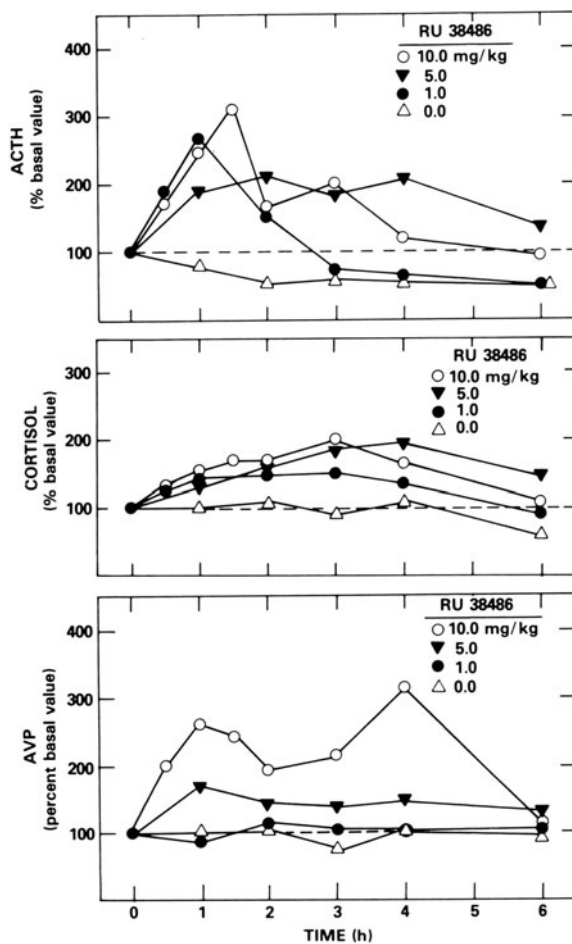


Fig. 5. Patterns of circulating levels of ACTH, cortisol, and AVP after RU 486, 10 mg/kg i.m. (open circle), 5.0 mg/kg (closed triangle), 1.0 mg/kg (closed circle), or vehicle (open triangle) at 0700 h to groups of 4 adult female cynomolgus monkeys. Hormone concentrations are expressed as percent of the mean ( $\pm$  SE) for 12 basal values: ACTH ( $11.7 \pm 1.9$  pg/ml), cortisol ( $44.7 \pm 2.4$  ng/ml) and AVP ( $0.4 \pm 0.3$  pg/ml).

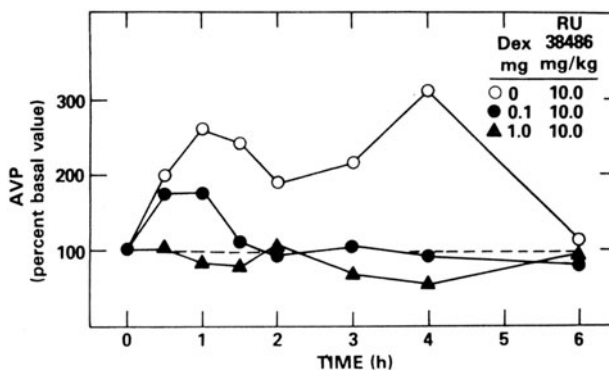


Fig. 6. Mean plasma AVP concentrations following RU 486 treatment, 10.0 mg/kg, without dexamethasone (n=4), or with dexamethasone, 0.1 mg, (n=3), or 1.0 mg (n=3) pretreatment in adult female cynomolgus monkeys. Plasma AVP concentrations are expressed as a percent ( $\pm$  SE) of the mean of 12 basal values ( $1.2 \pm 0.3$  pg/ml). Vertical bars indicate 1 SE.

#### Dexamethasone Pre-Treatment

Figure 6 shows the effect upon plasma AVP concentrations of dexamethasone administration before injection of 10 mg/kg RU 486. Note that 0.1 mg dexamethasone reduced the AVP response, while 1.0 mg dexamethasone abolished the increase in plasma AVP produced by RU 486. Peak AVP concentrations fell significantly ( $F_{3,48} = 21.3$ ;  $p < 0.025$ ) after 0.1 mg dexamethasone (0.03 mg/kg) pre-treatment when compared with RU 486 alone. Dexamethasone pre-treatment also prevented the increases in plasma ACTH and cortisol after RU 486.

#### DISCUSSION AND CONCLUSIONS

A major finding from our first study was that RU 486 consistently behaved as an antiprogesterone drug. This compound uniformly induced menstruation in castrated monkeys receiving exogenous  $E_2$  and P treatment on a schedule that mimicked the circulating steroid milieu of early pregnancy in intact cycling or in pregnant monkeys. Our observations establish that RU 486 can act directly upon endometrial tissue to produce uterine bleeding. Our studies also indicate that RU 486 does not bind to primate corpus luteal tissue and that endometrium is its only pelvic target organ.

Dose-response relationships were also examined in these experiments. Initial studies employed a concentration of 1 mg/kg RU 486. We have shown here that over a 100-fold dose range (10, 1, and 0.1 mg/kg), the RU 486 compound induced menstruation in all primates tested when injected daily for four days. Additional studies are required to 1) identify the minimum (threshold) effective dose, 2) assess the efficacy of single versus multiple daily dosage schedules, and 3) determine reliability of the antifertility actions of RU 486 in early pregnancy.

We have also demonstrated that familiar endometrial proliferation and withdrawal bleeding can redevelop acutely after administration of RU 486.

All monkeys menstruated within 72 hours after removal of all silastic E<sub>2</sub>- or P-filled capsules on day 49 of the treatment cycle (Fig. 1). Menstrual patterns in our randomized controlled trial were the same regardless of whether the placebo or RU 486 treatment regimens were applied first. Our study indicates that, at least in the short term, gross uterine and endometrial function in primates is normal following RU 486 exposure. These data are important safety prerequisites for clinical trials of this drug on a single or repetitive dose regimen; clearly, more extended tests are warranted.

RU 486 inhibition of pituitary PRL secretion occurred rapidly (<1 h), and the response to a single dose was short-lived (<6 h) compared with bromocriptine. Acute suppression of PRL by RU 486 confirmed and extended our previous reports upon the critical role of progesterone in the induction of hyperprolactinemia by sex steroids (Goodman and Hodgen, 1978; Williams et al., 1981; Healy and Burger, 1983). PRL increases noted during the luteal phase of the human menstrual cycle, pregnancy, combined oral contraceptive therapy, and with exogenous estrogen treatment of cycling women have been attributed to an estrogen action, without critical regard to the ambient progesterone concentrations. Although additional mechanisms may be operating in women, estradiol levels within or above the physiological range in non-human primates did not elevate circulating PRL levels (Goodman and Hodgen, 1978; Williams et al., 1981; Healy et al., 1983a). It is the introduction of progesterone into the estrogen-primed milieu that enhanced PRL secretion. In addition to supporting the physiological concept of an estradiol-progesterone synergy stimulating PRL release in primates, RU 486 or an analog may be an alternative to bromocriptine for treating hyperprolactinemia resulting from such a steroidal effect.

We have also shown (Figs. 4-6) that RU 486 treatment significantly elevates ACTH and cortisol for up to 4 hours and is therefore a glucocorticoid receptor antagonist in vivo. These effects are consistent with our in vitro data (Chrousos et al., 1984). Such an antiglucocorticoid action of RU 486 has clinical relevance for at least two reasons. First, an RU 486-produced blockage of the cortisol receptor may prevent the usual glucocorticoid stress response to anesthesia and surgery. In the patient who, for example, needs curettage after an RU 486-induced abortion, such an antiglucocorticoid effect may complicate anesthesia and recovery. Second, RU 486 deserves evaluation as an alternative treatment for patients with Cushing's syndrome, such as those with metastatic adrenal carcinoma or ectopic ACTH secretion, who fail to respond to conventional therapies, or for the pre-operative preparation of patients with Cushing's syndrome.

Our study of the effect of RU 486 upon the spontaneous midcycle LH surge is clearly preliminary but of obvious clinical interest. The failure to show a timely LH surge after RU 486 suggests that preovulatory P may augment E<sub>2</sub>-induced positive feedback and that, in a synergistic fashion similar to elevating PRL levels, preovulatory P may be required for full expression of the midcycle LH surge. An alternative explanation, that again highlights the importance of the anti-cortisol action of RU 486, is that the blunted LH surge reflects the removal of cortisol action upon the hypothalamus or pituitary. It is known that dexamethasone can block the preovulatory LH surge in rats (Hagino et al., 1969; Baldwin and Sawyer, 1974) though not in humans (Abraham, 1974) and that a single dose of triaminolone acetate given on day one or two of the menstrual cycle may delay folliculogenesis and ovulation (Cunningham et al., 1975). Further studies of RU 486 upon pulsatile gonadotrophin secretion are clearly warranted.

Our studies also define the dose- and time-dependent increases in ACTH, cortisol and AVP concentration and the endometrial response following RU 486 administration in non-human primates. The dose-finding studies suggest that

Table I. Area of Response of Plasma ACTH, Cortisol and AVP in Non-Human Primates Following Administration of 1.0, 5.0 or 10.0 mg/kg RU 486

RU 486 (mg/kg)	Areas of Response		
	ACTH (pg/ml/h)	CORTISOL ug/dl/h)	AVP (pg/ml/h)
1.0	19.6 $\pm$ 4.2	48.3 $\pm$ 8.6	1.4 $\pm$ 0.3
5.0	55.2 $\pm$ 7.8*	161.8 $\pm$ 19.7*	2.9 $\pm$ 0.9
10.0	70.2 $\pm$ 13.6	151.7 $\pm$ 18.3*	7.3 $\pm$ 1.3°

\* p<0.05 compared with 1.0 mg/kg dosage

° p<0.05 compared with 1.0 and 5.0 mg/kg dosages

Mean  $\pm$  SE

menstruation can occur following injection of as little as 0.1 mg/kg. The absence of a dose-menstruation response is consistent with a clinical report (Kovacs et al., 1984). By contrast, although parenteral administration of as little as 1.0 mg/kg RU 486 can elevate plasma ACTH levels, doses of 5.0 mg/kg and 10 mg/kg RU 486, respectively, are necessary to evoke significant increases in plasma cortisol and AVP (Table I).

This 50-fold difference (0.1 mg/kg vs. 5.0 mg/kg) between a significant endometrial response (menstruation) and a significant antiglucocorticoid action (hypercortisolemia) is clinically attractive for any intended use of RU 486 as an antiprogesterone steroid. RU 486 may prove clinically useful as a provocative test of the hypothalamic-pituitary-adrenal axis. Moreover, it may be therapeutically useful in hypercortisolism. Toward this end, data upon the pharmacokinetics of RU 486 are required. Extrapolation from this primate study, in which RU 486 was injected intramuscularly, to clinical investigations dependent upon oral absorption must be carefully drawn. Hepatic extraction and metabolic clearance of this compound are still unknown.

Our finding of an increase in plasma AVP concentrations after RU 486 also has clinical implications. The dose-response for AVP was up to an order of magnitude less sensitive than was the ACTH response to RU 486, and its time course was consistent with inhibition of glucocorticoid input to the supraoptic-paraventricular hypothalamic nucleus (Fig. 6). Dexamethasone pretreatment abolished this effect, demonstrating that RU 486-induced increases in plasma AVP levels are not due to a direct action of the drug and suggesting an action through the glucocorticoid receptor. The data indicate that glucocorticoids may exert an inhibitory effect on primate AVP secretion. Although further studies are needed to settle the issue, this conclusion would be consistent with the rise in AVP levels during glucocorticoid insufficiency in rats and dogs (Schwartz et al., 1983). RU 486 may be useful as a diagnostic test to examine for AVP reserve in suspected cases in diabetes insipidus, thereby replacing the water deprivation test.

## REFERENCES

- Abraham, G. P., 1974, Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle, J. Clin. Endocrinol. Metab., 39:340.
- Baldwin, D. M., and Sawyer, C. H., 1974, Effects of dexamethasone on LH release and ovulation in the cyclic rat, Endocrinology, 94:1397.
- Chrousos, G. P., Nieman, L., Healy, D. L., Spitz, I., Hodgen, G. D., Bardin, W., Cutler, G. B., and Loriaux, D. L., 1984, Anti-glucocorticoids: general aspects and clinical implications. in: "Aspects of Glucocorticoid Research and Therapy," M. Fehm, J. Koberling and H. Kruskemper, eds., Peri Med Fashbush, Verlagsgesellschaft, W. Germany (in press).
- Collins, R. L., Kreitmann-Gimbal, B., Kreitmann, O. L., and Hodgen, G. D., 1984, Blockade of the spontaneous midcycle LH surge by a progesterone antagonist, 7th International Congress of Endocrinology, Quebec City, Canada, July 1-7, abstract no. 375.
- Cunningham, G. R., Capterton, E. M., and Goldzieher, J. W., 1975, Antioviulatory activity of synthetic corticoids, J. Clin. Endocrin. Metab., 40:265.
- Dierschke, D. J., Yamaji, T., Karsch, F. J., Weick, R. F., Weiss, G., and Knobil, E., 1973, Blockade by progesterone of estrogen-induced LH and FSH release in the rhesus monkey, Endocrinology, 92:1496.
- Goodman, A. L., and Hodgen, G. D., 1978, Post partum patterns of circulating FSH, LH, prolactin, estradiol and progesterone in non-suckling cynomolgus monkeys, Steroids, 31:731.
- Hagino, N., Watanase, M., and Goldzieher, J. W., 1969, Inhibition by adrenocorticotrophin of gonadotrophin-induced ovulation in immature female rats, Endocrinology, 84:308.
- Healy, D. L., and Burger, H. G., 1983, Serum FSH, LH and PRL during the induction of ovulation with exogenous gonadotrophin, J. Clin. Endocrinol. Metab., 56:474.
- Healy, D. L., Baulieu, E. E., and Hodgen G. D., 1983a, Induction of menstruation by an anti-progesterone steroid (RU 486) in primates: site of action, dose-response relationships and hormonal effects, Fertil. Steril., 40:253.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen G. D., 1983b, Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863.
- Healy, D. L., Schenken, R. S., Lynch, A., Williams, R. F., and Hodgen, G. D., 1984a, pulsatile progesterone secretion: Its relevance to clinical evaluation of corpus luteum function, Fertil. Steril., 41:114.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Gold, P. W., and Hodgen, G. D., 1985, Increased ACTH, cortisol and arginine vasopressin (AVP) secretion in primates following the anti-glucocorticoid steroid RU 486 dose response relationships, J. Clin. Endocrinol. Metab., 60:1.
- Helmond, F. A., Simons, P. A., and Hein, P. R., 1980, The effects of progesterone on estrogen-induced luteinizing hormone and follicle-stimulating hormone release in the female rhesus monkey, Endocrinology, 107:478.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rose, P. J., 1984, Termination of very early pregnancy by RU 486 - an anti-progestational compound, Contraception, 29:399.
- Kreitmann-Gimbal, B., Goodman, A. L., Bayard, F., Hodgen, G. D., 1979, Characterization of estrogen and progesterone receptors in monkey endometrium: methodology and effects of estradiol and/or progesterone on endometrium of castrate monkeys, Steroids, 34:2512.
- Kreitmann-Gimbal, B., Kreitmann, O. L., Sopelak, V. M., Kurman, R. J., Baulieu, E. E., and Hodgen, G. D., 1983, Menstrual induction in the

- primate fertile and non-fertile cycle: anti-progesterone RU 486 binds to endometrial progesterone receptors without affecting luteal cells, J. Ster. Biochem., 19:1125.
- Schwartz, M., Keil, L. C. Masell, J., and Reid, I. A., 1983, Role of vasopressin in blood pressure regulation during adrenal insufficiency, Endocrinology, 112:234.
- Sopelak, V. M., Lynch, A., Williams, R. F., and Hodgen, G. D., 1983, Maintenance of ovulatory menstrual cycle in chronically cannulated monkeys: a vest and mobile tether assembly, Biol. Reprod. 28:703.
- Soules, M. R., Steiner, R. A., Clifton, D. K., Cohen, N. L., Aksel, S., and Brenner, W. J., 1984, Progesterone modulation of pulsatile luteinizing hormone secretion in normal women, J. Clin. Endocrinol. Metab., 58:378.
- Williams, R. F., Barber, D. L., Cowan, B. D., Lynch, A., Marut, E. L., and Hodgen, G. D., 1981, Hyperprolactinemia in monkeys: induction by an estrogen-progesterone synergy, Steroids, 38:2842.

STUDIES ON THE ANTIREPRODUCTIVE MECHANISMS  
OF ACTION OF RU 486

Francisco J. Rojas<sup>1</sup>  
James L. O'Connor<sup>2</sup>  
Ricardo H. Asch<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology  
The University of Texas Health Science Center  
San Antonio, Texas

<sup>2</sup>Department of Endocrinology  
Medical College of Georgia  
Augusta, Georgia

INTRODUCTION

In recent decades, special effort has been dedicated to the development of methods that will interfere with normal luteal function, in order to induce a postovulatory form of contraception. Both basic and clinical research have focused on altering the corpus luteum function, either by inducing a deficiency of alleged luteotropic substances or by administering substances having luteolytic activity.

Various approaches directed toward neutralizing the secretion and/or activity of endogenous luteinizing hormone (LH) were experimentally developed and proved to be successful in inducing luteolysis (Moudgal et al., 1972; Casper and Yen, 1979; Asch et al., 1981a). However, recent information has cast doubt upon the need for LH in the maintenance of luteal function in primates and, therefore, on the above mentioned approaches to inducing luteal insufficiency (Asch et al., 1982a; Balmaceda et al., 1983).

Other approaches have been based on the administration of substances that inhibit luteal steroidogenesis, thus inducing progesterone (P) deficiencies and the subsequent early onset of menses (Schane et al., 1978; Asch et al., 1980; Asch et al., 1982b). Prostaglandins also have been used to induce luteolysis in several animal species, including human and non-human primates (Kirton et al., 1970; Butler et al., 1975; Auletta et al., 1978; Balmaceda et al., 1980). However, neither of these last two approaches has proved to be a consistent, reproducible method of contraception, free of major side effects (Jones and Wentz, 1972; Lyneham et al., 1975).

More recently, a new method of luteal contraception, based on the interference of P activity at the endometrium by the use of antiprogestogen substances with competitive binding to the P receptor, has been theorized (Herrmann et al., 1982). In this report, we summarize our experiences on the effects of RU 486 (Philibert et al., 1982) on the luteal phase of the

rhesus monkey. In addition, we present experimental data exploring the cellular mechanisms by which RU 486 may exert its action.

#### EFFECTS OF RU 486 ON THE LUTEAL PHASE

To investigate the ability of RU 486 to affect the luteal phase, we selected sexually adult female rhesus monkeys (*Macaca mulatta*) experiencing regular menstrual cycles (Asch and Rojas, 1985).

##### Experimental Design

The animals were individually caged and exposed to centrally controlled temperature ( $23 \pm 1^\circ\text{C}$ ), humidity (20%) and light-dark photoperiod (0600-2000-0600). Details on housing, feeding and general husbandry practices have been described elsewhere (Asch et al., 1982a).

Ovulation was detected with an accuracy of  $\pm 12$  hours, using daily serum estradiol ( $E_2$ ) levels and serial laparoscopies, as described previously (Pauerstein et al., 1978). Both blood drawing and surgical procedures were performed with the animals under sedation induced with ketamine HCl (5-7 mg/kg; Vetalar<sup>R</sup>, Parke-Davis, Morris Plains, NJ).

From the day of ovulation, blood (3 ml) was drawn on a daily basis (0800) from a femoral or saphenous vein for up to 17 days. Blood was centrifuged, and the serum was stored at  $-20^\circ\text{C}$  until determination of follicle stimulating hormone (FSH), LH,  $E_2$  and P by radioimmunoassays (RIAs) described previously (Pauerstein et al., 1978; Asch et al., 1981b). All samples were assayed in duplicate and in only one assay for each hormone, to reduce variability in the determinations. Assay sensitivity was 800 ng/ml, 210 ng/ml, 20 pg/ml, and 0.2 ng/ml for FSH, LH,  $E_2$  and P, respectively. Intra-assay coefficients of correlation at a 70% maximum binding rate were 2.8%, 5.3%, 8.4% and 3.7% for FSH, LH,  $E_2$  and P, respectively.

Animals were divided into three groups ( $n = 5/\text{group}$ ), according to the schedule of administration of RU 486. RU 486 was administered by gavage in daily doses of 10 mg on the following schedule:

- Group 1 - From day 1 to day 5, postovulatory.
- Group 2 - From day 5 to day 9, postovulatory.
- Group 3 - From day 9 to day 13, postovulatory.

Each group was compared to a control consisting of animals ( $n = 5/\text{group}$ ) treated with the vehicle (lactose), instead of RU 486, in an experimental protocol identical to that explained above. Onset and duration of vaginal bleeding were determined by daily vaginal swabbings.

Group comparisons of luteal phase lengths and of hormone levels were carried out by Student's *t* test and analysis of variance. Differences were considered statistically significant when  $p < 0.05$ .

##### Effects on the Length of the Luteal Phase

Table I shows the length of the luteal phase in animals from Group 1 to 3 (treated with either vehicle only, or RU 486). It is clear that, whereas the vehicle appears to have no effect on the normal length of the luteal phase, the administration of RU 486 significantly induces early onset of menses in all groups tested. Furthermore, the earlier in the menstrual cycle RU 486 was administered, the earlier the onset of vaginal bleeding.

Table I. Length of the Luteal Phase in Animals of Groups 1-3 Treated with Either Vehicle or RU 486

	Vehicle	RU 486	p
Group 1	13 $\pm$ 2.0	4 $\pm$ 1.2	< 0.001
Group 2	14 $\pm$ 1.5	8 $\pm$ 0.6	< 0.001
Group 3	15 $\pm$ 2.1	10 $\pm$ 1.1	< 0.05

Data are expressed as days post-ovulation until the onset of vaginal bleeding  $\bar{x} \pm$  SEM.

#### Effects on Hormone Levels

Figures 1-3 show the hormone levels in animals of all groups, comparing the RU 486 treated monkeys with the control animals. No significant changes were observed for FSH, LH, E<sub>2</sub> or P. Serum P concentrations at the onset of vaginal bleeding induced by RU 486 ( $\bar{x} \pm$  SEM) were 2.1  $\pm$  0.3, 4.9  $\pm$  0.6, and 2.6  $\pm$  0.4 for Groups 1-3, respectively.

In addition, Figures 1-3 show the average onset of vaginal bleeding for each group of animals. We noted with interest that animals in Group 1 began to bleed approximately 72 hours after the initial dose of RU 486 and continued to bleed for approximately two days. After cessation of bleeding, they started experiencing vaginal bleeding again approximately on day 12 of the post-ovulatory period. Animals in Groups 2 and 3 presented vaginal bleeding in an average of 72 and 24 hours, respectively, after initiation of RU 486 administration.

No side effects (e.g. loss of hair, vomiting, diarrhea, hypotension or changes in body weight, appetite or daily activity) were observed in any of the animals during drug administration.

#### Comments

These data revealed that RU 486 consistently induces early onset of menses when administered during the luteal phase of regularly cycling adult rhesus monkeys. The present results confirm and extend those of Healy et al. (1983) in another non-human species, cynomolgus monkeys. These authors administered RU 486 to castrated, estrogen- and/or P-treated animals and observed consistently induced vaginal bleeding within 48 hours after drug administration. Similarly, Herrmann and co-workers (1982) demonstrated that, in humans, oral administration of RU 486 induces interruption of the luteal phase of the nonfertile menstrual cycle.

Our results conflict with those of Herrmann et al. (1982), since serum concentrations of FSH and LH were not affected by drug administration, as compared to levels observed in vehicle-treated monkeys. These findings agree with those of Healy et al. (1983) in that RU 486 exerts its effects on the luteal phase by a local action upon the endometrium.

#### EFFECTS OF RU 486 UPON GONADOTROPIN-STIMULABLE ADENYLYL CYCLASE ON HUMAN CORPUS LUTEUM

Conflicting data among groups of investigators concerning the effects of RU 486 upon hormonal levels during the luteal phase of the menstrual cycle

have led to the conjecture that this agent does not act exclusively at the endometrium level. Thus, studies indicating that RU 486 treatment decreases both LH and FSH and/or progesterone production (Herrmann, et al., 1982; Kovacs et al., 1984; Schaison et al., 1984) might imply that RU 486 can also interfere with pituitary and/or gonadal function. Furthermore, in a recent report, Herrmann et al. (1984) have suggested that RU 486 administration may affect the life span of the human corpus luteum when given in the absence of hCG after the 22nd day of a normal menstrual cycle.

In an attempt to explore the mechanisms by which RU 486 exerts its action, we have studied the effects of this steroid upon gonadotropin-

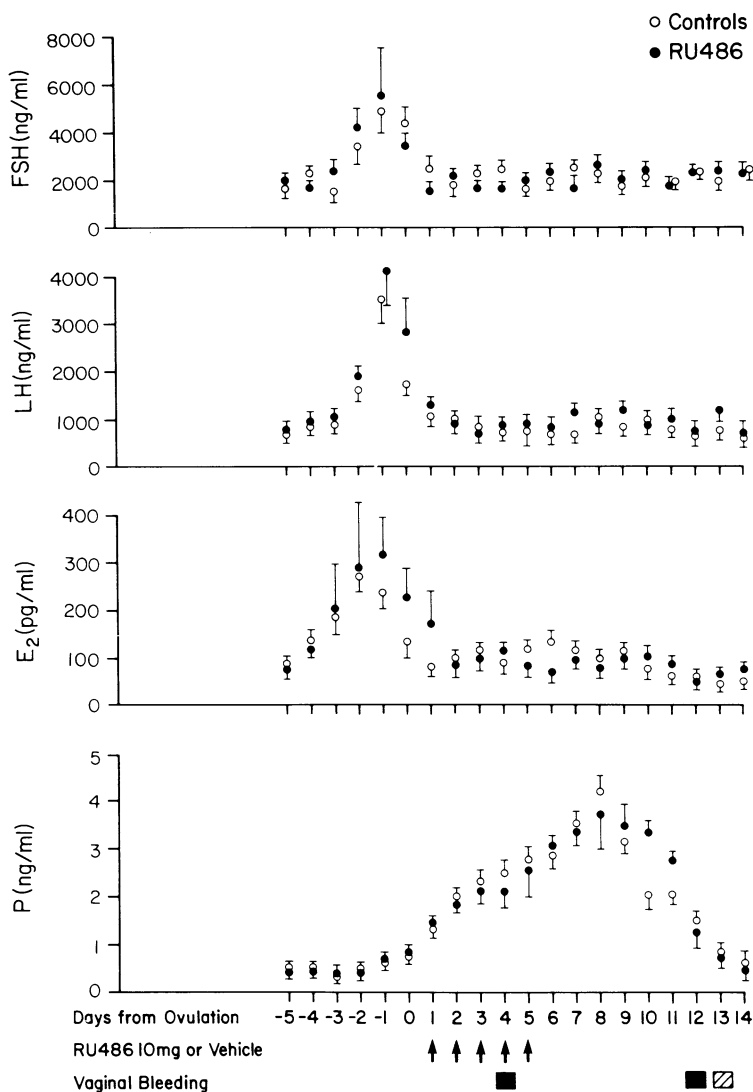


Fig. 1. Serum concentrations of FSH, LH, estradiol (E<sub>2</sub>) and progesterone (P) in animals from group 1 that received either RU 486 or vehicle (controls) from days 1-5 post-ovulation. Onset of vaginal bleeding is depicted by stripes in vehicle-treated animals and by solid in RU 486-treated animals (Asch and Rojas, 1985).

stimulable adenylyl cyclase in membrane preparations obtained from human corpus luteum. Since it is well established that the initiation of LH/hCG action in target cells is an increase in intracellular cAMP levels due to activation of adenylyl cyclase activity, this cell-free model provides the possibility to investigate the direct antigonadotropic effect of RU 486 at the corpus luteum level. The validity of such a model for studying the role of adenylyl cyclase in the regulation of luteal function in the human has been previously demonstrated (Rojas and Asch, 1984).

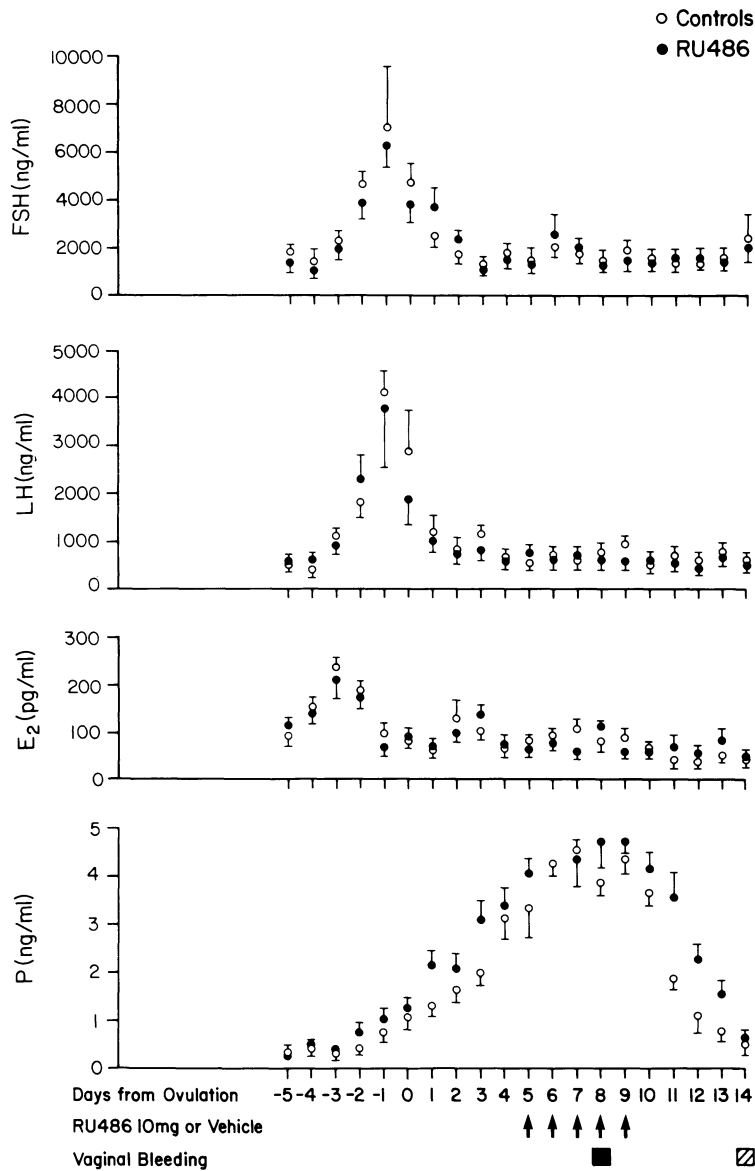


Fig. 2. Serum concentrations of FSH, LH, E<sub>2</sub>, and P in animals from group 2 that received either RU 486 or vehicle (controls) from days 5-9 post-ovulation. Onset of vaginal bleeding is depicted by stripes in vehicle-treated animals and by solid in RU 486-treated animals (Asch and Rojas, 1985).

Experimental Design

Corpora lutea were obtained from the ovaries of three regularly cycling women, aged 23 to 36 years, undergoing exploratory laparotomies at the Medical Center Hospital, San Antonio, Texas, for a variety of benign gynecological conditions. The study was approved by the Institutional Review Board. None of the women were pregnant nor hormonally medicated, and the ages of the corpora lutea were between 6 and 12 days, as assessed by histology and cycle dates. Immediately after removal, the luteal tissue was placed in iced Krebs-Ringer bicarbonate buffer, prepared with one half the recommended amount of  $\text{CaCl}_2$ , and transported to the laboratory.

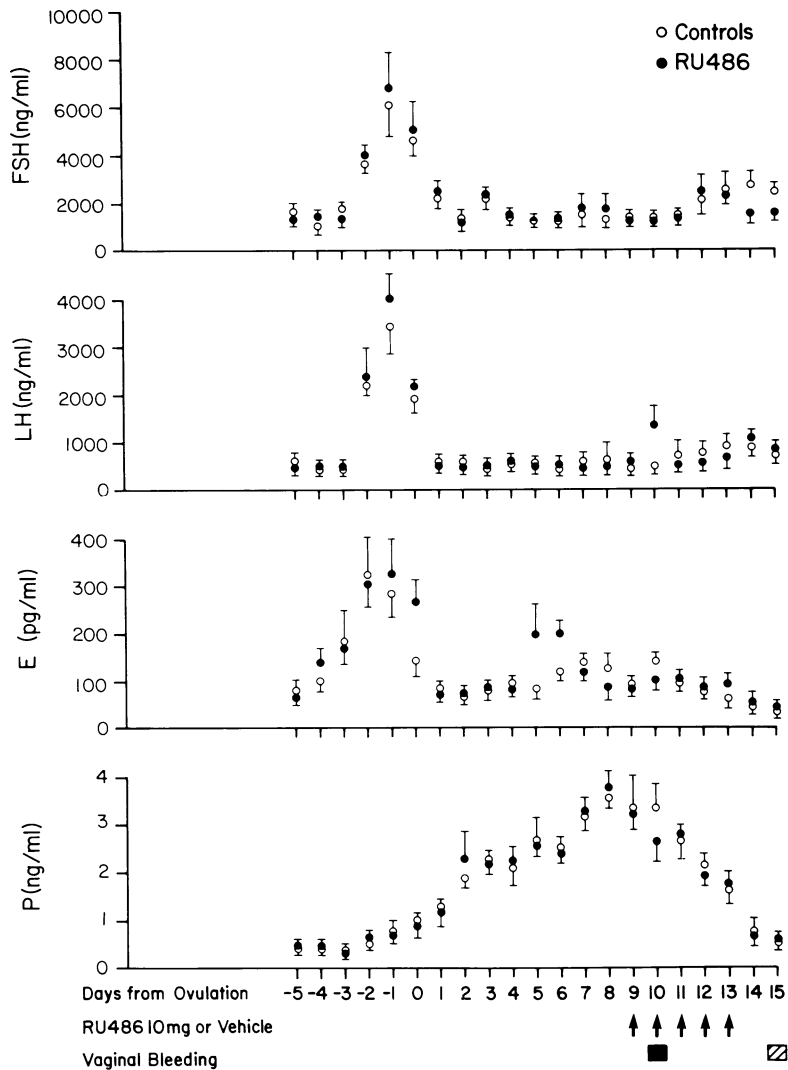


Fig. 3. Serum concentrations of FSH, LH, E<sub>2</sub>, and P in animals from group 3 that received either RU 486 or vehicle (controls) from days 9-13 post-ovulation. Onset of vaginal bleeding is depicted by stripes in vehicle-treated animals and by solid in RU 486-treated animals (Asch and Rojas, 1985).

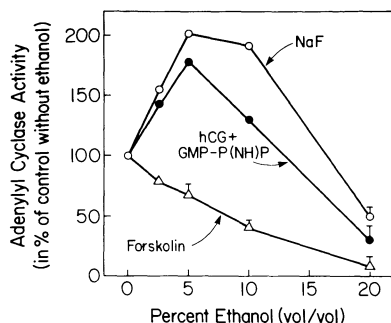


Fig. 4. Effects of increasing concentrations of ethanol on basal-, HCG-, NaF-, or forskolin-stimulated adenylyl cyclase activity from human luteal membranes. Gonadotropin responsiveness was determined under optimal conditions, i.e. in the presence of maximally effective concentrations of both hCG and GMP-P(NH)P. When present, hCG was 10 ug/ml, GMP-P(NH)P and forskolin were 100 uM, and NaF was 10 mM. Control values in the absence of ethanol were 20, 160, and 290 pmol/min/mg for hCG + GMP-P(NH)P, NaF, and forskolin, respectively.

Washed membrane particles were prepared as described previously (Rojas and Asch, 1984). Briefly, the corpora lutea were bivalved, teased from the surrounding stroma with forceps, and weighed. A small fraction was excised and placed in 10% formaldehyde solution for histological examination. The remaining tissue was then minced finely and homogenized in 20 vol (wt/vol) of 27% (wt/wt) sucrose, 10 mM Tris-HCl (pH 7.5), and 1mM EDTA in a Dounce homogenizer (Wheaton Industries, Millville, NJ). The homogenate was filtered through a silk screen, size 12 (G. F. Muth Co., Washington, D. C.), and centrifuged five minutes at 800 x g<sub>a.v.</sub>. The pellet was discarded and the supernatant recentrifuged at 10,000 x g<sub>a.v.</sub> for 45 minutes in the JA-20 rotor. The supernatant was discarded and the pellet (washed membrane particles) resuspended in 5 vol (with respect to original tissue weight) of homogenization medium with the loose pestle. After aliquoting, membranes were stored at -70°C.

Adenylyl cyclase activity was determined by the method of Birnbaumer et al. (1976). The optimal conditions for the assay of the enzyme from human luteal membranes have been described elsewhere (Rojas and Asch, 1984). Aliquots (10 ul) of membrane particle preparation (about 10 ug protein) were assayed in triplicate for adenylyl cyclase activity in the final volume of 50 ul containing 2.0 mM alpha-<sup>32</sup>P ATP (50 cpm/pmol), 5.0 mM MgCl<sub>2</sub>, 1.0 mM EDTA, 1.0 mM <sup>3</sup>H-cAMP (10,000 cpm), a nucleotide triphosphate regenerating system consisting of 20 mM creatine phosphate, 0.2 mg/ml creatine kinase (111 U/ml), 0.1 mg/ml myokinase (1950 U/mg) and 25 mM Tris-HCl buffer, pH 7.5. Incubations were performed at 32°C for ten minutes and terminated by the addition of 0.1 ml of a solution containing 40 mM ATP, 10 mM cAMP and 1% sodium dodecyl sulfate. The <sup>32</sup>P-cAMP formed and <sup>3</sup>H-cAMP present as recovery marker were isolated by double chromatography over Dowex 50 and alumina and quantified by liquid scintillation counting. The enzyme activities measured were linear with respect to time for at least 40 minutes and with respect to the amount of membrane protein used in the assay. In each experiment, a batch of membrane particles from a single corpus luteum was used. At least three determinations of the same tissue were assayed. In order to test tissue variability, the experiments were repeated with at least one batch of membranes from a different corpus luteum. All experiments showed a similar pattern of results. RU 486 was added as a dilution of a stock solution in absolute ethanol; final ethanol concentration (experimental and control groups) in the assay was 2%.

Table II. Effects of HCG, NaF and Forskolin on Adenylyl Cyclase Activity from Human Corpus Luteum Membranes

Additions	Adenylyl Cyclase Activity (pmol/min/mg)
None	8.2 $\pm$ 0.6
hCG	26.2 $\pm$ 2.8
hCG + GMP-P(NH)P	174.7 $\pm$ 4.5
NaF	130.5 $\pm$ 1.9
Forskolin	214.0 $\pm$ 14.3

When present, hCG was 10 ug/ml; GMP-P(NH)P and forskolin were 100 uM, and NaF was 10 mM.

#### Human Luteal Adenylyl Cyclase Activity

Table II shows activation of human luteal adenylyl cyclase by hCG, NaF and the diterpene forskolin. In agreement with reports in other mammalian adenylyl cyclase systems, the inclusion of a maximally effective concentration of guanyl nucleotide markedly enhanced the hormonal stimulation of the enzyme.

In addition, previous studies have demonstrated that under standard assay conditions, the human luteal adenylyl cyclase activity can be modulated by several other agents including calcium and ethanol. Thus, for example, Figure 4 illustrates the influence of acute exposure to ethanol on the enzyme activity. Up to a concentration of 5% (v/v), ethanol markedly potentiated stimulation of NaF and hCG responsiveness in a dose-dependent manner. In contrast, ethanol progressively inhibited forskolin stimulation at the same range of ethanol concentration. These data demonstrate that selective changes on the adenylyl cyclase activity of human luteal membranes can be induced by agents such as ethanol.

Under identical standard assay conditions, we observed that addition of up to  $10^{-6}$ M of RU 486 did not affect the hCG-stimulated adenylyl cyclase, nor did the steroid alone affect the activity of the enzyme in the absence of any stimulator (Table III). This failure to observe an effect on gonadotropin stimulation by RU 486 could not be attributed to the use of maximally effective concentration of guanyl nucleotide (which allows full gonadotropin responsiveness), since similar results were obtained when RU 486 was added in the presence of hCG alone (not shown). Similarly, further experiments showed that both NaF- and forskolin-stimulated activities were not altered by RU 486 (Table IV).

The data was in agreement with our finding that administration of RU 486 can induce early onset of menses in subjects simultaneously receiving doses of hCG that mimic early secretion of chorionic gonadotropin in the rhesus monkey (Asch and Rojas, 1985). In these experiments, one group of animals (n = 5/group) received RU 486 by gavage in daily doses of 10 mg on the following schedule:

Table III. Effect of RU 486 on Basal- and Gonadotropin-Stimulated Adenylyl Cyclase from Human Corpus Luteum Membranes

RU 486 (M)	Adenylyl Cyclase Activity (pmol/min/mg)	
	Basal	hCG responsiveness
0	8.7 $\pm$ 1.1	152.1 $\pm$ 1.8
10 <sup>-10</sup>	9.0 $\pm$ 5.6	148.0 $\pm$ 3.5
10 <sup>-9</sup>	8.9 $\pm$ 3.8	153.2 $\pm$ 9.4
10 <sup>-8</sup>	9.2 $\pm$ 1.0	155.1 $\pm$ 2.6
10 <sup>-7</sup>	8.2 $\pm$ 0.5	149.3 $\pm$ 1.0
10 <sup>-6</sup>	7.6 $\pm$ 0.9	140.5 $\pm$ 5.4

HCG responsiveness was determined under optimal condition, i.e. in the presence of maximally effective concentrations of both hCG (10 ug/ml) and GMP-P(NH)P (100uM).

From day 9 to day 13, postovulatory, plus increasing doses of hCG (30, 60, 90, 180 and 360 IU i.m. from day 6 to day 10, postovulatory) that mimic the early secretion of macaque chorionic gonadotropic (mCG) in the rhesus monkey gestation (Wilks et al., 1977; Asch et al., 1979).

Figure 5 shows the serum P level in animals that received either RU 486 or the vehicle from day 9 to day 13, postovulatory, in addition to increasing doses of hCG from day 6 to day 10, postovulation. Serum P concentrations at the onset of vaginal bleeding induced by RU 486 was 11.2 ng/ml. It is clear that, even in the presence of hCG, RU 486 was able to induce early onset of menses, despite high serum P levels. In addition, serum P levels did not differ among groups, suggesting no RU 486 effect at the luteal level.

#### Comments

These data demonstrate that RU 486 does not directly alter responsiveness of gonadotropin-sensitive adenylyl cyclase activity in membranes obtained from human corpus luteum. Failure to alter hCG-responsive adenylyl cyclase would imply that RU 486 does not affect gonadotropin receptor binding or cAMP synthesis and/or degradation in this membrane system. Such a failure strongly suggests that effects of RU 486 are unlikely to involve a direct antigonadotropic activity at the level of the human corpus luteum.

The observation that RU 486 administration can induce early onset of menses in subjects simultaneously receiving hCG (thus confirming earlier observations of Herrmann et al. (1982) during the early pregnancy of women) encourage the potential use of this antiprogesterone as a postcoital method of fertility control.

Table IV. Effect of RU 486 on NaF- and Forskolin-Stimulated Adenylyl Cyclase Activity from Human Corpus Luteum Membranes

Stimulator	Adenylyl Cyclase Activity (pmol/min/mg)	
	0 RU 486	10 <sup>-6</sup> M RU 486
NaF	144.7 ± 13.7	145.7 ± 4.2
Forskolin	168.7 ± 1.5	160.8 ± 7.2

When present, NaF was 10 mM and forskolin 100 uM.

#### EFFECT OF RU 486 ON GONADOTROPIN SECRETION OF DISPERSED RAT ANTERIOR PITUITARY CELL CULTURES

To explore the ability of RU 486 to directly interfere with pituitary function, we used a monolayer cell-culture system and investigated whether the agent affects LH and FSH secretion as well as response to LHRH.

#### Experimental Design

The monolayer cell-culture system was prepared by rat pituitary cell dispersal in trypsin followed by three days culture, as described by O'Conner et al. (1980). After three days, the cells were washed and exposed

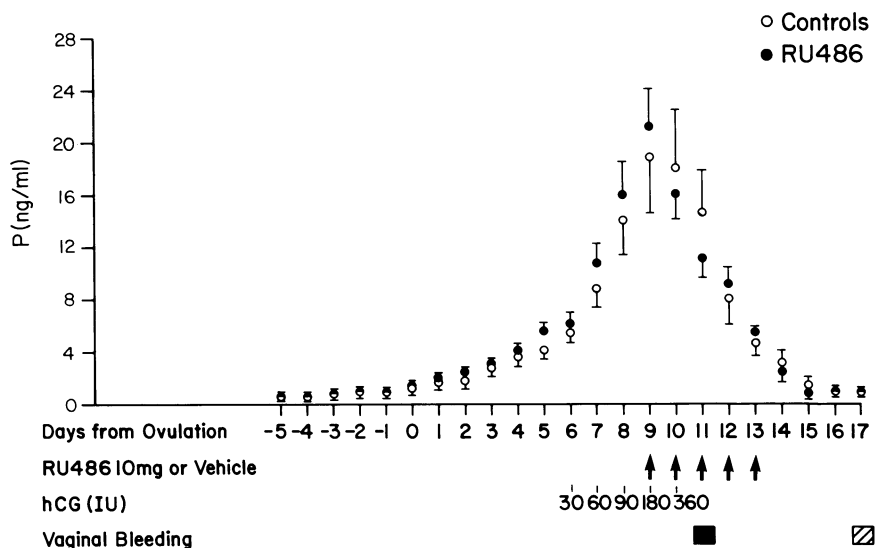


Fig. 5. Serum P concentrations in animals of group 4 that received either RU 486 or vehicle (controls) from day 9-13 post-ovulation and increasing doses of HCG from days 6-10 post-ovulation. Onset of vaginal bleeding is depicted by stripes in vehicle-treated animals and by solid in RU 486-treated animals (Asch and Rojas, 1985).

Table V. Effect of RU 486 Upon Basal LH and FSH Release  
in Dispersed Pituitary Cell Cultures

RU 486 (M)	Hormone Release (ng/culture)	
	LH	FSH
0	121 $\pm$ 12	242 $\pm$ 25
10 <sup>-12</sup>	142 $\pm$ 8	217 $\pm$ 8
10 <sup>-11</sup>	100 $\pm$ 5	258 $\pm$ 58
10 <sup>-10</sup>	105 $\pm$ 4	250 $\pm$ 37
10 <sup>-9</sup>	102 $\pm$ 7	291 $\pm$ 25
10 <sup>-8</sup>	140 $\pm$ 17	283 $\pm$ 25
10 <sup>-7</sup>	120 $\pm$ 16	295 $\pm$ 30

Table VI. Effect of RU 486 Upon LHRH Response of  
Dispersed Pituitary Cell Cultures

RU 486 (M)	Hormone Levels in Homogenates (ng/culture)	
	LH	FSH
0	767 $\pm$ 100	342 $\pm$ 17
10 <sup>-12</sup>	850 $\pm$ 42	429 $\pm$ 108
10 <sup>-11</sup>	833 $\pm$ 58	474 $\pm$ 67
10 <sup>-10</sup>	933 $\pm$ 87	417 $\pm$ 33
10 <sup>-9</sup>	1024 $\pm$ 167	499 $\pm$ 50
10 <sup>-8</sup>	1137 $\pm$ 60	450 $\pm$ 18
10 <sup>-7</sup>	871 $\pm$ 75	330 $\pm$ 35

LHRH (1ng/culture) is present in all cultures.

to RU 486 and/or LHRH. Following two hours, the culture media were removed and centrifuged to separate any unattached cells. The cells were covered with 0.01 M sodium phosphate-buffered saline. After freezing and thawing, the cells were hand homogenized with a glass homogenizer (Duell 21). Homogenates and incubation media were centrifuged at 1000 x g for ten minutes and stored at -20°C. Gonadotropin content was estimated by double antibody radioimmunoassay (O'Connor et al., 1980). The RU 486 ( $10^{-12}$ ,  $10^{-11}$ ,  $10^{-10}$ ,  $10^{-9}$ ,  $10^{-8}$ , and  $10^{-7}$ M, final concentration) was introduced into the cultures in 0.1 ml to make a final volume of 1 ml.

#### Activity of Dispersed Pituitary Cell Culture

Table V shows that addition of RU 486 has no significant effect upon basal LH and FSH release of the dispersed pituitary cell cultures. Similarly, RU 486 did not appreciably affect the response of the pituitary cells to LHRH, as determined by LH and FSH levels in either the homogenates (Table VI) or in the culture media (not shown). The concentration of LHRH used (1 ng/culture) has been previously demonstrated to sharply induce release of both gonadotropins in this cell-culture system (O'Connor and Lapp, 1984).

#### Comments

These data clearly show that RU 486 does not directly inhibit LH and FSH release by pituitary cells, nor does the steroid impair the response of these cells to the hypothalamic releasing hormone. It is unlikely, therefore, that RU 486 activity may involve a direct effect upon pituitary level. These observations agree with our data showing that RU 486 administration does not alter LH and FSH levels during the luteal phase of the menstrual cycle.

#### CONCLUSION

RU 486 induced vaginal bleeding when administered by gavage (10 mg daily) during the luteal phase of regularly cycling adult rhesus monkeys. Serum concentrations of FSH, LH, estradiol and progesterone were not affected during treatment. RU 486 also induced early onset of menses in subjects simultaneously receiving doses of hCG that mimicked early secretion of chorionic gonadotropin in the rhesus monkey.

We also showed in vitro studies indicating that RU 486 activity does not involve a direct antigonadotropic effect at the level of the human corpus luteum, as demonstrated by a failure to alter hCG-responsive adenyl cyclase in human luteal membranes. In addition, preliminary studies indicated that RU 486 does not affect basal LH and FSH release by pituitary cells in culture, nor does the agent impair the response of these cells to LHRH. Taken together, these data strongly suggest that RU 486 exerts its effects in the form of local action upon the endometrium. The availability of a target-action compound such as RU 486 opens a new approach to post-coital and interceptive contraception. It consistently induces early onset of vaginal bleeding without affecting the hormonal events of the menstrual cycle or inducing major untoward effects.

#### ACKNOWLEDGMENTS

We are grateful to Dr. Edouard Sakiz of Roussel Uclaf, Paris, France for the kind supply of RU 486. We also thank Ms. Rowena Bray, Mr. Arturo Moreno and Mr. Tom Turner for their technical assistance and Ms. Kim Francis and Ms. Gretta Small for their preparation of the original manuscript. This

study was supported in part by NIH Grant SP30 HD10202 (Radioimmunoassay Core) and by NIH Biomedical Research Grant RR05654.

## REFERENCES

- Asch, R. H., Smith, C. G., Siler-Khodr, T. M., and Pauerstein, C. J., 1979, Effects of delta-9-tetrahydrocannabinol administration of gonadal steroidogenic activity in vivo, Fertil. Steril., 32:576.
- Asch, R. H., Fernandez, E. O., Siler-Khodr, T. M., Bartke, A., and Pauerstein, C. J., 1980, Mechanism of induction of luteal phase defects by danazol, Am. J. Obstet. Gynecol., 136:932.
- Asch, R. H., Siler-Khodr, T. M., Smith, C. G., and Schally, A. V., 1981a, Luteolytic effect of D-Trp<sup>6</sup>-LH-RH in the rhesus monkey (*Macaca mulatta*), J. Clin. Endocrinol. Metab., 52:565.
- Asch, R. H., Balmaceda, J. P., Eddy, C. A., Siler-Khodr, T. M., Coy, D. H., and Schally, A. V., 1981b, Inhibition of the postcastration rise of LH and FSH in female rhesus monkeys (*Macaca mulatta*) by the administration of LH-RH inhibitory analogue ( $\frac{1}{2}$ N-Ac-D-Trp<sup>1,3</sup>, D-p-Cl-Phe<sup>2</sup>, D-Phe<sup>6</sup>, D-Ala<sup>10</sup><sub>2</sub>-LH-RH), Fertil. Steril., 36:388.
- Asch, R. H., Abou-Samra, M., Braunstein, G. D., and Pauerstein, C. J., 1982a, Luteal function in hypophysectomized rhesus monkeys, J. Clin. Endocrinol. Metab., 55:154.
- Asch, R. H., Smith C. G., Siler-Khodr, T. M., and Bartke, A., 1982b, Luteolytic effect of azastene in the nonhuman primate, Obstet. Gynecol., 59:303.
- Asch, R. H., and Rojas, F. J., 1985, The effects of RU 486 on the luteal phase of the rhesus monkey, J. Steroid. Biochem., in press.
- Auletta, F. J., Agins, H., and Scommegna, A., 1978, Prostaglandin F mediation of the inhibitory effect of estrogen on the corpus luteum of the rhesus monkey, Endocrinology, 103:1183.
- Balmaceda, J. P., Valenzuela, G. V., Eddy, C. A., and Asch, R. H., 1980, Prostaglandin production by rhesus monkey corpora lutea in vitro: effects of estrogen administration, Int. J. Gynecol. Obstet., 18:15.
- Balmaceda, J. P., Borghi, M. R., Coy, D. H., Schally, A. V., and Asch, R. H., 1983, Suppression of postovulatory gonadotropin levels does not affect corpus luteum function in the rhesus monkeys, J. Clin. Endocrinol. Metab., 57:866.
- Birnbaumer, L., Yang P-C, Hunzicker-Dunn, M., Bockaert, J., and Duran, J. M., 1976, Adenylyl cyclase activities in ovarian tissues. I. Homogenization and conditions of assay in Graafian follicles and corpora lutea of rabbits, rats and pigs: Regulation by ATP, and some comparative properties, Endocrinology, 99:163.
- Butler, R., Hotchkiss, J., and Knobil, E., 1975, Functional luteolysis in the rhesus monkey: ovarian estrogen and progesterone during the luteal phase of the menstrual cycle, Endocrinology, 96:1509.
- Casper, R. F., and Yen, S. S. C., 1979, Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor, Science, 205:408.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships, and hormonal effects, Fertil. Steril., 40:253.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, The effects of an antiprogesterone steroid on women: interruption of the menstrual cycle and of early pregnancy, C. R. Seances Acad. Sci. (III), 294:933.
- Herrmann, W., Schindler, A., Wyss, R., and Bischof, P., 1984, Antiprogesterone (RU 486), International Symposium: Future Aspects in Contraception, Heidelberg, Federal Republic of Germany, September, Abstract f 63.

- Jones, G. S., and Wentz, A. C., 1972, The effect of prostaglandin F<sub>2</sub> infusion on corpus luteum function, Am. J. Obstet. Gynecol., 114:393.
- Kirton, K. T., Pharriss, B. B., and Forbes, A. D., 1970, Luteolytic effects of prostaglandin F<sub>2</sub> in primates, Proc. Soc. Exp. Biol. Med., 133:314.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - An antiprogesterone compound, Contraception, 29:399.
- Lyneham, R. C., Korda, A. R., Shutt, D. A., Smith, I. D., and Shearman, R. P., 1975, The effect of intra-uterine prostaglandin F<sub>2</sub> on corpus luteum function in the human, Prostaglandins, 9:431.
- Moudgal, N. R., MacDonald, G. J., and Greep, R. O., 1972, Role of endogenous primate LH in maintaining corpus luteum function in the monkey, J. Clin. Endocrinol. Metab., 35:113.
- O'Conner, J. L., Allen, M. B., and Mahesh, V. B., 1980, Castration effects on the response of rat pituitary cells to luteinizing hormone-releasing hormone: Retention in dispersed cell culture, Endocrinology, 106:1706.
- O'Conner, J. L., and Lapp, C. A., 1984, Luteinizing hormone releasing hormone of fixed pulse frequency and duration, J. Pharmacol. Methods, 11:195.
- Pauerstein, C. J., Eddy, C., Croxatto, H. D., Hess, R., Siler-Khodr, T. M., and Croxatto, H. B., 1978, Temporal relationships of estrogen, progesterone and luteinizing hormone levels to ovulation in women and infrahuman primates, Am. J. Obstet. Gynecol., 130:876.
- Philibert, D., Deraedt, R., Tournemine, C., Mary, I., and Teutsch, G., 1982, RU 38486 -- A potent antiprogesterone, 6th International Congress of Hormonal Steroids, Jerusalem, Israel, Abstract f204
- Rojas, F. J., and Asch, R. H., 1984, Properties of gonadotropin-stimulable adenylyl cyclase of the human corpus luteum: Regulation of hormonal responsiveness by guanyl nucleotide and magnesium ion, J. Clin. Endocrinol. Metab., 59:219.
- Schaison, G., George, M., Lestart, N., Lagoguey, M., and Baulieu, E. E., 1984, Inhibitory effects of the antiprogesterone steroid RU 486 on gonadotropin secretion in women, 7th International Congress of Endocrinology, Quebec City, Canada, Abstract f2278.
- Schane, H. P., Creange, J. E., Anzalone, A. J., and Potts, G. O., 1978, Interceptive activity of azastene in rhesus monkeys, Fertil. Steril., 30:343.
- Wilks, J. W., Noble, A. S., Forbes, A. D. and Forbes, K. K., 1977, Steroidogenic responsiveness of the monkey corpus luteum to chorionic gonadotropin, Program for the 10th Annual Meeting of the Society for the Study of Reproduction, Austin, Texas, Abstract f8.

EFFECTS OF THE ANTIPROGESTERONE AGENT RU 486 ON THE  
NATURAL CYCLE AND GESTATION IN INTACT CYNOMOLGUS MONKEYS

G. Germain,<sup>1-4</sup> D. Philibert,<sup>2</sup> J. Pottier,<sup>2</sup> M. Mouren,<sup>2</sup>  
E. E. Baulieu<sup>3</sup> and C. Sureau<sup>1</sup>

<sup>1</sup>Clinique Universitaire Baudelocque  
I.N.S.E.R.M. U. 262  
123 Blvd. de Port-Royal  
75674 Paris Cedex 14, France

<sup>2</sup>Laboratoires Roussel-Uclaf  
102 Route de Noisy  
93230 Romainville, France

<sup>3</sup>Faculté de Médecine Paris-Sud  
Lab. Hormones, I.N.S.E.R.M. U. 33  
94270 Bicetre, France

<sup>4</sup>Station Centrale de Physiologie Animale  
C.N.R.Z. de l'I.N.R.A.  
78350 Jouy En Josas, France

ABSTRACT

This paper demonstrates that RU 486 is equally potent in inducing abortion and parturition in macaque monkeys at three stages of gestation (days 30, 80 and 140). The results suggest that progesterone probably plays a supportive role up to the end of gestation. However, erratic results were obtained when RU 486 was given orally, suggesting poor bioavailability of the compound when given by this route.

The second study, designed to assess the potential effect of RU 486 on luteolysis in macaques, shows that luteolysis can be chemically induced with high doses of RU 486. One early characteristic effect of the treatment was a transient increase in progesterone levels. Controlling bioavailability of RU 486 was the major problem in obtaining 100% success with the treatment. It was proved that ethanolic solutions are not appropriate for research on this problem.

INTRODUCTION

It is not yet clear whether progesterone is involved in the normal control of uterine motility in primates. Some information, obtained using non-pregnant macaque monkeys, points to a direct action by this hormone. Progesterone administration has been shown to induce a different pattern of motility in estrogen-primed castrated monkeys that exhibit a classical

pattern of coordinated motility. In this motility pattern, "desynchronized" activity also corresponds to a marked reduction of activity. In the same experimental conditions, progesterone withdrawal induces almost the same activity as during spontaneous menstruation, and coordinated uterine motility reappears (Sureau et al., 1983). However, when uterine activity was studied in the third trimester of pregnancy in macaques, there was no evidence of progesterone dominance. Clearly, the pregnant uterus showed synchronized activity despite the endogenous and local production of progesterone (Germain et al., 1982). Similar results were also found in humans (Lopes et al., 1984). Unfortunately, up to now it has not been possible to study the direct effect of progesterone withdrawal during pregnancy in macaque monkeys and thereby assess the role of progesterone in the control of uterine motility in the pregnant uterus of primates.

Acting at the receptor level and exhibiting antiprogestational activity in macaque monkeys (Philibert et al., 1982; Healy et al., 1982), RU 486 recently has provided a new tool that may help to answer questions such as: what would be the effects of blocking progesterone receptors if the hormone originates from an ovarian or placental source?

We report the effects of RU 486 on the time course of gestation and the menstrual cycle in macaques. Up to now most of the results obtained have been clinical. In this study we have tried to demonstrate a dose-response relationship for the effects of this compound. We used non-pregnant macaques because it was easier to perform such a study during the second half of the cycle rather than during advanced gestation.

#### EFFECT OF RU 486 ON GESTATION IN MACAQUE MONKEYS

##### Methodology

The 17 cynomolgus monkeys used in the study had all previously had at least one normal gestation in the colony where they were housed. They were naturally inseminated on days 10, 11 and 12 of the cycle (day 11 = day 0 of pregnancy), and gestation was assessed one month later by palpation of the enlarged uterus. After insemination, the animals were bled weekly (5 ml) between 09:30 and 11:30 am and then after RU 486 treatment daily until a response was observed. After ten days with no response the weekly regimen was readopted until the end of gestation. Endocrinological data was noted when available.

A single oral dose of 20 mg/kg/day (x 3 days) of RU 486 in 1% carboxymethyl cellulose in water (w:w) was given at 10:00 in the morning. After treatment, uterine bleeding was detected by daily vaginal swab. We palpated the uterus regularly to estimate the abortion day; a pelvic examination provided data on the obstetrical dynamics.

##### Results

Seven animals were used as controls. They delivered after a mean of  $160.14 \pm 5.60$  (SEM) days and all had live infants. The animals treated with RU 486 were divided into three groups (Fig. 1). In the first group treated during days 30 to 40 of gestation (n = 3), two subjects aborted within two days after the onset of the treatment. Two months later they had regular menstrual cycles. The pregnancy of the remaining animal (no. 104P) was unaffected by the treatment, in spite of a transient period of bleeding.

The second group (n = 2) was treated starting at day 85 of pregnancy. One subject (n.46) experienced bleeding one week after the treatment, and the bleeding lasted two weeks. Although uterine bleeding resumed two months

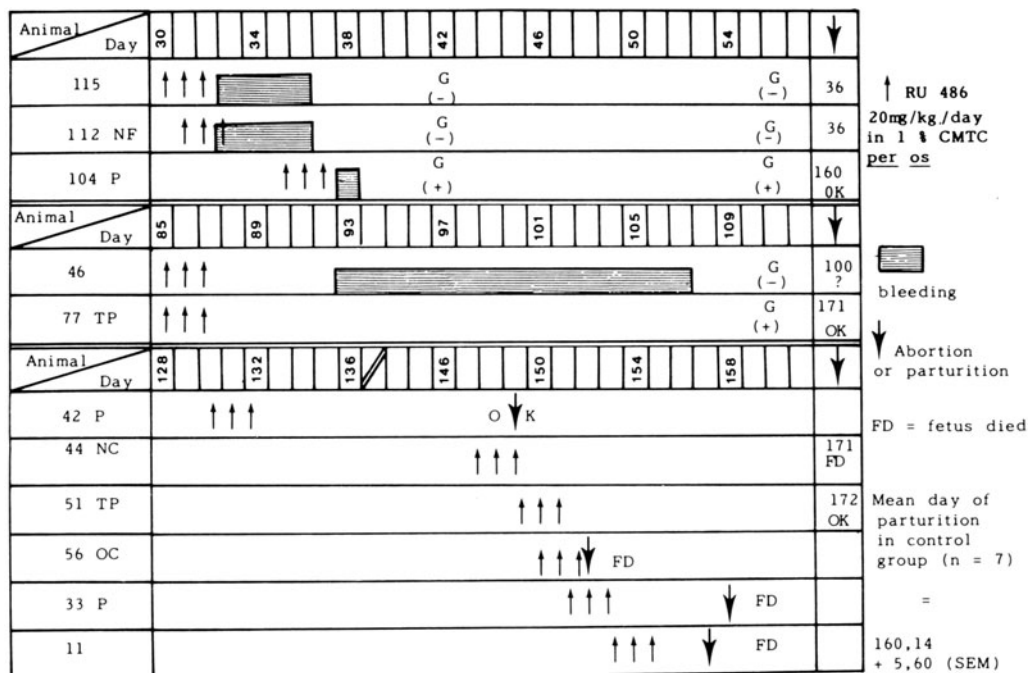


Fig. 1. Induction of abortion and parturition in macaque monkeys after treatment with RU 486 administered orally.

later, the uterus remained enlarged. We therefore decided to perform a hysterectomy to examine the uterine contents. The remnants of cranial bones were found in the uterine cavity. The other animal was not affected by the treatment and delivered a live infant.

The last group included six animals treated between days 130 and 155 of gestation. Four of the six (n. 42P, 44NC, 56OC and 11) were primed with 10 mg/day of estradiol benzoate in oil at day two, one and zero of RU 486 treatment. As shown in Figure 1, two gestations (n.42P and 51TP) were unaffected by the treatment. A third subject (n.44NC) did not abort, but the fetus died during delivery due to fetopelvic disproportion. The remaining animals (n.56OC, 33P and 11) delivered between one and seven days after the onset of the treatment; all the fetuses died. Two cases were clearly related to obstetrical problems because the fetuses had to be extracted by emergency cesarean section. In one case (n.11), there was no apparent clinical reason for fetal death.

#### Comments

As far as we know this is the first report of the effects of RU 486 in pregnant cynomolgus monkeys at various stages of gestation. This preliminary study demonstrates two important points. The first is that the results confirm the ability of RU 486 to cause abortion in primates, supporting what has already been demonstrated in humans (Herrmann et al., 1982). The second point is that RU 486 appears to be able to terminate pregnancy at any stage at which it is administered. Estrogen priming had no effect. In all groups, RU 486 caused abortion or the onset of labor with 50% success. Only acute responses occurring within one week were considered positive.

Thus our results support the theory that progesterone plays a large role up to the end of gestation in primates, as claimed by Csapo (1963) some time ago. RU 486 offers new perspectives in the study of the onset of labor and of associated uterine motility. Different mechanisms, though probably progesterone-dependent, might contribute to the reduction of uterine activity in the pregnant and non-pregnant primate uterus (Germain et al., 1982, Sureau et al., 1983).

The fetal deaths we report emphasize the limitations of such a study, inherent in the use of animal models. Because of the limited number of monkeys and the absence of technology adapted to monitor the course of labor in the current work, it was impossible to unequivocally connect the number of fetal deaths with the nature of the treatment. Clinical indications favor alterations in the obstetrical dynamics, and this will be studied in further work in our laboratory.

It is also clear from the present study that the single oral dose gave erratic results in terms of success; the first and simplest way to explain this is the poor, and therefore uncontrolled, bioavailability of the RU 486. This aspect of the problem was investigated in non-pregnant monkeys.

#### EFFECT OF RU 486 ON THE MENSTRUAL CYCLE

##### Methodology

Thirty seven cynomolgus monkeys weighing between 2.9 and 4.0 kg were used in this part of the work. Menstruation was detected by daily vaginal swab. In a previous study we showed that ovulatory cycles usually last less than 34 days and that no vaginal bleeding can be detected before day 26 of the cycle (Germain et al., 1982). Therefore, each experimental group consisted of ten animals that had previously experienced at least two cycles of normal duration (26-34 days).

Treatments were given at about 10:00 am. on day 19, 20, 21 or 22 of the cycle unless otherwise stated. RU 486 was administrated either orally (100 mg/kg 24 h) in 2 ml of 1% carboxymethylcellulose in water (w:w) or intramuscularly (1 to 35 mg/kg/24 h) after solubilization in 1 ml of 80% ethanol in water (V:V). Whatever the duration and intensity of the menses, if they occurred before day 26 and were not followed by a new bleeding period before day 40, the result was considered positive; otherwise it was noted as negative.

In the second half of the cycle, the monkeys were bled (5 ml) daily or every two days, depending on the experimental group. The blood was centrifuged for 10 min at 2000 x g, and the plasma was frozen at -20° C until assay. RU 486, progesterone (P), 17  $\beta$ -estradiol (E<sub>2</sub>) and cortisol (F) were measured by RIA (Raynaud et al., 1980; Abraham et al., 1972). Assay sensitivity was 0.2 ng/mg for RU 486, 0.2 ng/ml for P, 20 pg/ml for E<sub>2</sub> and 1 ng/ml for cortisol.

##### Results

Control group. As shown in Figure 2, cycle length ranged between 27 and 32 days. In one case (n. 46NC) menstruation occurred after day 40.

Single dose of RU 486 (100 mg/kg) given orally (Fig. 3). The treatment was positive in 4/10 cases. After treatment, the cycle lasted a mean 39.75 days in the positive group and 31.60 days in the negative group (Table I).

Table I. Duration of the cycle after an RU 486 treatment in relation to the dose of RU 486 used to induce menstruation in mid-luteal phase.

TREATMENT	DURATION OF THE CYCLE FOLLOWING THE TREATMENT CYCLE, IN DAYS ( $\bar{x} \pm SD$ , n)	
	GROUP OF ANIMALS WITH SUCCESS OF TREATMENT	GROUP OF ANIMALS WITH FAILURE OF TREATMENT
100 MG/KG in 1 % CMTC ORALLY	39.75 $\pm$ 6.65 (4)	31.60 $\pm$ 2.30 (6)
1 MG/KG in 80 % ETHANOL, I.M.	33.25 $\pm$ 6.45 (4)	38.00 $\pm$ 15.41 (5)
10 MG/KG in 80 % ETHANOL, I.M.	70.75 $\pm$ 27.10 (4)	84.60 $\pm$ 27.84 (6)
10 MG/KG/7H X 6 in 80 % ETHANOL, I.M.	$\geq$ 68.40 + 11.35 (8)	$\geq$ 88.00 + 7.07 (2)

Single dose of RU 486 (1mg/kg given i. m. (Fig. 4). Success was obtained in 4/10 cases; the others were negative. One of the latter (n. 125B) showed a biphasic bleeding period (days 22-25 and days 29-31) and another (n. 105) did not bleed before day 40. The mean duration of the cycle following treatment was 33.25 days in the positive group and 39.00 in

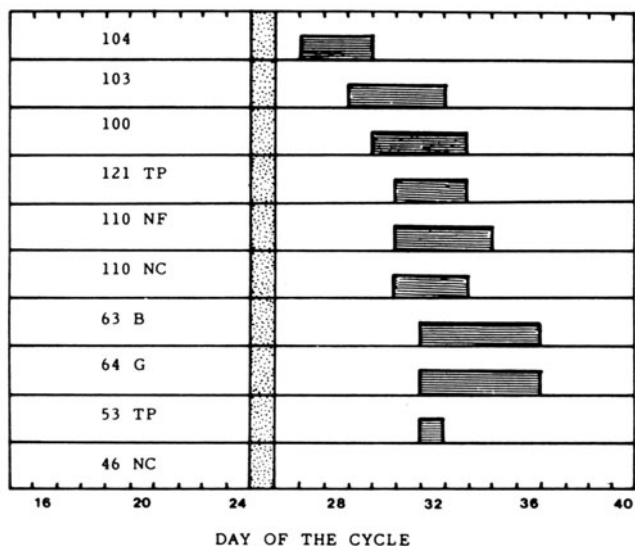


Fig. 2. Duration of the menstrual cycles in 10 intact macaque monkeys: control group. Shaded horizontal rectangles indicate length of the bleeding period.

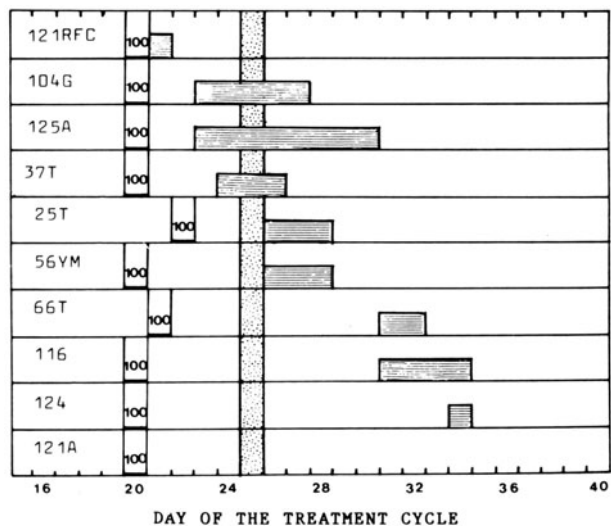


Fig. 3. Menstruation induced in intact macaque monkeys by RU 486 treatment (100 mg/kg in 1% CMTC) administered orally in mid-luteal phase.

the negative group (Table I). Figure 5 presents the kinetics of plasma RU 486 during the 48 hours after treatment. Low levels of around 10 ng/ml were recorded at 24 hours.

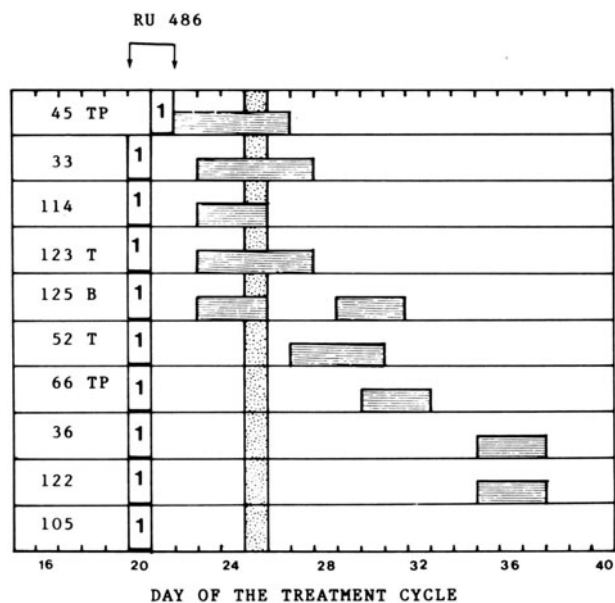


Fig. 4. Menstruation induced in intact macaque monkeys by RU 486 treatment (1 mg/kg in 80% ethanol) administered orally in mid-luteal phase.

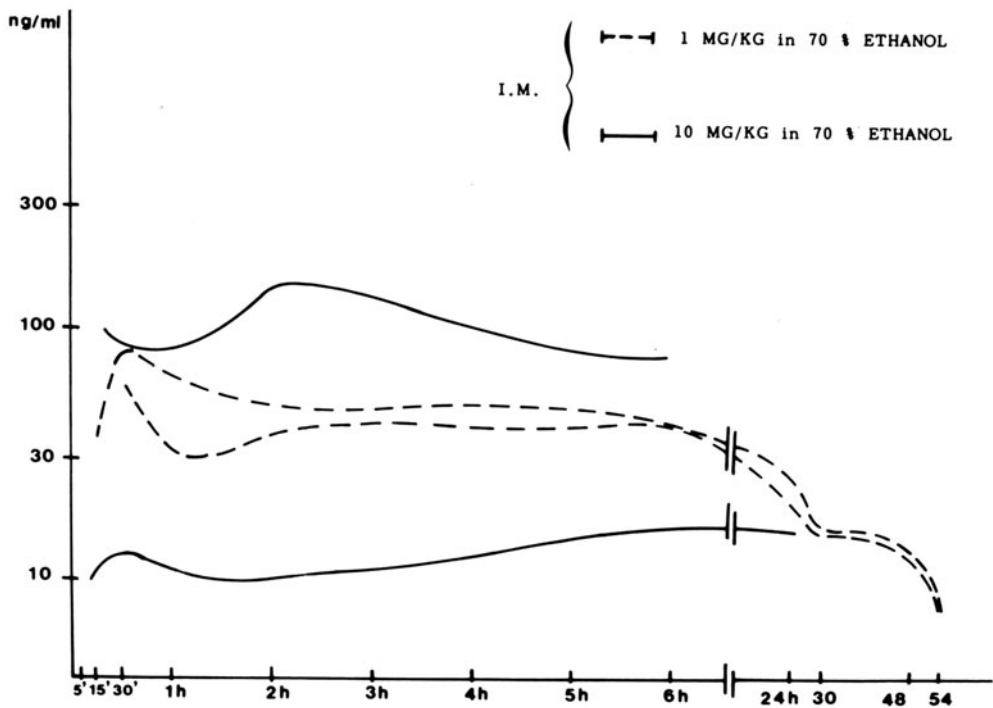


Fig. 5. Kinetics of plasma RU 486 in macaque monkeys after doses of 1 and 10 mg/kg in mid-luteal phase. RIA assay.

Single dose of RU 486 (10 mg/kg given IM, Fig. 6). Success was obtained in 4/10 cases; the remaining ones were negative. Two monkeys (n. 106 and 112 NF) had no menstruation until day 40. The mean duration of the cycle after the treatment was 70.75 days in the positive group and 84.60 days in the negative group (Table I).

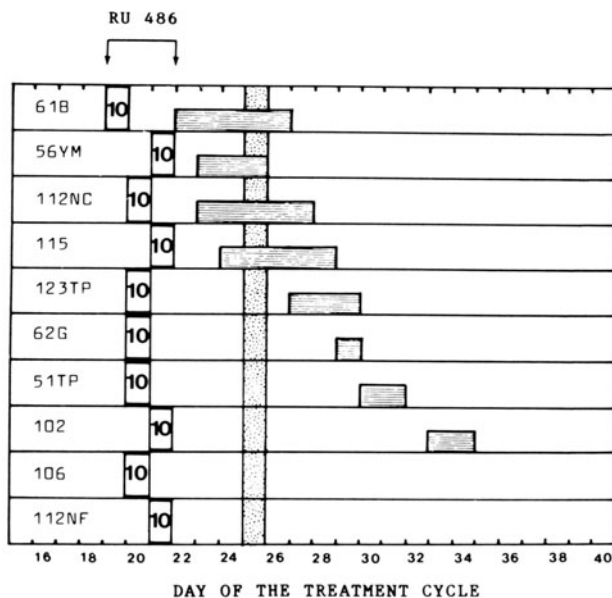


Fig. 6. Menstruation induced in intact macaque monkeys by RU 486 treatment (10 mg/kg in 80% ethanol) administered intramuscularly in mid-luteal phase.

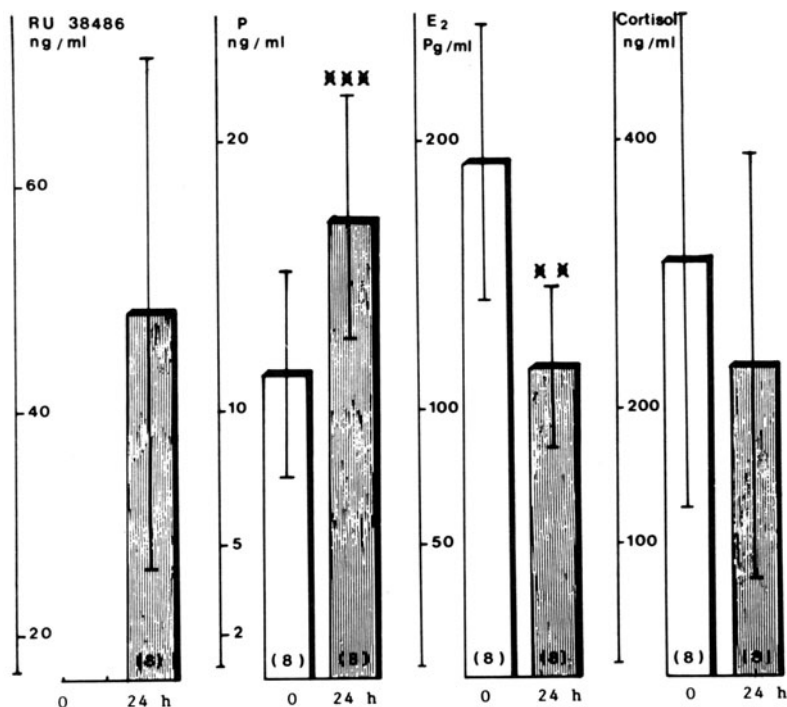


Fig. 7. Plasma levels ( $\pm$  SEM) of RU 486, progesterone (P), E<sub>2</sub> and cortisol in macaque monkeys at t=0 and 24 hours after RU 486 treatment (10 mg/kg in 80% ethanol) administered intramuscularly in the mid-luteal phase. P change,  $p < 0.001$ ; E<sub>2</sub> change,  $p < 0.01$ .

Figure 7 illustrates hormonal and RU 486 profiles before and 24 hours after treatment in eight monkeys that had an ovulatory cycle when treated. As in Figure 5, large variations in plasma-RU 486 levels were obtained with this regimen. Twenty four hours after treatment, the cortisol levels did not vary significantly. P levels were significantly elevated, whereas E<sub>2</sub> levels decreased with respect to zero time values.

Multiple doses of RU 486 (10 mg/kg/7h x 6 given IM) (Fig 8): The monkeys in this group were given six injections of 10 mg/kg each every seven hours. Success was recorded in 8/10 cases; the treatment was not positive in two animals (n. 66T and 25 TP), which did not bleed before day 40. Following the treatment, the cycle lasted 68.40 days in the positive group and 88.00 days in the negative group (Table I).

Figure 9 demonstrates that, with the multiple dose treatment, premature luteolysis was achieved in animals (n=8) that had ovulated prior to treatment.

Plasma levels of RU 486, P, E<sub>2</sub> and cortisol during the first 48 hours after the onset of the treatment are shown in Figure 10. High sustained levels of RU 486 (300-400 ng/ml) were obtained after the third injection. Interestingly, the P levels rose temporarily soon after treatment. This was followed by a decline until 48 hours, at which time the levels were significantly reduced vs. zero time levels. The time-course of E<sub>2</sub> levels was characterized by an increase at t=21-28 hours, and basal values were recovered at t=48 hours. At day 40 of treatment (data not shown), RU 486 levels ranged between 40 and 150 ng/ml. The E<sub>2</sub> and cortisol levels were not different from those at treatment zero time.

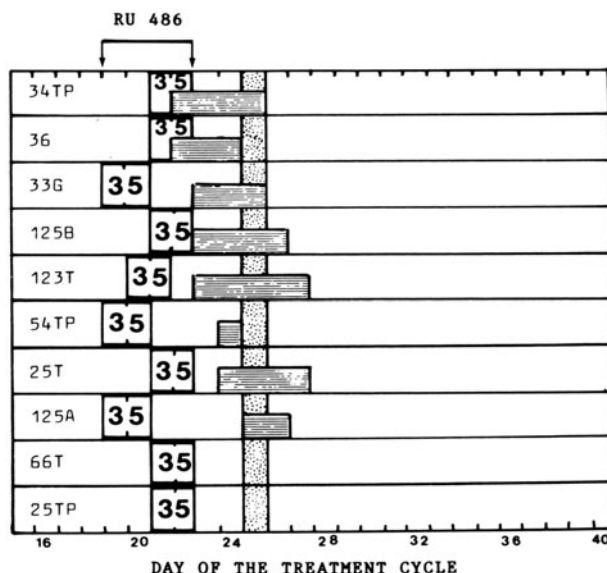


Fig. 8. Menstruation induced in intact macaque monkeys by RU 486 treatment (10 mg/kg 7h x 6 in 80%ethanol) administered intramuscularly in mid-luteal phase. The dose was nearly equivalent to 35 mg/kg/day x 2 days.

Summary of the results obtained in cycling monkeys treated with RU 486: Summing up the results described above, Figure 11 clearly demonstrates that when the dose of RU 486 was increased, the results were 100% positive.

Regardless of the route and dose of RU 486, the duration and intensity of induced bleeding were not different from those of the controls in any of the positive cases and occurred an average  $2.6 \pm 0.99$  (SEM) days after day one of treatment.

#### Comments

We decided to treat the monkeys at mid-luteal phase because it offered the most stable conditions for demonstrating the dose-dependent effect of RU 486. In macaques, plasma progesterone levels reach a plateau (Schoonmaker et al., 1982). Gonadotropin receptor binding sites (Cameron and Stouffer, 1982) and the steroidogenic capacities of the corpus luteum (Wilks and Noble, 1983) are at a maximum between days 17 and 23 of the cycle. This period of the cycle is the most sensitive for pharmacological induction of luteolysis with other agents such as estrogens alone (Schoonmaker et al., 1981; Sotrel et al., 1981) or combined with bromergocryptine (Castracane and Shaikh, 1980).

To our knowledge, this is the first report showing a dose-dependent effect at the termination of the natural menstrual cycle when RU 486 is used. High doses have been proven to produce actual premature luteolysis. Although speculative, a brief comment is necessary with respect to the putative mechanisms by which the demise of the corpus luteum is obtained. The major luteolytic agents that have been demonstrated to be efficient in macaques are exogenous estrogens and bromergocryptine. In this study, the high levels of RU 486 induced elevated levels of endogenous estrogens. It is unlikely that they could have caused luteolysis, because the suppression of estrogen synthesis during the luteal phase does not modify menstrual

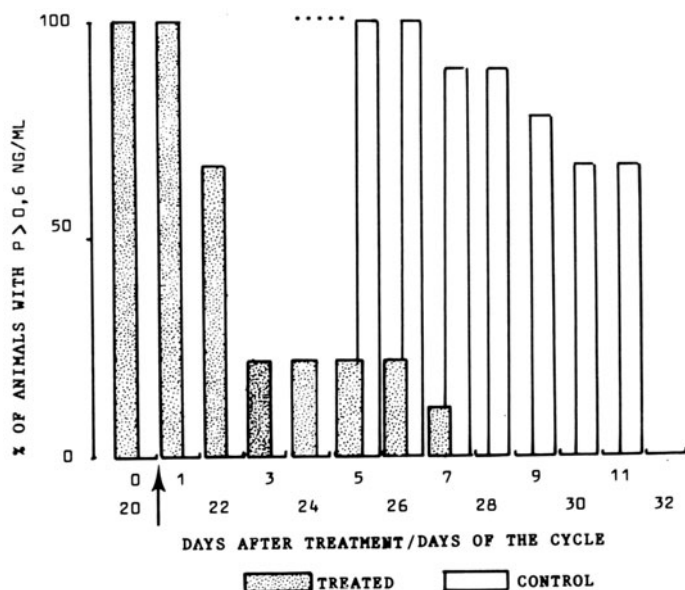


Fig. 9. Percentage of macaque monkeys (n=8) that experienced luteolysis (defined by plasma progesterone levels < 0.6 ng/ml in control group) and were treated with RU 486 (10 mg/kg/7h x 6) in mid-luteal phase. Luteolysis was advanced significantly ( $p < 0.02$ ) according to the Mann-Whitney test.

cycle length in macaques (Ellinwood and Resko, 1983). Indeed, a combination of estrogens and bromergocryptine is more potent for shortening the length of the cycle than estrogens alone (Castracane and Shaikh, 1980). This point raises the question of RU 486 interference with prolactin secretion. Healy et al., (1983), have shown that a dose of 10 mg/kg in ethanolic solution administered intramuscularly to hyperprolactinemic monkeys inhibits prolactin secretion for nearly 6 hours. Presumably, the multiple injection treatment in our study could have profoundly modified prolactin secretion for a long period of time. However, it is not yet clear whether prolactin is essential to the normal life-span of the corpus luteum in primates (Frawley and Neill, 1983). The temporal pattern of progesterone after RU 486 treatment was very interesting. In the two treatments (single dose of 10 mg/kg i.m. and multiple doses of 35 mg/kg i.m.), P was elevated over basal values after 24 hours, although when this occurred with the latter treatment it only elicited a brief response, followed by a decline towards zero levels. This response needs to be investigated further in connection with gonadotropic levels.

The last point of discussion concerns the long-term effects of RU 486 treatment on subsequent cyclicity in monkeys. These effects were not exhibited in the groups treated orally or with low doses of RU 486 administered intramuscularly. On the other hand, they appeared with higher doses injected intramuscularly in ethanolic solution. It is likely, at least in part, that this route produced an uncontrolled RU 486 "implant" that might have precipitated in the aqueous milieu where it was injected (i.e. in the muscle tissue) and have released small amounts of the product. The wide range of plasma RU 486 levels obtained after administration by this route favors this hypothesis (Fig. 5). Also supporting this theory is the fact that the plasma contained levels of 40 to 150 ng/ml up to 40 days after a dose of 35 mg/kg (data not shown). Some of the monkeys of this group have

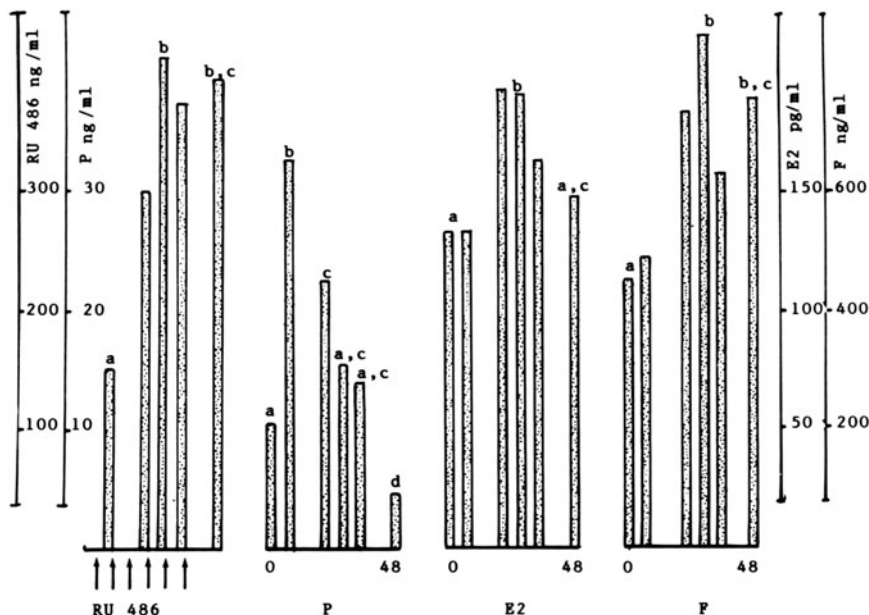


Fig. 10. Plasma levels of RU 486, progesterone, 17 beta E<sup>2</sup> and cortisol in macaque monkeys (n=8) at t=0 and after multiple injections (arrows) of RU 486 (10 mg/kg in 80% ethanol) in mid-luteal phase. Vertical columns with different superscripts indicate significant differences at the 0.05 level, according to the paired Student's t-test. For clarity, only significance at t=0 (or t=7h for RU 486, t=29 h and t=48h have been shown for RU 486, 17 beta-E<sub>2</sub> and cortisol.

already recovered their cyclicity, while others have not. Therefore, an ethanolic solution is not the best vehicle to use for potential application or for further research work with RU 486.

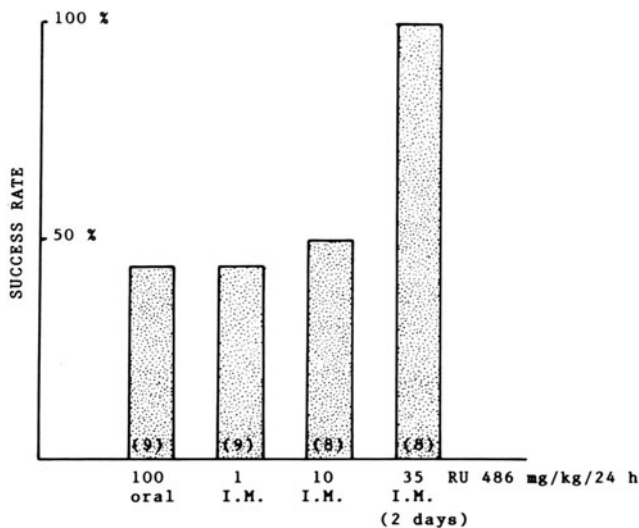


Fig. 11. Percentage of success according to the definition in methodology for the induction of menstruation using various routes and doses of RU 486 in intact cynomolgus monkeys treated in mid-luteal phase. The data only concern monkeys that had bleeding before D40.

## ACKNOWLEDGMENTS

We wish to thank M. Carpentier, D. Mauchand, C. Genty, B. Bonicel and M. C. Theron for excellent care of the primates. We are also indebted to the "Centre National de Recherches Zootechniques" (CNRZ) of the "Institut National de la Recherche Agronomique" (INRA) and to the "Fondation de Recherches en Hormonologie" which gave us the use of their facilities. The CNRZ housed the macaques and bred them. The financial support offered to G. G. by the "Fondation de Recherches en Hormonologie" is gratefully acknowledged. Ms. A. Daifuku (CNRZ-INRA) edited the original English manuscript.

## REFERENCES

- Abraham, G. E., Buster, J. E., and Teller R. C., 1972, Radioimmunoassay of plasma cortisol, Anal. Lett., 5:757.
- Cameron, J. L., and Stouffer, R. L., 1982, Gonadotropin receptors of the primate corpus luteum. I. Characterization of 125 I-labeled human luteinizing hormone and human chorionic gonadotropin binding to luteal membranes from the rhesus monkey, Endocrinology, 110:2059.
- Castracane, V. D., and Shaikh, A. A., 1980, Synergism of estrogen and bromergocryptine in the induction of luteolysis in cynomolgus monkeys (*Macaca fascicularis*), J. Clin. Endocrinol. Metab., 51:1311.
- Csapo, A. I., 1963, Model experiments and clinical trials in the control of pregnancy and parturition, Am. J. Obstet. Gynecol., 85:359.
- Ellinwood, W. E., and Resko, J. A., 1983, Effect of inhibition of estrogen synthesis during the luteal phase on function of the corpus luteum in rhesus monkey, Biol. Reprod., 28:636.
- Frawley, L. S., and Neill, J. D., 1983, Neuroendocrine regulation of prolactin secretion in primates, in: "The Anterior Pituitary Gland," A. S. Bhatnagar ed., Raven Press, New York.
- Germain, G., Cabrol, D., Visser, A., and Sureau, C., 1982, Electrical activity of the pregnant uterus in the cynomolgus monkey, Am. J. Obstet. Gynecol., 142:513.
- Germain, G., Cotinot C., Zorn, J. R., and Grenier, J., 1982, Réperage de l'ovulation chez le singe cynomolgus (*Macaca fascicularis*), Path. Biol., 30:650.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen G. D., 1983, Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Introduction of menstruation by an anti-progesterone steroid (Ru 486) in primates: site of action, dose-response relationships and hormonal effects, Fertil. Steril., 40:253.
- Herrmann, W., Wyss, R., Riondel, A., Philibert D., Teutsch, G., Sakiz E., and Baulieu E. E., 1982, Effet d'un stéroïde anti-progestérone chez la femme: interruption du cycle menstruel et de la grossesse au début, C. R. Acad. Sc. (Paris), 294:933.
- Lopes, P., Germain, G., Breart, G., Reitano, S., Le Houezec, R., and Sureau, C., Electromyographical study of uterine activity in the human during labour induced by prostaglandin-F2 alpha, 1984, Gynecol. Obstet. Invest., 17:96.
- Philibert, D., Deraedt, R., Tournemine, C., Mary, I., and Teutsch, G., 1982, RU 38486 a potent anti-progesterone, Sixth International Congress on Hormonal Steroids, Jerusalem, J. Ster. Biochem., 17:Abstract 204.
- Raynaud, J. P., Mary, I., Moguilewsky, M., Mouren, M., and Labrie, F., 1980, Inhibition of progesterone secretion in luteal phase by two luteinizing releasing hormone agonists in *Macaca fascicularis*, Fertil. Steril., 34:593.

- Schoonmaker, J. N., Victory, W., and Karsch, F., 1981, A receptive period for estradiol-induced luteolysis in the rhesus monkey, Endocrinology, 108:1874.
- Schoonmaker, J. N., Gergam, K. S., Steiner, R. A., and Karsch, F. J., 1982, Estradiol-induced luteal regression in the rhesus monkey: evidence for an extraovarian site of action, Endocrinology, 110:1708.
- Sotrel, G., Helvacioğlu, A., Dowers, S., Scommegna, A., and Auletta, F., 1981, Mechanism of luteolysis: effect of estradiol and prostaglandin-F<sub>2</sub> alpha on corpus luteum luteinizing hormone/human chorionic receptors and cyclic nucleotides in the rhesus monkey, Am. J. Obstet. Gynecol., 139:134.
- Sureau, C., Germain, G., Ferre, F., Breart, G., Goujard, J., Uzan, M., and Cedard, L., 1983, Therapeutic use of progesterone during the last two trimesters of pregnancy in: "Progesterone and Progestins," C. Wayne Bardin, E. Grom and P. Mauvais-Jarvis, eds. Raven Press, New York.
- Wilks, J. W., and Noble, A. S., 1983, Steroidogenic responsiveness of the monkey corpus luteum to exogenous chorionic gonadotropin, Endocrinology, 112:1256.

BEHAVIORAL AND ENDOCRINE CONSEQUENCES  
OF LONG-TERM ANTIPROGESTERONE (RU 486) ADMINISTRATION  
TO CYNOMOLGUS MONKEYS: PRELIMINARY RESULTS

Ronald D. Nadler,<sup>1</sup> Christian Roth-Meyer,<sup>2</sup>  
and Etienne-Emile Baulieu<sup>3</sup>

<sup>1</sup>Yerkes Regional Primate Research Center  
Emory University  
Atlanta, Georgia  
U.S.A.

<sup>2</sup>Centre International de Recherches Médicales de Franceville  
B.P. 769, Franceville, Gabon

<sup>3</sup>Laboratory Hormones  
INSERM U33  
94270, Bicetre, France

ABSTRACT

Menstrual cycle physiology and sexual behavior were studied in female cynomolgus monkeys following short- and long-term administration of RU 486 and a placebo, given by gavage at the end of the cycle. Some evidence of menstrual cycle irregularity associated with extended follicular phases was found in two of four females treated with RU 486, but interpretation is difficult because of the small number of subjects. No effect of RU 486 was apparent on the sexual behavior of the females or of the males with which they were tested.

INTRODUCTION

This study was prompted by the lack of information on the possible consequences of RU 486 administration with respect to menstrual cycle physiology and sexual behavior. The female cynomolgus monkey is a useful substitute for the human female in this regard, because previous research has demonstrated effects by RU 486 on the menstrual cycle and pregnancy in this species comparable to that found in women (Healy et al., 1983a, b; Kreitmann-Gimbal et al., 1984). Monkeys, moreover, show a greater responsiveness to hormonal regulation of sexual behavior than do human beings (Luttge, 1971; Beach 1976a,b). Monkeys, therefore, are sensitive models for evaluating possible adverse effects on sexual behavior, consequent to hormonal manipulations, which might be more obscure in human beings.

The objectives of this study were to determine whether RU 486 produces conspicuous adverse effects of menstrual cycle physiology and sexual

behavior during a) the first cycle following an initial treatment of 10 mg/day (about 3 mg/kg) which was given by gavage on two successive days at the end of the cycle and b) a second cycle following monthly treatments for approximately one year.

## MATERIALS AND METHODS

### Subjects

The subjects were 14 adult female and three adult male cynomolgus monkeys (Macaca fascicularis). The monkeys lived individually in conventional primate cages, 60 x 60 x 80 cm high, in an outdoor, covered facility subject to ambient equatorial light and temperature.

### Protocols

The females were divided into four groups, two experimental, receiving RU 486, and two control, receiving a placebo. Blood samples for hormone assay were obtained from all the females to assess hormone concentrations during the cycle. One experimental group (n = 4) and one control group (n = 2) were also given mating tests to assess measures of sexual behavior. RU 486 (10 mg) and placebo were given by gavage between 1430-1530 hours on days 26 and 27 of each cycle for 11 to 15 cycles (Mean  $\pm$  S.D. = 13.0  $\pm$  1.4). (Cycle days were numbered from the first day of menses which was designated day one.) Blood samples (5 ml) were obtained from the femoral veins during the first two cycles and a third cycle, approximately 13 months after the first. The samples were taken between 1430-1530 hours on alternate cycle days beginning on the day after the first day of menses to day 26 and then daily until the next onset of menses. The serum samples were stored at  $-60^{\circ}\text{C}$  until assayed.

The behavioral tests were conducted between 0900-1200 hours in a test cage, 290 x 60 x 80 cm high, by an observer positioned 150 cm from the center, in front of the cage. Two types of pair-tests were conducted. During the first two cycles, 10-minute tests were conducted six days a week throughout the cycle. On the last cycle, 13 months after the first, 30-minute tests were conducted daily during the ten day midcycle interval from day ten to day 19. The three males were used as partners for the females in the mating tests. They were vasectomized to prevent impregnation of the control females. The males were tested once or twice a day during cycles one and two but only once a day during the last cycles. The females were tested once a day, alternating among the males, in a paradigm that was balanced for order of males and daily testing time. The behavioral measures that were recorded were similar to those used by Zumpe and Michael (1983) and were similarly defined. In this paper, however, only data on the following measures are presented: female-initiated proximity (within 30 cm) to the male (including social grooming), female sexual presenting, precopulatory male contact of the female and male mounts, intromissions and ejaculations (behavioral ejaculatory patterns).

The data on menstrual cycle hormone concentrations have not been analyzed and will be presented elsewhere.

## RESULTS

### Parameters of the Menstrual Cycle

Table I presents the data on cycle length and the interval between the gavage (RU 486 and placebo) and menses for the six females that were given

mating tests. Because of the small number of subjects for which data are available, it is not possible to draw any firm conclusions, but certain aspects of the data are noteworthy. Two of the four females that received RU 486 (F94(E) and F132(E)) had relatively long cycles that were also more variable in length than those of the control females. These same females also had longer and more variable intervals between gavage and menses. Moreover, three of the experimental females experienced intervals without menses ranging from 1.5 to more than seven months duration. Although one of the control females (F84(C)) also had variable cycle lengths and gavage/menses intervals, neither had the extended intervals without menses that were found in the three experimental females.

It is possible that RU 486 induced long follicular phases in the two experimental females, F94(E) and F132(E). However, this may well be due to normal variability. In addition, there was inconsistency in the days that RU 486 was administered, making interpretation difficult. However, this inconsistency did enable us to examine the effect of RU 486 administration (on different cycle days) on the induction of menses (Table II). If RU 486 induced menses regardless of the day the gavage was administered, then the interval between the day of the gavage and the day of menses would be similar for all cycles, i.e., there would be a high correlation between the cycle day of the gavage and the cycle day of menses. This was not the case ( $r = -0.09$ ,  $n = 8$ ,  $df = 6$ ,  $P = n.s.$ ). If on the other hand, RU 486 had no effect on menses when given prior to the end of the cycle, then the interval between the day of the estradiol peak and the day of menses would be similar for all cycles regardless of the day the gavage was administered, i.e., there would be a high correlation between the cycle day of the estradiol peak and the day of menses. This was the case ( $r = +0.91$ ,  $n = 7$ ,  $df = 5$ ,  $P = 0.01$ ). This suggests that RU 486 did not induce menses in a nonfertile cycle irrespective of the cycle day on which it was administered.

#### Behavioral Measures

Table III summarizes the data on proximity and presenting in response to contact by the male for control and experimental females during ten minute tests in initial cycles with no treatment and the next cycle following RU 486 administration or placebo. There was reduced proximity and increased presenting to male contact in the cycle following RU 486 administration (and placebo), but this trend was apparent in control females as well as the experimental ones and was not significant. There was no indication, therefore, that RU 486 had any influence on these measures of female behavior. The considerable variability found in the proximity measures was mainly due to differences in the behavior of the males. The females maintained proximity with two of the males throughout the major portion of each test. The third male paced the cage a great deal, however, thereby preventing the females from establishing proximity. This resulted in relatively high proximity scores in two-thirds of the tests and mainly zero scores in one-third. Eliminating the third male from the analysis of proximity resulted in somewhat increased proximity scores and smaller standard deviations for most of the females, but no difference in interpretation (Table IV).

Table V summarizes the data on several measures of male sexual behavior obtained during the ten-minute tests on the first, control cycle and the cycle following treatment with RU 486 and placebo. There were no significant differences between the two cycles for the number of contacts, mounts or intromissions for either the control or experimental females. The males exhibited a higher percentage of tests with ejaculation during the second cycle, however, with both the control and experimental females. There was no indication that the males achieved fewer ejaculations with the experimental females after treatment. The data do not suggest, therefore,

Table I. Cycle Length and Interval Between Gavage and Menses for Non-treated Female Cynomolgus Monkeys (C) and Females Treated with RU 486 (E)

	Number of cycles	Cycle <sup>1</sup> length (days)	Interval <sup>1</sup> gavage/menses (days)
F 51 (C)	15	27.5 $\pm$ 1.6	2.2 $\pm$ 1.5
F 84 (C)	14	27.2 $\pm$ 4.8	3.1 $\pm$ 4.8
F 14 (E) <sup>2</sup>	12	29.3 $\pm$ 1.8	4.5 $\pm$ 2.0
F 94 (E) <sup>3</sup>	7	30.6 $\pm$ 6.0	6.4 $\pm$ 6.1
F 132 (E) <sup>4</sup>	11	33.5 $\pm$ 6.1	8.8 $\pm$ 6.3
F 152 (E) <sup>5</sup>	13	27.5 $\pm$ 2.8	2.9 $\pm$ 2.3

<sup>1</sup> Mean  $\pm$  S.D.

<sup>2</sup> No menses for 2 months after 10th cycle of treatment.

<sup>3</sup> No menses for at least 7 months after 7th cycle of treatment.

<sup>4</sup> No menses for 1.5 months after 2nd cycle of treatment.

<sup>5</sup> No treatment given at end of 2nd cycle.

that RU 486 adversely influenced any of these measures of male sexual behavior.

Table VI summarizes the data of the control and experimental females on proximity and presenting to contact by the male during 30-minute tests conducted during ten midcycle days following one year of monthly treatments with RU 486 or placebo. One experimental female (F94(E)) had not menstruated for seven months when these tests were initiated and was not tested. No data are presented for this female, therefore, in Table VI or Table VII. The proximity scores reflect individual differences among the females and among male-female pairs. Differences among the females, control and experimental, are difficult to interpret. It can be seen, however, that the experimental females did not differ in a conspicuous way from the control females. With respect to the measure, presenting to contact, all the females achieved high values and no differences between experimental and control females were apparent. These data, therefore, fail to demonstrate any adverse influence by RU 486 on these measures of female behavior following one year of RU 486 administration.

Table VII presents data on male sexual behavior in 30-minute tests during ten midcycle days with females that received monthly treatment with RU 486 or placebo for 13 months. It can be seen, despite the large standard deviations, that the number of male contacts, mounts, intromissions and ejaculatory patterns were not reduced in the experimental females, in comparison to the control females. There is no indication, therefore, that monthly treatment with RU 486 for 13 months adversely influenced these measures of male sexual behavior.

## DISCUSSION

The purpose of the research was to obtain preliminary indications of whether RU 486 administration at the end of one cycle subsequently influenced: 1) the female's menstrual cycle hormone patterns and/or 2) the sexual relations of the female and males with which the female was tested.

Table II. Cycle Day of the 17 $\beta$ -estradiol Peak, Gavage (Placebo and RU 486) and First Day of Menses for Two Cycles in Control (C) and Experimental (E) Female Cynomolgus Monkeys

	Cycle day of		
	<u>Estradiol peak</u>	<u>Gavage</u>	<u>Menses</u>
F51 (C)	9	26	28
	11	28	29
F84(C)	11	24	28
	11	27	28
$\bar{x} \pm \text{S.E.}$	10.5 $\pm$ 1.0	26.3 $\pm$ 1.7	28.3 $\pm$ 0.5
F14(E)	11	26	29
	12	24	30
F94(E)	11	26	26
	21	25	39
F132(E)	17	26	29
	25	23	39
F152(E)*	13	17	29
	13	27	29
$\bar{x} \pm \text{S.E.}$	15.4 $\pm$ 5.2	24.3 $\pm$ 3.2	31.3 $\pm$ 4.9

\*Gavage (RU-486) initiated on two separate cycle days in the same (first) cycle, i.e., days 17 and 27.

Data on length of the menstrual cycle in females treated and untreated for 13 months provide some suggestion of increased irregularity in the cycles of the females that received RU 486. Two of the four females that received RU 486 exhibited standard deviations in cycle length that were considerably longer than those of the control females. Three of the four treated females experienced disruptions of cyclicity with intervals between menses ranging from 1.5 to more than seven months. It is not possible to conclude from these data alone that the disruptions of the menstrual cycle were caused by RU 486 administration. Analysis of data on eight additional females in this study and on hormone concentrations during the cycles, however, should facilitate interpretation of these results. Preliminary hormonal data do not indicate profound disturbances of the cycle. However, it should be understood that the two-day 10 mg/day by gavage which was followed was established before present, more recent results were obtained, in particular those concerning the relatively uneven bioavailability of orally administered RU 486 (Deraedt et al., this volume; Germain et al., this volume). Consistency may be obtained by injection of an oil solution of RU 486 (Germain et al., this volume.) Also, it is likely that the relatively high dose (about 3mg/kg x 2 d) of RU 486 given at the end of the cycle may influence the beginning of the next cycle.

Cynomolgus monkeys were used in this study because of several attributes they possess that make them useful substitutes for human beings in such research. There is evidence, for example, that human sexual behavior is influenced in a similar way by hormonal fluctuations during the

Table III. Socio-Sexual Behavior of Female Cynomolgus Monkeys in Ten Minute Tests During a Control Cycle with no Treatment (NT) and Following Treatment with RU 486 (RU); C Denotes Control Females, E the Experimental Ones

	Proximity <sup>1</sup>		Present to Contact <sup>2</sup>	
	NT	RU	NT	RU
F 51 (C)	25 + 15 (n=22)	19 + 16 (n=26)	87 + 25 (n=21)	91 + 23 (n=25)
F 84 (C)	14 + 14 (n=22)	1 + 1 (n=24)	78 + 25 (n=21)	93 + 19 (n=24)
F 14 (E)	28 + 15 (n=24)	24 + 17 (n=25)	98 + 11 (n=22)	100 + 0 (n=25)
F 94 (E)	17 + 16 (n=23)	11 + 14 (n=32)	100 + 0 (n=16)	100 + 0 (n=26)
F 132 (E)	21 + 12 (n=24)	16 + 15 (n=34)	97 + 18 (n=24)	100 + 2 (n=32)
F 152 (E)	19 + 17 (n=25)	16 + 16 (n=27)	85 + 28 (n=18)	99 + 5 (n=21)

<sup>1</sup> Number of 15 second intervals out of 40 (Mean + SE) the female was proximate to the male.

<sup>2</sup> Percentage of times (Mean + SE) the female presented in response to male contact.

female cycle as is the behavior of monkeys (Adams, et al., 1978; Genazzani, et al., 1978). Although apparently influenced in a comparable way by hormones, human beings are less responsive to such influences than are monkeys (Luttge, 1979; Beach, 1976a,b). It was assumed, therefore, that any influences by RU 486 on sexual behavior that are relevant to human beings would be more readily apparent in the monkeys. It was also assumed that only effects that are conspicuous and clearly demonstrated in the monkeys would have significance for human beings.

Table IV. Proximity Scores (Mean + SD) of Female Cynomolgus Monkeys in Ten Minute Tests During a Control Cycle with no Treatment (NT) and Following Treatment with RU 486 (RU); C Denotes the Control Females, E, the Experimental Ones<sup>1</sup>

	NT	RU
F 51 (C)	30.9 + 12.1 (n=17)	28.1 + 12.4 (n=17)
F 84 (C)	15.6 + 14.2 (n=18)	2.3 + 3.8 (n=16)
F 14 (E)	37.1 + 2.5 (n=15)	36.3 + 3.1 (n=18)
F 94 (E)	25.9 + 13.9 (n=14)	16.4 + 13.7 (n=21)
F 132 (E)	32.1 + 11.0 (n=16)	24.2 + 10.3 (n=22)
F 152 (E)	21.0 + 16.0 (n=20)	23.4 + 14.5 (n=18)

<sup>1</sup> Data for one (aberrant) male eliminated from data presented in Table 2.

Table V. Sexual Behavior of Male Cynomolgus Monkeys in Ten-minute Tests During a Control Cycle with No Treatment of the Females (NT) and Following Treatment with RU 486 (RU). C Denotes the Control Females, E, the Experimental Ones

	Number of contacts <sup>1</sup>		Number of mounts <sup>1</sup>		Number of intromissions <sup>1</sup>		Percent tests with ejaculation	
	NT	RU	NT	RU	NT	RU	NT	RU
F 51 (C)	3.0 + 2.9 (n=22)	3.0 + 1.7 (n=26)	2.9 + 2.0 (n=22)	4.2 + 1.9 (n=26)	1.8 + 1.3 (n=22)	3.9 + 2.2 (n=26)	36 (n=22)	46 (n=26)
F 84 (C)	4.4 + 3.5 (n=22)	2.7 + 1.7 (n=24)	2.9 + 2.1 (n=22)	2.6 + 1.8 (n=24)	1.9 + 2.5 (n=22)	2.4 + 1.9 (n=24)	45 (n=22)	87 (n=24)
F 14 (E)	1.6 + 1.9 (n=24)	1.1 + 0.3 (n=25)	1.9 + 2.0 (n=24)	1.9 + 1.5 (n=25)	1.3 + 1.7 (n=24)	1.0 + 0.0 (n=25)	92 (n=24)	100 (n=25)
F 94 (E)	1.8 + 2.3 (n=23)	1.7 + 2.1 (n=32)	3.2 + 3.3 (n=23)	3.0 + 2.3 (n=32)	2.7 + 3.2 (n=23)	2.7 + 2.1 (n=32)	69 (n=23)	78 (n=32)
F 132 (E)	3.1 + 3.1 (n=24)	2.6 + 2.7 (n=34)	3.2 + 3.1 (n=24)	3.1 + 2.8 (n=34)	2.6 + 2.8 (n=24)	2.7 + 2.6 (n=34)	92 (n=24)	94 (n=34)
F 152 (E)	2.8 + 3.5 (n=25)	2.0 + 1.9 (n=27)	2.9 + 3.4 (n=25)	3.5 + 2.2 (n=27)	2.1 + 3.6 (n=25)	2.9 + 2.3 (n=27)	28 (n=25)	52 (n=27)

<sup>1</sup> Mean + SE

The measures of female behavior that were examined, proximity and presenting to male contact, reflect respectively, the female's affiliative relationship with the male and the female's receptivity to the male's sexual initiative. Although proximity was reduced during ten minute tests following the initial treatment with RU 486, there was no indication that the reduction was greater in the females that received RU 486. The treated females moreover, exhibited near-maximal receptivity scores on these tests. With respect to male sexual behavior during the ten minute tests, there was no effect by RU 486 apparent on the measures of male contacts, mounts, intromissions or ejaculations. These data suggest that a single administration of RU 486 had no significant influence on these measures of sexual behavior when assessed by a relatively short test.

Table VI. Socio-sexual Behavior of Female Cynomolgus Monkeys in 30 Minute Tests During 10 Midcycle Days Following Treatment with RU 486 or Placebo for a Period of 13 Months; C Denotes Control Females, E, the Experimental Ones

	Proximity <sup>1</sup>	Present to contact <sup>2</sup>
F 51 (C)	82.4 + 33.5	98.3 + 5.4
F 84 (C)	46.5 + 21.4	93.6 + 11.4
F 14 (E)	77.2 + 48.9	100.0 + 0.0
F 132 (E)	52.1 + 42.5	100.0 + 0.0
F 152 (E)	48.7 + 32.8	99.0 + 3.2

<sup>1</sup> Number of 15 second intervals out of 120 (Mean + SE) the female was proximate to the male.

<sup>2</sup> Percentage of times (Mean + SE) the female presented in response to male contact.

Table VII. Sexual Behavior (Mean  $\pm$  SD) of Male Cynomolgus Monkeys in 30 Minute Tests Conducted During 10 Midcycle Days with Females Treated Monthly with RU 486 or Placebo for 13 Months

	Number of contacts	Number of mounts	Number of intromissions	Number of ejaculations
F 51 (C)	4.9 $\pm$ 2.4	6.2 $\pm$ 3.1	4.6 $\pm$ 2.1	1.4 $\pm$ 0.5
F 84 (C)	4.8 $\pm$ 1.8	3.8 $\pm$ 1.9	2.6 $\pm$ 1.8	1.4 $\pm$ 0.7
F 14 (E)	5.2 $\pm$ 3.5	6.5 $\pm$ 5.2	3.7 $\pm$ 2.7	1.6 $\pm$ 0.5
F 132 (E)	7.0 $\pm$ 4.2	8.1 $\pm$ 5.1	5.7 $\pm$ 4.3	1.5 $\pm$ 0.5
F 152 (E)	8.5 $\pm$ 5.2	9.9 $\pm$ 5.6	6.5 $\pm$ 4.8	1.0 $\pm$ 0.5

Relatively short tests of sexual behavior have been reported to be less sensitive than tests of longer duration for detecting behavioral influences by hormones (Zumpe and Michael, 1983). The cumulative effect of repeated monthly treatment with RU 486 might be greater than that following a single treatment. The tests of longer duration (30 minutes) conducted after more than a year of monthly treatment with RU 486 were conducted to assess these possibilities. There was no indication that a cumulative effect of repeated treatment with RU 486 adversely influenced the behavioral measures that were examined from these tests of longer duration.

It is concluded that RU 486 had no conspicuous effect on sexual behavior of the cynomolgus monkey. This does not rule out the possibility of more subtle effects on the behavior of cynomolgus monkeys, but it suggests that no serious effects on human sexual behavior by RU 486, as administered, are likely. Whether or not the detected menstrual cycle irregularities occur more generally should be clarified when our data on additional females are analyzed. Other experiments with different regimens of RU 486 (changing doses, number of days of administration, injections) will be performed (see also Germain, et al., this volume and Nieman et al., this volume).

#### ACKNOWLEDGMENTS

This research was supported by the Centre International de Recherches Médicales de Franceville. RDN also received support from U.S. Public Health Service Grant RR-00165 (Division of Research Resources, National Institutes of Health). G. Affre, R. W. Cooper and M. Laumonier provided veterinary assistance.

#### REFERENCES

- Adams, D. B., Gold, A. R. and Burt, A. D., 1978, Rise in female-initiated sexual activity at ovulation and its suppression by oral contraceptives, New Eng. J. Med., 299:1145-1150.
- Beach, F. A., 1976a, Cross-species comparisons and the human heritage, Arch. Sex. Behav., 5:469-485.
- Beach, F. A., 1976b, Hormonal control of sex-related behavior, in: "Human Sexuality in Four Perspectives," F. A. Beach, ed., Johns Hopkins University Press, Baltimore.
- Deraedt, R., Bonnat, C., Busigny, M., Chatelet, P., Cousty, C., Mouren, M., Philibert, D., Pottier, J., and Salmon, J., Pharmacokinetics of RU 486, this volume.

- Genazzani, A. R., Devoto, M. C., Gianchetti, C., Pintor, C., Facchinetti, F., Mangoni, A. and Fioretti, P., 1978, Possible correlations between plasma androgen variations during the menstrual cycle and sexual behaviour in the human female, in: "Clinical Psychoneuroendocrinology in Reproduction," L. Carenza, P. Pancheri and L. Zichella, eds., Academic Press, New York.
- Germain, G., Philibert, D., Pottier, J., Mouren, M., Baulieu, E. E., and Sureau, G., Effects of an antiprogesterone (RU 486) on the time-course of the natural cycle and gestation in intact cynomolgus monkeys (*Macaca fascicularis*), this volume.
- Healy, D. L., Baulieu, E. E. and Hodgen, G. D., 1983a, Induction of menstruation by an anti-progesterone steroid (RU 486) in primates: site of action, dose-response relationships and hormonal effects., Fertil., Steril., 40:253-257.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen, G. D., 1983b, Pituitary and adrenal responses to the antiprogesterone and antiglucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863-865.
- Kreitmann-Gimbal, B., Kreitmann, O. L., Sopelak, V. M., Baulieu, E. E. and Hodgen, G. D., 1984, Menstrual induction in the primate fertile and non-fertile cycle: Anti-progesterone RU 486 binds to endometrial progesterone receptors without affecting luteal cells, J. Ster. Biochem., 19:1125, abstract no. 336.
- Luttge, W. G., 1971, The role of gonadal hormones in the sexual behavior of the rhesus monkey and human: A literature survey, Arch. Sex. Behav., 1:61-88.
- Nieman, L. K., Healy, D. L., Spitz, I. M., Merriam, G. R., Bardin, C. W., Loriaux, D. L. and Chrousos, G. P., Induction of menstruation in normal women by a single dose of the antiprogesterone steroid RU 486, this volume.
- Zumpe, D. and Michael, R. P., 1983, A comparison of the behavior of *Macaca fascicularis* and *Macaca mulatta* in relation to the menstrual cycle, Am. J. Primatol., 4:55-72.

## EFFECTS OF THE ANTIPROGESTERONE RU 486

### IN EARLY PREGNANCY AND DURING THE MENSTRUAL CYCLE.

W. L. Herrmann, A. M. Schindler, R. Wyss, and P. Bischof

Department of Obstetrics and Gynecology  
University of Geneva Medical School  
Geneva, Switzerland

#### SUMMARY

The antiprogesterone RU 486 produces an abortifacient effect during the early stages of most, but not all, pregnancies. The rate of complication appears similar to that occurring in spontaneous abortions. At this time there is no definitive explanation for failures.

The mechanism leading to abortion involves decidual necrosis and production of prostaglandin (PG). It is not known whether RU 486 has a direct effect on hCG production and/or secretion; this problem is currently being investigated. There is no real evidence for a luteolytic effect in early pregnancy.

The use of RU 486 for the treatment of extrauterine (tubal) pregnancy is not recommended.

Available evidence suggests that during the proliferative phase of the menstrual cycle the antiprogesterone blocks secretory action of the dominant follicle as well as of the pituitary. Recovery appears to be associated with a higher than normal LH surge.

During the earliest luteal phase, administration of the drug prevents the formation of a normal corpus luteum and is not followed by uterine bleeding.

In the midluteal phase, uterine bleeding is induced within 48 hours of the first dose, in spite of continued normal luteal function. Thus it is probably due to progesterone blockage in endometrial cells. A second episode of bleeding occurs at the expected time of menstruation. In the late luteal phase, the anti-P shortens the life of the corpus luteum which, under normal circumstances, requires LH stimulation in order to reach the expected duration of function.

Future work must be directed at elucidating the lack of uniform response during pregnancy, as well as finding means to combine the menses-inducing properties with a luteolytic action. The high rebound phenomenon observed in LH secretion might also predestine this drug to therapeutic use in problems of infertility.

## INTRODUCTION

In 1928, Corner & Allen wrote, "We have been able to prepare alcoholic extracts of the corpora lutea of swine which produce in spayed rabbits a condition of the uterus identical with normal progestational proliferation." In the following half century, the biosynthesis, sources and physiological and pharmacological properties of progesterone (P) have been well-defined, and its role during the menstrual cycle and pregnancy has been characterized. The likely hypothesis has emerged that selective blocking of P action will interfere with some events necessary for follicle maturation, the secretory phase of the cycle, and pregnancy.

In this chapter, we will present clinical and human pharmacological data from several series of experiments where RU 486, a strong anti-P, lacking progestomimetic and estrogenic properties, was administered during early pregnancy and during the proliferative and secretory phases of the cycle.

The biochemical and basic pharmacological properties of this compound have been previously described (Philibert et al., 1981) and several investigators have reported on its effect in animals, including primates (Healy et al., 1983).

Toxicological criteria to permit human experimentation have been satisfied (Roussel et al., internal report), as well as all legal and ethical conditions as required in Geneva, Switzerland.

### Oral Administration of RU 486 in Early Pregnancy

The first human data were obtained from a group of eleven women, 6-8 weeks pregnant, who requested abortion (Table I). All patients underwent a thorough physical examination, including routine pretreatment testing of blood and urine. In addition, fasting blood values were established for different metabolic and endocrine parameters (Table II). The duration of pregnancy was confirmed by clinical examination, ultrasound, and  $\beta$ -hCG levels.

The subjects were permitted to return to their homes during the treatment period. They were given the information necessary to contact the investigator responsible and were advised to report immediately all signs or symptoms, specifically those related to an impending abortion. Daily clinic visits were scheduled during treatment, which consisted of 200 mg of the drug in four divided doses per day, over a period of four days. Further controls were carried out on a weekly basis or as necessary.

Fasting early morning blood samples were obtained during each visit. Testing for metabolic parameters was done on a daily basis by the central laboratory at the University of Geneva Teaching Hospital. Hormonal values were established by immunoassays, using commercially available kits, in the laboratory of the Obstetrics and Gynecology Department.

## RESULTS

Nine patients reported the onset of uterine bleeding during the first three days of drug administration. This was followed by abortion within the following four days, one woman requiring a postabortion D&C. She was the only patient requiring hospitalization and blood replacement. No other side effects were reported, except for some lightheadedness experienced by three subjects early during treatment, thus probably not related to blood loss. Normal menstrual function returned within expected time limits.

Table I. Description of Patients

Patient Number	Age	G <sup>1</sup>	p <sup>2</sup>	Duration of pregnancy <sup>3</sup> (weeks)	Onset of bleeding hours after RU 486	Expulsion days after RU 486	Duration of bleeding including expulsion (days)	Side effects
1	26	1	0	7	36	4	7	dizziness
2	35	2	1	7	36	3	7	none
3	36	2	0	7.5	35	4	12	dizziness
4	34	2	0	6	36	7	14	none
5	27	6	1	6.5	48	5	10	heavy bleeding <sup>4</sup>
6	18	1	0	7	36	5	8	dizziness
7	32	6	3	6.5	24	3	16	none
8	21	1	0	7	34	3	8	none
9	21	1	0	6	54	4	5	none
10	21	1	0	7	72	-	-	no abortion aspiration
11	23	4	2	7.5	144	-	-	no abortion aspiration

<sup>1</sup>G = number of pregnancies. <sup>2</sup>p = number of deliveries.

<sup>3</sup>Judged by clinical examination and ultrasound.

<sup>4</sup>D & C performed immediately after expulsion.



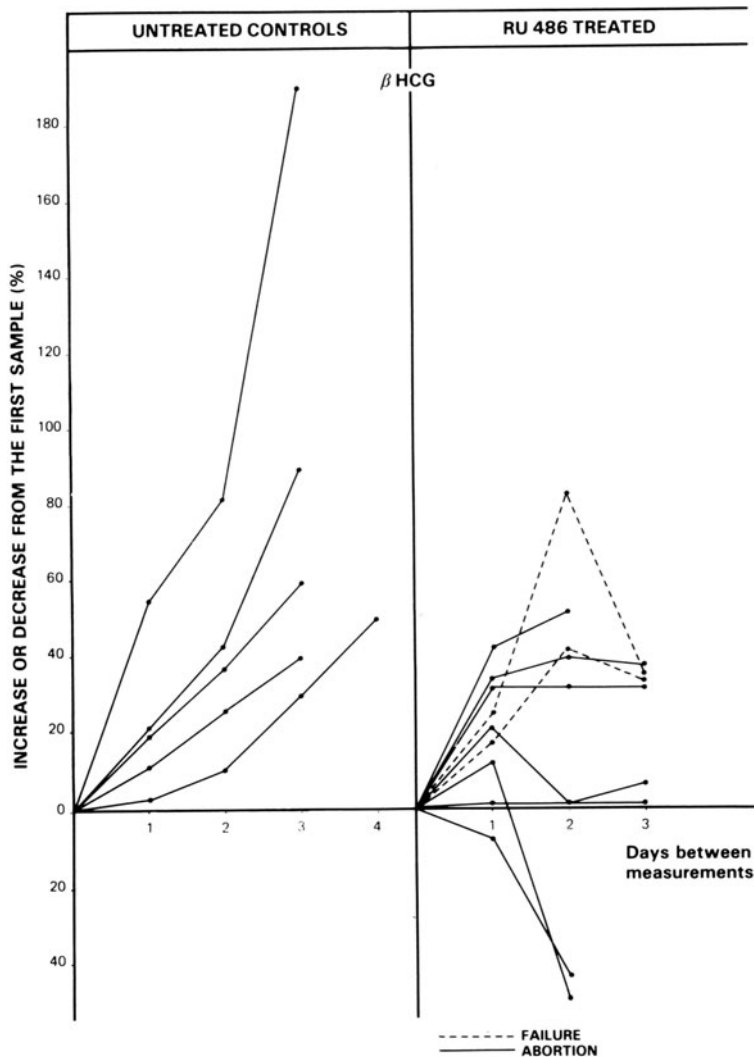


Fig. 1. Individual values before expulsion.

All laboratory values related to general health remained within pretreatment limits, with the exception of those reflecting upon blood loss (Table II). There was a decrease in hemoglobin from  $12 \pm 0.4$  to  $10.9 \pm 1.4$ . This amount is compatible with a hemorrhage experienced during miscarriage in patients not requiring hospitalization.

Two patients in this group did not abort. They experienced some bleeding on the 3rd and 6th days after the beginning of therapy. One week later, ultrasonic examinations confirmed the presence of normal intact intrauterine pregnancies, which were eventually interrupted by conventional means.

#### Endocrine Values

**$\beta$ -hCG.** Because the duration of pregnancy, as determined by available clinical parameters, varied from 6-8 weeks, a certain spread of pretreatment plasma hormone levels, particularly  $\beta$ -hCG, has to be expected. This is

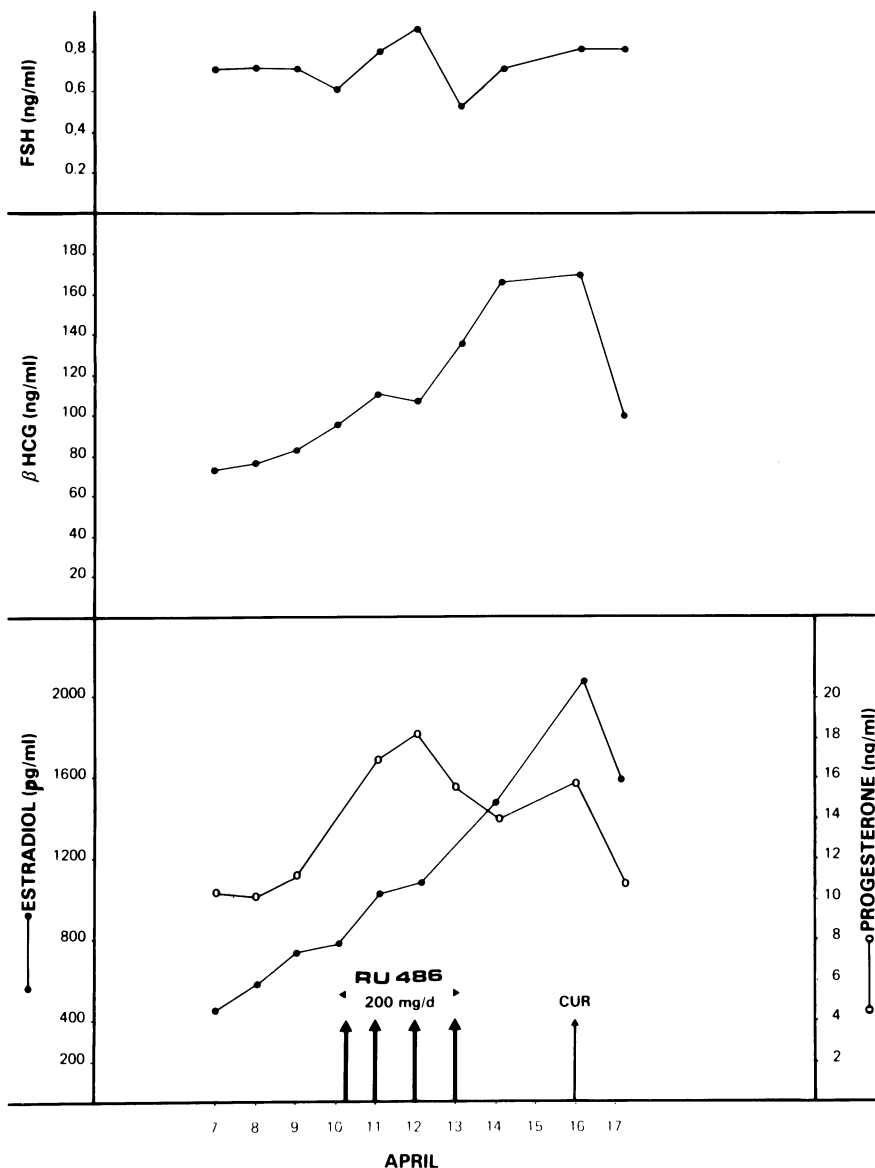


Fig. 2. J. B. L. M. P. 2/20/83. See text for explanation.

evident in five untreated cases where  $\beta$ -hCG plasma levels are shown as a percent increase on five consecutive days (Fig. 1).

In the treated cases, individual increases or decreases of  $\beta$ -hCG varied greatly, with the two women who failed to abort showing a more sustained rise during the first 48 hours after treatment. Whether the decline of chorionic gonadotrophin secretion is the result of the effect of the anti-P on hCG synthesis or release or merely reflects the beginning of trophoblast separation or necrosis is not apparent from these data.

The hormonal parameters of another patient, six weeks pregnant, who was not included in this first series, and whom we were able to study during a ten-day period, suggest that secretion of  $\beta$ -hCG was not affected by the daily administration during four days of 200 mg of RU 486 (Fig. 2).

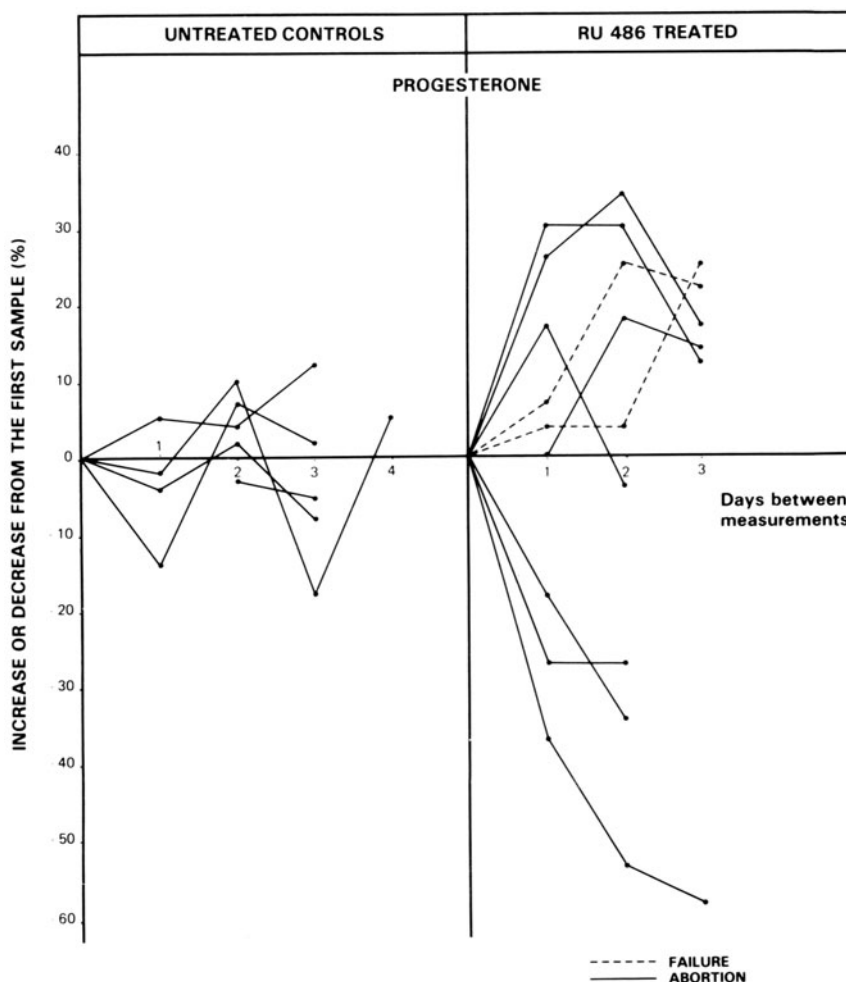


Fig. 3. Individual values before expulsion.

### Progesterone

In early pregnancy, most of the circulating P originates from the corpus luteum. Progesterone plasma level does not show a dramatic increase over a short period as does  $\beta$ -hCG. Furthermore, one must allow for a  $\pm 15\%$  rate of error when using standard radioimmunological laboratory methods. Hence, there was no appreciable increase in the control group.

In the treated group, both aborting and nonaborting patients showed a pattern suggesting that the anti-P had no luteolytic effect upon the corpus luteum of pregnancy, as there was no dramatic fall in progesterone levels that coincided with the onset of therapy. Negative changes may be related to decreased  $\beta$ -hCG stimulation of the corpus luteum (Fig. 3).

### Estradiol

As suspected, the control group showed only a minor estradiol increase during the four day period (Fig. 4). The treated patients had variable responses: a drop in the two patients who aborted within 72 hours and a pattern compatible with the controls in three patients who aborted on the 4th or 5th day.

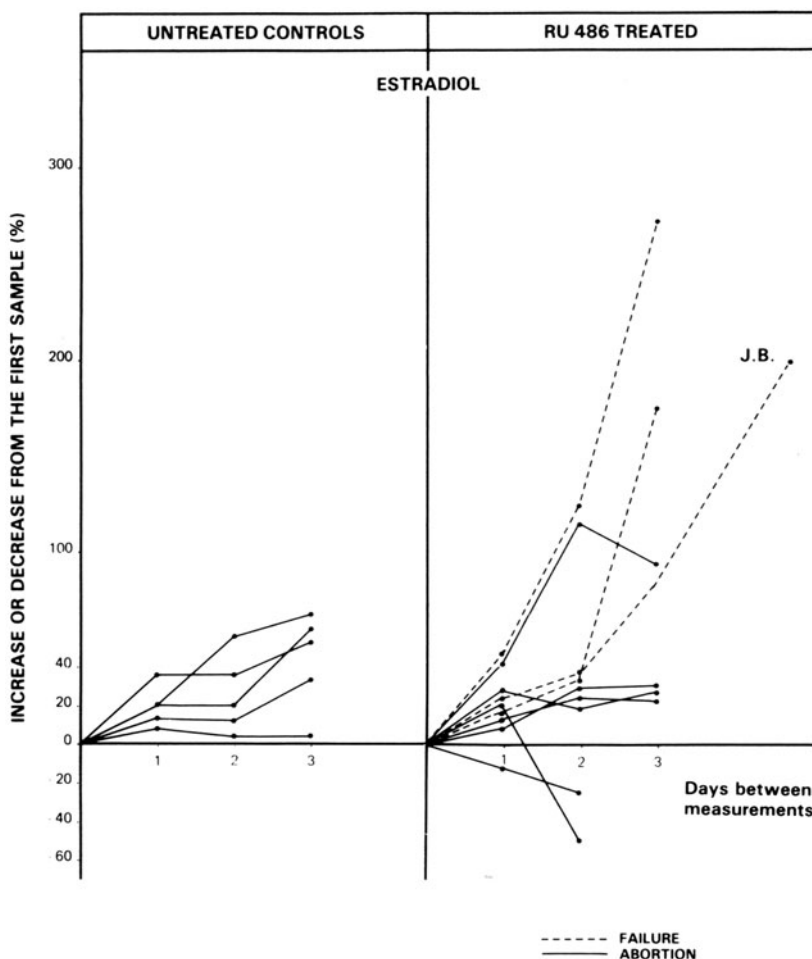


Fig. 4. Individual values before expulsion.

The two women whose pregnancies were not interrupted showed an unexpected increase in circulating estradiol ( $E_1$ ), as did patient J. B. (Fig 2).

At present, we cannot propose a mechanism for this observation. The appearance of cornified cells in the vagina and the typical fern pattern in drying cervical mucus has been looked upon by some clinicians as a sign of threatened abortion, explained however as due to the unopposed estrogen (E) rather than increased E. It is also conceivable that increased E secretion represents a defense mechanism directed at increasing the P receptor population. Our data require confirmation, and we need more knowledge about the effect of anti-P on steroid biosynthesis of the corpus luteum during pregnancy.

### Morphology

The mechanism by which RU 486 interrupts pregnancy is as yet not clear. Blockage of P action at receptor sites of the end organ is probably the first of a series of events, ultimately leading to expulsion of the products of conception. This part of our investigation was undertaken in order to learn more about the earliest morphological changes attributable to P withdrawal.

For details of this part of the study we refer to its original publication (Schindler et al., in press).

RU 486 was administered 24 or 48 hours prior to interruption of pregnancy by suction curettage (100 or 200 mg). After careful separation of the tissues under a dissecting microscope, trophoblast and decidua obtained were examined using light and electron microscopy.

The results showed no significant alterations of the trophoblast. Light microscopic examination of the decidua, however, showed vascular lesions consisting of edema and dissociation of the capillary wall. These effects were seen predominantly in RU 486-treated patients as compared to specimens obtained from an untreated control group.

Ultrathin sections (electron microscopy) showed frequent cytoplasmic lysis of decidual cells. The most marked alterations were found in capillaries, where the RU positive cases often showed a striking hyperplasia of the endoplasmic reticulum. The ER was sometimes dilated as well.

Other findings included the appearance of large vesicular spaces, with formation of multiple cytoplasmic protrusions on the endothelial surface.

A morphometric study undertaken to appreciate the validity of these observations yielded highly significant results ( $p < 0.001$ ) when endoplasmic reticulum surface density was considered.

#### Circulating Prostaglandin F<sub>2α</sub>

Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) of decidual origin plays an important role in initiating normal labor. Its use as an abortifacient following local or systemic administration has been successful, although it must be remembered that induction of uterine activity varies somewhat and seems to be dose dependent.

In order to determine whether the decidual necrosis described above was associated with an increase in PGF<sub>2α</sub> production, plasma levels of this hormone were determined before and 12 or 24 hours after anti-P administration.

There was indeed an increase of PGF<sub>2α</sub> in six of seven patients; however, this increase was variable (Fig. 5). This may be due to the timing of the experiments, 12 or 24 hours being too short an interval to allow for accumulation of PGF<sub>2α</sub> in the peripheral circulation. Nonetheless, the observed increases are statistically significant.

Finally, it must be remembered that individual responses in RU 486-induced abortions and the time required for onset of bleeding and/or expulsion are not uniform. The observed changes in PGF<sub>2α</sub> may thus represent merely another parameter reflecting variations in individual susceptibility to anti-P action.

#### Tubal Pregnancy

An attempt to interrupt a known tubal pregnancy with RU 486 was made in a 40 year-old nulligravida followed up to this point for a problem of primary infertility. The patient had agreed to participate in this experiment after she was given all pertinent information, including a description of all risks. She was also presented with the choice of submitting to the traditional procedure of conservative surgery after early diagnosis of her condition. Tubal implantation had been confirmed at six weeks gestation by laparoscopy and D&C (absence of trophoblastic villi).

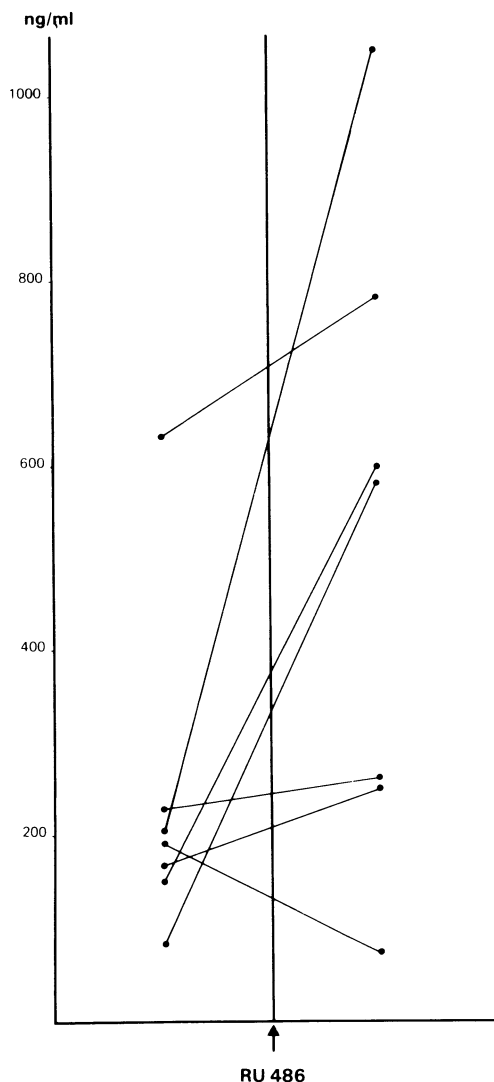


Fig. 5. Circulating  $\text{PGF}_{2\alpha}$  before and after the administration of RU 486.

After ten days of observation, when increasing titers of  $\beta$ -hCG indicate continued evolution of pregnancy, she was begun on an RU 486 treatment of 200 mg daily divided doses. She received this treatment for five days, during which  $\beta$ -hCG continued to rise (Fig. 6). The clinical symptoms of minor pain, spotting and minimal rebound tenderness remained unchanged during this time.

On the 6th day, the patient suffered an acute intraabdominal hemorrhage. Emergency laparotomy revealed a hemoperitoneum of 700 ml consequent to a fresh rupture of a right isthmic tubal pregnancy. A segmental tubal resection was performed. The postoperative course was uneventful.

Histological examination of the conceptus showed well preserved villi. Tubal modifications were compatible with those found in recently ruptured tubal pregnancy.

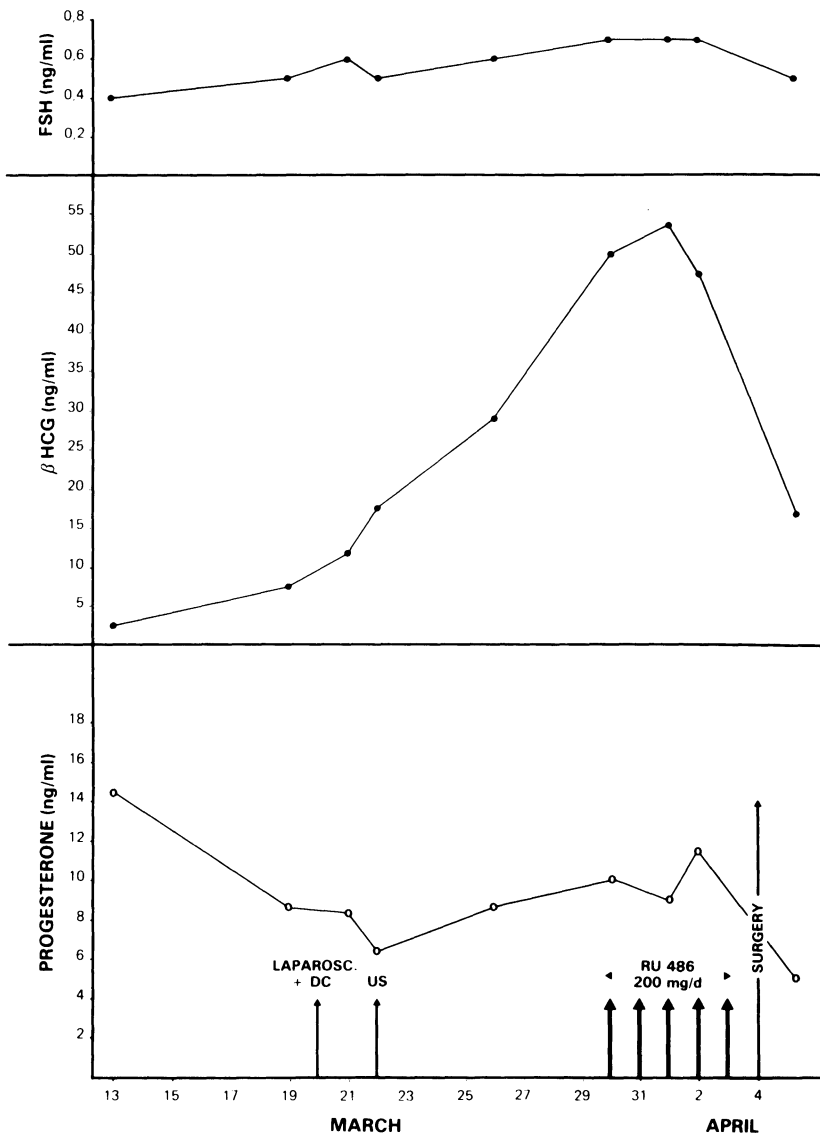


Fig. 6. Extra-uterine pregnancy L. M. P. 2/4/84.

After the outcome of this case, we have refrained from treating other patients with extrauterine pregnancies.

The undisturbed  $\beta$ -hCG secretion during treatment, the histologically unaffected trophoblast, as well as continued P secretion in this patient suggest that RU 486 has no direct effect on the trophoblast.

Since the endosalpinx does not assume the same function as the decidua in normal pregnancy, the absence of demonstrable hormonal alteration in peripheral blood appears to lend more credence to the idea that the effects of anti-P in early pregnancy are mediated by the early induction of decidual necrosis.

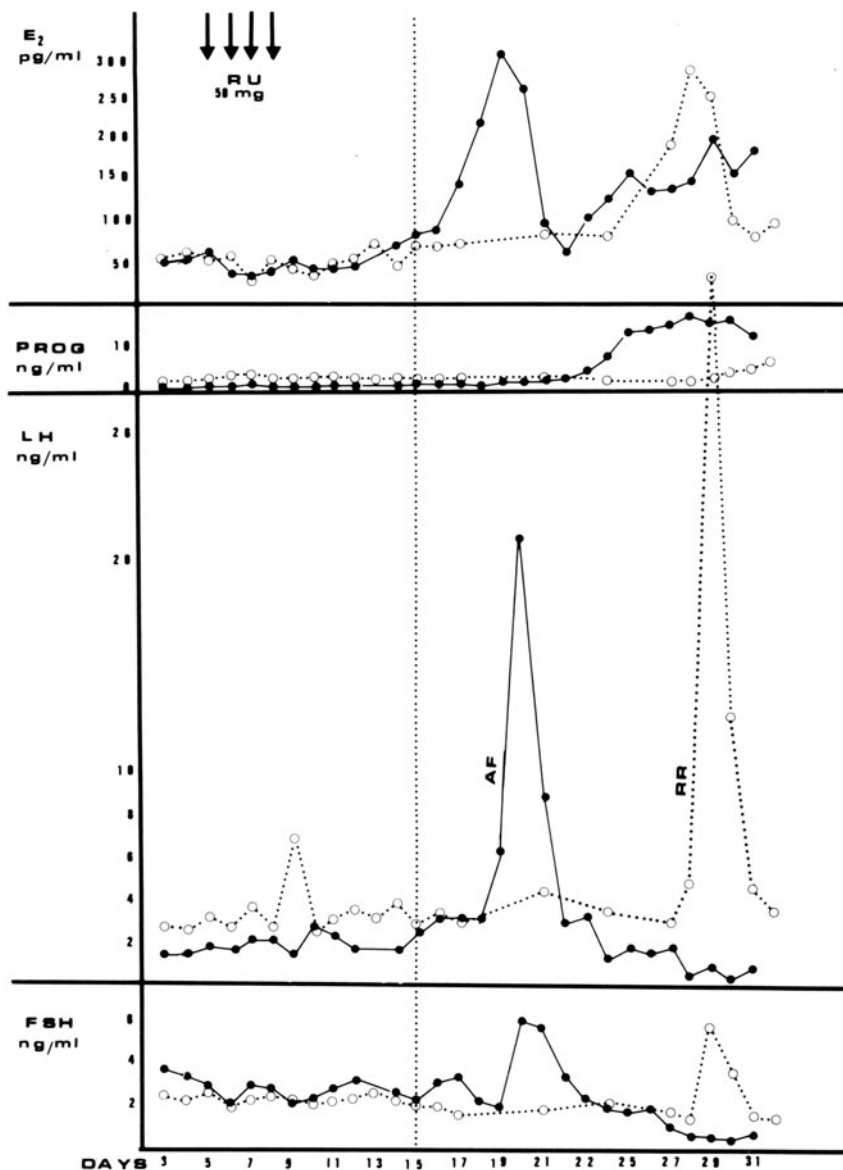


Fig. 7. See text for explanation.

#### The Administration of RU 486 During the Follicular Phase

In a series of experiments, Healy and his coworkers showed that intraovarian P plays an essential role in determining follicular growth and the selection of the dominant follicle in the primate menstrual cycle (Healy et al., 1983; Roussel Uclaf, internal report; Hodgen, 1982).

Experiments using human volunteers by several investigators have emphasized the importance of P in facilitating the positive feedback of E<sub>2</sub> upon LH release, as well as the induction of the FSH peak at midcycle. They also concluded that the impact of P on mid-cycle events is time and dose dependent. (March et al., 1979; 1981)

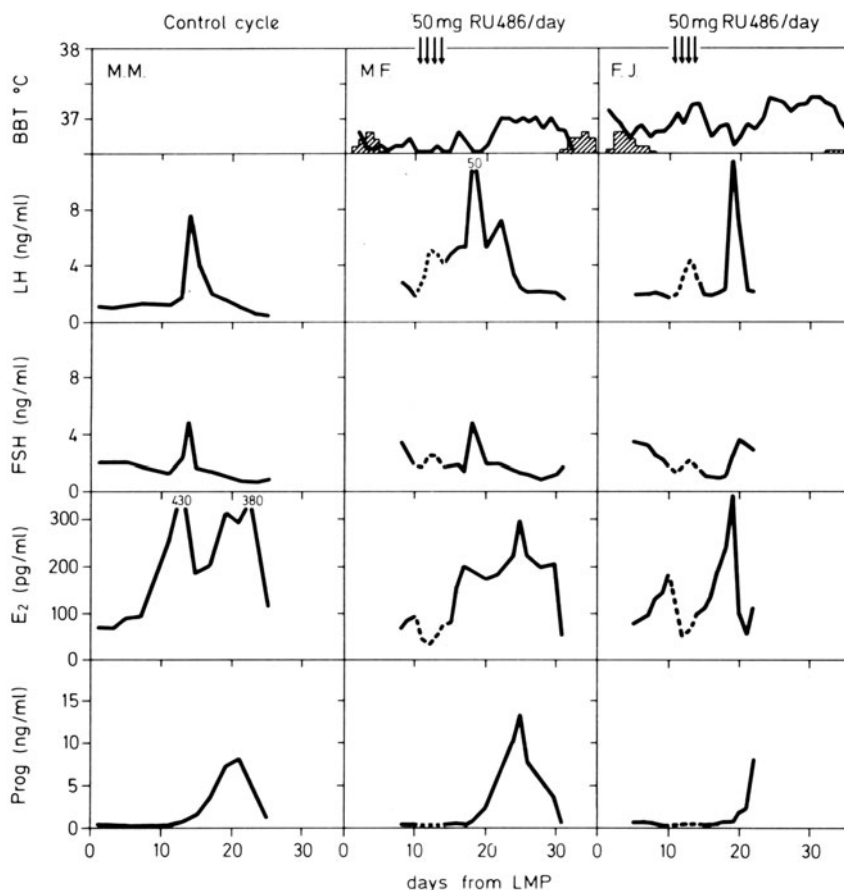


Fig. 8. See text for explanation.

In the following series of experiments, we have examined the role of P at different times of the follicular phase. Hormonal parameters were studied after P action was blocked with the anti-P in young, healthy women with regular cycles (28-30 days). Control data were obtained by daily samplings of untreated cycles. During control, as well as treatment periods, the subjects were asked to record their basal body temperature.

Administration of RU 486 (50 mg/day x 4) during the early proliferative phase (days 5-8 in patient R. R., Fig. 7), clearly inhibited the early E<sub>2</sub> surge that normally follows growth and development of the dominant follicle. Estradiol levels rose very slowly from 50 pg/ml to 80 pg/ml during 14 days following treatment; the beginning of the normal preovulatory rise was thus postponed until the 24th day of the cycle.

FSH remained essentially unchanged until the 29th day of the cycle, when a peak coinciding with a higher than normal LH surge (33 ng/ml) was noted. Interestingly, there was a smaller sharp LH discharge (8 ng/ml) on the day following treatment. This "rebound" phenomenon was observed repeatedly.

A second patient (A. F., Fig. 7), reacted identically when treated similarly, with the exception that ovulation occurred on the 20th day, a five day postponement, again after a higher than normal LH peak (21 ng/ml).

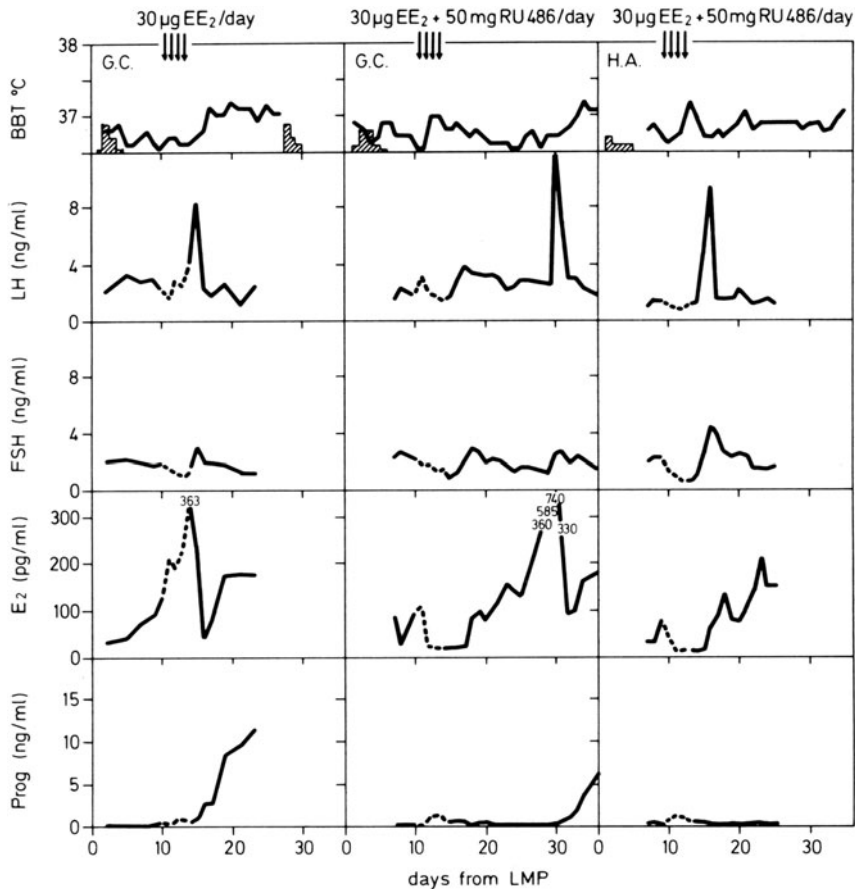


Fig. 9. See text for explanation.

When the beginning of the anti-P treatment was postponed to the 10th day of the cycle (Fig. 8), a time when the dominant follicle is at its maximal growth rate, the inhibitory action at the follicular level again became apparent. However, this time there was evidence of positive feedback on LH and FSH secretion while RU 486 was being administered. Also, follicle recovery seemed to be immediate, with ovulation taking place on day 19 following an abnormally high LH discharge.

In all patients, ovulation was followed by a secretory phase of normal duration.

These observations indicate that RU 486 has a negative effect on E secretions by the dominant follicle, which appears to be more sensitive before the onset of the preovulatory surge of E<sub>2</sub> secretion.

It is not clear whether the observed pattern of gonadotrophin secretion is the result of the absence of an E-dependent positive feedback or due to a direct effect of RU 486 on pituitary secretion.

To explore these possibilities, two experiments were conducted. In the first, 30 mg of ethinyl-E<sub>2</sub> were given simultaneously with RU 486 from day 10-13 of a normal cycle to two subjects. It was previously established that ethinyl-E<sub>2</sub> thus administered alone has no appreciable impact on FSH or LH secretion and ovulation (Fig. 9). In the presence of the anti-P, however,

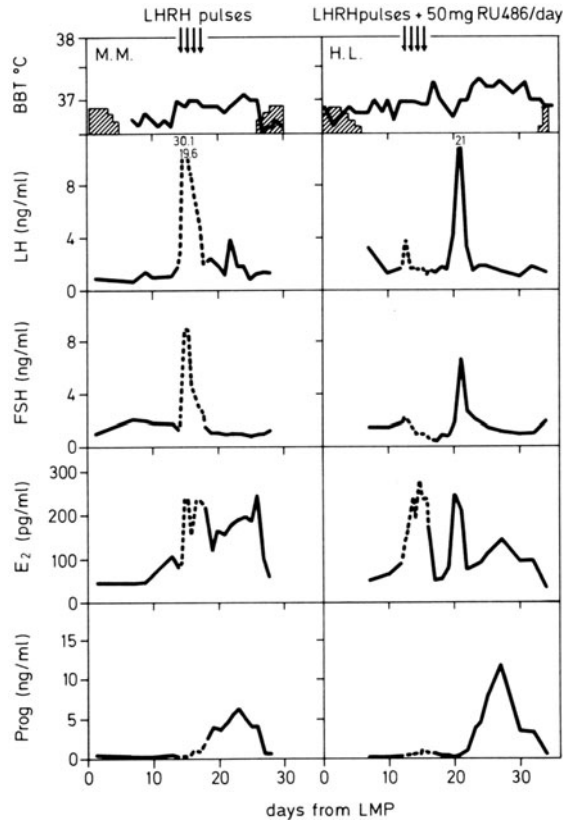


Fig. 10. See text for explanation.

there was again inhibition of follicular secretion, and ovulation was postponed until about day 30 in both subjects. This was in spite of a LH/FSH discharge on days 16-18, probably due to positive feedback of the exogenous E. Furthermore, ethinyl-E<sub>2</sub> apparently had potentiated the follicle blocking effect of the anti-P.

In the second experiment, gonadotrophin secretion was stimulated by pulsatile administration of GnRH during four days, starting 1-2 days before ovulation (Fig. 10). Patient M. M., serving as control, showed the expected exaggerated surge of LH and FSH followed by corpus luteum formation. In the presence of RU 486, pituitary response was markedly reduced. There was, however, an unexpected increase in circulating E<sub>2</sub>, which we have attributed, for want of a better explanation, to the secretory activity and recruitment of new follicles. The ovulatory LH surge occurred only six days after GnRH treatment.

In summary it can be stated that during the first half of the cycle, RU 486 exerts a blocking activity upon the follicle as well as the pituitary response to ovarian stimulation. The experimental evidence thus corresponds to that defining the early role of P during the follicular phase.

#### The Administration of RU 486 During the Secretory Phase

The potential use of this compound as an "end of the month pill" is the most interesting part of the study but also the most difficult to interpret.

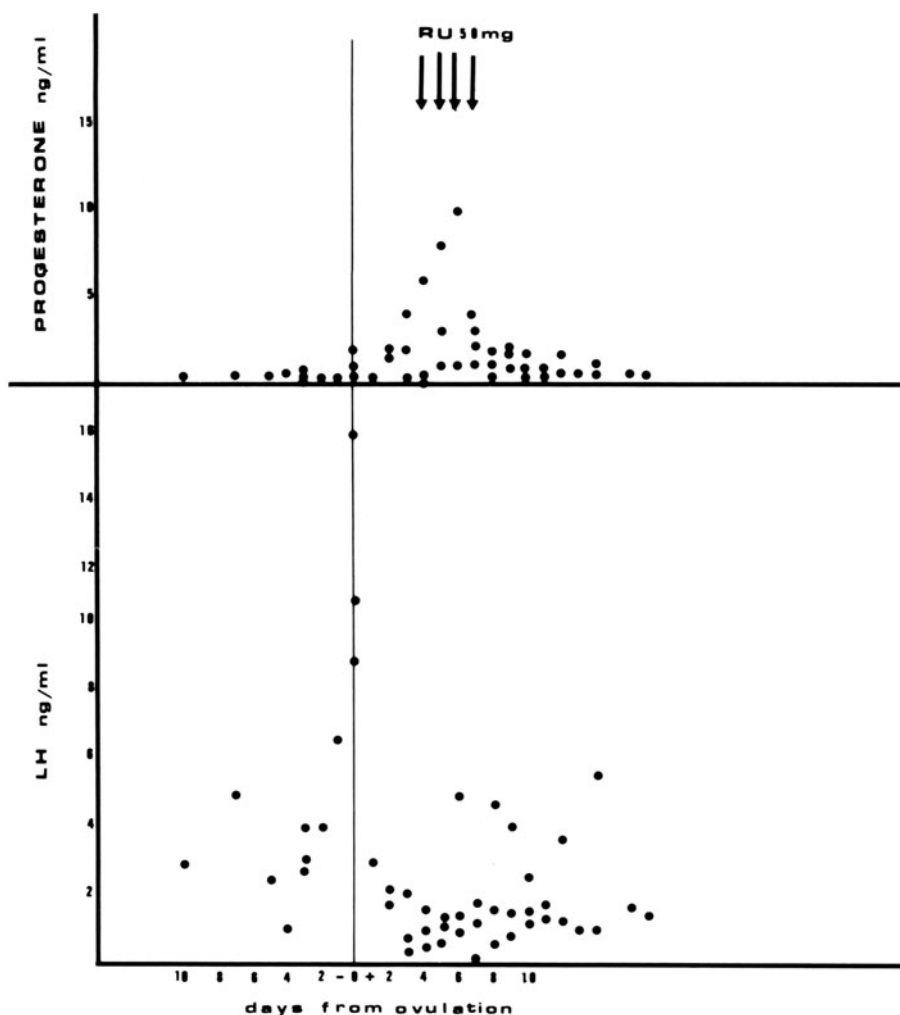


Fig. 11. See text for information.

The endocrine interactions determining formation, function and demise of the corpus luteum (as well as its continuing secretion in early pregnancy) are very complex (Fritz and Speroff, 1982). Progesterone production depends on preovulatory follicular development, LH secretion, LH receptors and their availability, E, the age of luteal cells, and other factors. It can thus be expected that the effects upon the corpus luteum by an anti-P will not be uniform throughout the luteal phase. The induction of uterine bleeding by P withdrawal (or blocking) might also depend upon the degree of secretory transformation of the endometrium.

To find answers to these questions, RU 486 (50mg/d x 4) was given to nine healthy young women with regular cycles during the early luteal phase, at the time of the P plateau and during the last week of the cycle.

In addition, there was one subject receiving 50 mg of the drug from the 23rd to the 26th day, in conjunction with 5000 IU of hCG q. d. during the same interval.

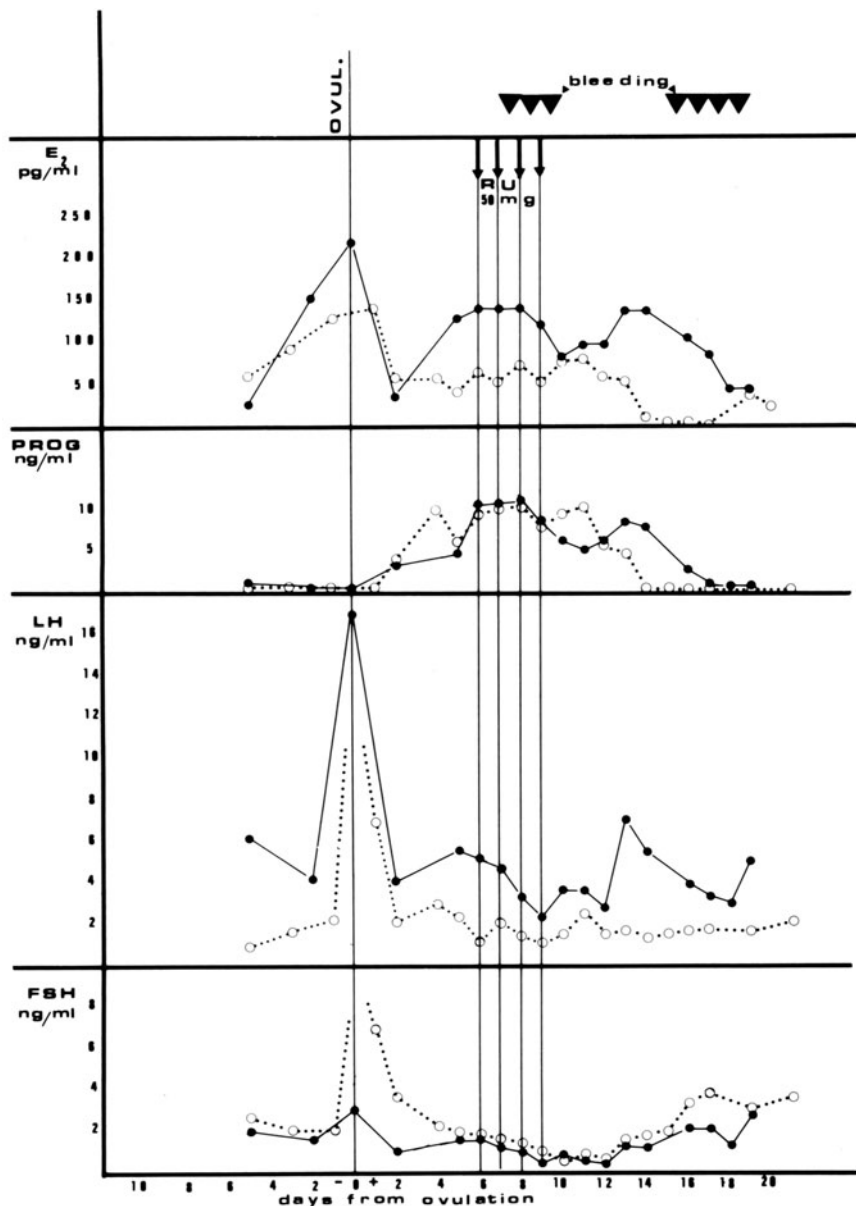


Fig. 12. See text for explanation.

## Results

Two women treated during early luteal phase experienced no vaginal bleeding during or following treatment; a third subject observed light spotting on one day. Plasma P after ovulation increased only a little, returning to preovulatory values by day 6-7 after ovulation. The LH pattern after ovulation was inconsistent (Fig. 11). Plasma  $E_2$  also remained low, indicating an early demise or malfunction of the corpus luteum. Menstruation or ovulation of the next cycle was delayed by several weeks.

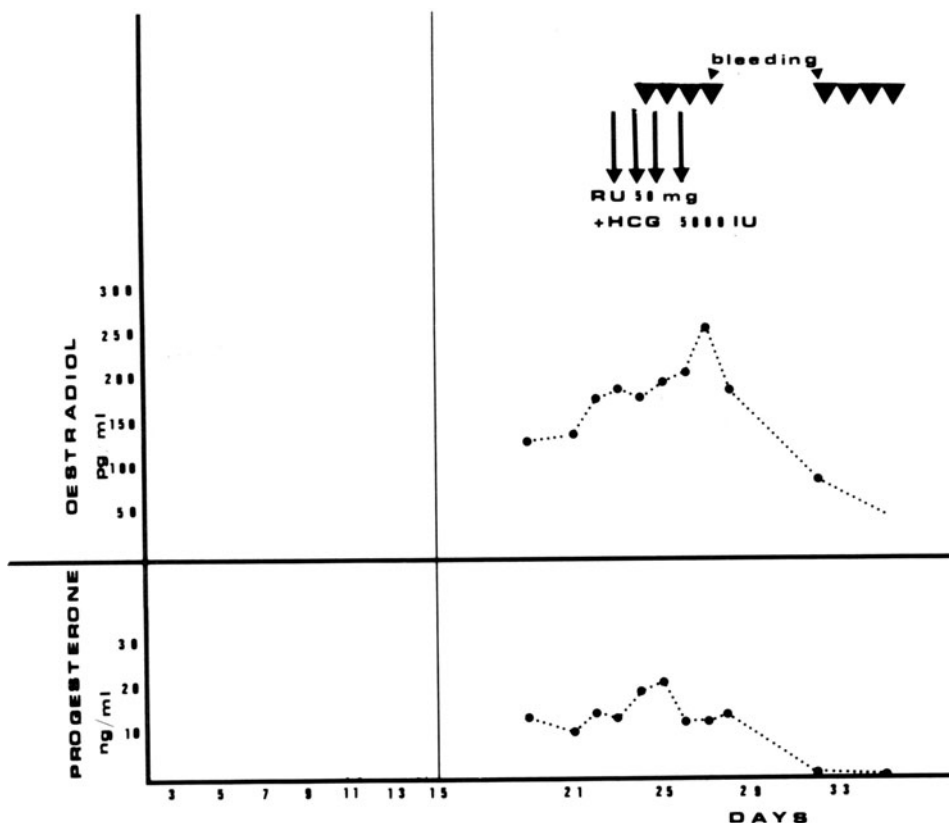


Fig. 13. See text for explanation.

Two other subjects were given the drug in midluteal phase, again as a single dose on four consecutive days. Both women experienced vaginal bleeding of moderate intensity for 3-4 days within 48 hours after the first dose of RU 486. They bled again at the time of the expected period for 4-5 days (Fig. 12). This was interpreted as P withdrawal bleeding in each instance, first due to the P-blocking effect of RU 486 upon the endometrium and then to the cessation of the luteal function. In contrast to the first group, the endometrium in these women seemed to have been more P dependent.

In this experiment, circulating hormone levels were not significantly affected by the treatment. In particular, there was no real evidence for luteolysis or LH inhibition. Estrogen remained within normal limits as did FSH, which rose toward the end of the cycle.

A similar pattern emerged in the subject who was given anti-P and hCG for four days, starting the 23rd day of the cycle. She too bled within 48 hours, in spite of the hCG-induced rise of plasma P. A second episode of vaginal bleeding occurred when the corpus luteum came to the end of its life span (Fig. 13).

One may conclude from these observations that the anti-P has no lytic effect upon the corpus luteum when it is at the height of its function or when it is stimulated by hCG.

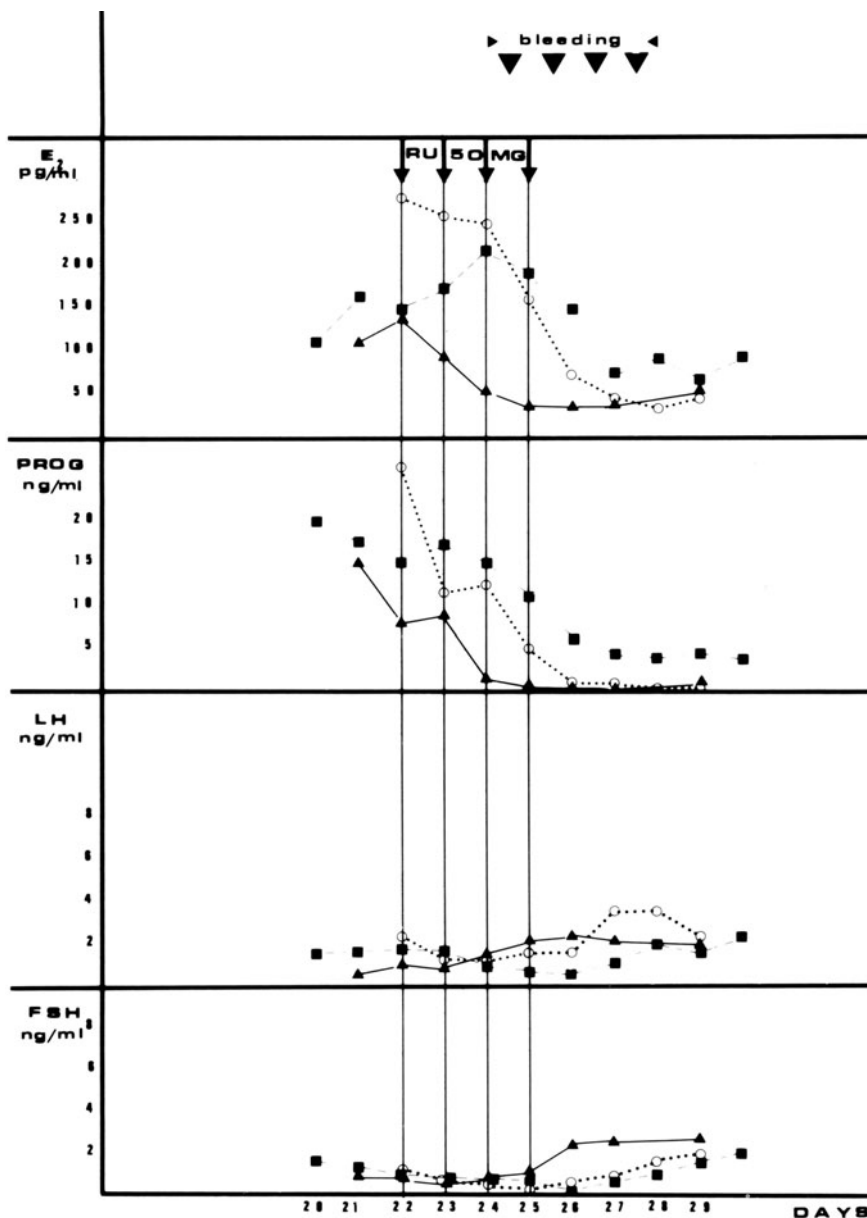


Fig. 14. See text for explanation.

In the last group of four women, anti-P was administered during the late luteal phase (50 mg q. d. x 4). This treatment was followed by a single episode of vaginal bleeding in all subjects, starting within 48 hours after the first dose and resembling a normal period. This time progesterone levels decreased to preovulatory values during the treatment. There was also a simultaneous drop of  $E_2$  as well as a moderate increase in FSH, signaling the beginning of events in preparation for the next cycle.

In all four women who normally had 28-30 day cycles, menstruation appeared at least 3-4 days earlier than expected, and their cycles had thus been shortened (Fig. 14).

## REFERENCES

- Corner, G. and Allen, W., 1928, Infertility diagnosis and management, in: "Clinical Perspectives in Obstetrics and Gynecology," J. Aiman, ed., Springer-Verlag, New York, 1984.
- Fritz, M. A., and Speroff, L., 1982, The endocrinology of the menstrual cycle: the interaction of folliculogenesis and neuroendocrine mechanisms, Fertil. & Steril., 38:509-529.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships, and hormonal effects, Fertil. & Steril., 40:253-257.
- Hodgen, G. D., 1982, The dominant ovarian follicle, Fertil. & Steril., 38: 281-300.
- Institut Roussel Uclaf, Paris, Internal report.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - an antiprogesterone compound, Contraception, 29:399-410.
- March, C. M., Goebelsmann, U., Nakamura, R. M., and Mishell, D. R., 1979, Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle stimulating hormone surges, J. Clin. Endocrinol. & Metab., 49:507-513.
- March, C. M., Marrs, R. P., Goebelsmann, R., and Mishell, D. R., 1981, Feedback effects of estradiol and progesterone upon gonadotrophin and prolactin release, Obstet. & Gynecol., 58:10-16.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 38486 a potent antiglucocorticoid in vivo, Eighth International Congress of Pharmacology, (Tokyo), 668:1463.
- Schindler, A. M., Zanon, P., Obradovic, D., Wyss, R., Graff, P., and Herrmann, W. L., Early ultrastructural changes in RU 486-exposed decidua, Gyneco. Obstet. Invest. (in press)

INTERRUPTION OF EARLY PREGNANCY BY THE  
ANTIPROGESTATIONAL COMPOUND RU 486

A. A. Haspels

Department of Obstetrics and Gynaecology  
University Hospital, Utrecht  
Netherlands

ABSTRACT

RU 486 was given to 33 women seeking termination of pregnancy. The patients were divided into two groups: 24 patients in group I, with amenorrhoea up to 7 6/7 weeks (55 days), and nine patients in group II, with amenorrhoea for 8-10 weeks. The patients received 200 mg of RU 486 daily for four days.

The start, duration and amount of bleeding were determined for 14 days. Plasma  $\beta$ -hCG, progesterone, estradiol and cortisol were determined at day zero and day seven. All patients started to bleed during treatment. In group I, frequency of complete abortion was 79% (19 out of 24 patients). In group II, 33% (3 out of 9 patients) experienced complete abortion. Most of the patients experienced only minor side effects--uterine pain and bleeding, as in a spontaneous abortion. However, two patients suffered from heavy bleeding, and required blood transfusion and curettage. Both of these patients were in group II and experienced 8 and 9 2/7 weeks amenorrhoea, respectively. In the patients who had complete abortion,  $\beta$ -hCG, estradiol, and progesterone decreased significantly within one week. Cortisol concentrations remained within the normal range at day zero and day seven.

Treatment with RU 486 may provide an acceptable method of early pregnancy termination, especially in women who refuse operative treatment and prefer a "spontaneous abortion."

In 14 women, RU 486 was administered from day 23-26 as a late "morning-after pill." All women except one started to bleed before day 28 and observed a normal menstrual period. One woman stayed amenorrheic for two months, but she was not pregnant, apparently experiencing an anovulatory cycle.

INTRODUCTION

About 15 years ago, prostaglandins were thought to be a good alternative to vacuum curettage for inducing early abortion. However, because side effects such as nausea, vomiting and diarrhea were common, prostaglandins were rarely used in the first trimester pregnancy except for ripening of the cervix prior to surgery. (Andriess et al, 1978). A few studies reported

successful self-administration of prostaglandin analogues (Haspels, 1982). Recently Herrmann (1983) reported that oral administration of a new antiprogesterational steroid caused abortion in nine out of eleven women with 6-8 weeks of amenorrhea.

## METHODS

Our study included 24 healthy women in group I (amenorrhoea up to 55 days or 7 6/7 weeks) and nine in group II (8-10 weeks amenorrhoea). All women admitted to the study were made aware of its nature and gave their informed consent. Before the start of the study, approval was obtained from the Ethical Committee of the University Hospital of Utrecht. Twelve patients in group I received the compound RU 486 orally in a dose of 100 mg twice daily for four days. From then on, the patients took 200 mg in one dose for four days. The patients attended three follow-up visits, three, seven and 14 days after the start of therapy. All tablets were taken at home. At day zero and day seven, blood samples were taken for the analysis of  $\beta$ -hCG, estradiol, progesterone and cortisol. Blood samples were also taken the same days to examine: hemoglobin, hematocrit, white blood cell count, differentiation, thrombotest, sodium, potassium, chloride and calcium, as well as urea, creatinine, alkaline phosphatase, SGOT, SGPT, bilirubin, gamma GT, LDH and glucose. At the follow-up visits, the outcome of therapy was classified as either complete or incomplete abortion, or uninterrupted pregnancy. The result was based on the duration and amount of bleeding; plasma  $\beta$ -hCG, progesterone, and estradiol levels; and ultrasound examination. If bleeding became excessive, or abortion was not complete at day 14, vacuum curettage was performed. As illustrated in Table I, 19 out of 24 patients or 79% with amenorrhoea for 5-7 weeks were regarded to have had completion abortions. Five had incomplete abortions and needed vacuum aspiration. Vaginal bleeding began in all patients except one within five days of the start of treatment. In the successfully treated patients, the bleeding had a duration of between one and two weeks. Two patients who had had amenorrhoea of 8 and 9 2/7 weeks, respectively, experienced heavy blood loss, resulting in hospital admission, blood transfusions and curettage. The last patient had had an unsuccessful vacuum aspiration by a gynecologist: her amenorrhoea was 9 2/7 weeks. On the second day of RU 486 treatment (200 mg per day), she started bleeding. On day four she needed two pints of blood and had an uneventful curettage.

Other side effects were of a mild nature. Almost all patients reported slight lower abdominal pain similar to the pain experienced during spontaneous abortion. A small amount of nausea and dizziness was reported.

Table II shows gravidity, parity, duration of amenorrhoea, age, weight and cycle length.

Table III shows the start of bleeding in relation to RU 486 treatment, bleeding intensity, and indicates whether expulsion has taken place. The results of the pregnancy tests on day zero and day seven are given in Table IV. Plasma levels of  $\beta$ -hCG, estradiol, progesterone and cortisol before and after one week are summarized in Tables V, VI, VII and VIII.

Kovacs observed an increase in plasma cortisol concentration during treatment, but values were not significantly different from pre-treatment levels at day seven (Table VIII). Progesterone values started to decrease on the first treatment day. The levels on day seven were significantly lower than they were before treatment ( $p < 0.0001$ ).

Patients were asked to indicate, on two scales of 0-100, their reaction to RU 486 - induced abortion. On the first scale, zero indicated "very

Table I Outcome of RU 486 Therapy  
for Termination of Pregnancy

	Amenorrhoea	No	Complete Absorption (%)	Incomplete Absorption (%)	Pregnancy continued
Group I	5- 7 weeks	24	19-79	5-21	-----
Group II	8-10 weeks	9	3-33	6-67	-----

Table II. Demography of Patients

Pat. no.	Age	Weight	Cycle	Grav.	Par.	Pregnancy week-days	
I							
1	38	69	28/5	4	2	5	4
2	40	68	26/4	4	2	6	0
3	37	52	28/3-5	3	2	5	3
4	20	56	26-28 days	1	0	6	6
5	37	58	28/5	4	3	5	4
6	30	70	30-37/5	2	0	6	1
7	21	63	28/6	2	0	7	0
8	21	61	29/5	1	0	5	1
9	33	65	14-32 days spotting	1	0	6	1
10	25	56	28/4	2	0	6	5
11	38	53	29/4	3	2	5	3
12	31	55	30/5	4	0	6	6
II							
13	27	70	reg.29/4	1	10	5	2
14	27	62	27/4	1	0	5	4
15	37	59	28/5	?	?	5	0
16	38	53	28/5	6	3	6	2
17	33	50	33/5	4	2	6	0
18	24	51	28/4	1	0	5	1
19	34	60	28/7	4	3	5	2
20	29	76	29/5	2	1	6	0
21	27	61	27/4	1	0	5	3
22	29	69	28/5	1	0	6	2
23	32	59	28/5	4	3	6	0
24	27	53	27/4	1	0	5	1

I: 100 mg b.i.d.

II: 200 mg o.d.

Table III. Bleeding, Time of Appearance and Nature

Pat. no	Start of bleeding	How long	Intensity of bleeding	Expulsion Aspiration	Vacuum
I					
1	3rd day	7 days		yes	no
2	1st day	3 days	became heavy on day four(during 12 hrs)	yes	no
3	2nd day	4 days	7 hrs. heavy then lessening	incompl.	yes
4	1st day	7 days	2.5 hrs. heavy then lessening	yes	no
5	2nd day	8 days	less than menstruation	yes	no
6	3rd day	ca 12 hrs	with clots	incompl.	yes
7	2nd day	6 days	first very little, later like menstruation		
8	2nd day	2 days	with clots	yes	no
9	1st day	4 days	little	yes	no
10	1st day	5 days	increasing	yes	no
11	3rd day	5 days	varying intensity	incompl.	yes
12	4th day	2 days	more than menstruation	yes	no
II					
13	4th day	10 days	first little, later with clots	yes	no
14	3rd day	7 days	like heavy menstruation	yes (day 5)	no
15	4th day	3 days	3 days heavy	yes (day 5)	no
16	3rd day	5 days	with clots	yes (day 3)	no
17	3rd day	8 days	with clots	yes (day 5)	no
18	4th day	6 days	with clots	yes (day 5)	no
19	3rd day	5 days	1 day strong	incompl.	yes
20	2nd day			yes (day 3)	no
21	2nd day	10 days	15th day prostagl. sulproston 250 mg twice	incompl.	no
22	2nd day	7 days	missed abortion 8th day prostagl. sulp. 250 mg twice	incompl.	P.G
23	3rd day	10 days	nausea	yes	no
24	3rd	5 days	----	yes	no

I: 100 mg b.i.d.

II: 200 mg o.d.

Table IV. Results of Pregnancy Test Day 0 and Day 7

Pat. no.	initial visit	2nd assessment	expulsion
1	pos	neg	yes
2	pos	neg	yes
3	pos		incomplete
4	pos	neg	yes
5	pos	pos(dubious)	yes
6	pos	pos	incomplete
7	pos	neg	yes
8	pos	neg	yes
9	pos	pos(dubious)	yes
10	pos	neg	yes
11	pos	pos	incompl.
12	pos	neg	yes
II			
13	pos	pos	yes
14	pos	neg	yes
15	pos	neg	yes
16	pos	neg	yes
17	pos	neg	yes
18	pos	neg	yes
19	pos	pos	incompl.
20	pos	neg	yes
21	pos	pos	incompl.
22	pos	pos	P.G.
23	pos	neg	yes
24	pos	neg	yes

I: 100 mg b.i.d.

II: 200 mg o.d.

unpleasant" and 100 "not unpleasant." On the second scale, zero equalled "worse than I expected," and 100 "not as bad as I expected." Mean scores were 75 and 82, respectively.

#### Amenorrhoea 8 - 10 Weeks

A smaller trial, using nine volunteers with 8 - 10 weeks of amenorrhoea, resulted in only three complete abortions. The same protocol was used as for the larger study. Patients later in pregnancy obviously have higher progesterone levels which would be sensitive only to higher doses of anti-progesterone, especially after the luteo-placental shift in progesterone production has occurred.

#### Incomplete Abortion and Prostaglandin Analogue

Treatment with an antiprogestational compound during early pregnancy may result in increased sensitivity of the uterus to exogenous prostaglandins.

It seems likely that some patients may need the addition of a uterotonic compound in order to obtain significant uterine contractions and a higher frequency of complete abortion. For this purpose, we administered a prostaglandin analogue (0.25 mg Sulproston) to three patients with incomplete abortions on the seventh day after the start of RU 486 treatment. After prostaglandin treatment, bleeding and contractions

Table V. HCG

	Pat. no	initial visit day 0	2nd assessment (day 7)	expulsion
I.	200	32	yes	
	2	200	23	yes
	3	200	200	incompl.
	4	200	283.3	yes
	5	200	26	yes
	6	200	200	incompl.
	7	200	20	yes
	8	200	25	yes
	9	200	140	yes
	10	200	22	yes
	11	200	200	incompl.
	12	200	20	yes
II.				
	13	1957	480	yes
	14	3120	389	yes
	15	391	67.6	yes
	16	8250	240	yes
	17	1640	55	yes
	18	391	67.6	yes
	19	22226	10451	incompl.
	20	200	200	yes
	21	200	200	incompl.
	22	200	180	yes + PG
	23	200	180	yes
	24	8250	270	yes

I: 100 mg b. i. d.

II: 200 mg o. d.

increased. Ultrasound examination revealed that the residual tissue had moved from the uterine cavity to the internal os of the cervix. It was removed by easy curettage.

#### Postcoital Treatment Day 24 - 27 of Menstrual Cycle

Postcoital antifertility treatment is readily available in the Netherlands (within 72 hours) after intercourse.

Treatment consists of high dosages of estrogens (Haspels, 1976) or combined steroids (Van Santen and Haspels, 1983). A post-coital I.U.D. can be inserted up to seven days after intercourse. A patient must wait until the expected date of menstruation until effectiveness can be determined. A daily dose of 200 mg of RU 486 was prescribed for four days to 14 patients from day 24 through 27 of the cycles. In 13 patients, a normal period occurred. In one patient amenorrhoea went on for two months; apparently she had an anovulatory cycle. In the future we will perform serum  $\beta$ -hCG and progesterone tests on days 24 and 26 to determine early pregnancy.

Table VI. Estradiol

	Pat. no	initial visit	2nd assessment	expulsion
I.	1	2100	101	yes
	2	1020	310	yes
	3	1019	1722	incompl.
	4	380	107	yes
	5	2091	410	yes
	6	3051	3782	incompl.
	7	2065	387	yes
	8	658	42	yes
	9	1645	360	yes
	10	1535	115	yes
	11	1075	75	incompl.
	12	1209	467	yes
II.	13	325	270	yes
	14	350	122	yes
	15	1210	220	yes
	16	370	117	yes
	17	1210	118	yes
	18	1210	425	yes
	19	1620	2270	incompl.
	20	1445	1155	yes
	21	650	275	incompl.
	22	990	265	yes + PG
	23	1365	285	yes
	24	1525	123	yes

I : 100 mg b. i. d.

II : 200 mg o. d.

Table VII Progesterone

	Pat. no	initial visit	2nd assessment	expulsion
I.	1	6.2	8.0	yes
	2	10	30	yes
	3	30	30	incompl.
	4	6.6	4.1	yes
	5	30	6	yes
	6	30	30	incompl.
	7	30	10.7	yes
	8	30	3	yes
	9	30	15	yes
	10	30	2.6	yes
	11	30	5.2	incompl.
	12	30	18.8	yes
II.	13	60.9	22.4	yes
	14	50.5	12.4	yes
	15	46.8	12.2	yes
	16	47.2	9.4	yes
	17	80	8.3	yes
	18	46.8	5.1	yes
	19	41.1	44.2	incompl.
	20	91.0	80	yes
	21	35.8	24.4	incompl.
	22	46.8	4.4	yes + PG
	23	49.9	5.7	yes
	24	64.3	8.3	yes

I: 100 mg b. i. d.

II: 200 mg o. d.

Table VIII. Cortisol

	Pat. no	initial visit	2nd assessment	expulsion
I.	1	.26	.27	yes
	2	.32	.30	yes
	3	.29	.35	incompl.
	4	.35	.47	yes
	5	.47	.45	yes
	6	.25	.43	incompl.
	7	.47	.48	yes
	8	.40	.45	yes
	9	.43	.47	yes
	10	.49	.51	yes
	11	.30	.36	incompl.
	12	.87	.51	yes
II.	13	.41	.35	yes
	14	.42	.37	yes
	15	.38	.37	yes
	16	.38	.37	yes
	17	.23	.26	yes
	18	.35	.41	yes
	19	.48	.49	incompl.
	20	.46	.38	yes
	21	.40	.35	incompl.
	22	.20	.32	yes + PG
	23	.29	.40	yes
	24	.32	.29	yes

I: 100 mg b. i. d.

II: 200 mg o. d.

Table IX. Patients with 8-10 Weeks of Pregnancy

Pat. no.	Age	Weight	Cycle	Grav.	Pari.	Pregnancy (week-days)
III-01	40	67	irreg. after 15 years OAC	2	1	9W OD
III-02	30	59	28/5-6	3	2	9W 3D
III-03	35	71	28/4-5	6	4	8W OD
III-04	38	54		4	2	8W 5D
III-05	38	61	28/6	3	2	8W 4D
III-06	36	59		3	2	8W 4D
III-07	40	69	28/5	3	2	9W 1D
III-08	29	56	28/5-6	3	1	8W OD
III-09	20	62	28/5-7	1	0	8W 1D

Table X. Patients with 8-10 Weeks of Pregnancy

Pat. no.	Start of bleeding	How long	Intensity of bleeding	Expulsion	Vacuum Aspiration
III-01	3rd day			incompl.	yes
III-02	8th day	2 days	decreasing	incompl.	yes
III-03	2nd day	5 days	2nd day little, 4th day heavy	yes	no
III-04	1st day	7 days	varying intensity	incompl.	yes
III-05	1st day	5 days		yes	no
III-06	4th day	2 days	with clots	yes	no
III-07	2nd day	3 days	needed 2 pints bl-transf. day 5	incompl.	yes
III-08	2nd day	3 days	needed 2 pints bl-transf. day 5	incompl.	yes
III-09	3rd day	5 days	prostaglandin  sulproston 2x250 mg 12th day curettage	incompl.	yes

## DISCUSSION

The antiprogesterone properties of RU 486 are clearly demonstrated in this study. In all 24 women with amenorrhoea up to seven weeks, bleeding was induced and resulted in 79% complete abortion. Five patients needed vacuum aspiration. We recently have completed abortions with 0.25 mg Sulproston, a prostaglandin analogue. Nulliparous women had a high success rate of 12 out of 13 (92%).

Our experience using prostaglandin analogues for termination of early pregnancy indicates that the frequency of incomplete abortion increases with gestational age, especially after the seventh week of amenorrhoea. In successfully treated patients, serum  $\beta$ -hCG, estradiol and progesterone decreased by day seven. It was not possible to judge whether the drug's effect was mainly on the corpus luteum, on the placenta, or both.

Herrmann et al. (1982) found an increase in plasma cortisol during treatment of women in early pregnancy with 200 mg RU 486. In the present study, cortisol levels were not significantly different at day seven compared to day zero. The increases in cortisol values are similar to those observed during prostaglandin-induced abortion and may be the result of stress rather than a drug-induced effect. However, this cannot be known for sure in view of RU 486's known anti-glucocorticoid activity at the receptor level (Herrmann et al., 1983).

RU 486 is superior to prostaglandin analogues in terms of uterine pain and gastrointestinal side effects. The efficacy of RU 486 and prostaglandin analogues is about the same for treatment during very early pregnancy. Prostaglandin analogues are superior to RU 486 in terms of the amount of blood loss. Improvement of results possibly can be realised by treating RU 486 patients having incomplete abortions with a prostaglandin analogue on day seven. The large majority of patients preferred the "spontaneous abortion" with RU 486 to instrumental vacuum aspiration.

## ACKNOWLEDGMENT

RU 486 was supplied by Roussel Uclaf, Paris, France.

## REFERENCES

- Andriesse, R., Hart, H., and Haspels, A. A., 1976, Pre-operative cervical dilation by small doses of prostaglandin F<sub>2</sub>, Contraception, 14:93-99.
- Haspels, A. A., 1976, Interception. Post coital estrogens in 3016 women, Contraception, 14:375-381.
- Herrmann, W., Wiess, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effect d'un steroide anti-progestérone chez la femme: interruption du cycle menstruel et de la grossesse au debut, Comptes Rendus Acad. Sc. Paris, 294:933-940.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - an antiprogesterone compound. Contraception, 29:399-410.
- Van Santen, M. R. and Haspels, A. A., 1983, Comparative randomized double blind study high dosage ethinyl-estradiol versus eth-estradiol-norgestrel combination in post coital hormonal contraception, Contrac. Deliv. Syst., 4:11.
- Van Santen, M. R. and Haspels, A. A., 1983, Interception by post coital IUD insertion, Contrac. Deliv. Syst., 4:36.

## CLINICAL STUDY OF RU 486 IN EARLY PREGNANCY

David Elia

Centre Planification M.N.E.F.  
Maternité - Hopital Rothschild  
2 rue Phalsbourg 75017  
Paris, France

### INTRODUCTION

This study was carried out in the family planning center of the Mutuelle National des Etudiants de France, with the collaboration of Rothschild Hospital Maternity, Paris and the Fondation de Recherches Hormonales. The aims of the study were: 1) to evaluate the efficacy of RU 486 in terminating early pregnancies; 2) to determine if any side effects could be observed.

### PROCEDURES

Eighteen healthy volunteers who requested legal abortion were treated with RU 486, after giving their informed consent to participate in the study. The subjects received 200 mg per day of the drug, orally, for four days.

Three days before the beginning of the treatment, each subject had a complete physical and gynecological examination. Ultrasound scan of the pelvic region was performed. Blood samples were taken for clinical chemistry and hematological tests. On the first day of treatment, a blood sample was taken (just before the first drug ingestion) for hCG and steroid hormone assays, including corticosteroids. These assays were repeated on days three and six. Gynecological examination was performed on day three and at the final follow-up visit, which was on day eight.

By day six, with the aid of ultra-sonic evaluation, the clinician in charge determined if the pregnancy had been terminated and whether or not the uterus had been totally evacuated. On the basis of this day six evaluation, the decision was made whether or not to perform vacuum aspiration.

### RESULTS

Table I describes the patients' age, height, weight, and gynecological and obstetrical history. The mean age was 28. The extremes were 20 and 38 years. Most volunteers were nulliparous; four women each had completed two term pregnancies; four women had already had a voluntary interruption of pregnancy.

Table II summarizes the cases by the age of pregnancy in weeks of amenorrhea. All were within weeks five and nine since their last menses. There is no clear relationship between the age of pregnancy and the result of treatment. The subjects refrained from using other medication, including aspirin, during the treatment.

## EFFECTIVENESS

In seven cases pregnancy was terminated and complete evacuation of the uterus followed spontaneously. These cases may be considered complete successes. Two cases experienced pregnancy termination but the uterus did not empty; vacuum aspiration was required. The other nine cases had no indication of pregnancy interruption during RU 486 treatment, and they underwent vacuum aspiration.

All women except two recorded having followed the prescribed treatment. One of these two had a successful pregnancy termination and the other did not.

### Predictive Factors

The duration of pregnancy had no significant influence on the outcome of treatment (Table II). The small number of cases, however, makes it impossible to draw a definite conclusion.

The initial plasma estradiol value appears to have a significant influence on the outcome of treatment. The successful cases tend to have lower initial values; the unsuccessful cases tend to have higher initial estradiol values, except case no. 17 (350 pg/ml).

There is no significant difference between the initial progesterone values of the cases in which treatment succeeded and of those that did not. The initial value for plasma hCG has a significant influence on the result ( $p < 0.01$ ). The successful cases have relatively low initial rates, while all the failures have high initial rates (Tables III and V).

### Effect of Treatment on Plasma Hormone Value

The estradiol rates, on an average, decline in successful cases and increase in unsuccessful cases. The difference between the two groups, however, are not significant (Tables IV and V).

Progesterone values vary significantly, depending on the outcome of treatment. When termination is complete, plasma progesterone usually falls significantly by day 6. In the cases of partial success, the progesterone rate remains unchanged or falls slightly. In unsuccessful cases, the progesterone rate usually remains unchanged or increases (Tables IV and V).

The course of  $\beta$ -hCG does not show a significant difference, according to whether the treatment results in complete or incomplete success (Tables IV and V).

Symptoms associated with treatment are shown in Table VI. Bleeding patterns, with treatment, are shown in Table VI. As would be expected, the bleeding appears significantly earlier in cases of success. On the average, the bleeding is longer and more abundant in successful cases.

### Side Effects

Reported side effects are shown in Table VIII. There was no significant variation in weight or blood pressure during treatment (Table IX). Blood

chemistry values before and after treatment are shown in Table X. Some values show minor variation, some bordering on statistical significance, but only the change in plasma cortisol is noteworthy. The elevation is probably associated with the anti-glucocorticoid activity of RU 486 at the level of negative feedback in the control of ACTH production.

The tables referred to in the text are presented on the pages following.

## CONCLUSION

Studies with RU 486 must continue to establish the appropriate dose and schedule of treatment to eliminate failures or partial successes in the termination of pregnancy. It is noteworthy that the drug causes no significant side effects. Since subjects received combinations of contraceptives after RU 486 therapy, we could not determine the effect of treatment on subsequent ovulation. One subject, however, did establish pregnancy six months after RU 486 treatment, having been using oral contraceptives during the interum period.

Table I. Patient Characteristics

General characteristics

Age (years)	Mean	28
	Minimum	20
	Maximum	38
Height (cm)	Mean	164
	Minimum	156
	Maximum	172
Weight (kg)	Mean	57
	Minimum	47
	Maximum	78

Gynecological history

Age at first menses (years)	Mean	13
	Minimum	11
	Maximum	17
Characterization of cycles	Regular	13
	Irregular	5
Duration of menses (days)	Mean	4
	Minimum	3
	Maximum	7
Nature of bleeding	Moderate	3
	Normal	15
	Important	0
Premenstrual syndrome	No	10
	Yes	8
Pain at ovulation	No	15
	Yes	3
History of more than 40 days of amenorrhea	No	14
	Yes	4

Obstetrical history

Term pregnancies	No	14
	Yes	4
Miscarriage	No	18
	Yes	0
Induced abortion	No	14
	Yes	4
Ectopic pregnancy	No	18
	yes	0

---

Table II. Weeks of Amenorrhea

Weeks of amenorrhea	Results		Total
	Success	Failure*	
5	1	0	1
6	4	3	7
7	2	3	5
8	2	0	2
9	0	3	3

\*Failure: amenorrhea continuing after treatment

Table III. Treatment Efficacy: Prognosis Factors

		Success (n = 9)	Failure <sup>1</sup> (n = 9)	
Weeks of amenorrhea	mean	6.7	7.3	
	s.d.	1.0	1.5	NS
	min-max	5 - 8	6 - 9	
Initial estradiol plasma level(pg/ml)	mean	400	700	
	s.d.	240	190	<0.05
	min-max	120-890	350-910	
Initial progesterone level (ng/ml)	mean	15.9	18.0	
	s.d.	5.6	6.1	NS
	min-max	7-26	12-28	
Initial $\beta$ -hCG level (mIU/ml)	mean	14.300	53.600	<0.01
	s.d.	19.000	30.300	
	min-max	600-59.000	25.500-114.000	

<sup>1</sup>Failure: amenorrhea continuing after treatment

Table IV. Hormone Plasma Levels

Hormone	Successful cases			Unsuccessful cases	
	Before treatment	After treatment		Before treatment	After treatment
Estradiol (pg/ml)	mean	320		770	1170
	s.d.	340		130	500
	min	50		510	450
	max	920		910	1950
<hr/>					
Progesterone (ng/ml)	mean	7.8		19.6	1.7
	s.d.	8.8		6.0	5.4
	min	1.0		12.0	5.0
	max	26.0		28.0	30.0
<hr/>					
β-hCG (mIU/ml)	mean	72		570	581
	s.d.	93		304	196
	min	100		270	390
	max	280		1140	1010

Table V. Individual Hormone Plasma Levels

Case number	Estradiol (pg/ml)	Progesterone (ng/ml)	β-hCG (mIU/ml)
Successful			
02	260	12	5300
06	230	20	59,000
07	120	14	600
08*	520	21	16,700
09	180	7	1900
11	430	13	14,900
13	580	26	4200
15	350	16	--
18	890	14	11,400
Unsuccessful			
01	910	15	44,500
03	510	28	92,500
04	770	24	43,000
05	800	17	52,500
10	760	16	51,500
12	870	25	114,000
14	780	12	31,500
16	530	13	27,000
17	350	12	25,000

\*Partial Success: pregnancy discontinued but products of conception were not spontaneously expelled within eight days.

Table VI. Symptoms

		<u>Present at Initiation</u>		<u>Not present at Initiation</u>	
		Disappeared	Persisted	Appeared	Did not appear
Nausea	Success	3	0	0	6
	Failure	2	4	1	2
	Total	5	4	1	8
<hr/>					
Dysuria	Success	3	0	0	6
	Failure	4	0	1	4
	Total	7	0	1	10
<hr/>					
Fatigue	Success	1	1	2	5
	Failure	1	3	2	3
	Total	2	4	4	8
<hr/>					
Breast Tenderness	Success	2	0	1	6
	Failure	5	2	0	2
	Total	7	2	1	8

Table VII. Vaginal Bleeding During Treatment

Start of bleeding	No bleeding	Day 2	Day 3	Day 4	Day 9
Success	0	5	3	1	0
Failure	3	0	4	1	1
<hr/>					
Duration of bleeding (days)	No	1	2	3	3
Success	0	1	1	5	1
Failure	3	1	3	1	1
<hr/>					
Amount of bleeding	No bleeding	Moderate	Normal	Important	
Success	2	2	3	4	
Failure	3	3	1	2	

Table VIII. Frequency of Symptoms

Symptom	Success (n = 9)	Failure (n = 9)	Total (n = 18)
Nausea	1	0	1
Lipothemia	2	2	4
Asthenia	6	8	14
Pelvic pain	4	3	7

Table IX. Weight and Blood Pressure

		Before treatment	After treatment	Difference	p*
Weight	Number	12	12	12	NS
	Mean (kg)	59.2	58.7	- 0.5	
	s.d.	9.3	9.5	1.8	
	Minimum	47	47	- 6	
	Maximum	78	78	+ 1	
Systolic B.P.	Number	14	14	14	NS
	Mean	116	118	2	
	s.d.	12	11	18	
	Minimum	100	90	25	
	Maximum	130	130	30	
Diastolic B.P.	Number	14	14	14	NS
	Mean	69	73	+ 4	
	s.d.	7	10	10	
	Minimum	60	60	- 10	
	Maximum	80	80	+ 20	

\*Paired Wilcoxon test

Table X. Serum Chemistry

	N	Before treatment		After treatment		p
		mean	+ s.d.	mean	+ s.d.	value
Hemoglobin (g/100ml)	14	13.4	+ 1.1	12.9	+ 1.1	0.05
Hematocrit (%)	14	39.5	+ 2.8	38.4	+ 2.7	0.05
Erythrocytes (x10/mm)	14	4.36	+ 0.35	4.19	+ 0.37	0.05
Leukocytes (x10/mm)	14	6.89	+ 1.80	8.46	+ 2.64	0.05
Neutrophils (%)	14	57.9	+ 9.4	67.6	+ 8.0	0.01
Eosinophils (%)	14	1.9	+ 1.1	2.5	+ 0.9	NS
Basophils (%)	14	0.1	+ 0.3	0.0	+ 0.0	NS
Lymphocytes (%)	14	35.9	+ 7.8	27.1	+ 7.1	0.01
Monocytes (%)	14	3.4	+ 2.3	2.8	+ 1.3	NS
Prothrombin time (%)	14	97.4	+ 6.1	98.4	+ 5.9	NS
Partial thromboplastin time (s)	14	36.9	+ 2.6	35.9	+ 1.6	NS
Blood glucose (mmol/l)	13	4.68	+ 0.54	4.84	+ 0.57	NS
Creatinine (umol/l)	14	61.2	+ 8.8	62.6	+ 14.1	NS
ASAT (U/l)	14	11.1	+ 6.6	16.9	+ 7.0	0.01
ALAT (U/l)	14	10.1	+ 11.5	14.7	+ 10.4	0.05
Alkaline phosphatase (U/l)	14	51.6	+ 14.3	46.2	+ 16.5	NS
Serum sodium (mmol/l)	13	136.5	+ 2.8	138.9	+ 3.6	0.05
Serum potassium (mmol/l)	13	3.89	+ 0.37	3.97	+ 0.35	NS
Serum chlorides (mmol/l)	12	103.9	+ 3.0	104.7	+ 4.0	NS
Total bilirubin (umol/l)	8	9.87	+ 3.12	9.4	+ 3.0	NS
Total proteins (g/l)	12	71.2	+ 5.7	68.2	+ 4.4	NS
Albumin (g/l)	9	37.4	+ 4.6	36.4	+ 2.5	NS
Cortisol (ng/l)	15	161.5	+ 57.6	246.1	+ 50.2	<0.001

TERMINATION OF VERY EARLY PREGNANCY WITH DIFFERENT  
DOSES OF RU 486: A PHASE I CONTROLLED CLINICAL TRIAL

Dr. Laszlo Kovacs

Department of Obstetrics and Gynaecology  
H-6725 Szeged  
Sемmelweis u. 1.  
Hungary

ABSTRACT

RU 486 was given to 37 healthy pregnant women with amenorrhea of 42 days or less and requesting termination of pregnancy. The patients received either 25, 50 or 100 mg of RU 486 orally twice daily for four days. The subjects attended the hospital on each of the treatment days and on three follow-up visits one, two and 5-6 weeks after the start of the treatment. All patients but three started to bleed during the treatment. On the second follow-up visit, one patient was found to have an ectopic pregnancy that was treated by surgery. Twenty two patients (61%) were regarded to have had a complete abortion, twelve subjects had an incomplete abortion, and in three patients (7.6%) the pregnancy remained intact. The clinical efficacy of the treatment was not dose dependent. Most of the patients experienced only minor side effects: mild uterine pain, headache, nausea. Two patients suffered from heavy bleeding, requiring blood transfusion and curettage. Plasma progesterone,  $\beta$ -hCG, 17  $\beta$ -estradiol, cortisol and ACTH were monitored throughout the study. RU 486 treatment proves to be a well-tolerated and reasonably effective method to terminate early pregnancy.

INTRODUCTION

The need is great for a simple and safe method of birth control to be used when menses are delayed for a few days. A non-surgical, preferably self-administered procedure without the risk of appreciable side effects would be an attractive alternative to vacuum aspiration for "menstrual induction," i.e. for termination of very early pregnancy.

Since progesterone plays an indispensable role during the implantation phase and during early pregnancy in animals (Csapo and Resch, 1979) and women (Csapo and Pulkkinen, 1978), withdrawal of progesterone, or blockage of its action, may result in abortion. Herrmann et al. (1982) have reported that oral administration of RU 486 to eleven women in the sixth to eighth week of pregnancy resulted in nine abortions.

The World Health Organization Special Programme of Research, Development and Research Training in Human Reproduction has always placed high priority on the development of menstrual regulators. On the basis of the findings of Herrmann et al. (1982), a phase I clinical study was organized by the

Special Programme in Hungary and Sweden to investigate the efficacy of three different doses of the compound in terminating very early pregnancy and to document any reported side effects. (Kovacs et al., 1984).

## PATIENTS AND METHODS

Healthy women with amenorrhea of 42 days or less were admitted to the study. They were judged, by plasma  $\beta$ -hCG and gynecological examination, to have apparently normal pregnancies; and all requested abortion. All subjects were made aware of the nature of the study and gave their informed consent.

The compound was administered orally at three different dose levels: in the first group 25 mg twice daily for four days (a total of 200 mg); in the second group 50 mg twice daily (400 mg total); and in the third group 100 mg twice daily for four days (800 mg total). The patients received the medication between 8 and 10 am., and between 8 and 10 pm., and fasted for four hours before and one hour after taking the drug.

The subjects attended the hospital on each of the treatment days and on three follow-up visits, one, two, and 5-6 weeks after the start of the treatment (Figure 1). On each treatment day before the administration of the compound and on each follow-up visit, blood samples were taken for hormone analyses ( $\beta$ -hCG, progesterone, 17  $\beta$ -estradiol, ACTH and cortisol), routine hematology and clinical chemistry. The data from the first treatment day thus represent the plasma concentration immediately prior to therapy.

At the second follow-up visit, the outcome of therapy was assessed and, if it was not obvious that the patient had aborted completely, the pregnancy was terminated by vacuum aspiration.

The statistical analyses were based on the logarithm of the plasma levels, and two-way analysis of variance was used to estimate the residual within-subject variation. Significance tests were based on selected contrasts between daily mean values.

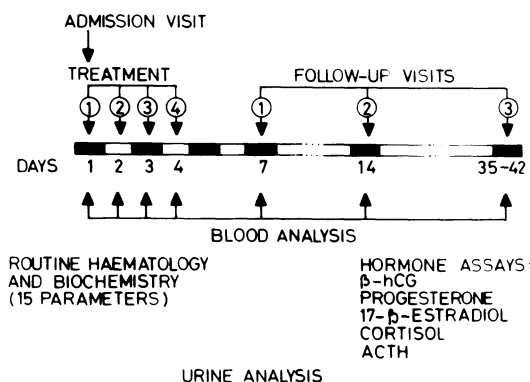


Fig. 1. RU 486 therapy for termination of very early pregnancy. Treatment and follow-up schedule.

## RESULTS

### Outcome of Pregnancy

The outcome of the therapy is summarized in Table I. One of the 37 patients was found, on the second follow-up visit, to have an ectopic pregnancy, which was treated by surgery.

Twenty two of 36 patients with normal pregnancies (61%) were regarded to have had a complete abortion. Eleven patients had an incomplete abortion and, in three patients (6.7%), the pregnancy remained intact.

The eleven incomplete abortions were evacuated by vacuum aspiration. Histopathologic examination of the removed tissue showed a necrotic ovum (missed abortion) or necrotic residual gestational tissue in all cases. The three intact pregnancies were terminated by dilatation and vacuum aspiration. In all three cases, the histopathology finding of the curettings was normal trophoblast.

In all cases of vacuum aspiration, a significant cervix dilating effect was experienced. In six of the 14 cases, dilation of the cervix was not necessary, while in the other eight cases, dilation from only 6th to 11th Hegar (mm) was needed.

All but three patients developed vaginal bleeding within five days of the start of the treatment. The patients receiving the two highest daily doses tended to start to bleed earlier (2nd-3rd day), while those who took the lowest dose started later (3rd-4th day). In the majority of the successfully treated patients, the bleeding had a duration of between one and two weeks, and the daily amount corresponded to that in their normal menstrual period.

Blood loss was measured in some of the subjects by use of the modified Nilsson-Hallberg method (Newton et al., 1977). Table II contains the blood loss measurements relating to twelve complete abortions. There was no difference with respect to the treatment dose, and blood loss was rather moderate. We were able to measure the blood loss of five patients in their next menstrual cycle. Taking this as 100%, blood loss during the treatment and complete abortion was 164%, less than twice the menstrual flow (Table III).

### Side Effects

Two patients experienced heavy bleeding: one in the 200 mg group on the sixth day of treatment, and one in the 800 mg group on the second day. They were admitted to the hospital and given blood transfusion and curettage. Other side effects were of a mild nature: headache, nausea and lower abdominal pain, as listed in Table IV.

### Plasma Hormone Levels

Plasma  $\beta$ -hCG concentrations before, during and after the treatment with 200 mg, 400 mg and 800 mg total doses of RU 486 are given for complete abortions in Figure 2 and Table V. In each of the three dose groups there was a slight (insignificant) increase during the first three treatment days. In the 200 mg group the values decreased on treatment day four, and there was a significant fall at the first follow-up visit ( $p < 0.001$ ). In the two higher dose groups, a significant fall in the  $\beta$ -hCG level commenced three days earlier, on treatment day 4 ( $p < 0.05$ ). Consequently there was a sharp decline in  $\beta$ -hCG plasma levels up to the first follow-up visit.

Table I. Outcome of RU 486 Treatment for  
Termination of Very Early Pregnancy

<u>Total dose (mg)</u>	<u>200</u>	<u>400</u>	<u>800</u>	<u>Total</u>
No. of patients	19	10	8	37
Complete abortion	12	5	5	22
Incomplete abortion	4	4	3	11
Ectopic pregnancy	1	0	0	1
Pregnancy continued	2	1	0	3

The changes in plasma progesterone values are very similar (Fig. 3 and Table VI). There was a significant decrease between values on days 1-4 and the first follow-up in all three groups. The decrease in progesterone values from days 1-4 was not significant in the 200 mg group, but there was a significant trend ( $p < 0.002$ ) in these values in the two higher dose groups.

The plasma 17  $\beta$ -estradiol values gradually decreased during treatment, and there was a sharp decline on day seven. The decline during the treatment was significant only in the 400 mg group. In the 800 mg group, the small number of samples ruled out the possibility of statistical significance (Fig. 4 and Table VII). Figure 5 shows the plasma estradiol values for all complete and incomplete abortions, regardless of the treatment dose. From treatment day four up to the first follow-up, there was an increase of estradiol values in the incomplete abortions. This was in contrast to the sharp decline observed in cases of complete abortions. From day one to the first follow-up, the estradiol values were consistently higher in the incomplete abortion group.

The plasma ACTH values increased during treatment, with the highest levels appearing on the second day (Fig. 6 and Table VIII). There was no dose-dependent difference in the changes. Values had returned to the pretreatment level by the first follow-up visit. Cortisol values for all treated patients are listed in Figure 7 and Table IX. Cortisol levels

Table II. Blood Loss in RU-486 Induced  
Complete Abortions

Total treatment dose (mg)	n	X (ml)	SE
200	5	88.0	15.2
400	5	86.4	17.8
800	2	87.5	9.5
Total	12	87.3	9.2

Table III. Comparison of Blood Loss in RU 486-Induced Complete Abortions and During Next Menstruation of Same Patients

Complete Abortions ml(%)	Menstrual Flow ml(%)
89 (171)	52 (100)
109 (170)	64 (100)
115 (180)	64 (100)
150 (176)	85 (100)
97 (121)	80 (100)
<hr/>	
X = 112.0 ml (163.8)	X = 69.0 ml (100)
SE = 10.5 (10.8)	SE = 6.0
n = 5	
Significance: p < 0.01	p < 0.01

increased significantly during the treatment, but the values had returned to the pretreatment levels by the time of the follow-up visit.

There were no appreciable changes in either the hemoglobin values and white blood cell count or the sodium, potassium, SGOT, SGPT and alkaline phosphatase levels in the blood samples.

#### DISCUSSION

The efficacy and safety of RU 486 treatment at three different dose levels for termination of very early pregnancy were evaluated. Of 36 intrauterine pregnancies, only three continued unaffected, whereas 22 ended in complete and 11 in incomplete abortion. These data are comparable with those reported by Herrmann et al. (1982). This efficacy is lower than those

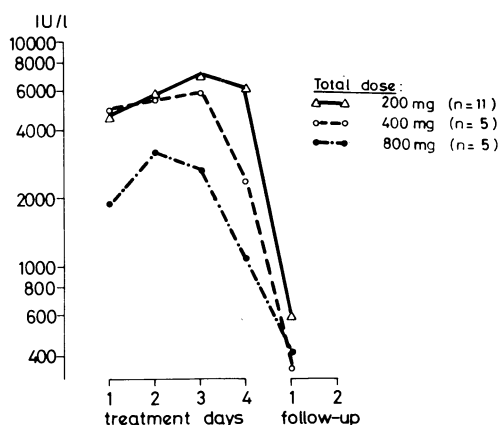


Fig. 2. Plasma  $\beta$ -hCG changes during RU 486 treatment. complete abortions.

Table IV. Side Effects of RU 486 Treatment

Heavy bleeding (blood transfusion)	2 cases
Headache	4 cases
Nausea	12 cases
Lower abdominal pain	21 cases

of the approved menstrual regulating methods: vacuum aspiration and prostaglandins. The frequency of complete abortion was not related to dose, although in the highest dose group no pregnancy remained intact, and two of the three women whose pregnancies continued received the lowest treatment.

The rates of complete and incomplete abortions may have been influenced by the study protocol. According to the study design, vacuum aspiration was performed two weeks following the start of the treatment if observation indicated that the abortion may not have been complete. It is likely that the frequency of complete abortion would have been higher if vacuum aspiration had been postponed in some of the cases, thereby allowing the opportunity for spontaneous expulsion of the product of conception. From a clinical point of view, such a delay of vacuum aspiration could have been possible in nine patients.

From clinical aspects, the treatment was more effective in disrupting development of the ovum (this was proved by the histopathology results on the curettings) than in expelling it from the uterus. The relatively high ratio of incomplete or missed abortions points to the low uterotonic effectiveness of the treatment. This may also be the explanation for the heavy bleeding, which appears to constitute the only major risk of the treatment. Apart from two heavy bleedings, our patients were free of any notable side effects; they tolerated RU 486 well. Treatment with RU 486 seems to be as safe or even safer to the other methods for termination of early pregnancy (vacuum aspiration or prostaglandins).

In the cases treated by vacuum aspiration, a significant softening and dilating effect on the uterine cervix was observed. This observation is in agreement with those reported by Elia (1984).

In the complete abortion cases, plasma  $\beta$ -hCG, progesterone and estradio- all had decreased by the first follow-up visit. With increasing doses of

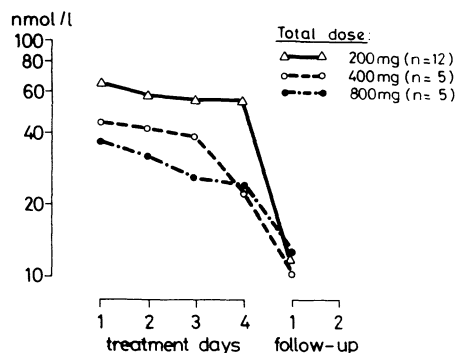


Fig. 3. Plasma progesterone changes during RU 486 treatment. Complete abortions.

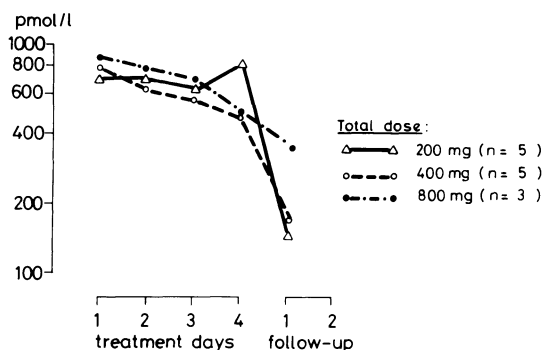


Fig. 4. Plasma 17-beta-estradiol changes during RU 486 treatment. Complete abortions.

RU 486, the fall in hormone values appeared earlier, by day three or four of the treatment, instead of day seven. The same was true for the commencement of vaginal bleeding. These results may indicate destruction of the conceptus began sooner when higher doses were given.

From the present study, it is not possible to judge whether the effect of RU 486 was exerted on the endometrium and/or the corpus luteum. It has been demonstrated that in the cynomolgus monkey the antiprogesterational compound acts directly upon the endometrium and not on the corpus luteum (Healy, 1983).

Analyses of our plasma estradiol results showed that pregnancies with higher estradiol levels were more resistant to the therapy. Elia (1984), too, regarded estradiol and hCG levels as important factors in determining the success rate of the treatment.

In the present study, increased ACTH and cortisol plasma levels were observed during treatment, but the values had returned to pretreatment levels by the follow-up visit. These observations are in agreement with those of Herrmann et al. (1982). The changes were not excessive and were not followed by any clinical symptoms. They may be a result of stress rather than a direct drug-induced effect, but the latter cannot be ruled out in view of the known glucocorticoid antagonist activity at the receptor level. The effect was transitory and completely reversible.

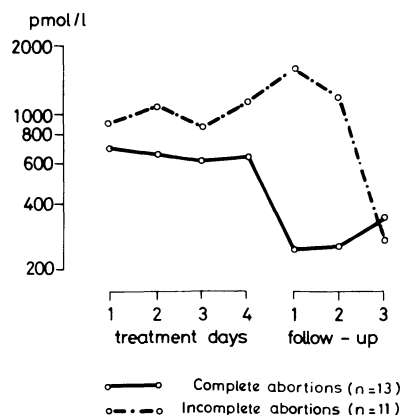


Fig. 5. Plasma 17- $\beta$ -Estradiol changes during RU 486 treatment.

Table V. Plasma  $\beta$ -hCG Concentrations (IU/l, mean and 95% confidence limits) in RU 486-Induced Complete Abortions

Dose (mg)	Treatment days				First follow-up visit
	1	2	3	4	
200 (n=11)	4760 (3120-7270)	5680 (3720-8680)	7070 (4630-10800)	6130 (4010-9350)	593xxx (389-906)
400 (n=5)	4950 (2640-9260)	5580 (2980-10400)	5960 (3180-11200)	2440 <sup>x</sup> (1300-4580)	359xxx (192-672)
800 (n=5)	1920 (1030-3600)	3300 (1760-6170)	2670 (1430-5000)	1100 <sup>x</sup> (585-2050)	420 <sup>x</sup> (224-786)

Significant difference compared to the preceding value:

x = p < 0.05

xxx = p < 0.01

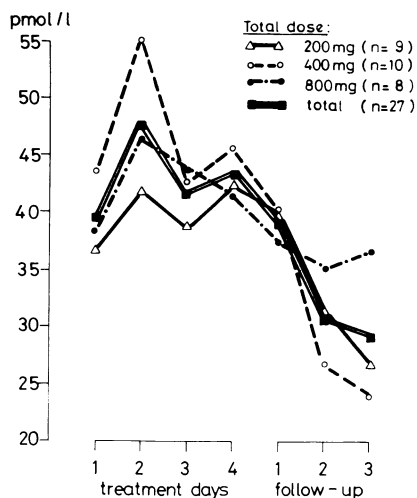


Fig. 6. Plasma ACTH changes during RU 486 treatment.

To summarize the results of the study, it can be concluded that treatment with an antiprogesterational drug is a promising new approach for fertility regulation in the human. RU 486 treatment proved to be a well-tolerated and reasonably effective method of menstrual regulation, and we hope subsequent clinical studies, modifying the treatment schedule will increase efficacy still further.

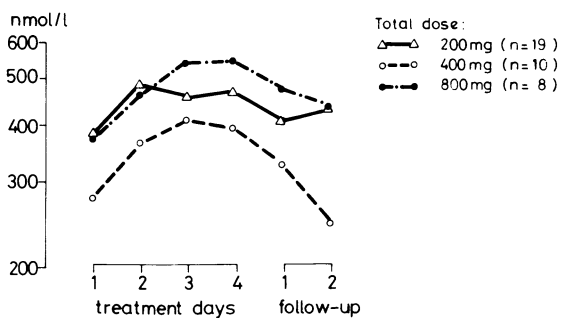


Fig. 7. Plasma cortisol changes during RU 486 treatment.

Table VI. Plasma Progesterone Concentrations (nmol/l, mean and 95% confidence limits) in RU 486-Induced Complete Abortions

Dose (mg)	Treatment days			First follow-up visit
	1	2	3	4
200 (n=12)	65 (52-80)	58 (47-72)	55 (45-68)	55 (44-68)
400 (n=5)	45 (32-62)	42 (30-58)	38 (27-53)	22 (16-31)
800 (n=5)	37 (27-52)	31 (23-44)	26 (18-36)	24 (17-34)

The differences between values on days 1 to 4 and those on the first follow-up were significant in all three groups ( $p < 0.001$ ).

Table VII. Plasma Estradiol Concentrations (pmol/l, mean and 95% confidence limits) in RU 486-Induced Complete Abortions

Dose (mg)	Treatment days				Follow-up visits	
	1	2	3	4	first	second
200 <sup>1</sup> (n=5)	674 (398-1142)	648 (341-1233)	632 (343-1166)	909 (565-1463)	179 (98-325)	261 (127-535)
400 <sup>2</sup> (n=5)	717 (469-1094)	607 (337-1092)	598 (308-1159)	460 (331-640)	189 (122-292)	290 (196-429)
800 <sup>3</sup> (n=3)	825 (203-2193)	765 (338-2213)	657 (225-1625)	479 (136-1404)	326 (287-465)	178 (151-828)

The differences were significant between the values: 1 on days 1 and 4 and those on follow-ups 1 and 2 ( $p < 0.001$ ); 2 on days 1 and 4 ( $p < 0.05$ ), day 1 and follow-up 1 ( $p < 0.001$ ), and day 4 and follow-up 1 ( $p < 0.01$ ); and 3 on day 1 and follow-up 2 ( $p < 0.05$ ).

Table VIII. Plasma ACTH Concentrations (pmol/l, mean and 95% confidence limits) during RU 486 Treatment for Termination of Very Early Pregnancy

Dose (mg)	Treatment days				Follow-up visits	
	1	2	3	4	first	second
200 (n=19)	37 (26-53)	42 (31-57)	39 (27-57)	42 (27-67)	40 (22-72)	31 (25-39)
400 (n=10)	44 (35-56)	55 (32-94)	42 (31-59)	46 (24-86)	40 (24-66)	27 (22-33)
800 (n=8)	38 (27-54)	46 (34-63)	44 (26--74)	41 (28-60)	37 (25-57)	35 (27-45)

The differences were not significant.

Table IX. Plasma Cortisol Concentrations (nmol/l, mean and 95% confidence limits) during RU 486 Treatment for Termination of Very Early Pregnancy

Dose (mg)	Treatment days				Follow-up visits	
	1	2	3	4	first	second
200 <sup>xxx</sup> (n=19)	371 (316-435)	487 (415-572)	452 (385-530)	474 (403-556)	401 (342-471)	419 (357-491)
400 <sup>xxx</sup> (n=10)	277 (222-346)	363 (291-453)	403 (323-502)	388 (311-484)	325 (261-405)	241 (193-301)
800 <sup>x</sup> (n=8)	351 (274-450)	450 (352-576)	517 (404-662)	525 (410-672)	467 (365-598)	441 (345-565)

The differences between values during treatment (days 2, 3, 3) and those on day 1 or those on follow-up visits were significant (xxx =  $p < 0.001$ , x =  $p < 0.05$ ).

## ACKNOWLEDGMENTS

This study was undertaken with the financial support of the WHO Special Programme of Research, Development and Research Training in Human Reproduction, Geneva, Switzerland. RU 486 was supplied by Roussel Uclaf, Paris, France. The following scientists participated in this study: Dr. M. Bygdeman and Dr. M. L. Swahn (Stockholm); Dr. P. J. Rowe (WHO, Geneva); Dr. M. Sas, Dr. B. A. Resch and Dr. Gy. Ugocsai (Szeged).

## REFERENCES

- Csapo, A. I., and Pulkkinen, M., 1978, Indispensability of the human corpus luteum in the maintenance of early pregnancy. Luteectomy evidence, Obstet. Gynecol. Surv., 33:69-81.
- Csapo, A. I., and Resch, B., 1979, Prevention of implantation by antiprogesterone, J. Steroid. Biochem., 11:963-969.
- Elia, D., 1984, Results obtained in early pregnancy termination, Minutes of the RU 486 Meeting, Paris, March 1984, p. 4.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships and hormonal effects, Fertil. Steril., 40:263-257.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effect d'un stéroïde anti-progesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut, C. R. Seances Acad. Sci., 294:933-938.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - an antiprogestational compound, Contraception, 29:399-410.
- Newton, J., Barnard, G., and Collins, W., 1977, A rapid method for measuring menstrual blood loss using automatic extraction, Contraception, 16:269-282.

CLINICAL EFFECTS OF RU 486 ADMINISTERED  
FOR SEVEN DAYS IN EARLY PREGNANCY

Lars Birgersson, Viveca Odland and Elov Johansson

Department of Obstetrics and Gynaecology, University Hospital  
751 85 Uppsala, Sweden

SUMMARY

RU 486 was given in doses of 50 mg twice daily for seven days to 28 healthy women applying for legal abortion before the end of the seventh gestational week. Clinical examinations, hCG/s and routine laboratory screenings were performed prior to treatment and again on day seven of treatment. Clinical examination and hCG/s were repeated 14 days after the onset of vaginal bleeding and again six weeks after the start of therapy. Of the 28 patients treated, five failed to respond to treatment and had surgical termination of pregnancy with vacuum aspiration. Of the remaining 23 patients, 20 were judged to have a complete abortion. Three patients had incomplete abortions as judged by inadequate involution of the uterus and sustained elevated hCG/s levels. The mean duration of bleeding in those women who had complete abortions was 10.9 days (range 7 to 18 days). A slight hemoglobin decrease was found at the first follow up visit after seven days. No woman, however, required blood transfusion or hospitalization. The serum levels of hCG dropped dramatically in those who aborted. Side effects reported were minimal. It is concluded that RU 486 is a potent abortifacient during very early pregnancy with a rate of complete abortions at about 70%, and is very well tolerated by women.

INTRODUCTION

RU 486 is a steroid with progesterone and glucocorticoid receptor antagonist properties (Philibert et al., 1982). It has been shown to induce interruption of luteal phase and early pregnancy in humans (Herrmann et al., 1982). The aim of the present study was to investigate the effectiveness of RU 486 as an abortifacient in humans at a dose of 50 mg twice daily for seven days and to quantify the side effects caused by the treatment. The preliminary results from the study are presented, including clinical data on 28 patients.

STUDY DESIGN

Twenty eight healthy women with a gestational length less than seven completed weeks were recruited among patients applying for legal abortion. The patients were 18 to 41 years old and had a history of regular menses (28±3 days). Patients with any symptoms of abnormal pregnancy, clinical evidence of cervical incompetence or recent (less than six months) use of glucocorticoids in any form were excluded from the study, as were patients

with a history of liver, gastro-intestinal or renal disease. The use of the drug has been approved by both the Ethical Committee of the Faculty of Medicine, University of Uppsala and by the National Drug Regulatory Authorities.

At the first visit to the clinic, the patients received information about the nature and purpose of the study. Before entering, each patient underwent a careful clinical examination and a laboratory screening including a full hematological profile, bilirubin, aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase, albumin, sodium, potassium, creatinine and urea in blood. HCG levels and estradiol and progesterone plasma concentrations were also determined. The patients were told to take the drug at 8:00 am and 6:00 pm and to withhold food for one hour before and after drug administration. Each patient was given a protocol to record the time of drug intake and any symptoms possibly related to the medication. A preliminary time for vacuum aspiration, approximately ten days after the beginning of therapy, was appointed in case of treatment failure or discontinuation of therapy for any reason. The patients then took 50 mg x 2 for seven days and returned to the clinic on treatment day seven for a pelvic examination and blood sampling as above.

At the third visit, approximately 14 days after the onset of vaginal bleeding, the patients were examined again to determine whether the abortion was complete. A third hCG/s was then obtained.

A final follow up visit took place six weeks after the beginning of treatment for the recording of the duration of bleeding, the patient's opinion of the treatment and her choice of family planning method for the future.

#### Criteria For Complete Abortion

Each woman was asked to give a history of bleeding and passage of products. At the clinical examination there should be vaginal bleeding and cervical dilation. The hCG/s should be less than 10% of the initial value seven days after the onset of bleeding. Fourteen days after the onset of bleeding, the hCG/s should again be less than 10% of the seven day level. By then the uterus should be back to normal size, and the cervix should be closed. Bleeding should be minimal.

In some cases, where the clinical picture was that of a complete abortion but the decrease in hCG levels was somewhat delayed compared to the limits stated above, additional sampling was done to assure that the hCG/s levels did decrease to zero. Statistical significance was assessed by Students' two-tailed t-test for dependent and independent observations.

## RESULTS

### Clinical Results

Out of 28 patients treated, 23 aborted (82.1%). The five patients that did not abort noted only a short period of vaginal bleeding, the amount less than a normal menstruation, and the uterus showed no sign of involution. In four patients the hCG/s levels increased during treatment; the hCG/s remained on the same level during treatment in the fifth case. A routine vacuum aspiration was performed in these cases. The histopathological examination showed ordinary abortion material.

In most of the 23 patients who responded to treatment vaginal bleeding started one day before passage of products and uterine contractions, the mode being treatment days two and three, respectively (Fig. 1).

Twenty of the 23 patients (71% of all patients treated) who aborted were judged at the second follow-up visit to have had complete abortions. The remaining three patients showed signs of incomplete abortion, with inadequate involution of the uterus, prolonged bleeding and sustained hCG/s levels. Exaeresis was performed in these cases, and histopathological examination of the curettage confirmed the diagnosis.

#### Levels of HCGs

Mean pretreatment hCG/s did not significantly differ between patients who subsequently had complete abortions and those who had incomplete abortions or only minor bleeding as a result of the treatment (Fig. 3). In the 20 patients who responded to treatment and had complete abortions the hCG/s levels were significantly reduced at the first follow-up visit (4-7 days after the onset of bleeding) and this reduction continued further at the second follow up visit (11-16 days after the onset of bleeding). There were pronounced interindividual variations concerning both initial serum levels of hCG and the speed at which the levels decreased. In 15 out of 20 patients there were no detectable amounts of hCG/s at the second follow up visit. In the remaining five patients the decrease was slower, though they all showed clinical signs of having aborted completely. Additional blood samples were taken and, subsequently, the serum levels of hCG decreased to zero (Fig. 2).

#### Hormonal Results

Plasma concentrations of progesterone and estradiol were determined before treatment and at one and two weeks after the onset of bleeding. The mean $\pm$ SD pretreatment level of progesterone was 48.3 $\pm$ 16.9 nmol/l in women who had complete abortions and 63.8 $\pm$ 16.2 nmol/l in those who did not respond to treatment or had incomplete abortions (Fig. 3). This difference was statistically significant ( $p < 0.05$ ). Progesterone concentrations decreased rapidly after the onset of bleeding (Fig. 4). The mean $\pm$ SD plasma concentration of estradiol was 1713 $\pm$ 569 pmol/l in the women who had complete abortions and 2532 $\pm$ 2047 pmol/l in those who did not respond or had incomplete abortions (Fig. 3). This difference was not statistically significant. Estradiol levels also decreased rapidly after the onset of bleeding (Fig. 5).

#### Bleeding Patterns

The duration of bleeding among the 20 patients with clinical and biochemical signs of complete abortion ranged from 7-18 days with a geometric mean ( $\pm$ SD) of 10.9 $\pm$ 2.82 days. In most cases, patients reported relatively heavy bleeding for 2-4 days, followed by a bleeding less in amount than ordinary menstruation during the following days. Prior to treatment mean ( $\pm$ SD) Hb values for the patients with clinical signs of complete abortion were 132.1  $\pm$  11.5 g/l and, at the first follow up visits, 123.4 $\pm$ 13.27 g/l, ( $p < 0.001$ ). The average percentual decrease in Hb was 6.6%, the largest noted difference being 20%. None of the patients treated needed blood transfusion.

#### Biochemical Results

Among the 28 patients treated, no significant changes were recorded in serum levels of bilirubin, ASAT, ALAT, alkaline phosphatase or sodium. There was a slight increase in leukocyte count ( $p < 0.05$ ), creatinine ( $p < 0.05$ ) and a decrease in albumin ( $p < 0.001$ ), potassium ( $p < 0.05$ ) and urea ( $p < 0.05$ ). All changes were within the normal limits.

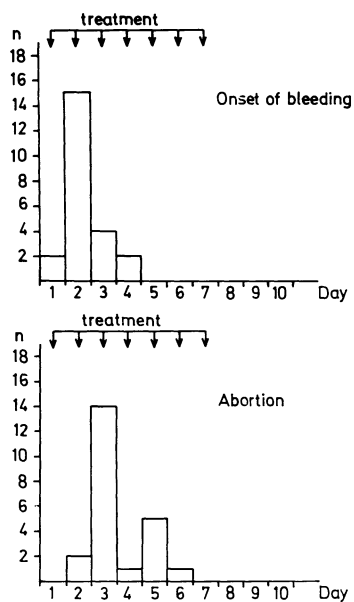


Fig. 1. Time of onset of bleeding and abortion, respectively, in 23 women who responded to treatment with RU 486, 50 mg twice daily for seven days.

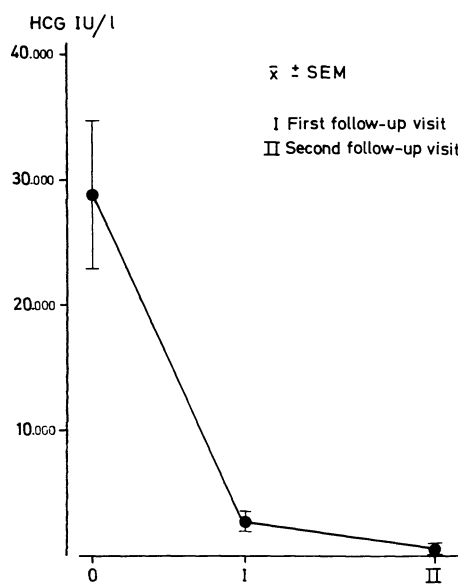


Fig. 2. Pretreatment levels of hCG/s, progesterone and estradiol in women with complete abortions (closed circles), nonresponders (open circles) and in women with incomplete abortions(x).

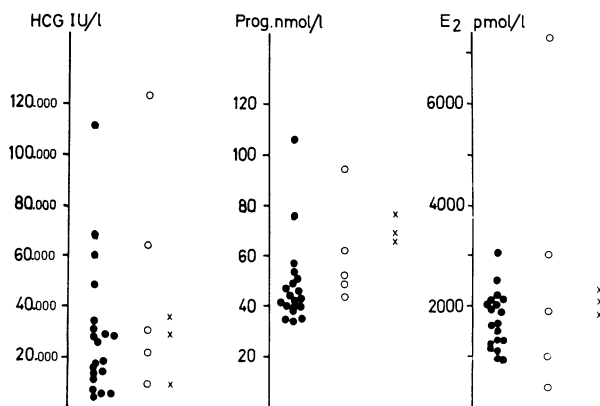


Fig. 3. Serum levels of hCG (mean  $\pm$  SEM) in 20 women who had complete abortions before and at the first and second follow-up visit after treatment with RU 486, 50 mg twice daily for seven days.

### Side Effects

Six of the 28 patients reported painful contractions exceeding normal menstrual pain. Seven patients complained of nausea during treatment, and three of these experienced vomiting as well. Two patients complained of bleeding more heavily than they had expected.

### DISCUSSION

RU 486 is a nonsteroid with substituted radicals in C<sub>11</sub> and C<sub>17</sub>, possessing strong antiprogestosterone properties at the receptor level (Philibert et al., 1982). We have evaluated its efficacy in terminating early first trimester pregnancies. The frequency of complete abortions in this study was 71%, a figure slightly higher than previously reported by Herrmann et al. (1982) and Kovacs et al. (1984), who accepted a gestational length of only six weeks. Kovacs did not report a dose dependency. In the present study, however, treatment duration was longer, and the higher incidence of complete abortions, despite longer gestational lengths, may indicate a duration-of-treatment dependency. The percentage of incomplete abortions was around 11%, which is lower than that of Kovacs et al. (1984), who reported around 30% incomplete abortions. As has been observed before, the hCG levels tend to remain fairly constant during treatment until abortion occurs, whereafter a rapid decline is seen (Herrmann et al. 1982). In some patients, however, the decrease in hCG levels appears slower even though the clinical signs are those of a complete abortion. When additional blood samples are taken, we observe that these patients also decline to zero hCG levels, implicating that a longer observation time probably would increase the number of complete abortions.

Five patients in this study showed no clinical sign of abortion, and three had incomplete abortions. They did not differ from the others in gestational length, or serum hCG and estradiol concentrations, but their progesterone concentrations were higher than those who responded to treatment. It is worth noting that even in those patients who did not have abortions, vaginal bleeding occurred, indicating some effect of the treatment. All patients claimed to have complied to the treatment protocol. This was not, however, biochemically tested.

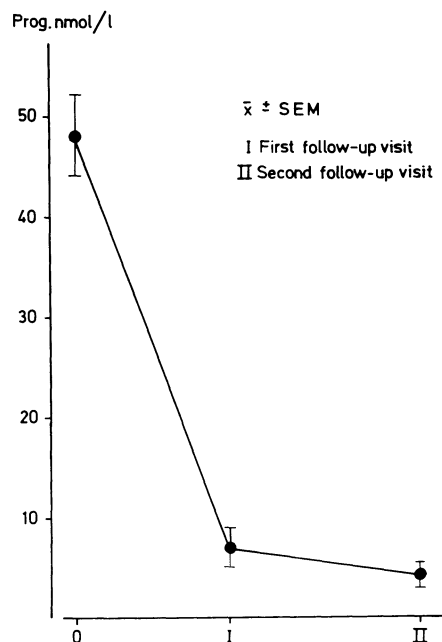


Fig. 4. Plasma concentrations of progesterone (mean  $\pm$  SEM) in 19 women who had complete abortions before and at the first and second follow-up visit after treatment with RU 486, 50 mg twice daily for seven days.

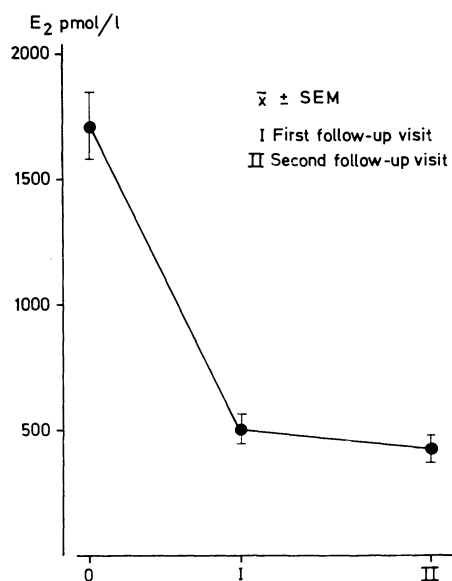


Fig. 5. Plasma concentrations of estradiol (mean  $\pm$  SEM) in 19 women who had complete abortions before and at the first and second follow-up visit after treatment with RU 486, 50 mg twice daily for seven days.

Reported side effects were mild. The slight reduction of hemoglobin one week after start of therapy and the average length of bleeding was comparable to earlier reports on vacuum aspiration and prostaglandin treatment (Bygdeman et al., 1977). Large amounts of bleeding necessitating blood transfusions as reported by Kovacs et al. (1984) were not seen in this study. These preliminary results suggest that with the dose regimen used RU 486 is less efficient than vacuum aspiration and probably than prostaglandin analogues (WHO Prostaglandin Task Force, 1982). On the other hand, patients spontaneously reported a preference for this method over vacuum aspiration, and side effects were less frequent and pronounced than has been reported with prostaglandin analogues (WHO Prostaglandin Task Force, 1982).

From the results presented in this study it is concluded that RU 486 is a promising approach in the search for an efficient abortifacient for early human pregnancy. Further studies with different dose regimens will better evaluate its efficiency. Detailed studies on the mechanisms of action are necessary to clarify why some patients exhibit only partial response to the treatment.

#### ACKNOWLEDGMENT

This work was supported by grants from the Rockefeller Foundation, the Ford Foundation, the Mellon Foundation, the G. Hecht Fund and the Swedish Medical Research Council. RU 486 was supplied by Roussel-Uclaf, Paris, France.

#### REFERENCES

- Bygdeman, M., Green, K., and Lundstroem, V., 1977, Interruption of first trimester pregnancy by prostaglandins, Int. J. Gynaecol. Obstet., 15:69-72.
- Herrmann, W., Wyss R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, The effects of an antiprogesterone steroid on women: Interruption of the menstrual cycle and of early pregnancy, Comptes Rendus, 294: 933-940.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P.J., 1984, Termination of very early pregnancy by RU 486 - an antiprogestational compound, Contraception, 29:399-410.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C. and Sakiz, E., 1982, RU 38 486 - a new lead for steroidal antihormones, Endocrine Society, 64th annual meeting, San Francisco, Abstract 668.
- WHO Prostaglandin Task Force, 1982, Termination of early pregnancy by vaginal administration of 16, 16-dimethyl-trans-delta-A<sup>2</sup>-PGE<sub>1</sub> methyl ester, Asia-Oceania J. Obstet. Gynaecol., 8: 263-268.

## THE USE OF RU 486 AS AN ABORTIFACIENT IN EARLY PREGNANCY

R. Sitruk-Ware, L. Billaud, I. Mowszowicz, H. Yaneva,  
P. Mauvais-Jarvis, C. W. Bardin,\* and I. M. Spitz\*

Department of Reproductive Endocrinology  
Hopital Necker, Paris, France

\*The Center for Biomedical Research  
The Population Council, New York, USA

RU 486 possesses a high affinity for the progesterone receptor and displays antiprogestational properties in animals (Philibert et al., 1982a, b). Two studies have also reported on its abortifacient properties in women. Complete abortion was reported in nine out of eleven subjects treated in one study (Herrmann et al., 1982) and 22 out of 38 in the second (Kovacs et al., 1984). Our laboratories recently commenced clinical trials with RU 486 in pregnant women in an attempt to induce abortion. The goal has been to evaluate the effect of various doses and duration of RU 486 treatment on the outcome.

### SELECTION OF SUBJECTS

Women aged 18 to 40 years with amenorrhea not longer than 49 days (calculated from the first day of the last menstrual period) were selected for the study. All had normal clinical examinations, previous regular menses, and a desire for pregnancy termination. Subjects with evidence of threatened or inevitable abortion, with any abnormality upon gynecological examination, or a uterine size consistent with greater than five weeks gestation were not studied. Also excluded were those in whom a previous attempt to terminate a pregnancy had failed, as well as those who had recently used corticosteroids.

### CRITERIA FOR COMPLETENESS OF ABORTION

The abortion was considered complete in the presence of sustained uterine bleeding associated with the passage of products of conception and dilation of the cervix, followed by progressive reduction in the levels of serum  $\beta$ -hCG. By day 14 there was a reduction in uterine size, and the cervical os was closed. Ultrasonography was performed on day one and then again 14 days after the start of bleeding. This was also used to assess the completeness of uterine evacuation. By day 14 if menses had not begun, evacuation was performed by suction or curettage. Blood samples were taken before commencing therapy, on day four (i.e., the last day of drug administration), and then at weekly intervals after bleeding commencement until  $\beta$ -hCG became undetectable or vacuum aspiration was performed.

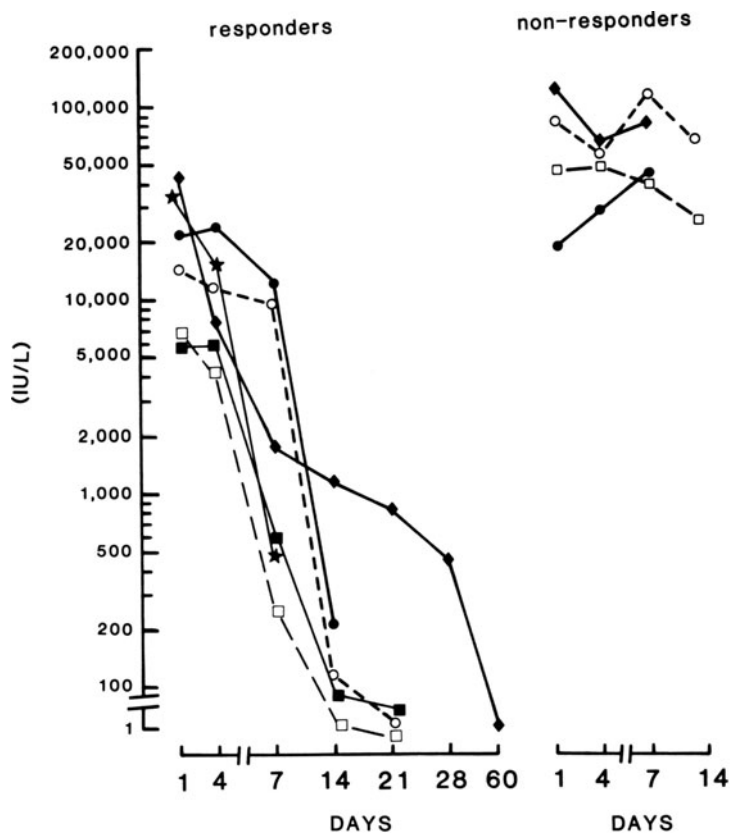


Fig. 1.  $\beta$ -HCG levels in the 6 responders who aborted (left panel) and the 4 non-responders who failed to abort (right panel). Each subject is shown by a different symbol.

#### DOSE SCHEDULE OF RU 486

In the present study RU 486 was administered on a sliding scale schedule of 400, 300, 200, and 100 mg/day for four successive days. The drug was given in two divided doses at 8 am and 6 pm. No food or fluid was allowed one hour prior or one hour after RU 486 administration. All subjects gave written consent after the nature of the study had been explained to them. Investigational Review Board approval had also been obtained.

#### RESULTS

Of the ten subjects who were treated, six had a complete abortion. Bleeding occurred within five days following commencement of drug administration and lasted from eight to 14 days. The blood loss was two or three times greater than the usual menstrual flow.

In the four remaining subjects, minimum bleeding occurred two to 12 days after commencement of therapy in all subjects and lasted from one to 9 days. In these four instances, vacuum aspiration was performed 14 to 21 days after the onset of bleeding.

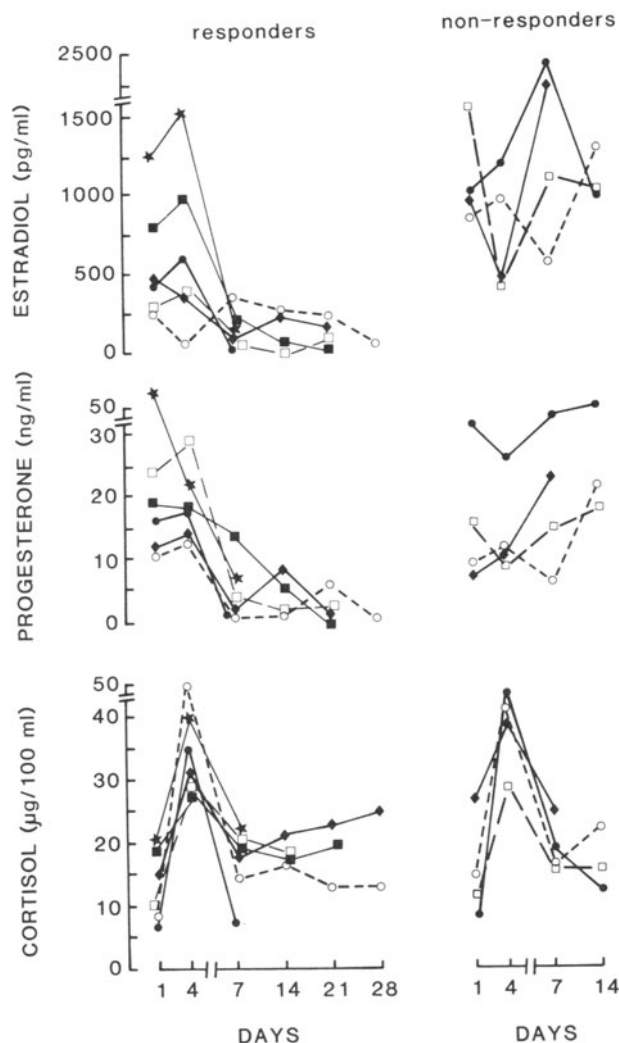


Fig. 2. Estradiol, progesterone and cortisol levels in the 6 subjects who aborted (left panel) and those who failed to abort (right panel). Symbols are the same as in Figure 1.

All ten subjects complained of asthenia and weakness. Nausea and vomiting were evident in four subjects, vertigo in two, and in five subjects there was mild pyrexia. No changes in blood pressure were evident.

In the six subjects in whom a successful outcome occurred, nothing was evident on the second ultrasonographic examination. In three of the four subjects who had an unsuccessful outcome, ultrasonography showed no further increase in conceptus size as compared to the initial examination.

$\beta$ -HCG values are shown in Figure 1. Basal values were not different in the responder and non-responder groups. In the subjects with successful outcomes,  $\beta$ -hCG decreased, but in one subject values were still detectable by 28 days. In all subjects who aborted, values were below 2000 mIU/ml 14 days after commencement of bleeding. In three of the four non-responders, the progressive anticipated increase in  $\beta$ -hCG was not evident over the time-period of the study.

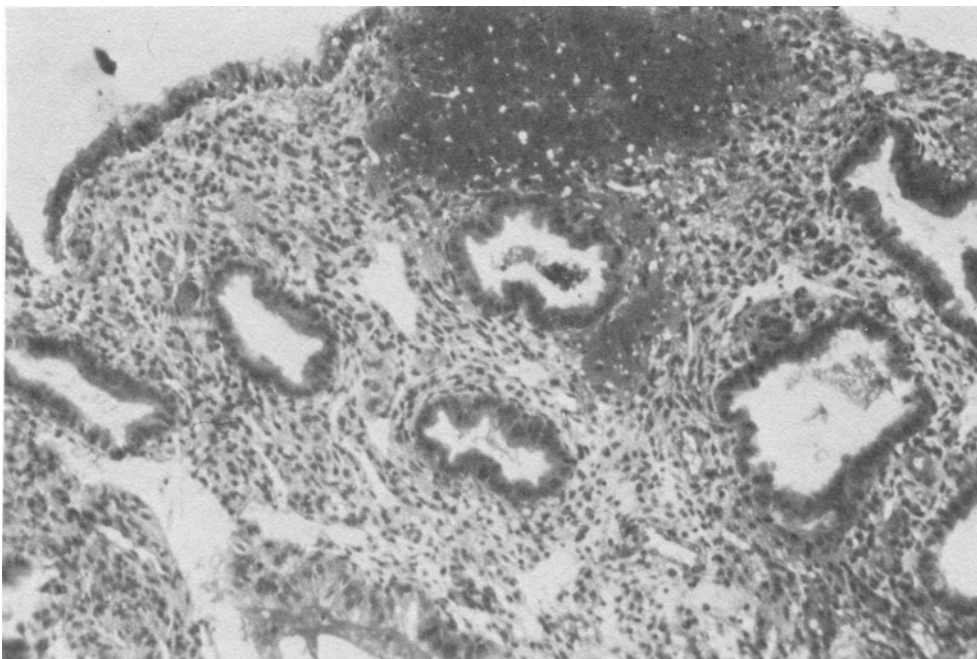


Fig. 3. Histological appearance of the decidua in a patient who failed to abort following treatment with RU 486. The sample was obtained by vacuum aspiration on the 8th week of amenorrhea. The decidua compacta is infiltrated with hemorrhagic foci. The decidua spongiosa remained intact with normal tortuous glands filled with secretion. There were neither hemorrhagic areas nor necrosis in this layer, and the architecture was normal.

Estradiol, progesterone, and cortisol values of these subjects are shown in Figure 2. Estradiol levels ranged from 250-1620 pg/ml and progesterone from 10-65 ng/ml. Basal levels of these two steroids were not different in the responder or non-responder groups. Whereas progressive decreases in estradiol and progesterone were evident in all six responders, transient decreases were also noted in 3 of the 4 non-responders.

In all ten subjects serum cortisol levels increased by the fourth day of drug administration, and values ranged from 25-50 ug/100 ml. This is above the normal range, which is 10 to 20 ug/100 ml. By day seven, cortisol levels had decreased to normal.

Histological studies of the products of conception were examined in three of the non-responders after vacuum aspiration. In none was there necrosis of the decidua spongiosa. The decidua compacta showed necrosis and hemorrhage in some areas; however, in other areas entirely normal architecture was evident (Fig. 3).

#### SUMMARY AND CONCLUSIONS

Using a sliding scale schedule, only six of the ten subjects (60%) aborted. Even in the remaining four subjects it is possible that the pregnancy was arrested, because in three ultrasonography showed no further increase in the size of the conceptus, and no rise in  $\beta$ -hCG was noted.

Neither  $\beta$ -hCG nor estradiol and progesterone were useful in predicting the outcome, and transient decreases were evident even in those subjects who failed to abort. In contrast to the findings observed in normal spontaneous abortion, only partial necrosis of the decidua compacta was evident.

It is not known if the asthenia and weakness noted in all subjects represent subjective evidence of glucocorticoid deficiency or if it is simply a consequence of the pregnancy and abortive process. RU 486 does act as a glucocorticoid antagonist (Philibert et al., 1981); Proulx-Ferland et al., 1982; Gaillard et al., 1984), and a transient rise in cortisol was evident in all cases. Studies with dogs have shown that these animals display increased cortisol levels but do not develop objective signs of glucocorticoid deficiency, as evidenced by their ability to excrete a water load after ten days of RU 486 administration (Spitz et al., 1984).

The incidence of complete abortion achieved in this study was 60%. Factors such as dose and duration of treatment, as well as case selection (including length of gestation), may well account for differences in incidence of abortion in the various studies. In this series relatively high doses of RU 486 were given. In another trial (see Birgersson et al., this volume), using the same patient selection criteria, a success rate of 71% occurred. This study used a dose schedule of 100 mg for seven days, an amount lower than that used for the presently described subjects. Recent studies by Gravanis et al. (1985) have shown that RU 486 may also display progesterone agonistic properties. It is possible that this phenomenon may explain why 40% of our subjects failed to abort. Further studies are currently in progress to evaluate the effect of lower doses of RU 486 as an abortifacient.

#### ACKNOWLEDGMENT

This work was supported by grants from the Ford Foundation and the Mellon Foundation. The drug was supplied by Roussel Uclaf, Paris, France.

#### REFERENCES

- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., 1984, RU38486: A Steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day, Proc. Natl. Acad. Sci. USA, 81:3879-3882.
- Gravanis, A., Schaison, G., George M., deBrux, J., Satyaswaroop, P. G., Baulieu, E. E., and Robel, P., 1985, Endometrial and pituitary responses to the steroidal antiprogesterin RU 486 in postmenopausal women, J. Clin. Endocrin. Metab., 60:156-163.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effect d'un-steroïde anti-progestérone chez la femme: interruption du cycle menstruelle de la grossesse au debut, C. R. Acad. Sc., 194:933-938.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU38486 - An antiprogesterational compound, Contraception, 29:399-410.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU38486, a potential antiglucocorticoid in vivo, 8th International Congress of Pharmacology, Abstract 1463.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, RU38486 - A new lead for steroidal anti-hormones, Endocrine Society, 64th Annual Meeting, Abstract 668

- Philibert, D., Deraedt, R., Tournemine, C., Mary I., and Teutsch, G., 1982, RU38486 - A potent antiprogesterone, J. Ster. Biochem., Sixth International Congress on Hormonal Steroids, Abstract 20417.
- Proulx-Ferland, L., Cote, J., Philibert, D., and Deraedt, R., 1982, Potent anti-glucocorticoid activity of RU38486 on ACTH secretion in vitro and in vivo in the rat, J. Steroids, Abstract 80.
- Spitz, I. M., Wade, C. E., Krieger, D. T., Lahteenmaki, P., and Bardin, C. W., 1984, The effect of RU 486 in the dog, this volume.

## PHARMACOKINETIC AND CLINICAL STUDIES OF RU 486 FOR FERTILITY REGULATION

M. L. Swahn,<sup>1</sup> S. Cekan,<sup>2</sup> G. Wang,<sup>2</sup> V. Lundstrom<sup>1</sup> and  
M. Bygdeman<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology and  
<sup>2</sup>Reproductive Endocrinology Unit  
Karolinska Hospital S-104 01  
Stockholm, Sweden

### ABSTRACT

Previous clinical results indicate that oral administration of RU 486 can be highly effective in terminating very early pregnancy, but that the frequency of incomplete abortion is too high to be acceptable. The outcome of the therapy is unrelated to the dose amount, at least within a dose range of 50-200 mg RU 486 daily for four days.

The aim of the present investigation was to evaluate the possibilities of improving treatment efficacy by studying: a) the pharmacokinetics of the compound during early pregnancy; b) the effect of the drug on uterine contractility and sensitivity and c) the effect of supplementing RU 486 treatment with prostaglandin when used for termination of early pregnancy.

Radioimmunoassay of the drug plasma concentration following oral administration of 50 mg to two patients indicated that the peak plasma level is reached within one to two hours and that the half-life of the compound is approximately 24 hours. The resulting plasma levels were also dose dependent. Treatment with 25 mg twice daily for four days in eleven patients resulted in a mean plasma level of 3.5 umol/l. If the dose was increased to 25 mg four times daily, the corresponding mean level was 5 umol/l. The increase in plasma level in the 12 hour period between the second and the third 25 mg dose was between 2.5 and 3.5 umol/l. The spontaneous uterine activity and the sensitivity of the myometrium to prostaglandin (one i.m. injection of 0.25 mg 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide) was recorded in three early pregnancy patients on the fourth day of treatment with RU 486 (25 mg twice daily) and compared with the same parameters in three nontreated early pregnancy patients. The local blockage of the progesterone receptor in the uterus resulted in a change from practically no activity to regular coordinated contractions and an increased sensitivity to prostaglandin.

The clinical study included 33 healthy women in early pregnancy. The duration of amenorrhea was 49 days or less. Each patient received either 25 mg RU 486 twice daily for four or six days, or 25 mg RU 486 four times daily for four days. In 16 of the 33 patients, one intramuscular injection of 0.25 mg of the PGE analog was given on the morning of the fourth day of RU 486 treatment. In the 17 women who received RU 486 alone, frequency of complete abortion, incomplete abortion and uninterrupted pregnancy was 76.5%, 5.9% and 17.6%, respectively. Neither the duration of treatment,

four or six days, the number of administrations per day, 2 or 4, or the daily dose, 50 or 100 mg, seemed to influence the outcome of the therapy. If the RU 486 treatment was supplemented with a low dose of the PGE analogue, all 16 patients had complete abortions.

The results of the present study indicate: a) that the observed lack of dose effect (complete abortion) is not due to insufficient absorption of the drug; b) that withdrawal of the intrinsic uterine suppressor progesterone will induce regular, coordinated uterine contractions and an increased sensitivity to prostaglandin; and c) that sequential therapy of RU 486 and a low dose of prostaglandin has more potential as a non-surgical method to terminate very early pregnancy than does either compound used alone.

## INTRODUCTION

It was recently reported that oral administration of RU 486, a new synthetic steroid with the properties of a receptor level progesterone and glucocorticoid antagonist, resulted in abortions when administered to 11 women in the sixth to eighth week of pregnancy (Herrmann et al., 1982). That the steroid is able to terminate early pregnancy was confirmed in a joint study in Szeged and Stockholm (Kovacs et al., 1984). The latter study included 37 pregnant women with amenorrhea of 42 days or less. The patients received either 25 mg, 50 mg or 100 mg RU 486 twice daily for four days. The results of the study suggested: a) that the outcome of the therapy was not dose dependent (in the range of doses tested); b) that although the treatment was highly effective in terminating early pregnancy (the pregnancy remained intact in only three patients, 7.6%), the frequency of complete abortion (31.4%) was too high to be clinically acceptable; and c) that while the risk of heavy bleeding was not negligible, other side effects generally were of a mild nature.

The high frequency of incomplete abortion and the occurrence of heavy uterine bleeding in association with the therapy indicated that, although the compound effectively interfered with the viability of the pregnancy, the direct or indirect effect of RU 486 alone on uterine contractility was insufficient to result in expulsion of the conceptus.

In order to develop a non-surgical outpatient procedure based on treatment with RU 486 to compete with the surgical alternative (vacuum aspiration), we need to obtain pharmacokinetic data on the compound in humans as a base for the development of more effective treatment schedules and to evaluate the advantages and disadvantages of complementing the RU 486 therapy with a uterotonic compound. Such studies are ongoing in our department, and some preliminary results will be presented.

## PATIENTS AND METHODS

### Pharmacokinetic Studies

Three pharmacokinetic studies were performed using volunteers in early pregnancy: a) Two women in the sixth week of pregnancy received one single oral dose of 50 mg RU 486. Repeated blood samples were taken for 120 hours following treatment. b) From eleven patients receiving 25 mg twice daily, and from seven patients receiving 25 mg four times daily for four days, blood samples were obtained before and on each treatment day prior to intake of the first tablet of that day. c) Repeated blood samples were also taken from two patients treated with 25 mg twice daily for four days during the 12 hour interval between the second and the third 25 mg dose.

The levels of RU 486 in peripheral blood plasma were measured by means of a radioimmunoassay involving chromatography of plasma ether extracts on Sephadex LH 20. A parallelism test and a coincidence of chromatographic peaks of  $^3\text{H}$ -labelled and non-radioactive RU 486 indicated a high degree of purity of RU 486 assayed. Details of the methodology will be published elsewhere. The reagents (antibody,  $^3\text{H}$ -labelled and non-radioactive RU 486) were donated by Roussel-Uclaf Co., Romainville, France.

### Uterine Contractility and Sensitivity

Three patients in early pregnancy who were admitted to the hospital for termination of pregnancy by vacuum aspiration and three patients treated with RU 486 (25 mg twice daily) volunteered to record uterine contractility prior to vacuum aspiration and on the fourth day of RU treatment, respectively. In the three patients treated with RU 486, slight uterine bleeding had started, but in none was abortion imminent. A pressure transducer connected to a Grass polygraph was introduced through the cervical canal into the extra-amniotic space and up in the fundal part of the uterus. After an observation period of at least 30 minutes, 0.25 mg 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide (Schering AG, Berlin) was administered intramuscularly.

### Clinical Studies

The study included 33 healthy women with apparently normal pregnancies as judged by plasma  $\beta$ -hCG and gynecological examination. Their amenorrhea was 49 days or less. The compound, RU 486, was administered orally in different dose levels: 1) 25 mg twice daily for four or six days; 2) 25 mg four times daily for four days. In 16 patients one intramuscular injection of 0.25 mg 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide was given on the morning of the last day of RU 486 treatment. The subjects fasted at least one hour after taking the drug. All patients attended three follow-up visits one, two and six-eight weeks after the start of treatment.

On each treatment day, before the administration of the compound, and again at the same time at each follow-up visit, between 8:00 and 10:00 a.m., blood samples were taken for analysis of  $\beta$ -hCG, progesterone and cortisol. The data from the first treatment day thus represented the plasma concentration immediately prior to therapy.

At the second follow-up visit, the outcome of therapy was preliminarily assessed as complete abortion, incomplete abortion, or uninterrupted pregnancy. The assessment was based on the duration and amount of bleeding, plasma  $\beta$ -hCG concentration, gynecological examination and, if it was not obvious that the patients had aborted completely, ultrasound examination. If curettage was found to be necessary, the outcome of therapy was based on the histopathological examination. Side effects or complications were carefully noted on each treatment day and at the follow-up visits.

## RESULTS

### Pharmacokinetic Studies

These preliminary studies indicated that following oral administration of a single dose of 50 mg RU 486, the peak plasma level was reached within one to two hours. Forty eight hours after therapy the concentration of RU 486 in plasma was no longer detectable. The half life was about 24 hours.

Treatment with 25 mg twice daily resulted in steady plasma levels of 3.5  $\mu\text{mol/l}$ . Four daily doses of 25 mg resulted in plasma levels of 5  $\mu\text{mol/l}$ . No signs of accumulation were found (Figs. 1 and 2).

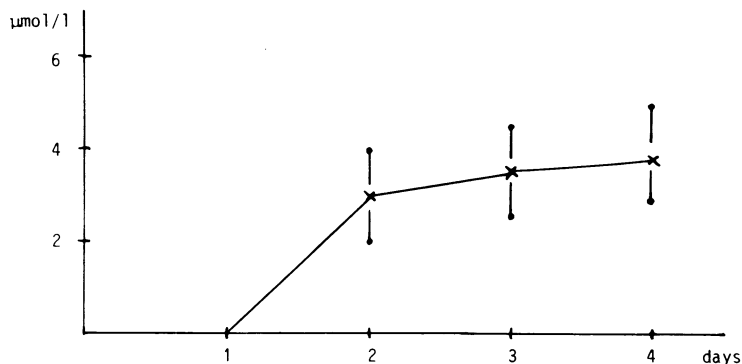


Fig. 1. Plasma levels of RU 486 before and during treatment with 25 mg twice daily in 11 early pregnant patients. The blood samples were taken in the morning prior to the first tablet of the day. (Geometric means and 95% confidence limits).

In the 12 hour interval between the second and the third 25 mg dose in patients treated with 25 mg twice daily for four days, the maximal plasma level was reached after two hours. The increase was between 2.5 and 3.5  $\mu\text{mol/l}$  (Fig. 3).

#### Uterine Contractility and Sensitivity

Figure 4 shows representative recordings from two of the six patients. The first patient belonged to the control group and had received no

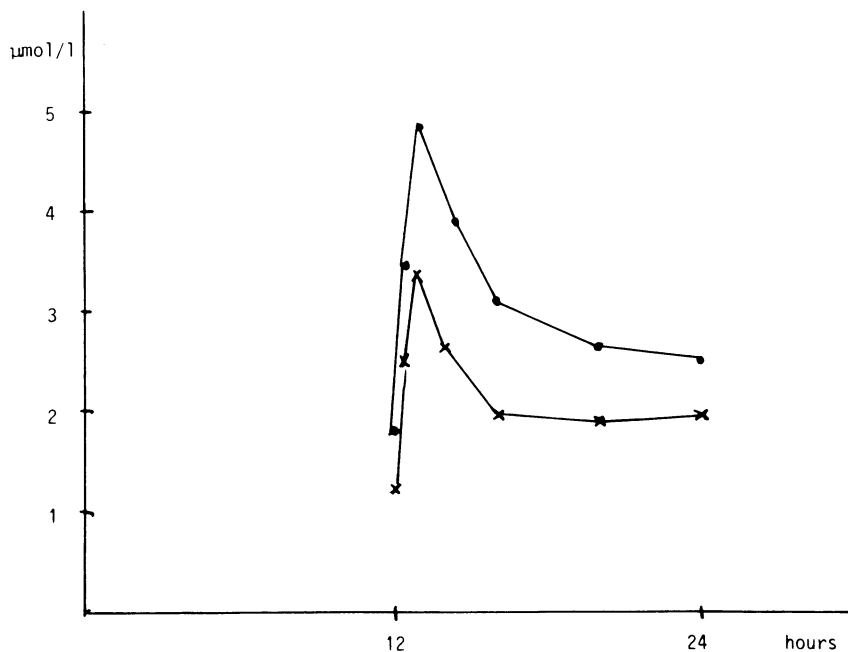


Fig. 2. Plasma levels of RU 486 before and during treatment with 25 mg four times daily in seven early pregnant patients. The blood samples were taken in the morning prior to the first tablet of the day. (Geometric means and 95% confidence limits).

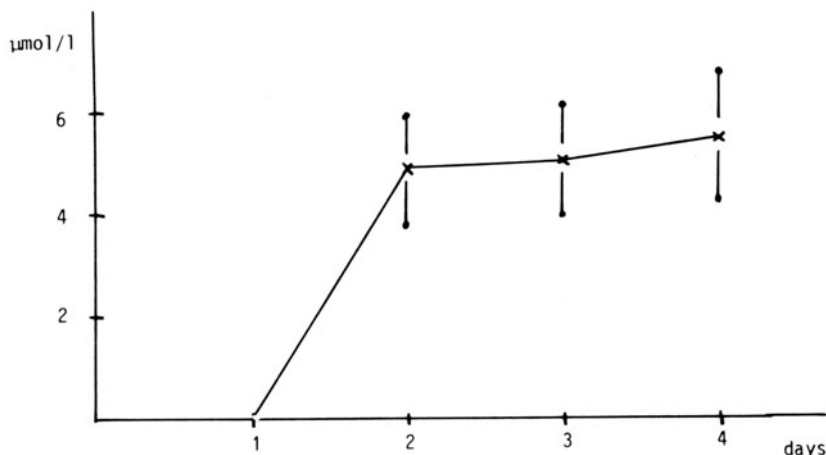


Fig. 3. The figure illustrates the plasma levels of RU 486 in the 12 hour interval between the second and third 25 mg dose in two early pregnancy patients.

pretreatment, while in the second patient the uterine recording was performed on the morning of the fourth day of treatment with RU 486 (25 mg twice daily). In the control patient there was practically no spontaneous uterine contractility. One intramuscular injection of 0.25 mg of the PGE analogue resulted in a slow increase in uterine tone, followed by the development of irregular uterine contractions of low amplitude. In the patient pretreated with RU 486, the contractility pattern was quite different. Prior to prostaglandin treatment, regular uterine contractions with an amplitude of approximately 25 to 40 mm Hg could be observed. Following intramuscular administration of the same amount of the PGE analogue, there was a rapid increase in uterine tone and an obvious stimulation of both the frequency and amplitude of the contractions. The stimulation of uterine contractility was associated with slight or moderate pain.

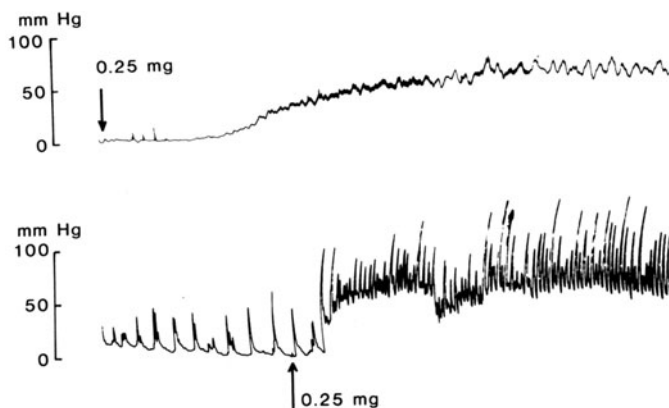


Fig. 4. The figure shows the spontaneous uterine contractility and the effect of an intramuscular injection of 0.25 mg 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide in two early pregnant women. The first patient received no pretreatment (upper curve), while in the second patient, the recording was performed on the morning of day 4 of treatment with 25 mg RU 486 twice daily (lower curve).

Table I. Termination of Early Pregnancy  
by RU 486 Alone (group A) or in Combination  
with One Intramuscular Injection of 0.25 mg  
16-Phenoxy-Tetranon PGE<sub>2</sub> Methyl Sulfonylamide  
on the Last Day of Treatment (group B)

Patient group	Dose (mg)	No. of patients	Complete abortion	Incomplete abortion	Pregnancy continued
A	25 x 2	11	8	1	2*
	25 x 4	6	5	0	1
	Total	17	13 (76.5%)	1 (5.9%)	3 (17.6%)
B	25 x 2	9	9	0	0
	25 x 4	7	7	0	0
	Total	16	16 (100%)		

\* Includes one extrauterine pregnancy

#### Clinical Study

As illustrated in Table I, 13 out of the 17 patients treated with RU 486 alone (group A), i.e. 76.5%, were regarded to have had a complete abortion. The data did not indicate any relation between the frequency of complete abortion and the dose given. One patient had an incomplete abortion (5.9%), and in three patients (17.6%) the pregnancy remained intact. In 16 patients the therapy was supplemented with one intramuscular injection of 0.25 mg 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide on the morning of the last day of treatment with RU 486. All patients were observed to have had a complete abortion. In one patient the decrease of plasma  $\beta$ -hCG was slow, indicating placental residue, but since ultrasound examination was negative,

Table II. Plasma Concentrations of  $\beta$ -HCG and Progesterone  
Before Day 1 and on Day 4 of Treatment with RU 486

Group of patients	Dose (mg)	No. of patients	hCG (U/l)		Progesterone (nmol/l)	
			Day 1	Day 4	Day 1	Day 4
A	25 x 2	8	18493	26314	76.6	64.1
B	25 x 2	9	14451	20545	71.5	38.5
A	25 x 4	5	25780	8560	69.6	34.8
B	25 x 4	7	29048	32846	85.0	71.9

Patients in group A received no additional therapy; in group B the patients were given one intramuscular injection of 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide on the last day of treatment. Only patients in whom the treatment resulted in a complete abortion are included.

Table III. Side Effects and Complications Associated with Termination of Early Pregnancy by RU 486

Patient group	Dose (mg)	No. of patients	Vomiting & Diarrhea	Pain Analgesic injection	Excessive bleeding
A	25 x 2	17	0	1 (5.9%)	1 (5.9%)
	25 x 4				
B	25 x 2	16	0	1 (6.3%)	0
	25 x 4				

Group A was treated with RU 486 alone. Group B was treated with RU 486 plus one intramuscular injection of 0.25 mg 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide.

curettage was not performed. Since the further clinical course was uneventful, this case was also considered a complete abortion. A second patient experienced a normal follow-up period and return of first menstruation, but thereafter inter-menstrual spotting appeared. Curettage was performed three months after treatment. The microscopic examination revealed minor residues of decidua but no chorionic villi. The patients in groups A and B were similar with regard to age, parity and duration of pregnancy. As illustrated in Table II, there were no differences in plasma  $\beta$ -hCG or progesterone before start of treatment or on day four of treatment with RU 486, indicating that the patients in group B would not have been more likely to abort than the patients in group A if the treatment with PGE analogue had been excluded.

All patients who had complete abortions started vaginal bleeding within five days after the start of treatment. In the majority of the successfully treated patients, the bleeding had a duration of between one and two weeks and a daily amount corresponding to or slightly more than their normal menstrual period. One patient in group A experienced heavy bleeding resulting in hospital admission, blood transfusion and removal of the conceptus by forceps six days after the start of treatment.

Other side effects were generally of a mild nature. Almost all patients reported slight low abdominal pain but only two patients, one in each treatment group, experienced strong uterine pain. In the patient in group A, the conceptus was trapped in the cervical canal. After one analgesic injection and removal of the conceptus by forceps, the pain rapidly waned. In the patient belonging to group B, the pain reaction followed the intramuscular injection of the PGE analogue. One intramuscular injection of meperidine chloride was sufficient to alleviate the pain (Table III).

The plasma levels of cortisol increased slightly during treatment with RU 486 but had returned to pretreatment level at the first follow-up visit. There was no apparent difference between the patients who received the intramuscular injection of 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide and those who did not.

## DISCUSSION

A non-surgical procedure suitable for self-administration is an attractive alternative to vacuum aspiration for termination of very early

pregnancy. So far, prostaglandin analogues are the only compounds with this potential that have been investigated clinically on a large scale (WHO Prostaglandin Task Force, 1982; Bygdeman et al., 1983). The prostaglandins have the unique ability to stimulate uterine contractility, resulting in abortion during very early pregnancy. Several studies have shown that administration of certain E analogues can be as effective as a surgical procedure (Rosen et al., 1984). Although the E analogues are less likely to cause gastrointestinal side effects than the naturally occurring prostaglandins, the treatment is nonetheless associated with an incidence of vomiting and diarrhea, as well as uterine pain, thereby limiting its clinical usefulness. Another possibility for non-surgical abortion is to interfere with progesterone activity because of progesterone's indispensable role during the implantation phase and during early pregnancy in animals and women.

RU 486 is a synthetic steroid acting as a progesterone antagonist at the receptor level; it is the first compound of this kind to reach clinical testing. Both the initial clinical study by Herrmann et al. (1982) and that of Kovacs et al. (1984) have demonstrated that the compound acts as an abortifacient during early pregnancy. However, the clinical efficacy in terms of frequency of complete abortion in these studies was insufficient to compete with vacuum aspiration. To increase the "success rate" by changing the treatment schedule so far has not been successful. Kovacs et al., (1984) used doses of 25 mg, 50 mg and 100 mg twice daily for four days and found no difference in frequency of complete abortion. Plasma progesterone and  $\beta$ -hCG concentration declined, but only slowly, during RU therapy (Kovacs et al., 1984). It was therefore possible that extension of therapy from four to six days may improve efficacy. Although the number of patients in the present study is limited, this does not seem to be the case.

The peak plasma levels also seemed unrelated to clinical efficacy. In the present study, the frequency of administration was increased from two to four per day without improvements in success rate. To further extend either the duration of treatment or the number of administrations per day is impractical from a clinical point of view.

The apparent lack of dose-effect relationship does not seem to be due to insufficient absorption of the drug. The results of the pharmacokinetic studies showed a dose related increase in plasma levels. Although the half life time of the compound is long (24 hours), no accumulation was observed, at least not if the daily dose was limited to 100 mg.

Other possible explanations for the limited success rate are: 1) there is insufficient binding to the progesterone receptor in the uterus or 2) an anti-progestational action at the receptor level is not sufficient to induce a complete abortion in all cases. Other studies indicate that the first alternative is unlikely (Healy et al., 1983; Philibert et al., 1982).

The uterus in early pregnancy is inactive, uterine contractility is restricted to small oscillations in intrauterine pressure as illustrated in Fig. 4 (upper curve). Csapo has suggested that the pregnant human uterus is an active but endogenously suppressed organ and that the uterine activity is regulated by the balance between the intrinsic uterine suppressor, progesterone, and the stimulant, PGF<sub>2</sub> (Csapo, 1973). After treatment with RU 486 the spontaneous uterine activity changed into a pattern of coordinated contractions (Fig. 4, lower curve). A likely reason is the mere withdrawal of progesterone suppression. The high frequency of incomplete abortion found following RU treatment can then be explained by either a lack of or too low an increase in the endogenous production of the stimulant PGF<sub>2</sub> resulting in insufficient uterine contractility. If this concept is correct sequential treatment with RU 486 and prostaglandin would better

Table IV. Comparison of RU 486 alone, RU 486 in Combination with Prostaglandin, and Prostaglandin Alone for Termination of Early Pregnancy

Type of treatment	Percent of patients			
	Complete abortion	Vomiting & Diarrhea	Pain (analgesic inj)	Excessive bleeding
RU alone <sup>1</sup>	76.5	0	5.9	5.9
RU + Pg <sup>1</sup>	100	0	6.3	0
Pg alone <sup>2</sup> (vaginal administration)	92-97	40-50	4-34 <sup>3</sup>	0

<sup>1</sup>This study. <sup>2</sup>Bygdeman et al., 1983, 1984

<sup>3</sup>The lower figure is for home-treated and the higher for hospital-treated patients. The same criteria were used in all studies for the evaluation of success, side effects and complications.

mimic the natural events and improve the efficacy of RU 486 as a non-surgical abortion method. Pretreatment with RU 486 should increase the sensitivity of the myometrium to prostaglandin so that the amount of prostaglandin needed to induce an effective uterine contractility can be kept below the level causing side effects. An increased sensitivity to the prostaglandin analogue 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonamide could also be demonstrated following pretreatment with RU 486. The effect was also quite different. In the control patient, an increase in uterine tone was found, while after treatment with RU 486, the increase in tone was accompanied by coordinated contractions with increasing amplitude and frequency.

The effectiveness of sequential therapy using RU 486 and prostaglandin was evaluated in the clinical study. The prostaglandin was administered on the last day of RU treatment. It seemed natural in this pilot study to use one of the new E analogues that are less likely to cause gastrointestinal side effects than the natural prostaglandins, and among those 16-phenoxy-tetranor-PGE<sub>2</sub> methyl ester which is most suitable for intramuscular administration. Other E analogues, i.e. 9-methylene-PGE<sub>2</sub> mainly have been administered vaginally, a route of administration regarded less suitable for the present study because the majority of patients had started to bleed at the time of treatment.

The dose of the analogue used, 0.25 mg, is the highest dose that normally does not cause vomiting and diarrhea but is still below the one needed to induce an abortion during early pregnancy (0.5 mg twice or three times)(Bygdeman et al., 1983).

Additional treatment with a low dose of the PGE analogue increased the frequency of complete abortion from 76.5% to 100% and also seemed to reduce the risk of excessive bleeding, while the frequency of gastrointestinal side effects remained low. The patients in the two treatment groups were similar with regard to age, parity, duration of pregnancy, and hormonal status at the time of prostaglandin treatment, indicating that the improved efficacy was not due to uneven patient selection.

The result of this and other studies indicates that to lessen frequency and intensity of uterine pain and gastrointestinal side effects, treatment with RU 486 alone is superior to treatment with prostaglandin analogues for termination of early pregnancy. However, prostaglandin therapy is more effective. Excessive blood loss is also a very rare phenomenon with prostaglandin induced early abortion (Table IV).

The present preliminary study indicates that sequential therapy with these two drugs may be developed into a highly effective non-surgical method to terminate early pregnancy, without the drawbacks each compound possesses if used alone.

#### ACKNOWLEDGMENTS

RU 486 was supplied by the Roussel-Uclaf Co., Paris, France, and 16-phenoxy-tetranor-PGE<sub>2</sub> methyl sulfonylamide by Schering AG, Berlin, FRG. This study was undertaken with the partial financial support of the WHO Special Programme of Research, Development and Research Training in Human Reproduction, Geneva, Switzerland. We would also like to acknowledge the cooperation of Dr. A. R. Aedo in the development of the radioimmunoassay of RU 486, the nurses of our research unit for supervising the patients, and A. Hagblad for typing the original manuscript.

#### REFERENCES

- Bygdeman, M., Christensen, N., Green, K., Zheng, S., and Lundstrom, V., 1983, Termination of early pregnancy - future development, Acta Obstet. Gynecol. Scand., (Suppl.), 113:125-129.
- Bygdeman, M., Christensen, N. J., Green, K., and Vesterquist, O., 1984, Self administration at home of prostaglandins for termination of early pregnancy, in: "Prostaglandins and Fertility Regulation," E. S. E. Hafez, M. Bygdeman, and M. Topozada, eds., MTP Press, Lancaster.
- Csapo, A. I., 1973, The prospects of PGs in postconceptional therapy, Prostaglandins, 3:245-289.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships, and hormonal effects, Fertil. Steril., 40:253-257.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, The effects of an antiprogesterone steroid in women: Interruption of the menstrual cycle and of early pregnancy, Comptes Rendus, 294:933-940.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - an antiprogestational compound, Contraception, 29:399-410.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, RU 486: a new lead for steroidal anti-hormones, Presented at the Sixty-Fourth Annual Meeting of the United States Endocrine Society, San Francisco, Abstract 668.
- Rosen, A. S., von Knorring, K., Bygdeman, M., and Christensen, N. J., 1984, Randomized comparison of prostaglandin treatment in hospital or at home with vacuum aspiration for termination of early pregnancy, Contraception.
- WHO Prostaglandin Task Force, 1982, Termination of early pregnancy by vaginal administration of 16, 16-dimethyl-transdelta<sup>2</sup>-PGE<sub>1</sub>methyl ester, Asia-Oceania J. Obstet. Gynecol., 8:263-268.

RU 486 STIMULATION OF PGF<sub>2</sub>-ALPHA PRODUCTION IN ISOLATED  
ENDOMETRIAL CELLS IN SHORT TERM CULTURE

R. W. Kelly,<sup>1</sup> D. L. Healy,<sup>2</sup> M. J. Cameron,<sup>1</sup>  
I. T. Cameron,<sup>2</sup> and D. T. Baird<sup>2</sup>

M. R. C. Reproductive Biology Unit<sup>1</sup> and  
Department of Obstetrics and Gynaecology<sup>2</sup>  
University of Edinburgh Centre for Reproductive Biology  
Edinburgh, Scotland

ABSTRACT

RU 486 stimulates PGF<sub>2</sub>α production in isolated human endometrial stromal cells incubated for 25 hours. The increase is dose-dependent over the 10-1000 nmol/l concentration range of RU 486 and is competitively inhibited by progesterone. This effect of RU 486 upon PGF<sub>2</sub>α release is not seen when isolated endometrial glands are cultured. The data suggest that RU 486 induces menstruation by acting on the endometrial stroma to generate prostaglandin production.

INTRODUCTION

There is extensive evidence that prostaglandin (PG) production in the human uterus is under the control of ovarian steroids and that progesterone (P) exerts an inhibitory action on PG production (Abel & Baird, 1980). Induction of menstruation in primates by an antiprogesterin such as RU 486 (Healy et al., 1983) might therefore proceed via a stimulatory action on PG production. Accordingly, we have examined the action of RU 486 on the production of prostaglandin F<sub>2</sub>α (PGF<sub>2</sub>α) by human endometrial cells dispersed by collagenase and cultured for 24 hours in a defined medium.

MATERIALS AND METHODS

Endometrium was obtained at curettage from seven women at the time of tubal sterilization. The women were of reproductive age, were not taking the oral contraceptive pill and were between days six and 25 of the menstrual cycle. Four of the women were in the secretory phase of the cycle (days 18-25), as assessed by gynecological history and as judged from subsequent histological study using the criteria of Noyes, Hertig and Rock (1950).

Endometrium was collected on ice in Hank's balanced salt solution (Flow Labs.), weighed and chopped finely with a scalpel blade. The tissue was then shaken for 20-30 minutes at 37° in collagenase (2.5 mg/ml) and DNAase (0.2 mg/ml) in Hank's balanced salt solution. The tissue was next filtered through glass wool, and the eluate was filtered through a 75 uM nylon mesh

to give the preparation of endometrial cells. The glass fiber filter was back-flushed with medium (Hepes buffered M199 with 50 units/ml penicillin, 50 ug/ml streptomycin (Gibco), 5 ug/ml fungizone (Flow Labs.), 3% ultrosor (L.K.B.) and 2 ug/ml arachidonic acid). The obtained endometrial glands were sedimented. The glands were then washed once with medium and dispensed with a broad-ended pipette. The filtered endometrial cell preparation was also washed with fresh medium, and the preparation was counted in a hemocytometer. Steroids were dissolved at 200x final concentration in ethanol, and 25 ul of this stock solution was added to 1.25 mls medium. Fifty microliters of this medium was dispensed into 0.35 ml microtiter wells, and 150 ul of cell suspension in the above supplemented M199 medium was added to give a final concentration of  $1.0 \times 10^6$  cells/ml. Aliquots of the gland preparation were washed and treated with fresh collagenase in Hank's balanced salt solution, and cell numbers were measured from the digested material. All incubations in this study contained  $17\beta$ -estradiol ( $E_2$ ) in a concentration of 100 nM/l.

All cells were incubated under a pressure of 2 psi of  $O_2/CO_2$  (95:5 v/v) for 24 hours. After the incubation period, cell viability was assessed by counting cells excluding trypan blue dye. At this time, an aliquot of medium (150 ul) was removed from each microtiter well, and the  $PGF_{2\alpha}$  content of the medium was assessed by radioimmunoassay using  $^3H$  labelled  $F_{2\alpha}$  (Amersham Ltd.) and a specific antisera raised in rabbits. The B/Bo of 50% binding was 22.9 pg and B/Bo 85% binding was 5.0 pg. Cross reactivities were as follows:  $PGF_1$ , 7.2%;  $PGF_3$ , 2.9%;  $PGF_{2\beta}$ , 3.5%;  $E_2$ , 1.1%; 6-OXO- $F_1$ , 1.05%. All other PGs tested showed less than 0.1% cross-reactivity.

## RESULTS

Cell viability as assessed by vital dye exclusion ranged from 72% to 94% after 24 hours of culture.

$PGF_{2\alpha}$  production in control wells ranged from 98 to 2,520 pg per well, with a coefficient of variation within one batch of cells of 7% to 32% ( $n=5$  in all cases). Of the seven cell preparations, six showed a decline in  $PGF_{2\alpha}$  production in the presence of P (1.0 nM to 1 uM).  $PGF_{2\alpha}$  production was significantly lower at 1 uM and 100 nM than when isolated endometrial cells were cultured without P ( $p < 0.05$ ;  $n=7$ ; Wilcoxon's Signed Rank test; Fig. 1).

All cell preparations incubated with RU 486 gave significantly increased  $PGF_{2\alpha}$  production, as shown in Figure 1 ( $p < 0.02$ ). In the absence of added P, RU 486 stimulated  $PGF_{2\alpha}$  production at all doses (10 nM to 1 uM) in six out of the seven endometria; in the seventh, the production was within 17% of control values at all doses.

Endometrial glands were obtained from four women in the secretory phase of the menstrual cycle. By contrast to results from isolated endometrial cells, no significant rise in  $PGF_{2\alpha}$  production was seen in these glands as a response to RU 486.

## DISCUSSION

The main effect of progesterone on prostaglandin production by human endometrium is inhibitory (Cane & Vilee, 1975; Abel & Baird, 1980), although the rise in PG levels found in secretory phase endometrium (Downie Poyser and Wunderlich, 1974; Maathuis & Kelly, 1978) suggest that progesterone has a priming effect on PG production. This effect has been

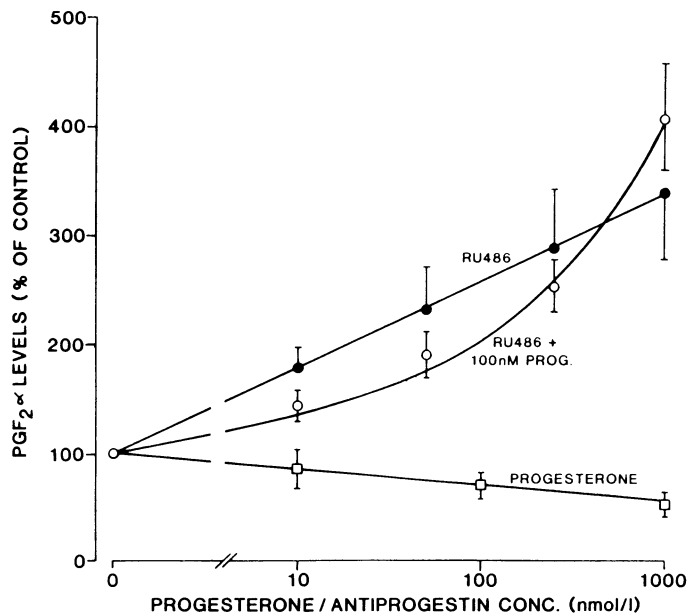


Fig. 1.  $\text{PGF}_2\alpha$  concentrations in media after 24 hours. incubation of endometrial cells after separation of the endometrial glands. Each datum point is the mean of pentuplicate cultures from each of the seven patients. Results are expressed as a % of control incubations without added P or RU 486. Data is represented as mean  $\pm$  SE.

found in extensive work using guinea pigs (Blatchley & Poyser, 1974) and sheep (Caldwell et al., 1972; Scaramuzzi et al., 1977).

The action of an antiprogestin upon endometrial PG production is of interest, both for its potential as a tool for elucidating the mechanism by which P alters PG production and for the vital role this action may play in the induction of menstruation by RU 486 (Healy et al., 1983).

The rise seen in  $\text{PGF}_2\alpha$  production by isolated endometrial cells in response to RU 486 was not matched by an increase in production of PG by the endometrial glands from the same patients. This result was seen despite the fact that the glands produced more  $\text{PGF}_2\alpha$  than the filtered endometrial cells. Thus, any action of an antiprogestin on PG production in uterine tissue is likely to be on the endometrial cells other than those in the glands. One possible endometrial stromal target cells is the decidual cell, which is known to secrete prolactin in a P-dependent fashion (Healy, 1984).

In this study P, in the presence of  $\text{E}_2$ , showed the expected inhibition of PG production. RU 486 stimulated  $\text{PGF}_2\alpha$  production both in the presence and absence of exogenous progesterone. Moreover, the production of  $\text{PGF}_2\alpha$  in the isolated endometrial cells treated with RU 486 (10 to 1000 nM) was elevated above control levels, showing that the antiprogestin apparently did more than merely counteract the inhibitory effect of progesterone (Fig. 1).

These observations suggest that either P receptors are occupied prior to incubation and RU 486 displaces bound progesterone, or that the action of the antiprogestin is via a non-genomic mechanism. The former possibility is unlikely, since the effect is seen in proliferative endometrium (days 6-11) as much as in secretory endometrium (days 18-25). These periods have

different P receptor levels and markedly different previous exposure to progesterone. The latter possibility cannot be discounted at this stage; there is good evidence that progesterone acts at the cell membrane of Xenopus laevis oocytes to modulate calcium flux (Baulieu et al., 1978), but this has not been demonstrated in mammals.

#### REFERENCES

- Abel, M. H., and Baird, D. T., 1980, The effect of 17 $\beta$ -estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture, Endocrinology, 106:1599.
- Baulieu, E. E., Godeau, F., Schordevet, M., and Slatkine, S. S., 1978, Steroid induced meiotic division in Xenopus laevis oocytes: surface and calcium, Nature, 275:593.
- Blatchley, F. R., and Poyser, N. L., 1974, The effect of oestrogen and progesterone on the release of prostaglandins from the uterus of the ovariectomized guinea pig, J. Reprod. Fertil., 40:105.
- Cane, E. M., and Villee, C. A., 1975, the synthesis of prostaglandin F by human endometrium in organ culture, Prostaglandins, 9:281.
- Downie, J., Poyser, N. L., and Wunderlich, M., 1974, Levels of prostaglandins in human endometrium during the normal menstrual cycle, J. Physiol., 236:465.
- Healy, D. L., Baulieu, E. E., and Hodgen, G., 1983, Induction of menstruation by an antiprogestosterone steroid (RU 486) in primates: Site of action, dose-response relationships and hormonal effects, Fertil. Steril., 40:253.
- Healy, D. L., 1984, The clinical significance of endometrial prolactin, Aust. N. Z. J. Ob. Gyn., 24:117.
- Maathuis, J. B., and Kelly, R. W., 1978, Concentrations of prostaglandins F<sub>2</sub> $\alpha$  and E<sub>2</sub> in endometrium throughout the menstrual cycle, after the administration of clomiphene or an oestrogen, progestagen pill and in early pregnancy, J. Endocrinol., 77:361.
- Noyes, R. W., Hertig, A. T., and Rock, J., 1950, Dating the endometrial biopsy, Fertil. Steril., 1:3.
- Scaramuzzi, R. J., Baird, D. T., Boyle, H. P., Land, R. B., and Wheeler, A. G., 1977. The secretion of prostaglandin F from the transplanted uterus of the ewe, J. Reprod. Fertil., 49:157.

THE DEMONSTRATION OF THE ANTIPROGESTIN EFFECTS OF RU 486 WHEN  
ADMINISTERED TO THE HUMAN DURING HCG-INDUCED PSEUDOPREGNANCY

Horacio B. Croxatto, Irving M. Spitz,  
Ana Maria Salvatierra and C. Wayne Bardin

Consultorio de Planifacion Familiar, Santiago, Chile and Center for  
Biomedical Research, The Population Council, New York, U.S.A.

INTRODUCTION

In the absence of fertilization, there is a progressive decrease in circulating progesterone levels towards the end of the luteal phase which leads to endometrial bleeding. If implantation occurs, however, human chorionic gonadotropin (hCG) from the developing trophoblast stimulates progesterone secretion from the corpus luteum. This prevents shedding of the endometrium during early pregnancy. The administration of exogenous hCG to normal women during the luteal phase simulates early pregnancy. This model has been used to study the action of drugs that interfere with progesterone synthesis, secretion or peripheral action.

RU 486, a synthetic 19-norsteroid derivative, has been observed to show antiprogestational and antiglucocorticoid actions in both man and experimental animals (Philibert, et al., 1981; Herrmann et al., 1982; Philibert et al., 1982a; Philibert et al., 1982b; Proulx-Ferland et al., 1982; Gaillard et al., 1984). We reasoned that women with hCG-induced prolongation of the luteal phase would be ideal for studying the *in vivo* action of RU 486 and other antiprogestins. Utilizing this model, we have administered hCG in combination with RU 486 in an attempt to demonstrate the antiprogestational activity of the latter. The results in this report show that during exogenous hCG treatment, RU 486 induces menstrual bleeding despite high circulating levels of progesterone. Since RU 486 is also a glucocorticoid antagonist (Philibert et al., 1981; Proulx-Ferland et al., 1982; Gaillard et al., 1984), we also measured serum cortisol. Although levels of this steroid increased, they remained within the normal range.

The Study of Antiprogestins in Women

This study was performed on ten regularly cycling women aged 32 to 40 years of age who had previously undergone surgical sterilization. The nature and aims of the study were explained to all the subjects who then gave their consent. Four subjects received hCG alone, and one subject received hCG combined with RU 486. The other five subjects had two treatment cycles, each separated by two untreated menstrual cycles. In two of these subjects the hCG study was performed first and in three the hCG-RU 486 combination was given initially. In all treatment cycles, the precise day of the LH surge was determined by measuring LH daily in early morning

urine samples, using Higonavis supplied by Mochida Pharmaceutical Co., Tokyo, Japan. This day was designated as day 0. On day 9 following the LH surge, daily hCG administration was commenced. Progressively increasing doses (500, 1000, 1500, 3000, 6000, 10,000 and 15,000 IU) were administered each morning from days 9 to 15.

In cycles where hCG was combined with RU 486, the latter compound was administered on days 12 to 15 inclusive, in a dose of 100 mg/day. Fifty mg were given at the time of the hCG injection and again in the evening. In all treatment cycles, blood samples were taken each morning, before drug administration, from day 9 to day 16 and on alternate days from day 16 to 22 for measuring LH, FSH, estradiol, progesterone, and cortisol.

These hormones were measured in plasma by utilizing the reagents and procedures supplied by the World Health Organization Programme for the Provision of Matched Assay Reagents for the RIA of Hormones in Reproductive Physiology (Hall, 1978). HCG levels were determined using a cross reacting LH RIA.

#### The Effect of RU 486 on Gonadotropin and Sex Steroid Levels In HCG-Treated Women

During treatment there was a progressive increase in immunoreactive hCG levels (as measured in the LH assay) in all cases; the peak was evident on the day following cessation of hCG administration. Thereafter, hCG levels decreased progressively with a half disappearance time of approximately 48 hours. By day 23 following the LH surge, mean values were below 25 mIU/ml, which is within the range of basal LH levels seen in the normal luteal phase (Figs. 1 & 2). FSH showed a slight but significant increase during treatment ( $p < 0.001$  with the hCG alone and  $p < 0.002$  with the hCG-RU 486 combination). There were no differences in FSH response in the 2 tests (Figs. 1 & 2).

On day nine, following the LH surge, the mean ( $\pm$  SD) progesterone levels were  $12.3 \pm 3.7$  and  $15.8 \pm 6.2$  ng/ml in the hCG-RU 486 cycles, respectively. A progressive increase in progesterone levels occurred during hCG administration in both treatment cycles. Peak levels of  $34.6 \pm 9.0$  ng/ml occurred on the day following cessation of hCG administration when the later was given alone and on the fifth day of hCG when it was administered in combination with RU 486. In the latter instance the peak was  $28.5 \pm 4.6$  ng/ml. There were no differences in progesterone levels in hCG treated or hCG - RU 486 treated cycles. In both instances progesterone levels gradually decreased to mean levels below 2.5 ng/ml by day 23 following the LH surge (Figs. 1 and 2).

Mean ( $\pm$  SD) estradiol levels were  $141 \pm 104$  pg/ml on the first day and  $198 \pm 155$  pg/ml on the last day of RU 486 treatment. These values were not different from one another nor from the estradiol levels on the equivalent days of the hCG treatment cycles, where levels were  $112 \pm 35$  pg/ml and  $108 \pm 40$  pg/ml, respectively.

#### The Effect of RU 486 On Menstrual Bleeding

When hCG was given alone, bleeding started on days 21 to 24, and the mean duration ( $\pm$  SD) was  $5.3 \pm 1.8$  days (Fig. 1). With the hCG-RU 486 combination, bleeding began in all instances on the fourth day of RU 486 administration and lasted for  $2.5 \pm 1.4$  days. Further bleeding occurred on days 23-28, after the LH surge, and the duration was  $2.2 \pm 0.8$  days (Fig. 2).

#### The Effect of RU 486 On Serum Cortisol

Mean ( $\pm$  SD) cortisol levels were  $13.4 \pm 4.6$  and  $12.6 \pm 3.8$  ug/100 ml during days nine to twelve of the two tests. These values were not

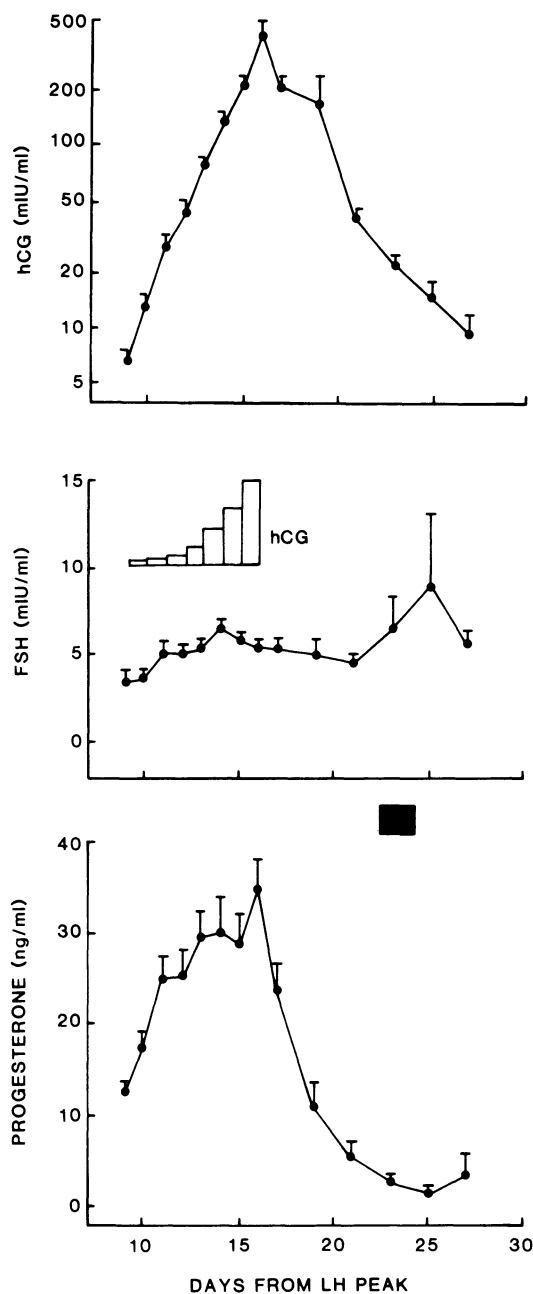


Fig. 1. The effects of hCG on the mean ( $\pm$  SEM) LH, FSH and progesterone levels in women. Increasing doses of hCG were given from days 9 to 15 following the LH surge as indicated in the text. The hCG values are shown on a logarithmic scale. The time and duration of the menstrual bleeding are indicated by the black rectangle.

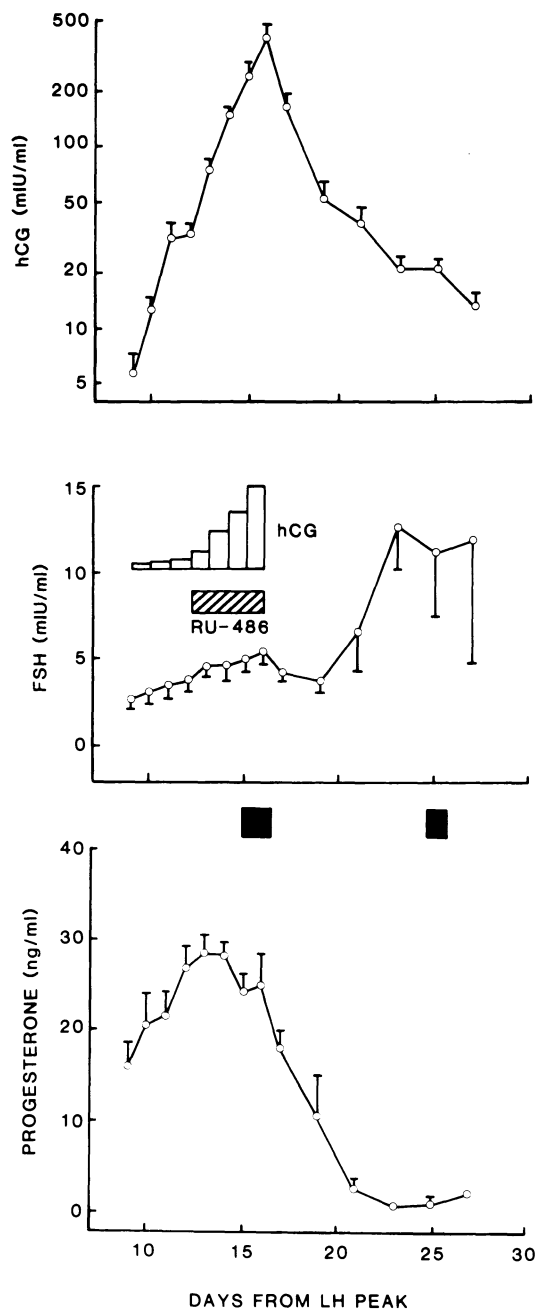


Fig. 2. The effects of hCG and RU 486 on hCG, FSH and progesterone levels in women. HCG was given as indicated in Fig. 1 and RU 486 in a dose of 100 mg per day from days 12 to 15 following the LH surge. The two episodes of menstrual bleeding are indicated by the black rectangles.

different from each other. There were no changes in cortisol during treatment with hCG alone. By contrast, during the hCG-RU 486 combination, there was a significant rise in cortisol levels ( $p < 0.02$ ). The peak was  $18.2 \pm 3.3$  ug/100 ml and this occurred on the day after completion of RU 486 administration (Fig. 3). This value however was still well within the normal range. Cortisol returned to base line after two days.

#### SUMMARY AND CONCLUSIONS

These results confirm that increasing doses of hCG administered during the normal luteal phase induce a state of pseudopregnancy prolonging the functional lifespan of the corpus luteum (Kaiser and Geiger, 1971). This is associated with a progressive rise in progesterone, to levels higher than those seen in the normal luteal phase and are compatible with those observed during early pregnancy (Mishell et al., 1974). Following cessation of hCG treatment, progesterone levels decreased, and bleeding occurred 21 to 24 days after the midcycle LH surge, when progesterone values had decreased substantially. This indicates that the luteal phase had been prolonged by eight or more days. When RU 486 was administered with hCG, menstrual bleeding was evident on the 4th day of RU 486 administration. Thus, at the time of menstrual bleeding, mean progesterone levels were at their peak and comparable to those of early pregnancy. Estradiol levels at the time of bleeding were similar to those observed in the early follicular phase.

The results of the present study show that circulating progesterone levels were not altered by RU 486 treatment. This suggests that the latter has no detectable effect on the secretion or metabolism of progesterone and implies that the action of RU 486 was directly on the endometrium. The further bleeding on days 23-28 (when progesterone levels had decreased) suggests that the endometrial shedding induced by RU 486 was incomplete.

Several investigators have shown that RU 486 also has a high affinity for the uterine progesterin receptor and that it antagonizes the effect of progesterone on the endometrium (Philibert et al., 1982 a & b; Herrmann et al., 1982). Moreover, its ability to inhibit progesterone-induced decidual formation has been observed in the ovariectomized, adrenalectomized pseudopregnant rat, which is further evidence that it acts at the level of the uterus (Chang, et al., 1984). Other studies have shown that RU 486 acts directly on the endometrium to inhibit expression of the uteroglobin gene and uteroglobin secretion induced by progesterone (Chen et al., 1984). These studies in animals support the concept that RU 486 induced endometrial bleeding in the present study by inhibiting the action of progesterone on the endometrium.

An FSH rise was seen during hCG administration in both cycles. A possible explanation is that this represents cross-reaction of exogenous hCG with the FSH antibody used in the radioimmunoassay. The fact that hCG administration often leads to FSH suppression in men argues against the possibility of a stimulatory effect of hCG on FSH secretion during the luteal phase (Reiter et al., 1972). Alternatively, the rise in FSH may be unrelated to the hCG and could represent the rise in FSH seen normally at the end of a normal luteal phase, stimulating follicular recruitment and maturation for the subsequent cycle. However, in the normal cycle the rise in FSH occurs at the same time as a decline in progesterone levels (Ross et al., 1981). In the present study, progesterone levels were elevated when FSH levels rose.

There was also a slight but significant rise of plasma cortisol levels during RU 486 administration. This finding has been observed previously in man as well as in experimental animals and is in keeping with the

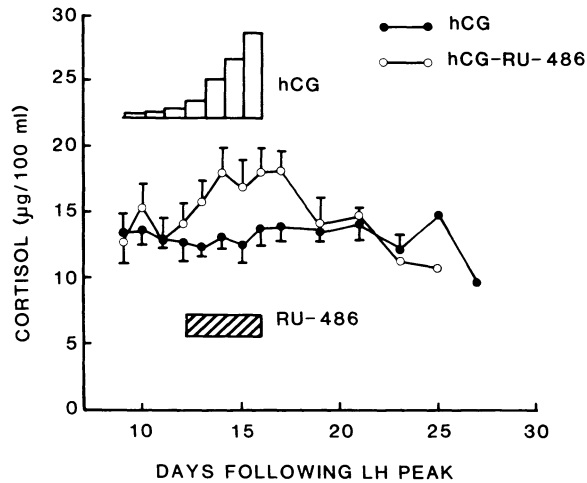


Fig. 3. The serum cortisol response to hCG alone and the hCG-RU486 combination. The duration of treatment was the same as in Figs. 1 and 2.

antiglucocorticoid properties of the drug (Gaillard, et al., 1984; Bertagna, et al., 1984; Healy et al., 1984). Despite the rise in cortisol, levels were still within the normal range encountered in women during the morning hours. This is a good indication that adrenal function has not been compromised. Moreover, studies in dogs have shown that the ability to excrete a water load remains normal, despite increases in basal cortisol level with doses of RU 486 as high as 50 mg/kg for ten days (Spitz et al., 1984).

In conclusion, the results of this study indicate that RU 486 can induce endometrial bleeding in women with hCG-induced prolongation of the luteal phase. Bleeding occurred in these subjects when progesterone was elevated.

#### ACKNOWLEDGMENT

This work was supported by grants from the Ford Foundation and the Mellon Foundation. The drug was supplied by Roussel Uclaf, Paris, France.

#### REFERENCES

- Bertagna, X., Bertagna, C., Luton, J. P., Husson, J. M., and Girard, F., 1984, The new steroid analog RU 486 inhibits glucocorticoid action in man, Clin. Endocrinol. Met., 59:25-28.
- Chang, C. C., Segal, S. J., and Bardin, C. W., 1984, Efficacy of an antiprogesterone steroid (RU 38486) by different routes of administration in the rat, 7th International Congress of Endocrinology, Abstract 355.
- Chen, C., Chang, C. C., Bardin, C. W., and Janne, O. A., 1984, Inhibition of uteroglobin gene expression by an anti-progestin, RU 38486, 7th International Congress of Endocrinology, Abstract 519.
- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., 1984, RU 486: A steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day, Proc. Natl. Acad. Sci. USA, 81:3879-3882.

- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effect d'un steroide anti-progestérone chez la femme: interruption du cycle menstruelle de la grossesse au debut., C. R. Acad. Sc., 294:933-938.
- Kaiser, R. and Geiger, W., 1971, Die ostrogen-und pregnandiolausscheidung bei hCG-pseudograviditäten und nachfolgenden normalen fruhgraviditäten., Acta Endocrinol., 67:331-336.
- Mishell, D. R., Thornycroft, I. H., Nagata, Y., Murata, T., and Nakamura, R. M., 1974, Hormone patterns in early human gestation, in: "Physiology and Genetics of Reproduction," E. Coutinho and F. Fuchs, eds., Plenum Press, New York.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 38486: a potent antiglucocorticoid in vivo, 8th International Congress of Pharmacology, Tokyo, abstract 463.
- Philibert, D., Deraedt, R., and Teutsch, G., Tournemine, C., and Sakiz, E., 1982a, RU 38486 - a new lead for steroidal anti-hormones, Endocrine Society, 64th Annual Meeting, San Francisco, Abstract 668.
- Philibert, D., Deraedt, R., Tournemine, C., Mary, I., and Teutsch, G., 1982b, Ru 38486 - a potent antiprogestosterone, J. Ster. Biochem., Sixth International Congress on Hormonal Steroids, Jerusalem, Abstract 204:17.
- Proulx-Ferland, L., Cote, J., Philibert, D., and Deraedt, R., 1982, Potent anti-glucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat, J. Steroids, Abstract 204:17.
- Reiter, E. O., Kulin, H. E., and Loriaux, L. D., 1972, FSH suppression during short term hCG administration: A gonadally mediated process, J. Clin. End. Metab., 34:1080-1084.
- Ross, G. T., Vande Wiele, R. L., and Frantz, A. G., 1981, the Ovaries and the Breasts, in: "Textbook of Endocrinology," R. H. Williams, ed., W. B. Saunders Col., Philadelphia, London, Toronto.
- Spitz, I. M., Wade, C. E., Krieger, D. T., Lahteenmaki, P., and Bardin, C. W., 1984, this volume.

## RU 486 IN WOMEN WITH NORMAL OR ANOVULATORY CYCLES

Gilbert Schaison, Martine George, Nelly Lestrat and  
Etienne-Emile Baulieu

Department of Endocrinology and Reproductive Medicine  
Inserm U 33, Hopital de Bicetre  
94270 Bicetre, France

### ABSTRACT

The antiprogesterone steroid RU 486 was given orally to 32 normally cycling women for four days, starting on the fourth day after the basal body temperature shift. Uterine bleeding occurred on the third day of RU 486 administration in all 14 women treated with 100 mg per day, in seven out of the eight women treated with 50 mg, and in eight out of ten women receiving 25 mg per day. Luteal regression was observed in eight women treated with 100 mg per day, in three treated with 50 mg, and in two receiving 25 mg per day. Plasma LH was measured every 15 minutes from 0800 h to 1200 h for five days in 17 patients. Mean levels decreased, and its computerized pulsatile release disappeared in seven of the eight subjects treated with 100 mg, in two out of four receiving 50 mg, and in one out of five treated with 25 mg. RU 486 had no effect when given to five patients with anovulatory cycles for four days, starting on day 18 of their cycle.

In conclusion: 1) RU 486, given to normally cycling women at mid-luteal phase, induces uterine bleeding. 2) This effect occurs whether or not luteal regression is induced by the compound, indicating that RU 486 acts directly upon the endometrial tissue, very likely at the progesterone receptor level. 3) The drug may impair both luteal function and gonadotropin secretion in a dose-dependant manner. 4) At the dosage of 100 mg per day, the lack of antigluccorticosteroid activity suggests that RU 486 may be promising as a new agent for fertility control.

### INTRODUCTION

In this study, we administered RU 486 at several dosages (100, 50 and 25 mg per day for four days) to 32 normally cycling women during their mid-luteal phase. Our aim was to correlate the occurrence of endometrial bleeding with the circulating levels of ovarian steroids and therefore with the status of the corpus luteum. We have also assessed the effects of the drug on gonadotropin secretion and the pituitary-adrenal functions. In addition, we have evaluated its actions in five patients with anovulatory cycles.

### SUBJECTS AND METHODS

Thirty-two normal women, aged 21-37 years, with regular menstrual cycles (28-32 days), volunteered for the study. Oral informed consent was obtained

Table I. Effects of RU 486 in 32 Normally Cycling Women

Subject n°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Dose per day	100 mg														50 mg							25 mg										
Uterine bleeding	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-
Luteal regression	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Gonadotropin inhibition	-					+	+			+	+	+	+	+	-	+	+	-					+						-	-	-	-

Uterine bleeding occurred in 29 cases. Plasma progesterone and estradiol levels decreased (luteal regression) in 13 cases. Gonadotropin inhibition was observed in 10 out of 17 cases studied.

from all subjects before the investigation. None of the subjects used any contraceptive method during the study. All women had previous ovulatory cycles, as indicated by basal body temperature (BBT) shift and plasma progesterone levels above 7 ng/ml measured on days 20, 22 and 24 of the cycle. The administration of RU 486 was started on the fourth day after the BBT shift. In 10 women (nos. 23-32) a dose of 25 mg was given once a day at 0800 h for four days (Table I).

Five patients aged 24-30 years with anovulatory cycles also participated in this study. They had plasma estradiol levels between 40 and 70 pg/ml and displayed menstruation after a progestin-induced withdrawal bleeding test. RU 486 was administered at a dose of 50 mg at 0800 h and 2000 h for four days, starting on the 18th day of the anovulatory cycle.

In all cases, blood samples were obtained over a six day period the day before RU 486 administration (day 0), during the treatment (days 1, 2, 3 and 4) and the following day (day 5). Samples collected at 0800 h, 1000 h and 1200 h were pooled to determine estradiol and progesterone plasma levels. In seventeen subjects (nos 1, 5, 6, 9-13, 15-18, 24, and 29-32), LH and FSH plasma levels were measured on days 0-4 at 15 minutes intervals from 0800 h to 1200 h. Cortisol and ACTH plasma levels, aldosterone and plasma renin activity after overnight recumbancy were determined at 0800 h, on days 0-5 in seven subjects receiving a constant sodium daily diet and 100 mg of RU 486 per day.

Results were expressed as mean  $\pm$  SE. Statistical analysis was performed using the Wilcoxon non parametric test for paired observations to compare estradiol, progesterone, cortisol, ACTH, aldosterone and plasma renin activity levels before and after RU 486 administration. Since LH and FSH plasma levels exhibited pulsatile oscillations, the cosinor method was used both to validate and to quantify the rhythms (Halbert et al., 1972). Computerized cosinor analyses were made for each of the subjects, as well as for the group on days 1, 2, 3, and 4. To find the best fitting sine function, all data were approximated for a given experimental situation with a period of 4, 2, 1.33 and 1 hours. The least squares method was used. A rhythm was detected for a given period when the rhythm amplitude differed from zero (test of a non nil amplitude with  $p < 0.05$ ). The amplitude was defined as half of the total variability, i.e. the difference between peaks and troughs. In addition, the cosinor method provided the rhythm adjusted mean  $\pm$  SE. The fitness of the pulsatile rhythm approximation can be visualized and complemented with the calculation of residuals.

Table II. Estradiol (pg/ml) and Progesterone (ng/ml) Plasma Levels Before (day 0), During (days 1-4) and after (day 5) RU 486 Administration (100 mg per day)

Subject n°	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5	
	E	P	E	P	E	P	E	P	E	P	E	P
1	83	8.4	140	11.3	107	10.9	50	4.9	40	0.8	20	0.3
2	105	10.4	122	12	96	4.9	47	1.3	32	0.6	27	0.4
3	111	7.9	118	5.9	84	3.7	64	1.6	48	0.5	37	0.3
4	73	8.6	80	6.8	53	3.9	29	0.5	25	0.4	21	0.2
5	165	15.3	160	12.4	139	7.2	103	4.1	36	0.6	22	0.3
6	120	9.4	88	7.5	102	5.7	68	2.7	47	2.4	39	0.6
7	95	19.0	149	15.6	135	23.0	173	16.1	127	7.4	39	1.3
8	100	9.2	110	9.5	107	9.8	96	7.2	49	5.2	51	3.9
9	150	12.1	150	13.9	140	12.8	160	11.0	115	7.5	100	4.8
10	107	7.3	110	9.3	140	8.3	136	9.4	153	8.0	140	6.4
11	70	7.5	70	9.1	70	8.0	75	11.4	78	11.0	65	6.9
12	150	7.1	115	6.2	160	9.6	170	13.5	117	10.3	125	11.0
13	90	7.9	95	7.5	109	12.5	116	14.3	108	13.7	104	11.0
14	198	11.1	119	11.4	106	7.1	141	12.5	94	9.1	101	8.2

## RESULTS

### Uterine Bleeding Induced by RU 486

In all 14 normal cycling women (nos. 1-14) treated with 100 mg per day, uterine bleeding occurred on day three of treatment (Table I). In eight of these subjects (nos 1-8), bleeding persisted for at least 72 hours and was similar in abundance and length to the patient's normal menses. In 6 women (nos 9-14), the bleeding on day three was lighter and stopped after 36 hours. Normal menstruation occurred between days eight and eleven after the first intake of RU 486. Thus, in these women, the overall length of the cycle was not shortened.

In eight normally cycling women receiving 50 mg per day (nos. 15-22), uterine bleeding occurred on day three in seven cases (Table I), followed by a second bleeding seven to ten days later in four of them. In ten women treated with 25 mg per day (nos 23-32), normal uterine bleeding or spotting for two days occurred on day 3 or 4 in eight cases. Six women menstruated again at the expected date of the cycle.

Thus 29 of the 32 normally cycling women who received RU 486 had uterine bleeding within four days after initiation of the drug. However, among them, 16 had a second bleeding at the time of expected menses.

### Corpus Luteum Regression Induced by RU 486

The effects of RU 486 on the luteal function were dose-dependent. However for the same dose, two different patterns of response were observed. In the 14 women treated with 100 mg per day (Table II), RU 486 induced either a decrease of plasma estradiol and progesterone levels between days 3 and 5 (nos. 1-8) or no luteal regression (nos. 9-14). Concurrently, in women 1-8, BBT decreased within the three days following the onset of the bleeding. The next ovulation occurred after a 15-18 day span with reference to the bleeding. In spite of the bleeding on day three the BBT of subjects 9-14 did not decrease before the second bleeding at the expected date of the menses. The following ovulation occurred 14 to 17 days later.

Table III. Estradiol (pg/ml) and Progesterone (ng/ml) Plasma Levels Before, During (days 1-4) and After RU 486 Administration (50 mg per day)

Subject n°	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5	
	E	P	E	P	E	P	E	P	E	P	E	P
15	180	8.2	109	5.7	62	2.9	34	0.3	40	0.3	32	0.2
16	142	7.3	150	5.8	94	2.5	42	0.6	20	0.3	18	0.2
17	126	11.8	159	13.1	154	11.4	90	4.2	62	2.6	33	0.7
18	90	9.2	65	11.7	68	10.8	63	11.4	61	8.3	62	5.3
19	108	7.1	102	7.4	152	12.2	184	14.7	170	15.9	164	13.8
20	225	19.1	217	21.7	225	17.8	146	14.8	128	18.1	134	16.3
21	118	12.6	146	13.2	208	18.3	173	19.8	191	29.3	180	26.1
22	136	10.1	160	13.4	125	13.8	141	11.7	176	21.0	186	14.0

In the eight women receiving 50 mg per day, RU 486 decreased plasma estradiol and progesterone levels in only three cases (nos. 15-17; Table III). In these patients, the decrease of the BBT occurred on day 3 or 4. In cases 18-21, the luteolysis was delayed, and the BBT decreased several days later at the onset of the normal menses. In the eight women mentioned above the next ovulation occurred in agreement with the decrease of the BBT and luteal regression.

In the ten women treated with 25 mg per day (Table IV), the compound induced a luteal regression and a decrease of the BBT in only two cases (nos. 23, 24). In cases 25-30, decrease of the BBT occurred at the expected time of the cycle.

Thus, in eight women receiving 100 mg per day (nos. 1-8), three receiving 50 mg per day (nos. 15-17), and two, receiving 25 mg per day (nos. 23-24), RU 486 induced luteal regression. However, in 16 women (nos. 9-14, 18-21 and 25-30), the drug produced uterine bleeding without luteal regression.

#### Effects of RU 486 on Gonadotropin Secretion

The effects of RU 486 on gonadotropin secretion were studied in 17 women (Table I). Of the eight subjects treated with 100 mg per day, RU 486 impaired gonadotropin secretion in seven cases (nos. 5, 6, 9-13). In these women, the mean concentration of LH decreased from  $3.2 \pm 0.6$  mIU/ml on day zero to  $1.1 \pm 0.2$  mIU/ml ( $p < 0.05$ ). The decreases in rhythm adjusted means

Table IV. Estradiol (pg/ml) and Progesterone (ng/ml) Plasma Levels Before, During (day 1-4) and After RU 486 Administration (25 mg/day)

Subject n°	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5	
	E	P	E	P	E	P	E	P	E	P	E	P
23	136	14.5	102	12.6	80	6.3	24	0.9	23	0.6	39	0.4
24	320	20.6	258	13.1	199	9.5	77	3	36	0.6	26	0.4
25	90	7.5	156	10.9	127	7.8	130	4.7	84	5.6	64	5.8
26	240	8.6	395	7.2	276	9.1	152	8.7	161	6.4	148	5.6
27	80	18.5	167	18.5	196	26.3	182	29.3	148	12.7	152	11.6
28	92	7.6	105	6.1	135	8.4	127	7.8	151	9.3	191	16.0
29	110	11.7	141	17.0	105	7.9	281	16.9	204	21.4	156	18.9
30	144	8.6	80	12.3	75	17.2	106	19.7	99	14.5	88	12.7
31	106	19.0	131	26.1	127	25.0	129	26.2	108	28.1	96	18.8
32	88	10.1	107	11.4	114	10.9	100	15.2	138	17.2	139	19.4

Table V. Estradiol (pg/ml) and Progesterone (ng/ml) Plasma Levels Before, During (days 1-4) and After RU 486 Administration (100 mg/day) in Five Subjects with Anovulatory Cycles

Subject n°	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5	
	E	P	E	P	E	P	E	P	E	P	E	P
33	59	0.29	54	0.24	35	0.26	38	0.21	40	0.22	36	0.28
34	66	0.16	69	0.16	41	0.20	78	0.19	75	0.18	49	0.20
35	45	0.13	42	0.13	22	0.15	26	0.12	31	0.15	28	0.16
36	52	0.18	42	0.28	38	0.20	39	0.17	44	0.19	50	0.22
37	46	0.26	71	0.30	71	0.25	42	0.22	37	0.28	30	0.22
Mean	53	0.2	55	0.2	41	0.2	44	0.2	45	0.2	38	0.2
±SE	±4	±0.1	±6	±0.1	±8	±0.1	±8	±0.1	±7	±0.1	±5	±0.1

were associated with changes of the estimated rhythm period provided by the cosinor method. On days 0 and 1, the prominent period was usually equal to four hours. On days 2, 3 and 4, the period shortened to 2, 1.33 or 1 hour, and thereafter the ultrarhythm was obliterated (no rhythm detection in subject no. 9).

It should be noted that, in spite of gonadotropin inhibition, no luteal regression was observed in five women (nos. 9-13). In addition, in subject no. 1, a decrease of plasma estradiol and progesterone levels occurred without any apparent antigonadotropic activity of the drug (Table I). In the four women receiving 50 mg per day, the compound impaired gonadotropin secretion in only two cases (nos. 16, 17). In subject no. 15, no suppression of gonadotropin secretion was demonstrated, but luteolysis was obvious on day three. In the five women treated with 25 mg per day, the antigonadotropic activity of the drug was evident in only one case (no. 24).

Thus, RU 486 induced gonadotropin inhibition in ten women, five of which showed no luteal regression. In two cases (nos. 1, 15), the corpus luteum regression was not related to a significant change of gonadotropin secretion.

#### Effects of RU 486 in Patients with Anovulatory Cycles

In the five subjects with anovulatory cycles, RU 486 at a dose of 100 mg per day induced no bleeding and no BBT change. Mean plasma estradiol levels before and after treatment were  $53 \pm 4$  and  $38 \pm 5$  pg/ml respectively. Plasma progesterone levels remained below 1 ng/ml (Table V).

#### Effects of RU 486 on Pituitary-Adrenal Functions

The effects of RU 486 on pituitary-adrenal functions were investigated in seven women receiving 100 mg per day (nos. 5, 6, 9-13). The compound did not increase circulating levels of either cortisol or ACTH. On days zero and four of RU 486 administration, the mean plasma cortisol and ACTH levels were  $14.2 \pm 1.3$  and  $16.8 \pm 2.8$  ug/dl,  $43 \pm 12$  and  $48 \pm 16$  pg/ml respectively. Likewise, the differences observed for plasma aldosterone and plasma renin activity before and after treatment were not statistically significant. On days zero and four, after overnight recumbancy, the mean plasma aldosterone and plasma renin activity were  $12 \pm 4$  and  $11 \pm 2$  ng/dl,  $28 \pm 7$  and  $27 \pm 8$  ng/l/min respectively.

#### DISCUSSION

The present study clearly demonstrates that, in 14 women with normal ovulatory cycles, 100 mg per day of RU 486 administered for four days,

starting on the fourth day of the luteal phase, induced uterine bleeding with no exceptions. In addition using this protocol for eight women treated with 50 mg per day and 10 treated with 25 mg per day, bleeding occurred in seven and eight subjects respectively.

As noted in earlier reports, RU 486 has been used to induce menstruation. In three normal women, this compound at a dose of 50 mg per day administered on the 22nd day of the cycle, promptly induced menstruation (Herrmann et al., 1982). Our results confirm that RU 486 behaves as a progesterone antagonist at the receptor level, since in six subjects treated with 100 mg per day, four treated with 50 mg, and six treated with 25 mg, uterine bleeding occurred in spite of persistent high plasma progesterone and estradiol levels. Thus, RU 486 may act directly upon endometrial tissue regardless of any effect upon plasma ovarian steroid levels. In the five women with anovulatory cycles, RU 486 had no effect. These results are expected of a compound having the properties of a progesterone antagonist.

There is no general agreement concerning the antigonadotropic activity of the drug. In castrated adult female monkeys receiving replacement exogenous estradiol and progesterone treatment, the daily mean serum gonadotropin profile was not modified after four days of RU 486 administration (Healy et al., 1983a). Likewise, no acute suppression of gonadotropin secretion occurred within eight hours after RU 486 injection (Healy et al., 1983b). In contrast, in the three previously studied normal women having received RU 486 on day 22 and following, plasma LH and FSH had decreased after antiprogesterone steroid administration. The discrepancies between these results may be related to different experimental conditions. In the present study, RU 486 decreased the mean levels of FSH and LH in ten women (seven out of eight treated with 100 mg, two out of four treated with 50 mg and one out of five treated with 25 mg). The compound appears to be an inhibitor of gonadotropin secretion, and its effects are dose-dependent. Furthermore, RU 486 induced changes of plasma ultradian rhythm parameters and shortened the LH period from four hours to two, 1.33 or 1 hour. Recent studies have demonstrated that progesterone slows LH pulse frequency and reduces mean plasma LH levels (Soules et al., 1984; Filicori et al., 1984). In normal women, the LH interpulse interval increased from the early luteal phase to the late luteal phase. These modifications in LH pulse frequency correlated with the duration of exposure to progesterone. Thus, the compound increasing the frequency of pulsatile release of LH behaves as a progesterone antagonist. However, RU 486 also inhibits gonadotropin secretion. In the mid-luteal phase, progesterone progressively reduces the mean amplitude of LH and, in this study, the drug has the same effect. In postmenopausal women, Gravanis et al., (1984) have recently shown that RU 486 (100 mg twice a day) given during the last six days of a 15-day estradiol benzoate treatment had some progesterone like activity. In addition, in these estrogen primed postmenopausal women, the compound further decreased plasma gonadotropins to premenopausal levels (Schaison et al., 1984). Thus, at present, the presumed mechanism of action of RU 486 upon LH secretion is not well understood. Taken together our data are compatible with progesterone partial agonist-antagonist properties of the compound, which are more complex than those initially reported.

The effects of RU 486 on luteal function have not yet been conclusively described so far. Given on days 22-25 of the cycle, the drug produced rapid luteolysis (Herrmann et al., 1982). In the present study, RU 486 was given early in the mid luteal phase, excluding a physiological luteal regression. In eight women receiving 100 mg per day, three 50 mg and two 25 mg per day, plasma progesterone and estradiol levels promptly decreased during the treatment. Thus, the effects of the drug on the corpus luteum function are also dose-dependent.

Global analysis of the effects of RU 486 upon gonadotropic and luteal functions indicates that both gonadotropin inhibition and corpus luteum regression occurred in only five cases. In five other cases, plasma progesterone and estradiol levels remained unchanged, though pulsatile gonadotropin release was suppressed. Contradictory information exists in the literature as to the role of gonadotropins in the support of the corpus luteum. In the rhesus monkey, it has been previously shown that the suppression of postovulatory gonadotropin levels did not affect corpus luteum function (Balmaceda et al., 1983). In women, the early corpus luteum shows an apparent insensitivity to LH. Its sensitivity to gonadotropins appears later in the mid luteal phase (Filicori et al., 1984). However, our results argue that, on the fourth day of the luteal phase, the corpus luteum is not highly LH dependent. In some women, a partial autonomy from the pituitary may persist as a consequence of the rapid pulsatile release of LH in the early luteal phase.

Finally, in two other subjects, the drug induced luteolysis in spite of unchanged plasma LH ultradian rhythm parameters. The exact mechanisms of control of corpus luteum life span remain unknown. In the present study the decrease in plasma ovarian steroid levels associated with a normal gonadotropin function cannot be satisfactorily explained. Up to now, there is no evidence of any effect of RU 486 on progesterone biosynthesis by luteinized cells *in vitro* (Kreitmann-Gimbal et al., 1983). However, other mechanisms than gonadotropin inhibition have been proposed for the corpus luteum regression (Rothchild, 1981).

RU 486 is also a glucocorticosteroid antagonist. The compound at a dosage exceeding 200 mg per day administered to humans had antiglucocorticosteroid effects and antagonized the negative pituitary feed-back of the nocturnal administration of dexamethasone (Gaillard et al., 1983; Bertagna et al., 1984). Recently, RU 486 at a dose of 5 to 20 mg/kg has been successfully used in a case of Cushing's syndrome (Nieman et al., 1984). In the present study, 100 mg per day did not increase circulating levels of either ACTH, cortisol, aldosterone or plasma renin activity. Thus, it appears that the antiglucocorticosteroid effects occur at doses larger than those able to induce uterine bleeding.

## CONCLUSION

The data provided by this study establish that RU 486 at a dose of 100 mg per day given during the mid luteal phase to normal women uniformly induces uterine bleeding. This drug may impair both gonadotropin secretion and luteal function in a dose-dependent manner. However, the data indicate that the uterine bleeding results from *in situ* action upon the endometrium, very likely at the progesterone receptor level. No effect attributable to the antiglucocorticosteroid activity was observed, confirming the potential use of RU 486 as a "contragestive" agent. However, in subjects with anovulatory cycles, RU 486 is not a menses inducer. Thus, further studies will be required to determine the clinical use of RU 486 as a drug for fertility control.

## REFERENCES

- Balmaceda, J. P., Coy, Borghi, M. R., Schally, A. V., and Asch, R. H., 1983, Suppression of post ovulatory gonadotropin levels does not affect corpus luteum function in rhesus monkeys, J. Clin. Endocrinol. Metab., 57:866.
- Bertagna, X., Bertagna, C., Luton, J. P., Husson, J. M., and Girard, F., 1984, The new steroid analog RU 486 inhibits glucocorticoid action in man, J. Clin. Endocrinol. Metab., 59:25

- Filicori, M., Butler, J. P., and Crowley, W. F., 1984, Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion, J. Clin. Invest., 73:1638.
- Gaillard, R. C., Riondel, A., Herrmann, W., Muller, A. F., and Baulieu, E. E., 1983, The antifertility steroid RU 486 is an antiglucocorticosteroid depressing the pituitary-adrenal system in the human but only at a specific time during the day, 65th Annual Meeting of the Endocrine Society, San Antonio, Texas, Abstract 219.
- Gravanis, A., Schaison, G., George, M., DeBrux, J., Satyaswaroop, P. B., Baulieu, E. E., and Robel, P., 1984, Endometrial responses to the antiprogesterone steroid RU 486 in post menopausal women, 7th International Congress of Endocrinology, Quebec, Canada, Abstract 1183.
- Halberg, F., Johnson, E. A., Nelson, W., Runge, W., and Sothorn, R., 1972, Autorhythmometry procedures for physiologic self measurements and their analysis, Physiologic Teacher, 1:1
- Healy, D. L., Baulieu, E. E., and Hogden, G. D., 1983a, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates; site of action, dose response relationships, and hormonal effects, Fertil. Steril., 40:253.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen, G. D., 1983b, Pituitary and adrenal response to the antiprogesterone and antiglucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effet d'un stéroïde antiprogesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut, CR Acad Sci Paris, 294:933.
- Kreitmann-Gimbal, B., Kreitman, O. L., Sopelak, V. M., Kurman, R. J., Baulieu, E. E., and Hodgen, G. D., 1983, Menstrual induction in the primate fertile and non fertile cycle: antiprogesterone RU 486 binds to endometrial progesterone receptors without affecting luteal cells, J. Steroid. Biochem., Supp. 19: 1 2 S, abstract 336.
- Nieman, L. K., Chrousos G. P., Spitz, I., Nisula, B. C., Cutler, G. B., Merriam, G. R., Bardin, C. W., and Loriaux, D. L., 1984, Successful treatment of Cushing's syndrome with the glucocorticoid-antagonist RU 486, 7th International Congress of Endocrinology, Quebec, Canada, Abstract 1718.
- Rothchild, I., 1981, The regulation of the mammalian corpus luteum, Rec. Prog. Horm. Res., 37:183.
- Schaison, G., George, M., Lestrat, N., Lagoguey, M., and Baulieu, E. E., 1984, Inhibitory effects of the antiprogesterone steroid RU 486 on gonadotropin secretion in women, 7th International Congress of Endocrinology, Quebec, Canada, Abstract 2278.
- Soules, M. R., Steiner, R. A., Clifton, D. K., Cohen, M. L., Aksel, S., and Bremner, W. J., 1984, Progesterone modulation of pulsatile luteinizing hormone secretion in normal women, J. Clin. Endocrinol. Metab., 58:378.

USE OF SINGLE DOSES OF THE ANTIPROGESTERONE STEROID RU 486 FOR  
INDUCTION OF MENSTRUATION IN NORMAL WOMEN

Lynnette K. Nieman,<sup>1</sup> David L. Healy,<sup>2</sup> Irving M. Spitz,<sup>3</sup>  
George R. Merriam,<sup>1</sup> C. Wayne Bardin,<sup>3</sup> D. Lynn Loriaux,<sup>1</sup> and  
George P. Chrousos<sup>1</sup>

Developmental Endocrinology Branch,<sup>1</sup> National Institute of Child  
Health and Human Development, Bethesda, Maryland; the M.R.C. Centre  
for Reproductive Biology,<sup>2</sup> Edinburgh, Scotland and the Center for  
Biomedical Research,<sup>3</sup> Population Council, New York, New York

ABSTRACT

Progesterone supports secretory endometrium during the luteal phase of the menstrual cycle. We tested the hypothesis that a single mid-luteal dose of a progesterone antagonist, RU 486, could cause menstruation and thus potentially function as a single dose contraceptive agent. RU 486 was given in increasing doses to 11 regularly cycling women on day 21. Menses was induced in all subjects with an established luteal phase at doses of RU 486 > 5 mg/kg. Thus, RU 486 may be an effective menstrual cycle regulator and contraceptive agent.

INTRODUCTION

There is considerable demand, and clinical appeal, for an orally active, estrogen-free contraceptive that could be taken once monthly or after sexual intercourse. In theory, a progesterone antagonist could interrupt the support of endogenous progesterone upon the endometrium and cause withdrawal bleeding. Various antiprogesterone compounds have been evaluated to this end, but none has been widely used as a contraceptive either because of low efficacy or unacceptable side effects. (Sakiz et al., 1974; Mora et al., 1975; Gu and Chang, 1979; Hahn et al., 1981; Creange et al., 1981). Recently a new antiprogesterone has become available that holds promise as a prototype antiprogestational contraceptive agent.

RU 486 is a synthetic steroid that antagonizes progesterone competitively at the receptor level. (Philibert et al., 1982). In women or nonhuman primate females RU 486 acts locally upon the endometrium to induce menstruation when given orally or injected daily for four days during the luteal phase (Hermann et al., 1982; Healy et al., 1983). Ideally a contraceptive agent would be given once monthly and would cause menses at the expected time (day 28) without changing the interval to the next menstrual period (28 days later). We have therefore evaluated the effects of a single oral dose of RU 486 in eleven normal women during the mid-luteal phase of the menstrual cycle (Nieman et al., 1985). This medication induces menstruation at doses greater than or equal to 5 mg/kg, without changing the

expected interval to the next menstrual period. Serum progesterone and estradiol concentrations decrease following treatment, suggesting that luteolysis occurs in addition to the antiprogesterin effect on the endometrium.

#### EXPERIMENTAL PROTOCOL AND RESULTS

Approval for the study was obtained from the United States National Bureau for Drugs and Biologics and the Human Investigation Committee of the NICHHD Clinical Research Sub-Panel of the National Institutes of Health. Eleven subjects (mean age 30 years, range 19-42) were studied. All women had regular menstrual cycles (mean interval 28 days, range 26-30), were within 15% of their ideal body weight, and were not at risk for pregnancy. No volunteer was taking other drugs or hormonal contraceptives.

A blood sample was drawn from each subject for measurement of serum progesterone by established radioimmunoassay. RU 486, provided by Roussel-Uclaf, Paris, France, was administered at 8 AM after an 8 hr fast at a dose of 2.5 mg/kg to one woman, 5 mg/kg to three, 10 mg/kg to four and 25 mg/kg of the drug to three women. Each woman kept a menstrual record chart to note onset, duration and quality of menstruation over the next three months. In addition, alternate day blood samples were drawn from two women for 1 or 2 menstrual cycles following RU 486 for measurement of progesterone, estradiol, LH and FSH by radioimmunoassay.

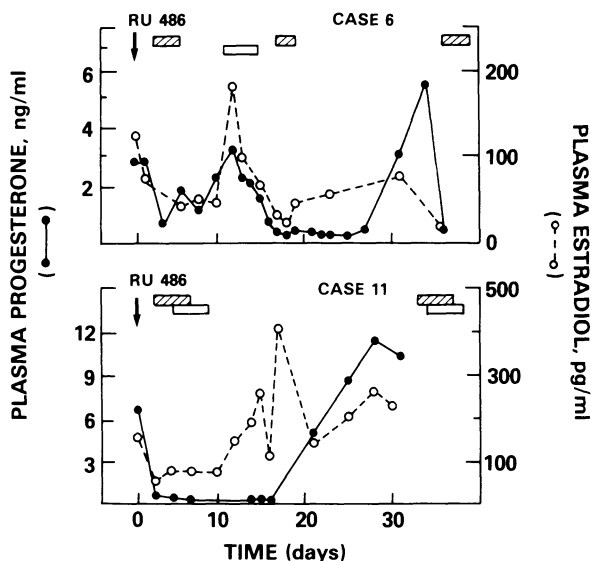


Figure 1. Plasma concentrations of progesterone and estradiol in two women (cases 6 and 11, see Table 1). Progesterone and estradiol levels decreased following RU 486. Open bars indicate the time of expected menses in the absence of RU 486. Shaded bars indicate actual menses.

TABLE I. Clinical Profile of Women Who Received RU 486

Case no.	Age (years)	Dose of RU 486 (mg/kg)	Plasma P <sub>4</sub> (ng/ml)	Post RU 486 menses <sup>1</sup>	Duration of post-RU 486 Menses (days)	Interval to Ensuing Menses (days)
1	37	2.5	6.4	-	np <sup>2</sup>	np
2	20	5	0.2	-	np	np
3	21	5	1.5	-	np	np
4	31	5	16.8	+	3	28
5	30	10	13.9	+	4	29
6	19	10	2.8	+	3	14
7	38	10	10.8	+	3	30
8	30	10	24.7	+	3	26
9	22	25	0.8	-	np	np
10	42	25	2.8	+	7	30
11	37	25	13.9	+	4	29

<sup>1</sup>Within 72 hr of RU 486 administration.

<sup>2</sup>np, not pertinent

Administration of RU 486 caused menstruation in 7 of 11 subjects within three days of ingestion (Table I). All women who had ovulated, as indicated by a mid-luteal plasma progesterone concentration greater than 1.5 ng/ml, and who received 5 mg/kg or more RU 486, menstruated within three days. Menstruation persisted for an average of 4 days (range 3-7 days) and was not excessive in any volunteer. In women who did menstruate after RU 486, the next menses began  $28 \pm 2.4$  days (mean  $\pm$  SE) later (range 14-33 days). In the four women who did not menstruate following RU 486 ingestion, menstruation occurred at the expected time in two (days 29 and 30), but was delayed until day 39 and 60, respectively, in the remaining subjects.

We measured plasma levels of progesterone and estradiol every other day for 1 or 2 menstrual cycles in two women who had RU 486-induced menses. The results are shown in Figure 1. Estradiol and progesterone plasma concentrations decreased from luteal phase levels to follicular phase levels within 3 days of RU 486 administration in both women; this was temporally associated with post-RU 486 menses. Estradiol and progesterone concentrations in case 11 then followed a normal ovulatory pattern, with 14 days of follicular phase levels followed by luteal phase levels and a second menstrual period. Luteolysis was less complete in case 6, in whom progesterone concentrations rose to 3.3 ng/ml and then fell within 14 days in association with a second menstrual period. She then had plasma estradiol and progesterone levels consistent with a short ovulatory cycle (19 days) followed by a third menstrual period. Plasma concentrations of LH and FSH remained within the normal female range throughout (data not shown).

## COMMENT

RU 486 appears to be an effective progesterone antagonist without clinically important progesterone agonist activity or toxicity. A single mid-luteal dose of at least 5 mg/kg consistently caused menstrual bleeding in normally cycling women. Although RU 486 is also a potent glucocorticoid antagonist at the receptor level, no signs of adrenal insufficiency were seen at the doses used in our subjects. The absence of apparent antiglucocorticoid activity may be dose-dependent, since the antiprogestational effects of RU 486 occur at a lower dose than the antiglucocorticoid effects (Healy et al., 1983a, 1983b; Nieman et al., 1985). Therefore, it may be possible to avoid antiglucocorticoid side effects during RU 486 administration for gynecologic indications by giving the medication as a single dose or multiple doses over a short time period.

Four women did not menstruate within three days of receiving RU 486 (Table I). Each had either low progesterone levels or had received less than 5 mg/kg of RU 486. One (case 1), who had luteal levels of plasma progesterone, received only 2.5 mg/kg, probably a suboptimal dose, and had menses when expected on day 28. Another woman (case 3) had evidence of corpus luteum function and menstruated at her expected time (day 29) despite administration of 5 mg/kg of RU 486. It is unclear whether she did not respond because of inadequate absorption of the drug or because the antagonist was given before true luteal levels of progesterone were achieved. Measurement of plasma concentrations of RU 486 may help to explain a response failure such as this and to define the optimal dose of RU 486. The two remaining individuals (cases 2 and 9) received optimal doses of RU 486 but had not yet ovulated. One menstruated on day 39, 16 days after RU 486, and probably had a long follicular phase unrelated to RU 486 administration. The other had menses on day 60, or 40 days after she received RU 486. Dose-response studies during various phases of the menstrual cycle are needed to evaluate whether RU 486 administration during the follicular phase will delay menses.

Plasma progesterone and estradiol levels in two women having RU 486-induced menses suggest that partial or complete luteolysis occurs following drug administration. This finding explains both the early vaginal bleeding seen on day 14, in case 6, and the normal length of the ensuing menstrual cycle seen in the remainder of the women with RU 486-induced menses. Thus, induction of luteolysis by RU 486, in addition to its antiprogestational activity on the endometrium, provides an additional explanation for the RU 486-induced menses. Dependence of the corpus luteum upon progesterone has been suggested by I. Rothchild (Rothchild, 1981).

Thus, it appears that RU 486 has promise as a contraceptive agent, menstrual cycle regulator, and pharmacologic probe for understanding progesterone action. Further dose-response studies, including measurement of RU 486 blood concentrations, are needed to define the lowest optimal dose of RU 486 to reliably induce menses in normally cycling women.

## REFERENCES

- Bertagna, X., Bertagna, C., Luton, J. P., Husson, J. P. and Girard, F., 1984, The new steroid analog RU 486 inhibits glucocorticoid action in man, J. Clin. Endocrinol. Metab., 59:25-28.
- Creange, J. E., Anzalone, A. J., Potts, G. O., and Schane, H. P., 1981, Win 32,729, A new, potent interceptive agent in rats and rhesus monkeys, Contraception, 24:289-299.
- Gu, Z., and Chang, M. C., 1979, A-nor steroids as post-coital contraceptives in the hamster with special reference to the transport and degeneration of eggs, Contraception, 20:549-555.

- Hahn D. W., McGuire, J. L. and Chang, M. C., 1980, Contragestational agents. in: "Research frontiers in fertility regulation," G. I. Zatuchni, M. H. Labbok, and J. J. Sciarra, eds. Hagerstown, Harper and Row, 173.
- Healy, D. L., Baulieu, G. D., and Hodgen, G. D., 1983, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose response relationships and hormonal effects, Fertil. Steril., 40:253-257.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, R. W., Baulieu, E. E., and Hodgen, G. D., 1983, Pituitary and adrenal responses to the antiprogesterone and anti-glucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863-865.
- Hermann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, The effects of an antiprogesterone steroid in women: interruption of the menstrual cycle and pregnancy, Comptes Rendu Acad. Sci. Paris, 294:933-938.
- Mora, G., Faundes, A., and Johansson, E. D. B., 1975, Lack of clinical contraceptive efficacy of large doses of R2323 given before implantation or after a missed period, Contraception, 12:211-216.
- Nieman, L. K., Healy, D. L., Spitz, I. M., Merriam, G. R., Bardin, C. W., Loriaux, D. L. and Chrousos, D. L., Induction of menstruation in normal women by a single dose of the antiprogesterone steroid RU 486, Submitted for publication.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, RU 486: A new lead for steroidal antihormones, 64th Annual Meeting U.S. Endocrine Society, San Francisco, abstract 688.
- Rothchild, I., 1981, The regulation of the mammalian corpus luteum, Rec. Progr. Horm. Res., 37:183-298.
- Sakiz, E., Azadian-Boulanger, G., Larague, F., and Raynaud, J. P., 1974, A new approach to estrogen-free contraception based on progesterone receptor blockade by mid-cycle administration of ethynorgestrienone (R-2323), Contraception, 10:467-472.

## ENDOCRINOLOGIC EFFECTS OF THE ANTIPROGESTERONE RU 486

### IN THE LUTEAL PHASE OF NORMAL WOMEN

Donna Shoupe, Daniel R. Mishell, Jr., Maria Lacarra,  
Elia Gutierrez, Pekka Lahteenmaki, and Irving M. Spitz,

Department of Obstetrics and Gynecology  
Section of Reproductive Endocrinology  
University of Southern California, Los Angeles, California  
Center for Biomedical Research, Population Council, New York  
Steroid Research Laboratory, University of Helsinki  
Helsinki, Finland

### ABSTRACT

RU 486 is a new synthetic antiprogesterone with anti-glucocorticoid activity. This study was designed to assess the tolerance of a single oral dose of RU 486 in normal cycling women during the luteal phase and to assess its effect on bleeding, gonadotropin and steroid patterns. Either 200, 400, 600 or 800 mg of the compound was orally administered to groups of five women at 0800 hours, and blood was sampled over a 48 hour period. There were no untoward clinical effects. Menses were induced in all but one patient within three days after ingestion of RU 486. This bleeding occurred in association with high levels of estradiol and progesterone. There was a further bleeding episode at the time of the expected menses. Plasma levels of RU 486 were maximum between 1 and 4 hours and remained elevated for 48 hours, indicating a prolonged disappearance time. Significant increases in prolactin were noted between 4 and 10 hours. Luteinizing hormone and estradiol were unchanged with the two lower dosages, but there was a transient decrease after the 600 and 800 mg dosage. Cortisol remained unchanged except for a late rise at 24 and 48 hours after the 400 mg and 800 mg dosage. There were no changes in follicle stimulating hormone, progesterone or ACTH.

We conclude that RU 486 is well-tolerated as a single oral dose in normal women and causes induction of menses. It also produces a transient elevation in serum prolactin, a delayed elevation in cortisol, and in higher dosages a decrease in luteinizing hormone and estradiol.

### INTRODUCTION

RU 486 is a new synthetic 19-norprogesterone with an affinity for the progesterone receptor five times higher than progesterone (Philbert et al., 1981). It acts as a progesterone antagonist and has been reported in preliminary studies to induce interruption of the luteal phase in women (Herrmann et al., 1982) and primates (Healy et al., 1983), and termination of early pregnancy in women (Herrmann et al., 1982).

RU 486 also has an affinity for the glucocorticoid receptor which is about three times higher than dexamethasone and has been shown to have anti-glucocorticoid activity (Philbert, 1981; Proulx-Ferland, 1982). In humans (Bertagna et al., 1984) and primates, (Healy et al., 1983, 1985) it produces a moderate but transient increase in ACTH and cortisol after a single oral dose. In both in vivo and in vitro conditions, this compound has been shown to inhibit the dexamethasone-induced suppression of ACTH (Proulx-Ferland et al., 1982; Gaillard et al., 1984).

The aim of this study was to investigate the tolerance of a single oral dose of RU 486 and its effect on the bleeding, gonadotropin and steroid patterns when given to healthy women in the mid-luteal phase. In addition, pharmacodynamics of this compound were studied using a recently developed radioimmunoassay.

## MATERIALS AND METHODS

Healthy volunteers, whose ages ranged from 25 to 32 years, were selected. They had regular menstrual cycles ( $28 \pm 3$  days) with weights between 45 and 70 kg and had either undergone surgical sterilization or were using an IUD for contraception. They were excluded from the study if they had taken a steroid medication within the last six months.

Beginning on day ten of the menstrual cycle, each subject had daily blood sampling for luteinizing hormone (LH), to document the day of the LH surge. Five to seven days after ovulation the volunteers were asked to report to the clinic at 7:30 a.m. after an overnight fast and to refrain from smoking after midnight.

There were four groups of five patients. Each received either 200, 400 600 or 800 mg RU 486 (50 tablets provided by Institut Roussel Uclaf, Paris, France), at 8:00 a.m. No food or smoking was permitted for two hours after treatment. Vital signs were recorded at 30 minute intervals for six hours. Blood samples were obtained using an in-dwelling catheter at -1/2, 0, 1/2, 1, 2, 4, 6, 10, 24, and 48 hours after ingestion of the medication. Serum samples were assayed for LH, follicle stimulating hormone (FSH), prolactin (Prl), estradiol ( $E_2$ ) and progesterone (prog) and plasma samples were tested for ACTH, cortisol and for RU 486. The radioimmunoassay for RU 486 was developed by the Steroid Research Laboratory, University of Helsinki, Helsinki, Finland. The antibody used was provided by Institut Roussel Uclaf, Paris, France. The paired t-test was used for statistical analysis.

## RESULTS

Menses were induced in all subjects within one to three days after ingestion of the medication. Several days after cessation of this bleeding episode, and corresponding to the normal time of the expected menses for each particular subject, another bleeding episode occurred. The following cycle was normal. No adverse side effects were noted, and the electrolytes renal and hepatic function and full blood count did not change in any subject.

One subject who received the 800 mg dose did not bleed for 56 days following ingestion of the medication. Her menses subsequently returned to normal.

With all dosages of RU 486, plasma levels of RU 486 reached a maximum between 1 and 4 hours after ingestion. By 48 hours, circulating levels still remained elevated indicating a long half disappearance time (Fig. 1).

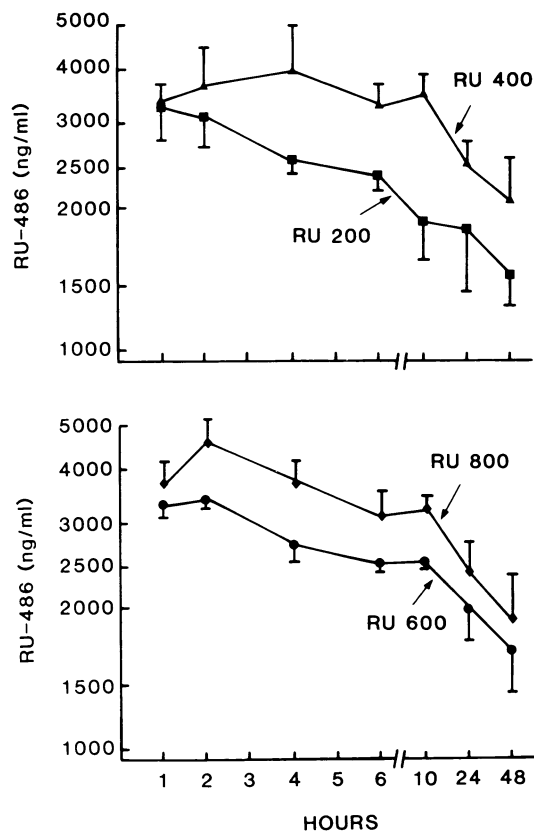


Fig. 1. Plasma levels of RU 486 after oral ingestion of 200, 400, 600 and 800 mg. The values on this and all the following figures are mean  $\pm$  SEM. In this figure there is a logarithmic scale on the ordinate.

There was a transient but significant increase in Prl levels with all dosages. The increase was maximal between four and six hours and values returned to normal levels by 24 hours (Fig. 2). When compared to basal values, the maximal increase was between 115-136 percent, and this was significant at ( $p < 0.05$ ).

There was no significant change in LH with the two lower doses (Fig. 3). At the 600 mg dose only one point (2 hours), was significantly lower ( $p < 0.05$ ) when compared to baseline values. After the 800 mg dose, all points between 2 and 10 hours were significantly lower ( $p < 0.05$ ), except for four hours. Compared to baseline samples, the percent ranged from 29-60 percent. There were no significant changes in FSH with all dose schedules (Fig. 4).

There was no change in  $E_2$  with the two lower doses of RU 486. There was a transient but significant decrease in  $E_2$  ( $p < 0.05$ ) at six hours with the 600 mg dose and at 2, 4 and 6 hours after the 800 mg. This represented a 13-15% decrease in  $E_2$  levels (Fig. 5). There were no significant changes in prog with any of the doses (Fig. 6).

Cortisol (Fig. 7) and ACTH (Fig. 8) were unchanged except for a significant increase in cortisol at 24 and 48 hours after the 400 and 800 mg dose. The normal a.m. cortisol range is between 275-700 nMol/L. Therefore,

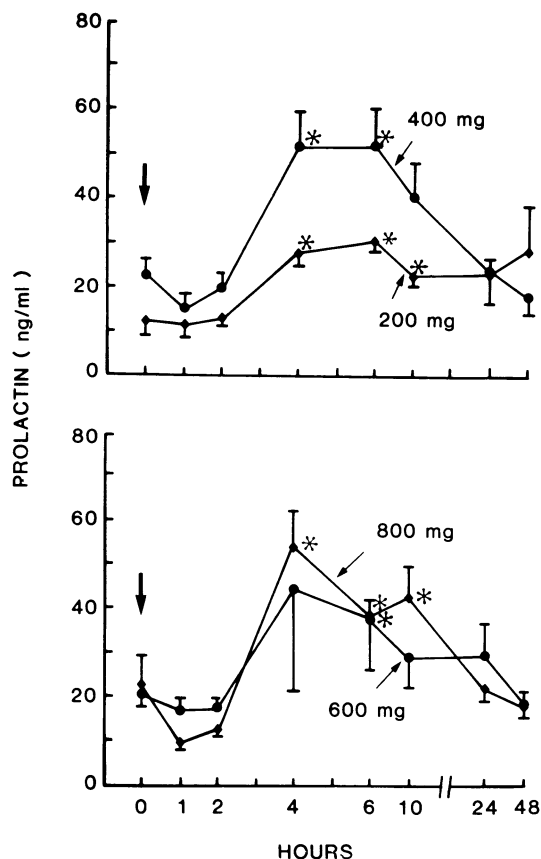


Fig. 2. Serum levels of prolactin after ingestion of 200, 400, 600 and 800 mg RU 486. \* =  $p < 0.05$  compared to basal values.

the levels at 24 and 48 hours after the 800 mg dose exceeded the normal range.

## DISCUSSION

Administration of this compound as a single oral dose was well-tolerated in our subjects at all dosage levels. This confirms previous studies using a single dosage of up to 400 mg (Bertagna et al., 1984).

The immunoassayable RU 486 persisted in the circulation for 48 hours implying there is a long half disappearance time. However, the radioimmunoassay used for measurement of RU 486 utilizes a non-specific antibody, and several of the metabolic degradation products are known to compete for binding sites. These byproducts are also known to have decreased biological activity. Further studies using a more specific antibody, or high pressure liquid chromatography, are needed to define more precisely the pharmacodynamics of this compound.

There were no significant changes in ACTH in any of our patients, although a late rise in cortisol was noted at 24 and 48 hours after ingestion of the 400 and 800 mg dose. With the 400 mg dosage, these values were all within the normal range. However, basal hypercortisolemia was

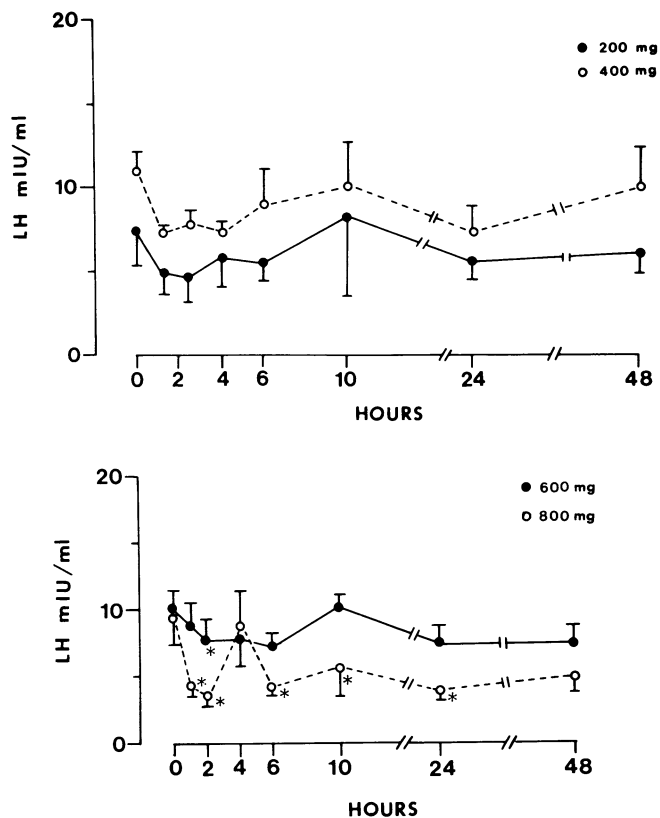


Fig. 3. Serum levels of LH after ingestion of 200, 400, 600 and 800 mg RU 486. \* =  $p < 0.05$ , compared to basal values.

apparent at both 24 and 48 hours with the 800 mg dosage, suggesting the presence of antiglucocorticoid activity. The reason for our failure to observe an associated increase in ACTH is not known, but may possibly be related to inadequate blood sampling or assay insensitivity. A similar pattern was seen by Spitz (this volume), who observed, in dogs, a rise in

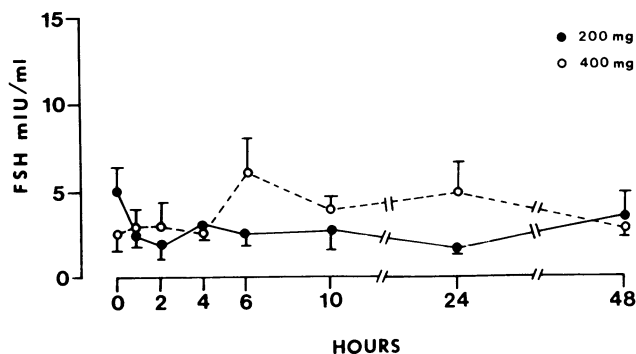


Fig. 4. Serum FSH levels after ingestion of 200 and 400 mg RU 486. Levels after 600 and 800 mg not shown.

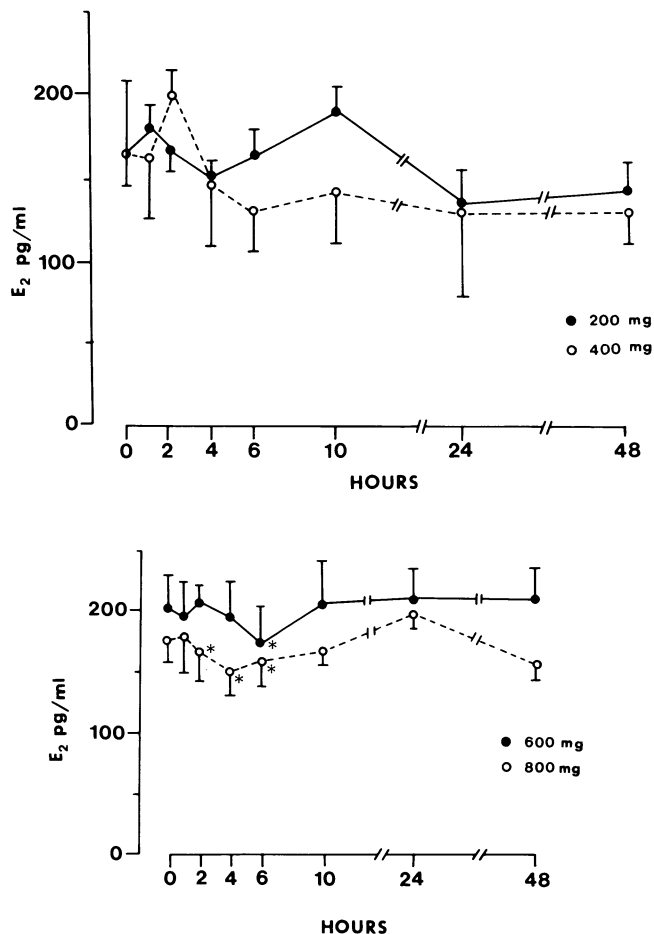


Fig. 5. Serum estradiol levels after ingestion of 200, 400, 600 and 800 mg RU 486. \* =  $p < 0.05$  compared to basal values.

cortisol before an increase in ACTH, in response to RU 486. This elevation had no adverse consequences, and the dogs maintained the ability to excrete a water load.

Our results are in variance with Healy et al. (1983, 1985), who reported a transient increase in ACTH and cortisol secretion four hours after an intramuscular injection of RU 486 using dosages of 1.0 and 5.0 mg/kg in primates. Bertagna et al. (1984) also saw an increase in ACTH and cortisol after oral administration of 400 mg in humans at 0100 in the morning. However, these workers noted no increase in cortisol when RU 486 was administered in the afternoon (1400 hours). From these results they concluded that the antigluco-corticoid action of RU 486 occurs during periods of active ACTH secretion. Gaillard et al. (1984) also observed that the action of RU 486 is time-dependent.

The induction of menses confirms the work of Herrmann et al. (1982), and also of Healy et al. (1983), who concluded that RU 486 acts directly upon endometrial tissue to produce bleeding regardless of any effect it may have on progesterone secretion. Indeed, in our subjects, bleeding occurred with  $E_2$  levels over 100 pg/ml and prog levels which ranged from 10-20 pg/ml.

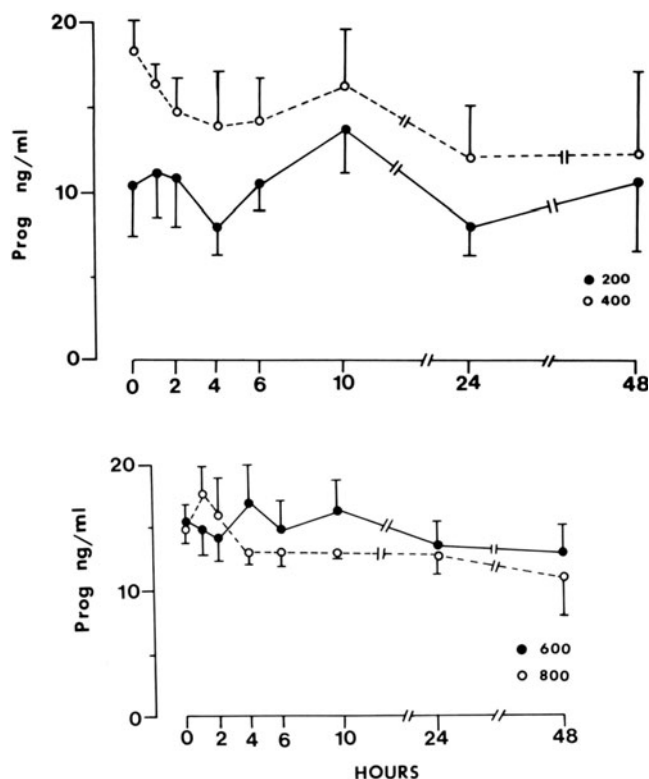


Fig. 6. Serum levels of progesterone after ingestion of 200, 400, 600 and 800 mg RU 486.

Further support of this direct action of RU 486 on the endometrium is the report of Croxatto (this volume), in which nonpregnant women demonstrated withdrawal bleeding during concomitant administration of hCG and RU 486 during the luteal phase. At the time of bleeding circulating progesterone levels were also high.

Previous investigators have looked for an effect of progesterone on gonadotropin secretion. While the pulse frequency of LH during the luteal phase is diminished, the amplitude of each pulse is increased (Reame et al., 1984). Our study was not designed to assess the effect of RU 486 on LH pulsatility; more frequent sampling would be required. However, we did note a transient reduction in LH with higher doses of RU 486. This is compatible with the data of Rakoff and Yen (1979), who described an acute release of LH in estrogen-primed ovariectomized women receiving intramuscular progesterone.

In the same study, Rakoff and Yen (1979) also observed a transient increase in Prl. In contrast, in our current study with RU 486, we observed an increase in Prl from four to ten hours after ingestion of the tablet. Although luteal phase levels of Prl are usually slightly above follicular phase levels, none of the baseline Prl values would be considered to be hyperprolactinemic.

In contrast to our study, Healy et al. (1983) did see a 20% decrease in Prl levels after RU 486 (10 mg/kg) in castrated monkeys on steroid replacement with estradiol and progesterone capsules. This replacement regimen produced a moderate hyperprolactinemia. Within one hour of intramuscular administration of RU 486, these hyperprolactinemia levels were

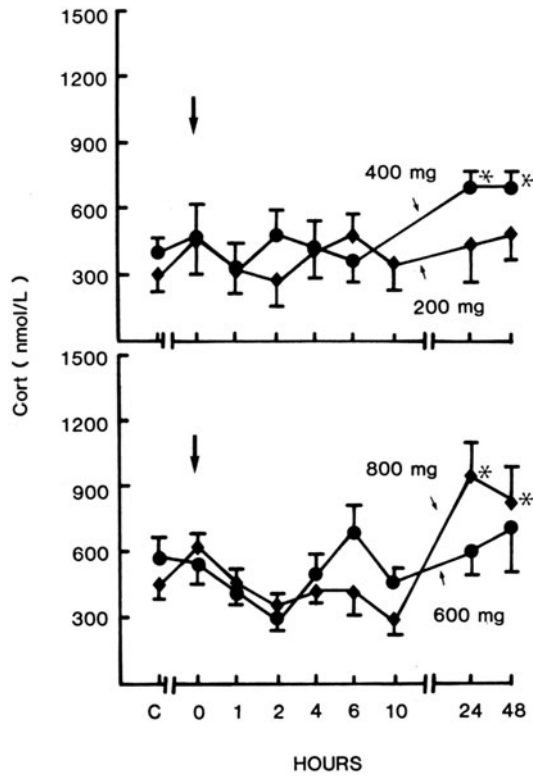


Fig. 7. Plasma levels of cortisol after ingestion of 200, 400, 600 and 800 mg RU 486. \* =  $p < 0.05$  compared to basal values.

significantly decreased. Their conclusion was that this short-lived decrease in Prl levels supported the importance of progesterone in the induction of hyperprolactinemia in the monkey. It is thus evident that the effect of an antiprogesterone on Prl secretion may indeed be dependent on th

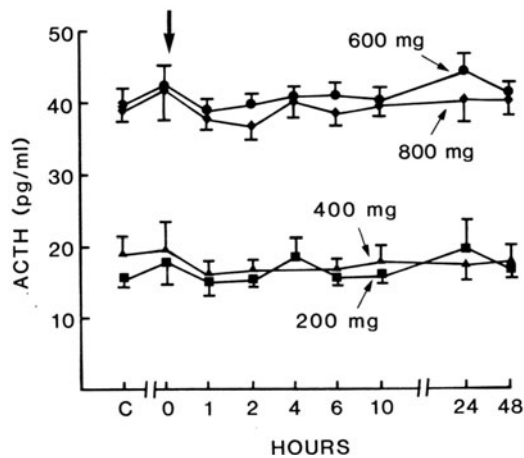


Fig. 8. Plasma levels of ACTH after ingestion of 200, 400, 600 and 800 mg RU 486.

species, on the time of exposure and may vary according to the prevailing estrogen and progesterone concentrations.

The mechanism whereby RU 486 produces these changes in LH, progesterone, and E<sub>2</sub> levels are currently not known, and future studies are in progress in order to evaluate these phenomena as well as the potential therapeutic benefits of this compound.

#### ACKNOWLEDGMENT

This work was supported by grants from the Ford Foundation and the Mellon Foundation. The drug was supplied by Roussel Uclaf, Paris, France.

#### REFERENCES

- Bertagna, X., Bertagna, C., Luton, J. P., Husson J. M., and Girard, F., 1984, the new steroid analog 486 inhibits a glucocorticoid action in man, J. Clin. Endocrinol. Metab., 59:25.
- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., 1984, RU 486: A steroid with antiglucocorticoid steroid that only disinhibits the human pituitary - adrenal system at a specific time of day, Proceedings of the National Academy of Science, 1:3879.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983, Induction of menstruation of an antiprogesterone steroid (RU 486) in primates: Site of action, dose-response relationships, and hormonal effects, Fertil. Steril., 40:253.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P.W., Baulieu, E. E. and Hodgen, G. D., 1983, Pituitary and adrenal responses to the antiprogesterone and antiglucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, W., and Baulieu, E. E., 1982, The effects of an antiprogesterone steroid on women: Interruption of the menstrual cycle and of early pregnancy, Comptes Rendus, 294:933.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, RU 486: a new lead for steroidal anti-hormones, Presented at the Sixty-Fourth Annual Meeting of the United States Endocrine Society, San Francisco, June 16-18, Abstract 668.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 38486 a potent antiglucocorticoid in vivo, 8th International Congress of Pharmacology, Tokyo, Japan, p. 14631 (abstract).
- Proulx-Ferland, L., Cote, J., Philibert, D., and Deraedt, R., 1982, Potent antiglucocorticoid activity of RU 38486 on ACTH secretion in vitro and in vivo in the rat. Presented at the Sixth International Congress of Hormonal
- Rakoff, J. S., and Yen, S. S. C., 1979, The simultaneous release of prolactin and gonadotropins in response to progesterone administration in estrogen-primed women, J. Clin. Endocrinol. Metab., 47:918.
- Reame, N., Saunders, S. E., Kelch, R. P., and Marshall, J. C., 1984, Pulsatile Gonadotropin Secretion during the Human Menstrual Cycle: Evidence for Altered Frequency of Gonadotropin-Releasing Hormone Secretion, J. Clin. Endocrinol. Metab., 59:328.

ENDOMETRIAL AND PITUITARY RESPONSES TO THE STEROIDAL ANTIPROGESTIN RU 486  
IN POSTMENOPAUSAL WOMEN

Paul Robel,<sup>1</sup> Achille Gravanis,<sup>1</sup> Gilbert Schaison,<sup>2</sup>  
Martine George,<sup>2</sup> Jean de Brux,<sup>3</sup> Pondichery G.  
Satyaswaroop,<sup>4</sup> and Etienne-Emile Baulieu<sup>1</sup>

<sup>1</sup>CNRS ER 125 and INSERM U 33, Lab. Hormones  
94270 Bicetre, France

<sup>2</sup>Department of Endocrinology, Bicetre Medical School  
94270 Bicetre, France

<sup>3</sup>Fondation d'Enseignement et de Recherche en Histo et  
Cytopathologie, 75016 Paris, France

<sup>4</sup>Present address: The Milton S. Hershey Medical Center  
Hershey, Pennsylvania 17033, USA

ABSTRACT

The effects of the antiprogesterone RU 486 on the human endometrium were investigated. Seventeen postmenopausal women were injected with estradiol benzoate (0.625 mg/d) for 15 days. Progesterone (25 mg/d) and/or RU 486 (100 or 200 mg/d) were given to groups of 2-3 women during the last six days of estradiol benzoate treatment. Serial blood samples were drawn for the measurement of plasma estradiol ( $E_2$ ), progesterone (P) and LH and FSH. An endometrial biopsy was performed on the last day of treatment and processed either for histology or for assays of DNA polymerase alpha (DNA pol), estradiol dehydrogenase ( $E_2$ DH), and progesterone receptor (PR).

Treatment with estradiol benzoate alone resulted in a marked decrease of plasma gonadotropins. In those patients who received either P, RU 486, or both, in addition to estradiol benzoate, the concentrations of plasma LH and FSH were further decreased to premenopausal levels.

In absence of glycerol, the affinity of RU 486 for the endometrium PR ( $K_d=0.8$  nM) was higher than that of P ( $K_d = 1.2$  nM). Glycerol markedly decreased the affinity of RU 486, whereas the affinity of P for the PR was unchanged. RU 486 had negligible affinity for plasma transcortin.

Either P or RU 486, but not both, induced secretory changes in the endometrium, as determined from histologic sections of tissue biopsies. Either P or RU 486 decreased DNA pol and increased  $E_2$ -DH activities in the endometrium. Unexpectedly, when P and RU 486 were given together,  $E_2$ -DH activity remained at the level of  $E_2$ -treated women. In vitro cultures of proliferative endometrium treated with the synthetic progestagen R 5020 or with RU 486 also had increased  $E_2$ -DH activity; RU 486 counteracted R 5020 effects.

We conclude that, contrary to previous results with experimental animals, the anti-progesterone RU 486 has some progestomimetic activity in humans, though only under specific conditions. Paradoxically, when given together with progesterone, RU 486 loses most of its progestomimetic activity in the endometrium and behaves as a "pure" antagonist.

## INTRODUCTION

No progestomimetic effect of RU 486 has been reported in any mammalian species investigated so far. It behaves as a "pure" P antagonist. However, the affinity of the progesterone receptor (PR) for progestagens and analogs are known to differ markedly among species (Smith et al., 1974). In addition, the biological properties of the endometrium and their hormonal control are quite variable among mammalian species. Therefore, the results of pharmacological studies with P derivatives in rats and rabbits may not be correctly assumed to be equal to expected results in humans. Our purpose was to use a human model to confirm the progesterone antagonist properties of RU 486 at the endometrial level (with morphological and biochemical criteria), and to seek progestomimetic properties of RU 486. We wanted to avoid any physiological situation with endogenous progesterone secretion, namely the secretory phases of normal menstrual cycles or early pregnancies. We therefore decided to study the effects of RU 486 in postmenopausal women. They often need treatment with estrogens or estrogen-progestin combinations to relieve their menopausal symptoms; the investigations conducted by King et al., (1979) and Robel et al. (1984) have provided better understanding of the biochemical responses of the endometrium to estrogen-progestin combinations.

The search for P agonistic and antagonistic properties in the endometria of women pre-treated with E<sub>2</sub> was conducted under conditions that resembled those reported previously for rats, rabbits (Philibert et al., 1982) and monkeys (Healy et al., 1983a). Besides plasma gonadotropins and the histology of endometrial biopsies, we investigated several biochemical parameters of the human endometrium that may be relevant to progestomimetic activity: 1) the total PR and the fraction thereof in the nuclear compartment, 2) the activity of DNA polymerase alpha (DNA pol) (Robel et al., 1984) and 3) the activity of estradiol-dehydrogenase (E<sub>2</sub>-DH) (Tseng and Gurpide, 1975).

## MATERIALS AND METHODS

### Patients

Seventeen women (age range 35-58 years) were investigated. Two of them had been ovariectomized; all others had spontaneously menopausal. None of them had any history of uterine disease or obesity, and none had received any hormonal treatment for menopausal symptoms within three months before the present study. They were treated with estradiol benzoate im, 0.625 mg/d, for 15 days. This dose pattern was previously reported to increase plasma E<sub>2</sub> concentrations above 200 pg/ml (Schaison, 1983). The duration of estrogen therapy was too short to cause full regrowth of the atrophic endometrium. However, King et al. (1979) reported that a three-week treatment with estrogen results in less pronounced effects when compared to two-week treatments. For practical reasons, it was decided to limit the treatment period with estrogen, either alone or combined, to 15 days.

Although it would be best to study the same women in a serial fashion during repeated treatment cycles, most of our patients were unwilling to undergo more than one endometrial biopsy. Therefore P and/or RU 486 were

Table I. Plasma Estradiol, Progesterone and Gonadotropins

Treatment groups	E <sub>2</sub> d 15 (ng/ml)	P d 15 (ng/ml)	LH d 0 (mU/ml)	FSH d 15 (mU/ml)
1. E <sub>2</sub>				
A	410	< 0.1	40	13
B	508	< 0.1	39	16
C	564	< 0.1	27	12
(mean ± sem)	494 ± 78	< 0.1	36 ± 7	13 ± 2
2. E <sub>2</sub> + P				
D	764	9.0	63	9
E	577	17.6	49	9
F	497	16.6	21	6
(mean ± sem)	612 ± 137	14.4 ± 4.7	44 ± 21	8 ± 2
3. E <sub>2</sub> + (RU 486 (100))				
G	523	0.2	26	< 1
H	540	0.2	45	5
I	465	0.1	39	5
(mean ± sem)	509 ± 39	0.2 ± 0.1	36 ± 10	4 ± 2
4. E <sub>2</sub> + (RU 486 (200))				
J	785	0.2	16	1
K	527	ND	51	2
L	320	0.3	21	< 1
(mean ± sem)	544 ± 236	0.2 ± 0.1	29 ± 19	1 ± 1
5. E <sub>2</sub> + P + (RU 486 (100))				
M	835	8.0	15	< 1
N	580	10.1	20	< 1
(mean ± sem)	708 ± 180	9.1 ± 1.5	18 ± 4	< 1
6. E <sub>2</sub> + P + (RU 486 (200))				
O	550	9.5	6	< 1
P	328	10.2	28	< 1
Q	263	7.8	45	1
(mean ± sem)	447 ± 160	9.1 ± 1.2	23 ± 20	< 1

Seventeen patients with climacteric symptoms were treated with estradiol benzoate (E<sub>2</sub>), 0.625 mg i.m. daily for 15 d. The patients in treatment groups 2, 5 and 6 were also treated with progesterone (P) in oil solution, 25 mg i.m. daily from d 9-15. Patients in treatment groups 3,4,5 and 6 were treated orally with RU 486, either 50 or 100 mg twice daily, from d 9-15. Day 0 = before treatment; day 15 = end of treatment interval.

given to groups of 2 to 3 women after 9 days of estrogen treatment. P in oil was injected by the intramuscular route, in a dose of 25 mg daily. This dose produced plasma steroid concentrations larger than 8 ng/ml, to mimic the circulating steroidal milieu of the secretory phase of normal menstrual cycles (Table I). RU 486 was taken orally in doses of 50 or 100 mg twice daily. This amount has been reported to induce abortion in early pregnancy (Herrmann et al., 1982), or to result in menstruation when given during the secretory phase of the cycle (Herrmann et al., 1982; George et al., 1983). Both progesterone and antagonist were given for 6 days, either alone or together. This time interval was selected because it was optimal for obtaining a full progestational response of the endometrium in post-menopausal women pretreated with estrogen (Whitehead et al., 1981).

Blood samples were drawn by venipuncture, and plasma was stored for the assay of E<sub>2</sub>, LH and FSH on days zero and 15 of estradiol benzoate treatment. Plasma P was measured in the last plasma sample.

An endometrial biopsy was performed on the morning of day 15, one hour after the last dose of hormone(s), immediately after the removal of blood for hormone assays. The samples were stored frozen in liquid nitrogen and kept for less than one month prior to the biochemical assays. The remaining tissue was fixed in Bouin's solution and processed for histological examination.

### Progesterone Receptor Binding Assays

For the assay of PR in endometrial biopsy samples, the technique utilized allowed measurements of total (filled and unfilled) receptor sites in the cytosol and nuclear fractions as reported in detail by Bayard et al. (1978). The measurement of nuclear receptor sites was improved by the use of a glass fiber filter exchange technique permitting both exchange and measurement of bound radioactivity without transfer of nuclear suspensions (Levy et al., 1980a).

Samples of proliferative human endometrium from several women were pooled and homogenized in 6 vol of buffer A with a motor-driven Teflon-glass Potter homogenizer. Buffer A consisted of 10 mM Tris-HCl, 1.5 mM EDTA, 0.5 mM dithiothreitol, 20 mM Na molybdate, pH 7.8. The homogenate was divided into two equal parts. One was diluted with an equal volume of buffer A, the other with an equal volume of buffer A containing 60% glycerol. High speed supernatants were prepared, adjusted to 2 mg protein/ml, and stripped of endogenous non-radioactive progestins by adsorption to a pellet of dextran-coated charcoal, prepared in buffer A containing 30% glycerol, at 0-4°C for 30 min. The cytosol samples were centrifuged at 1,500 g for 10 minutes to pellet the charcoal. The supernatant was divided into two equal parts for the measurement of equilibrium binding constants and relative binding affinities.

Increasing concentrations of (1,2,6,7)-<sup>3</sup>H-progesterone (96 Ci/mmol, Amersham, England), or (6,7)-<sup>3</sup>H-RU 486 (37.5 Ci/mmol, Roussel-Uclaf, France), range 2-50 nM, were used for the measurement of equilibrium binding constants of cytosol PR. The incubations with 50 nM radioinert ligand served for the determination of non specific binding. For the measurement of relative binding affinities, 2 nM <sup>3</sup>H-P was incubated with increasing concentrations of non-radioactive P or RU 486 (range 2-1,000 nM). All incubations were done in a total volume of 250 µl in the presence of 1 µM cortisol to prevent binding of either ligand to glucocorticosteroid receptor and/or to plasma CBG.

The relative binding affinities of cortisol, P, and RU 486 to the CBG of 50-fold diluted human pregnancy plasma also were investigated. <sup>3</sup>H-P was used at a 6nM concentration, and each of the nonradioactive competitors at 5 to 1,000 nM concentrations.

After incubation at 0-4°C for three hours (equivalent results were obtained with 18-hour incubations), 200 µl of each incubation mixture was treated with an equal volume of dextran-coated charcoal (0.025%, o.25%, w/v) in buffer A containing 30% glycerol, at 0-4°C for 30 min. After centrifugation at 1,500 g for 10 minutes, the supernatants were removed and counted for radioactivity. Scatchard analysis, with the Rosenthal correction for non-specific binding (Rosenthal, 1967), was used for the calculation of equilibrium binding constants.

### Estradiol-Dehydrogenase

Enzyme activity was measured in the 800 g supernatants or 105,000 g pellets of endometrial samples, according to the method of Tseng and Gurpide (1975). Results were expressed in nmol estrone formed per 30 minutes per mg protein.

The in vitro induction of E<sub>2</sub>-DH activity also was investigated as described previously (Tseng and Gurpide, 1975; Satyaswaroop and Mortel, 1982). Explants of normal late proliferative human endometrium were incubated in MEM culture medium containing 10% FCS, 10 µg/ml insulin, D-glucose (final concentration 5 mg/ml), and 1% antibiotic-antimycotic mixture, under 95% air, 5% CO<sub>2</sub>, at 37°C for 48 h. Non-radioactive R 5020 (500 ng/ml) was used as inducer, whereas RU 486 (50 to 5,000 ng/ml) was used either as inducer or as R 5020 antagonist. R 5020 was used because, contrary to progesterone, it is not metabolized during the 48 hour incubation (Satyaswaroop and Mortel, 1982).

### DNA Polymerase Alpha

About 1/20th volume of each endometrial homogenate was diluted to 1.2 mg protein/ml, 20 mM Tris-HCl, pH 7.6, 0.5 M KCl, 2 mM dithiothreitol, 0.5% Triton X 100, sonicated, and kept at 0°C for 60 min. Thereafter triplicate 20 µl samples were used for the assay of DNA pol activity according to Bertazzoni et al. (1977). The results were expressed in pmol thymidine triphosphate incorporated per min per mg protein.

### Other Methods

Radioimmunoassay of plasma hormones. E<sub>2</sub>, P, LH and FSH were determined according to established procedures (Brailly et al., 1981; Schaison et al., 1984).

DNA and Protein Assays. DNA was measured using the Burton (1956) procedure. Protein was measured by the Bradford (1976) Coomassie blue dye procedure using BSA as standard.

Statistical analysis. Statistical comparisons of mean values were performed using Student's t test for unpaired sample populations.

## RESULTS

### Plasma Estradiol, Progesterone, and Gonadotropins

Plasma estradiol and gonadotropins were measured before and after the treatment period. P was measured only in the last sample. RU 486 did not interfere in the progesterone radioimmunoassay. Control values for plasma E<sub>2</sub> (range 0-67 pg/ml), LH (range 6-63 mU/ml) and FSH (range 29-133 mU/ml) were within the limits for post-menopausal or castrated women. Treatment with estradiol benzoate resulted in a marked increase of plasma E<sub>2</sub> (range 263-835 pg/ml Table I.) In those patients treated with P, plasma P was elevated and within the range for the mid-secretory phase of normal menstrual cycles (7.8 to 17.6 ng/ml). LH and FSH concentrations were markedly decreased after estradiol benzoate. In those patients who received either P, RU 486 or both compounds together, the decrease of plasma gonadotropins was much more marked than after estradiol benzoate alone.

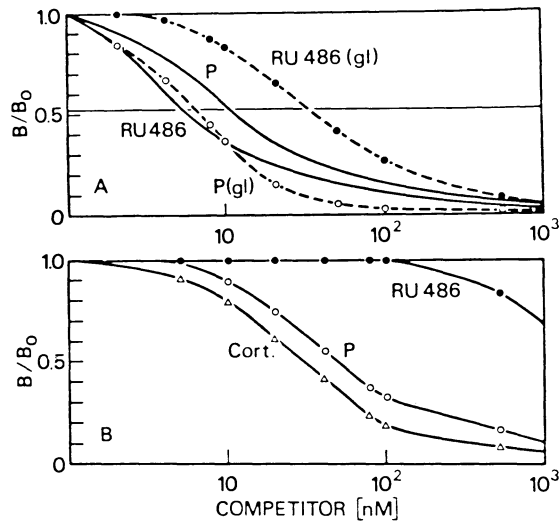


Fig. 1. Relative binding affinities of progesterone and RU 486 for human PR and plasma CBG. Top (1a): Human endometrium cytosol (2 mg protein/ml) was prepared in buffer A without or with 30% glycerol (gl). Incubation was done in a total volume of 250  $\mu$ l with 2 nM  $^3$ H-P and increasing concentrations of nonradioactive P or RU 486 (range 2-1,000 nM), in the presence of 1  $\mu$ M cortisol. Bottom (1b): Human pregnancy plasma was diluted 50 fold with buffer A, then incubated with 6 nM  $^3$ H-P and increasing concentrations of nonradioactive P, RU 486, or cortisol (cort.) at 5 to 1,000 nM concentrations.

#### Binding of RU 486 to the human progesterone receptor; Effects of glycerol.

The relative binding affinities of RU 486 and P for the PR of human endometrium were determined either indirectly by competition experiments, or directly by the measurement of equilibrium binding constants of tritiated ligands. In absence of glycerol, the  $K_d$  of RU 486 (0.8 nM) was lower than that of P (1.2 nM). The addition of 30% glycerol to the incubation buffer did not significantly change the  $K_d$  of P (1.4 nM), whereas it decreased markedly (8.7 nM, about one order of magnitude) the affinity of RU 486 for the PR.

Competition experiments also were performed with  $^3$ H-P as radioactive ligand and nonradioactive P or RU 486 as competitors (Fig. 1a). In the absence of glycerol, RU 486 had a greater affinity for the PR than did P, whereas the reverse situation was found in presence of 30% glycerol.

Contrary to P or cortisol, RU 486 had negligible binding affinity for human plasma transcortin (Fig. 1b).

#### Histology of the Endometrium

In thirteen of seventeen patients, sufficient tissue was taken to permit evaluation of hormonal stimulation by the same pathologist, who had no previous knowledge of the treatment given to individual patients. All the biopsies examined showed signs of estrogenic influence, but the glands were scanty and the glandular cells were "hypotrophic." The biopsies taken from

Table II. Effects of Progesterone and RU 486 on Progesterone Receptor in Human Endometrium

Group	Treatment		Number of patients	PR <sub>T</sub>	PR <sub>N</sub> /PR <sub>T</sub>
	Dose (mg)	Duration (days)			
1. E <sub>2</sub>	0.625	1-15	(3)	2.9 ± 0.7 <sup>a</sup>	0.12 ± 0.06 <sup>b</sup>
2. E <sub>2</sub> P <sub>2</sub>	0.625 25	1-15 10-15	(3)	2.1 ± 0.8	0.35 ± 0.17
3. E <sub>2</sub> RU 486	0.625 100	1-15 10-15	(3)	2.2 ± 0.4	0.44 ± 0.17
4. E <sub>2</sub> RU 486	0.625 200	1-15 10-15	(3)	2.1 ± 0.2	0.44 ± 0.05
5. E <sub>2</sub> P <sub>2</sub> RU 486	0.625 25 100	1-15 10-15 10-15	(2)	1.9 ± 0.2	0.36 ± 0.18
6. E <sub>2</sub> P <sub>2</sub> RU 486	0.625 25 200	1-15 10-15 10-15	(3)	1.8 ± 0.3	0.60 ± 0.06

PR concentrations were measured in cytosol and nuclei as previously reported (Bayard et al., 1978; Levy et al., 1980a) and expressed in pmol/mg DNA. PR<sub>T</sub> = total PR (cytosol + nuclei). PR<sub>N</sub>/PR<sub>T</sub> = fraction of total receptor found in nuclei. Results are expressed as mean ± SEM for each treatment group. Student's t test: (a) significantly different from groups 5 and 6 at p < 0.05. (b) significantly different from groups 2-6 at p < 0.05. Results are mean ± SEM.

patients treated with P showed persistent mitoses and mild signs of secretory transformation. They corresponded to histological dating days 15 to 16, according to the criteria of Noyes et al. (1950). The biopsies taken from patients treated with RU 486 contained very few mitotic cells; the glands and spiral arteries were more developed than with P. They corresponded to histological dating days 21 to 22, although the secretory activity was subnormal. The biopsies taken from patients treated with both P and RU 486 were characterized by persistent proliferative activity of stromal cells, with almost undetectable secretory activity.

#### Progesterone Receptor

PR concentrations were determined in the cytosol and nuclear tissue subfractions and expressed in pmol/mg DNA. They were relatively high, within the range reported by Levy et al. (1980b) for proliferative human endometrium, likely the result of the treatment with estradiol benzoate. In the treatment groups who received either P or RU 486, total PR concentrations (i.e. cytosol + nuclei) tended to decrease, although not significantly. Only in the patients who received combined P and RU 486 was the concentration of total PR significantly decreased (Table II).

P administration resulted in a significant increase of the fraction of total PR associated with endometrial nuclear fraction. RU 486 had the same effect. The largest concentration of nuclear PR was found in the biopsies of patients who received P together with the 200 mg dose of RU 486.

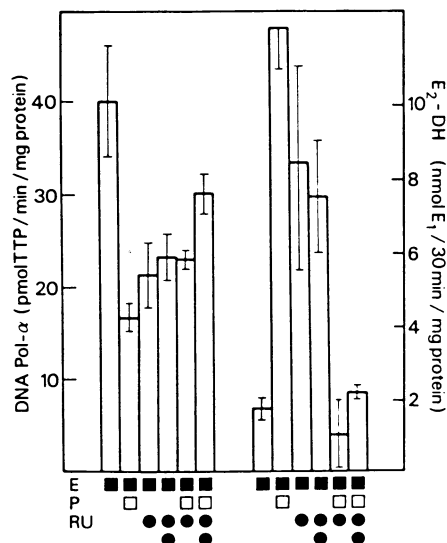


Fig. 2. Effects of estradiol benzoate, progesterone and/or Ru 486 on DNA pol and E<sub>2</sub>-DH activities. The treatment groups of patients were as indicated in Table I. DNA pol and E<sub>2</sub>-DH activities were determined in endometrial biopsies performed on the last day of treatment. E = estradiol benzoate, 0.625 mg/d for 15 d (solid squares). P = progesterone, 25 mg/d from d 9-15 (open squares). RU 486, 100 (single dot) or 200 (double dot) mg/d from d 9-15.

#### DNA Polymerase Alpha

The activity of endometrial DNA pol was markedly and significantly lower in the groups of patients who received either P or RU 486 than in those who received estradiol benzoate alone (Fig. 2).

In the group of patients treated with P and 100 mg RU 486 daily, DNA pol activity also was significantly decreased, whereas in the last group of patients treated with P and 200 mg RU 486, DNA pol activity was higher, and was not statistically different from the value in estrogen-treated patients.

#### Estradiol-Dehydrogenase

Endometrial E<sub>2</sub>-DH activity was very low in patients treated with estradiol benzoate alone (Fig. 2). P produced a 5 to 6 fold increase of E<sub>2</sub>-DH activity ( $p < 0.001$ ). RU 486, either at the 100 mg or at the 200 mg daily doses, also was a very efficient inducer of E<sub>2</sub>-DH (about 4 fold,  $p < 0.05$ ), but not as potent as P. Combined treatments with P and RU 486 gave unexpected results. E<sub>2</sub>-DH activity was not stimulated as with either compound administered alone and remained at the level of that in the estrogen treated patients.

E<sub>2</sub>-DH activity can be directly stimulated by progestagens added to the medium of human endometrium organ culture (Tseng and Gurpide, 1975). Two experiments were performed with explants of premenopausal proliferative endometrium, exposed to the synthetic progestagen R 5020 and/or RU 486 for 48 h, then E<sub>2</sub>-DH activity was assayed in these explants (Table III). Both R 5020 and RU 486 were efficient inducers of E<sub>2</sub>-DH activity. The effect of RU 486 seemed inversely correlated with dose. Again, enzyme activity was less increased by the combination of both compounds than by either one incubated separately.

Table III. Effects of RU 486 on Estradiol-Dehydrogenase Activity in Human Endometrium Organ Culture

( Experiment : Treatment : E <sub>2</sub> -DH )		
( N° : (μmol x l <sup>-1</sup> ) : (nmol E <sub>1</sub> /30 min/mg prot) )		
( : : : )		
( 1 : Control : 1.7 )		
( : R 5020 (1.5) : 9.2 )		
( : RU 486 (11.6) : 4.8 )		
( : R 5020 (1.5) : 3.1 )		
( : + RU 486(11.6) : )		
( : : : )		
( 2 : Control : 1.8 )		
( : R 5020 (1.5) : 5.4 )		
( : RU 486 (0.2) : 4.1 )		
( : RU 486 (1.2) : 2.4 )		
( : RU 486 (11.6) : 2.0 )		
( : R 5020 (1.5) : 1.3 )		
( : + RU 486(11.6) : )		
( : : : )		

Fragments of pooled proliferative endometrium biopsies, about 40 mg per incubation, were kept in organ cultures in the presence of the indicated concentrations of R 5020 and/or RU 486, at 37°C for 48 h (Satyaswaroop and Mortel, 1982). E<sub>2</sub>-DH activity was measured in the 800 g supernatants of tissue homogenates as indicated under METHODS. Each number represents a single incubation.

## DISCUSSION

RU 486 shares structural features with several synthetic progestagens. It has higher affinity than P for the human PR, thus confirming previous observations in experimental animals (Philibert et al., 1982). Glycerol has a stabilizing influence on progesterone-receptor complexes and decreases their dissociation rate constant (Feil et al., 1972; Bayard et al., 1978). Glycerol stabilizes the folded native state of proteins in solution, and may hold the ligand-binding site of PR in a more rigid conformation (Ogle, 1983). This conformation does not favor the binding of RU 486, since its K<sub>d</sub> for PR was markedly increased by glycerol.

RU 486 was effective in increasing the nuclear fraction of PR, which was at least as high in the patient groups treated with combined P and RU 486 as in patients treated with P alone. The nuclear transfer of <sup>3</sup>H-RU 486 PR complexes previously was reported, using T47D human breast cancer cells in culture (Horwitz et al., 1983).

Although the treatment of climacteric patients with estradiol benzoate resulted in high concentrations of plasma E<sub>2</sub> (at least equal to those during the preovulatory surge or early pregnancy), the duration of this treatment (15 days) was insufficient for full regrowth of the endometrium, which was reported to be "hypotrophic" by the pathologist. Nevertheless, the levels of PR concentrations and DNA pol activity in endometrial biopsies of patients treated with estradiol benzoate alone were probably the consequence of the stimulatory effects of estrogens on these parameters (King et al., 1979; Edwards et al., 1980).

In the mammalian species investigated so far, (rats and rabbits; Herrmann et al., 1982), RU 486 behaved as a P antagonist devoid of any agonist activity. Our results strongly suggest that RU 486 has some progestational activity in estrogen-primed, post-menopausal women: DNA pol activity was decreased, E<sub>2</sub>-DH activity was markedly increased, and histological examination of endometrial biopsies showed secretory

transformation of glandular epithelium. RU 486 may be considered a partial P agonist, since the response after 200 mg per day was not larger than those after 100 mg per day and probably were maximal, although lower than those elicited by 25 mg per day P. Finally RU 486 strongly potentiated the decrease of plasma LH and FSH produced by estradiol. Our results are in accordance with those obtained by Horwitz et al. (1983) on human breast cancer cells in culture; they found that progestins inhibited cell growth, and that RU 486 also had a weak, dose-dependent, growth inhibitory action.

RU 486 also was given in combination with P to investigate its antagonistic properties. The highest dose clearly counteracted the inhibition of DNA pol activity by P. Moreover, P's stimulatory effect on E<sub>2</sub>-DH activity was completely abolished by RU 486. This was unexpected, since each of these compounds given separately strongly increased this enzyme activity. Such inhibitory effect of RU 486 cannot be explained by mere competition for the binding to PR. In contrast, RU 486 was more potent than P in inhibiting gonadotrophin secretion and did not counteract the decrease of plasma gonadotrophins produced by P (Healy et al., 1983a). Acute administration of RU 486 also failed to have any effect on FSH and LH levels in cynomolgus monkeys pretreated with an estradiol-progesterone replacement regimen that mimicked the fertile menstrual cycle (Healy et al., 1983b).

The biochemical and histological results obtained from this study are compatible with P partial agonist-antagonist properties, which are more complex than those described in experimental animals. These results should be taken into account for the clinical use of this P antagonist. Further work will be needed, however, before extending this postmenopausal model to the women of reproductive age.

#### ACKNOWLEDGMENTS

We thank Roussel-Uclaf, Romainville, for providing us with radioactive and non-radioactive RU 486; and J. C. Lambert, L. Outin and F. Boussac for their help in the preparation of the original manuscript. The material included in this report is reproduced with the kind permission of the editor of the Journal of Clinical Endocrinology and Metabolism.

#### REFERENCES

- Bayard, E., Damilano, S., Robel, P., and Baulieu, E. E., 1978, Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium, J. Clin. Endocrinol. Metab., 46:635.
- Bertazzoni, U., Scovassi, A., and Brun, G., 1977, Chick embryo DNA polymerase, Eur. J. Biochem., 81:237.
- Bradford, M. M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of proteins utilising the principle of protein-dye-binding, Anal. Biochem., 72:248.
- Brailly, S., Gougeon, A., Milgrom, E., Bomsel-Helmreich, O., and Papiernik, E., 1981, Androgens and progesterone in the human ovarian follicle: differences in the evolution of the preovulatory, healthy non ovulatory and atretic follicles, J. Clin. Endocrinol. Metab., 53:128.
- Burton, K., 1956, A study of the conditions and the mechanism of the diphenylamine reaction for the colorimetric determination of DNA, Biochem. J., 62:315.
- Edwards, D., Murthy, S., and McGuire, W., 1980, Effects of estrogen and antiestrogen on DNA polymerase in human breast cancer, Cancer Res., 40:1722.

- Feil, P. D., Glasser, S. R., Toft, D. O., and O'Malley, B. W., 1972, Progesterone binding in the mouse and rat uterus, Endocrinology, 90:1071.
- George, M., Lagoguey, M., Reinberg, A., Baulieu, E. E., and Schaison, G., 1983, Action d'un antiprogesterone (RU 486) chez la femme normale, Program of the 4th Annual meeting of the French Endocrine Society, Marseille, 44:178, abstract no. 64.
- Healy, D. L., Baulieu, E. E., and Hodgen, G. D., 1983a, Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships, and hormonal effects, Fertil. Steril., 40:253.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen, G. D., 1983b, Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:683.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E.E., 1982, Effet d'un stéroid antiprogesterone chez la femme. Interruption du cycle menstruel et de la grossesse au début, C. R. Acad. Sci., Paris, 294:933.
- Horwitz, K. B., Freidenberg, G. R., Sheridan, R. L., Alexander, P. S., 1983, Progestins and the antiprogesterone RU 38486: effects on progesterone receptors and on proliferation of human breast cancer cells. Program of the 65th Annual meeting of the Endocrine Society, San Antonio, TX, abstract No. 581.
- King, R. J. B., Whitehead, M. I., Campbell, S., and Minardi, J., 1979, Effects of estrogen and progestin treatments on endometria from post-menopausal women, Cancer Res., 39:1094.
- Levy, C., Eychenne, B., and Robel, P., 1980a, Assay of nuclear estradiol receptors by exchange on glass fiber filters, Biochem. Biophys. Acta, 630:301.
- Levy C., Robel, P., Gautray, J. P., De Brux, J., Verma, U., Descomps, B., and Baulieu, E. E., 1980b, Estradiol and progesterone receptors in human endometrium: normal and abnormal menstrual cycles and early pregnancy, Amer. J. Obstet. Gynecol., 136:646.
- Noyes, R. W., Hertig, A., and Rock, J., 1950, Dating the endometrial biopsy, Fertil. Steril., 1:3.
- Ogle, T. F., 1983, Action of glycerol and sodium molybdate in stabilization of the progesterone receptor from rat trophoblast, J. Biol. Chem., 158:4982.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982, RU 38486 a new lead for steroid antihormones. Program of the 64th Annual Meeting of the Endocrine Society, San Francisco, abstract no. 668.
- Robel, P., Gravanis, A., Roger-Jallais, L., Catelli, M. G., Binart, N., George, M., Laval, C., and Baulieu, E. E., 1984, Female sex steroid receptors in post menopausal endometrial carcinoma. Biochemical responses to antiestrogen and progestin, in: "Hormones and Cancer," volume 142, E. Gurpide, R. Calandra, C. Levy, and R. J. Soto, eds., Alan R. Liss, Inc., New York, pp. 167-80.
- Rosenthal, H. E., 1967, A graphic method for the determination and presentation of binding parameters in a complex system, Anal. Biochem., 20:526.
- Satyaswaroop, G., and Mortel, R., 1982, Failure of progestins to induce estradiol dehydrogenase activity in endometrial carcinoma in vitro, Cancer Res., 42:1322.
- Schaison, G., 1983, Contraception et galactorrhée. Aspects biologiques, in: "Contraception et Sein," R. Renaud and B. Gairard, eds., Masson, Paris, 70-6.

- Schaison, G., Brailly, S., Vuagnat, P., Bouchard, P., and Milgrom, E., 1984, Absence of a direct inhibitory effect on the GnRH agonist Buserelin on testicular steroidogenesis in men, J. Clin. Endocrinol. Metab., 58:885.
- Smith, E., Smith, G., Toft, O., Neergaard, R., Burrows, P., and O'Malley, B. W., 1974, Binding of steroids to progesterone receptor proteins in chick oviduct and human uterus, J. Biol. Chem., 249:5924.
- Tseng, L., and Gurpide, E., 1975, Induction of human endometrial estradiol dehydrogenase by progestins, Endocrinology, 94:419.
- Whitehead, M. E., Townsend, P. T., Pryse-Davies, J., Ryder, T. A., King, R. J. B., 1981, Effects of estrogens and progestins on the biochemistry and morphology of the post-menopausal endometrium, New Engl. J. Med., 305:1599.

## RU 486: A FULL PROGESTIN ANTAGONIST IN HUMAN BREAST CANCER CELL LINES

Henri Rochefort and Dany Chalbos

Unité d'Endocrinologie Cellulaire et Moléculaire  
U 148 Inserm  
34100 Montpellier, France

### SUMMARY

To evaluate agonist or antagonist activities of the progestin analog RU 486, we have assayed proteins that are specifically induced by progestin in the progesterone receptor positive human breast cancer cell lines MCF7 and T47D. After the labelling of newly synthesized proteins by <sup>35</sup>S Methionine, followed by SDS polyacrylamide gel electrophoresis, two proteins can be defined with molecular weights of 48,000 dalton (released by T47D cells) and 250,000 dalton (in MCF7 and T47D cell extracts). The biosynthesis of these proteins is specifically increased by progestins via the progesterone receptor. We show here that RU 486 alone has no agonist activity on the production of these proteins, while it inhibits their induction when added to R5020. We conclude by these simple criteria that RU 486 behaves as a full progestin antagonist in human breast cancer cell lines.

Specific markers of response to progesterone are required in order to estimate the progestin and antiprogestin activities of a drug. General responses to a hormone, such as a general modulation of protein synthesis and of cell proliferation, cannot be used as specific markers, because they can be triggered by several classes of hormones (Tomkins et al., 1966). In contrast, specific proteins situated by a single class of hormones, or more precisely by one class of activated hormone receptors, are valuable as markers for defining the pharmacological activity of a drug (Rochefort & Garcia, 1984).

We will first show evidence that two proteins recently found by our laboratory in human breast cancer cell lines are specifically regulated by progestins. We will then show how these proteins have been used to define the degree of agonist and antagonist activities of RU 486.

### TWO PROGESTIN-INDUCED PROTEINS IN HUMAN BREAST CANCER CELL LINES

Hormone-responsive human metastatic breast cancer cell lines (MCF7, T47D, ZR75-1...) offer many advantages in demonstrating hormone-specifically regulated proteins and RNA in humans, and in specifying the degree of agonist and antagonist activity of a drug. They contain receptors for estrogen, progesterone, and androgens. The T47D cell line, isolated from a pleural metastasis of a mammary ductal carcinoma (Keydar et al., 1977), is particularly suitable for studying progestin and antiprogestin activities, since it contains very high concentrations of the progesterone receptor (Horwitz et al., 1982), even in the absence of added

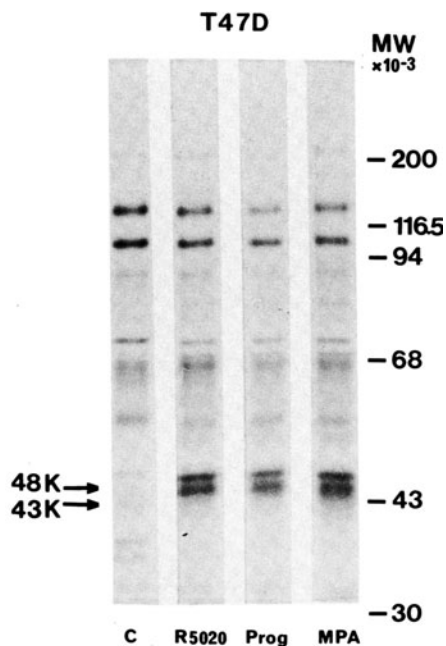


Fig. 1. Effects of progestins on proteins released by T<sub>47</sub>D cells. T<sub>47</sub>D cells. (clone 11) were withdrawn from steroids by incubation in medium + 10% fetal calf serum stripped with charcoal (FCS-DCC) for 3 days, then with 3% FCS-DCC for 2 days. They were plated in 96 microwell-dishes (10,000 cells per well) in medium + 3% FCS-DCC without added insulin. After two days, the cells were incubated for five days without hormone (C) or with R 5020 (0.1nM), progesterone (Prog. 10nM), and medroxy progesterone acetate (MPA, 0.1 nM). Media were changed every 12 hours in the case of cells incubated with progesterone and every two days in the case of other cells. The cells were then labeled with <sup>35</sup>S methionine for 6 hours, and 10 ul aliquots were precipitated with trichloroacetic acid. The released proteins (5,000 trichloroacetic acid precipitable cpm) were loaded onto a 12% polyacrylamide gel, and labeled proteins were revealed by flurography. The molecular weights of the hormone-regulated proteins (48 K and 43 K) were estimated according to their mobilities relative to standard proteins of known molecular weight indicated on the right side. Adapted from the J. Biol. Chem. (1984), by permission of the editor.

estrogens. The progesterone receptor (RP) binding sites are regulated by progestins in these cells, where both the activation and processing of this receptor have been described (Horwitz et al., 1983), as well as other metabolic and morphological responses (Rochefort & Chalbos, 1984). Recently, by labeling newly synthesized proteins with <sup>35</sup>S methionine and by analyzing them with SDS gel electrophoresis, we have described two progestin-regulated proteins, which were defined according to their molecular weight under denaturing conditions. One of these proteins was found in a medium conditioned by T<sub>47</sub>D cells and has a molecular weight of 48,000 (48K) (Chalbos and Rochefort, 1984a) (Fig. 1). The other was found in cell extracts of both MCF7 and T<sub>47</sub>D cells and has a molecular weight of 250,000 (250K) (Chalbos & Rochefort, 1984b). The function of these proteins is still unknown, but their hormone specificity gives them great potential for defining progestin agonist and antagonist activities in vitro.

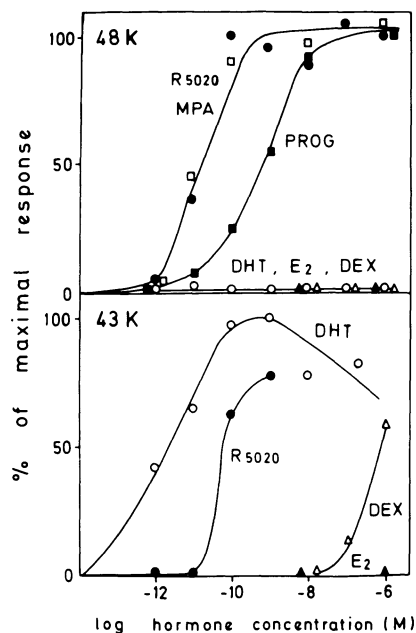


Fig. 2. Dose-response curves of the hormonal regulation of progestin (48 K) and androgen (43 K) specific proteins released by T<sub>47</sub> cells. The T<sub>47</sub> cells were withdrawn from steroids and plated in microwells, as described in Fig. 1. They were then treated for 5 days with the indicated concentrations of R 5020 (solid circles), medroxyprogesterone acetate (MPA, open squares), progesterone (PROG, closed squares), estradiol (E<sub>2</sub>, closed triangles), 5 alpha-dihydrotestosterone (DHT, open circles), or dexamethasone (DEX, open triangles). After <sup>35</sup>S methionine labeling, proteins released into the medium (5,000 cpm) were analyzed in SDS-12% polyacrylamide gel. The fluorographs were then scanned using a Vernon densitometer. The amounts of 48 K and 43 K proteins were estimated from the traces and expressed as a percentage of the response obtained in each experiment with 1 nM R 5020 for the 48 K protein, and 1 nM DHT for the 43 K protein (maximal responses). Reproduced by permission from Mol. Cell. Endocrinol. (1984).

#### Evidence for a 48 K Progestin-Regulated Protein Released by T<sub>47</sub>D Cells (Chalbos & Rochefort, 1984a)

When T<sub>47</sub>D cells were treated with increasing concentrations of progestins (R5020, medroxyprogesterone acetate or progesterone), proteins labeled by <sup>35</sup>S methionine and released into the medium were altered. In addition to a general decrease in the amount of proteins released into the medium (Chalbos & Rochefort, 1984a; Bignon et al., 1983), the amounts of certain proteins with molecular weights of 48 K, 43 K, (Fig. 1) and 22 K (not shown) increased markedly. The production of the 48 K protein appeared to be very specifically stimulated by progestins via the progesterone receptor (RP). In fact, the 48 K protein was not regulated in the RP-negative cell line BT 20; moreover, in the T<sub>47</sub>cell line, other classes of steroids (androgens, estrogens, and glucocorticoids) had no effect on the production of this protein. The dose-response curve of the hormonal

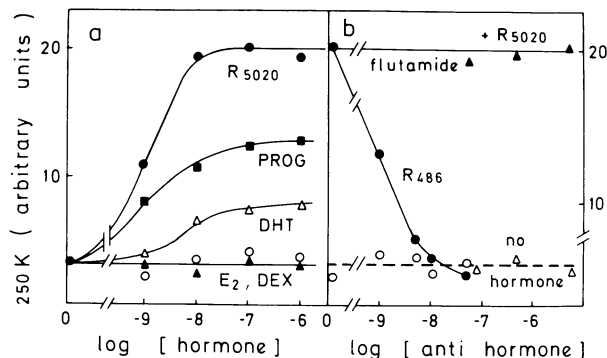


Fig. 3. a) The progestin-regulated 250 K protein. Cells were treated for 4 days with various concentrations of R 5020 (closed circles), progesterone (open squares), 5  $\alpha$ -dihydrotestosterone: DHT (open triangles), dexamethasone: DEX (open circles) or estradiol: E<sub>2</sub> (closed triangles). Media were changed twice a day in the case of progesterone and every two days in the case of the other hormones. After <sup>35</sup>S methionine labeling, the cellular proteins were analyzed on SDS polyacrylamide gel. Proteins were visualized by autoradiography. The films were scanned and the amount of labeled 250 K protein was estimated in arbitrary units from the traces. b) Effect of anti-hormones on the induction of the 250 K protein. Cells were treated for 5 days with the indicated concentration of RU 486 (circles) or flutamide (triangles) in the absence (open symbols) or presence (closed symbols) of 1nM R 5020. The cells were then labeled with <sup>35</sup>S methionine and the amount of the 250 K protein was evaluated as in Fig. 2. Reproduced by permission of the Editor of Biochem. Biophys. Res. Commun. (1984).

regulation of the 48 K protein (Fig. 2) was correlated with the progressive saturation of the RP by R5020 (Raynaud, 1977). In contrast, the 43 K protein appeared to be an androgen-regulated protein, since it was stimulated by lower concentrations of androgens (Fig. 2) and since it was inhibited by flutamide, which had no effect on the 48K protein. These results indicate that the 48 K protein could be used to assay the progestin and antiprogestin activities of a hormone or drug, and the 43 K and 22 K proteins to assay the androgen and antiandrogen activities.

#### Evidence for a 250 K Progestin Regulated Protein in MCF7 and T47D Cells (Chalbos and Rochefort, 1984b)

When MCF7 cells or T47D (clone 11) cells were withdrawn from steroids in medium containing charcoal stripped FCS, and subsequently treated by R 5020 (10nM), the pattern of cellular proteins labeled by <sup>35</sup>S methionine and extracted was modified compared to that of control cells. There was a marked increase in a protein migrating at approximately 250,000 daltons (Fig. 3). This protein was also seen after silver staining of the electrophoregram, indicating its high cellular concentration (6% of the total cellular proteins). Figure 3a shows the steroid specificity of induction of the 250 K protein. Progesterone was less active than R 5020, but its efficacy could be increased by changing the medium more frequently. This is consistent with the extensive metabolism of progesterone in these

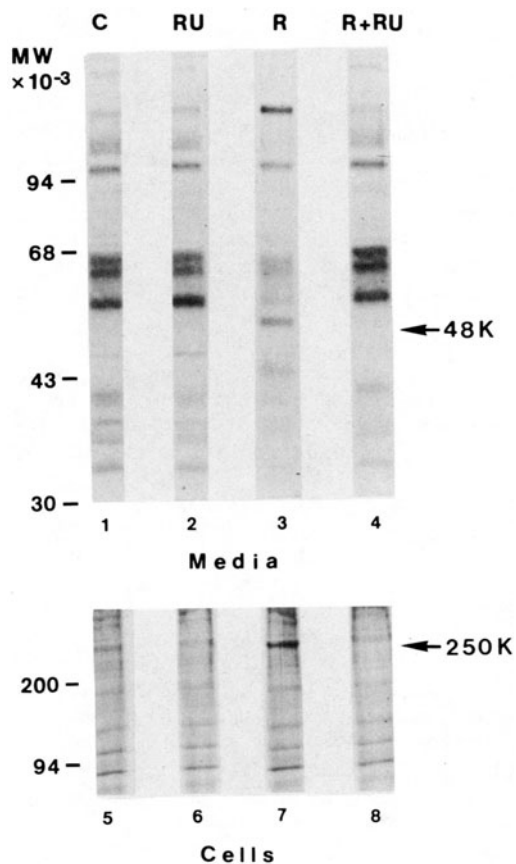


Fig. 4. Effect of RU 486 on Two Progesterin-Specific Proteins: SDS Polyacrylamide Gel. T<sub>47</sub> cells (clone 11) were withdrawn from steroids and plated in microwells (20,000 cells/wells), as described in Fig 1. After two days, they were incubated for 4 days, either with vehicle (C: 1, 5), 10nM RU 486 (RU: 2, 6), 1 nM R 5020 (R: 3,7) or 10 nM RU 486 plus 1 nM R 5020 (R+RU: 4, 8). The cells were then labeled with <sup>35</sup>S methionine. Equal amounts of TCA-precipitable methionine-labeled protein released into the media (5,000 cpm 1 to 4) and present in the cells (150,000 cpm 5 to 8) were analyzed on SDS 12% polyacrylamide gels and labeled proteins were revealed by fluorography. Migrations of standard proteins are indicated on the left and that of 48 K and 250 K proteins on the right. Reproduced by permission of the Editor of J. Clin. Endocrin. Met. (1985).

cells. Other classes of steroids (estradiol and dexamethasone) were unable to stimulate the 250 K protein. The androgen 5 alpha dihydrotestosterone was partially active, but at higher concentrations than R 5020, suggesting that its effect was mediated by the progesterone receptor. The concentration of R 5020 inducing 50 % of maximal response in MCF<sub>7</sub> cells (0.5 to 1 nM) was close to the K<sub>D</sub> of R 5020 for the progesterone receptor. The antiprogestin RU 486 completely inhibited the induction of the 250 K protein by R 5020, whereas the anti-androgen flutamide was ineffective (Fig. 3b).

## RU 486 IS A FULL PROGESTIN ANTAGONIST IN BREAST CANCER CELL LINES

RU 486 is an analogue of progestin that has a high affinity for the progesterone and glucocorticoid receptor (Philibert et al., 1982) and is being used as an anti-progestin agent (Hermann et al., 1982). Its pharmacological activity has been defined mostly *in vivo* using complex parameters to define its progestin and antiprogestin activities (Clauberg test, etc.). These systems do not preclude indirect effects of the drug, and the complex responses measured (tissue proliferation, uterine weight, histological and cytological modifications of endometrium) do not always prove to be specifically and exclusively triggered by progestins (Chalbos, et al., 1984; Rochefort & Chalbos, 1984).

Using the two specific progestin regulated proteins (48 K and 250 K) as markers of progestin activity, we have studied the *in vitro* effect of RU 486 alone, in order to determine its possible progestin activity and its effect when added to R 5020 in order to define its antagonist activity.

Figure 5 shows that RU 486 alone was unable, at any concentration, to increase the synthesis of the 48 K or 250 K protein. It also prevented the stimulation of these two proteins by R 5020 (Fig. 4). Therefore, when the biosynthesis of these two specific proteins (one secreted, the other cellular) was studied *in vitro*, RU 486 was clearly a full progestin antagonist. Other proteins regulated by androgen (43 K, 22 K) were also inhibited by RU 486. However, as mentioned above, these proteins are also inhibited by flutamide and this seems to be the consequence of the activation of the androgen receptor rather than that of the progesterone receptor. It is known that R 5020 and several progestins can also bind to the androgen receptor and possibly trigger androgenic responses. The 43 K and 22 K proteins are stimulated by R 5020 and inhibited by RU 486, which indicates that RU 486 also displays antiandrogenic activity. We have not defined a specific marker of response to glucocorticoids in these cells and were therefore unable to study the antiglucocorticoid activity of the drug in this system.

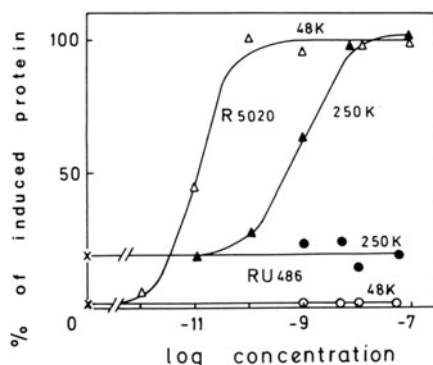


Fig. 5. Effect of RU 486 on two progestin-specific proteins: dose-response curves. T<sub>47</sub>D cells were incubated with vehicle (x) or the indicated concentrations of R 5020 (triangles) or RU 486 (circles). After <sup>35</sup>S methionine incorporation, proteins released into the media and cellular proteins were analyzed on SDS 12% polyacrylamide gels. The amounts of cellular 250 K protein (closed symbols) and of released 48 K protein (open symbols) are expressed as percentages of the maximal response obtained with R 5020.

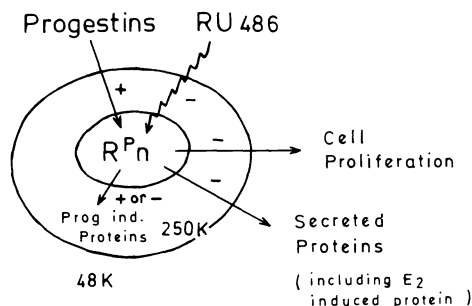


Fig. 6. Summary of the 3 series of effects of RU 486 on progestin regulated responses in human breast cancer cell Lines. The antagonist RU 486 totally inhibits the production of the two progestin-regulated (48 K and 250 K). Moreover, as in the case of R 5020 and Tamoxifen, RU 486 inhibits the total production of released proteins, including that of estrogen-regulated proteins in the culture medium, and subsequently inhibits cell growth. These effects appear to be mediated by the progesterone receptor ( $R^{Pn}$ ) (Bardon et al., 1985). The relationship between these three series of effects is under current study.

## CONCLUSIONS

It is clear from the assays of these two well-defined progestin-regulated proteins, that RU 486 alone displays no agonist activity and is therefore a full antiprogestin, even though it binds with a high affinity to the progesterone receptor (Philibert et al., 1982). The concept of a partial agonist activity described after *in vivo* administration (Schaison et al., this volume) is based on criteria that are less clearly defined biochemically (i.e. histology or cytology of the endometrium). Moreover, the action of RU 486 *in vivo* may involve several targets and indirect responses, and it is conceivable that some of its activity may be partially agonist. In these *in vitro* studies, we have not found this partial agonist activity, and have confirmed, in human cells, that this steroid analogue is a full progestin antagonist when two specifically stimulated proteins are assayed. A comparison can be made with Tamoxifen, which displays some agonist activity *in vivo* (in the vagina for instance), but is a full estrogen antagonist in the regulation of the 52 K protein (Westley & Rochefort, 1980). It remains a partial agonist for the progesterone receptor in the same MCFG7 cells (Horwitz et al., 1978). The relationship between the cell growth inhibitory effects of progestin and RU 486 (Bardon et al., 1985) (Fig. 6), the regulation of the two progestin-specific proteins, and the inhibition of estrogen-regulated proteins (possible decreased production of growth factors), is currently under study in our laboratory. It is expected that the use of human hormone-responsive cell lines and of specific markers regulated by sex steroid hormones will allow us to define the pharmacological activities of other drugs and antagonists.

## ACKNOWLEDGMENTS

We would like to thank C. Rougeot and C. Prebois for their excellent technical help and E. Barrie for her skillful preparation of the original manuscript. We are grateful to Drs. Sakiz and Philibert (Roussel

Laboratories) and to Dr. E. E. Baulieu for providing us with RU 486 and R 5020.

## REFERENCES

- Bardon, S., Vignon, F., Chalbos, D., and Rochefort, H., 1985, RU 486, a progestin and glucocorticoid antagonist inhibits the growth of breast cancer cells via the progesterone receptor, J. Clin. Endocrin. Metab., in press.
- Chalbos, D., and Rochefort, H., 1984a, Dual effects of the progestin R 5020 on proteins released by the T47D human breast cancer cells, J. Biol. Chem., 259:1231.
- Chalbos, D., and Rochefort, H., 1984b, A 250-kilodalton cellular protein is induced by progestins in two human breast cancer cell lines MCF7 and T47D, Biochem. Biophys. Res. Commun., 121:421.
- Chalbos, D., Bardon, S., Vignon, F., and Rochefort, H., 1984, Use of hormon responsive cell lines to study the mechanism of action of progestin an anti-progestin, in: "Medical Management of Endometriosis," T. Ojasoo e al., eds., Raven Press, in press.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effet d'un stéroïde anti-progestérone chez l femme: Interruption du cycle menstruel et de la grossesse au debut, C. R. Acad. Sc. Paris. 294:933.
- Horwitz, K. B., Kosedi, Y., and McGuire, W. L., 1978, Estrogen control of progesterone receptor in human breast cancer: Role of estradiol and antiestrogen, Endocrinology, 103:1742.
- Horwitz, K. B., Mockus, M. B., and Lessey, B. A., 1982, Variant T47D human breast cancer cells with high progesterone receptors levels despite estrogen and antiestrogen resistance, Cell, 28:633.
- Horwitz, K. B., Mockus, M. B., Pike, A. W., Fennessy, P. V., and Sheridan, R. L., 1983, Progesterone receptor replenishment in T47D human breast cancer cells: Roles of protein synthesis and hormone metabolism, J. Biol. Chem., 258:7603.
- Keydar, I., Chen, L., Karby, S., Weiss, F. R., Delarea, J., Radu, M., Chaitcik, S., and Brenner, H. J., 1979, Establishment and characterization of a cell line of human breast carcinoma origin, Eur. J. Cancer, 15:659.
- Philibert, D., Deraedt, R., Tournemine, C., Mary, I., and Teutsch, G., 1982 RU 38486: A potent antiprogesterone, J. Steroid. Biochem., 17:IXVIII.
- Raynaud, J. P., 1977, R 5020, a tag for the progestin receptor, in: "Progress in Cancer Research and Therapy, Vol. 4: Progesterone Receptors in Normal and Neoplastic Tissues," W. L. McGuire, J. P. Raynaud, and E. E. Baulieu, eds., Raven Press, New York.
- Rochefort, H., and Garcia, M., 1984, The estrogenic and antiestrogenic activities of androgens in female target tissues, Pharmacol. Ther., 23:193.
- Rochefort, H., and Chalbos, D., 1984, Progestin specific markers in human cell lines: Biological and pharmacological applications, Mol. Cell. Endocrinol., 36:3.
- Schaison et al., this volume.
- Tomkins, G. M., Thompson, E. B., Hayashi, S., Gelehrter, T., Granner, D., and Peterkofsky, B., 1966, Tyrosine aminotransferase induction in mammalian cells in tissue culture, Cold Spring Harbor Symp. Quant. Biol., 31-349.
- Vignon, F., Bardon, S., Chalbos, D., and Rochefort, H., 1983, Antiestrogeni effect of R 5020, a synthetic progestin in human breast cancer cells i culture, J. Clin. Endocrin. Metab., 56:1124.
- Westley, B., and Rochefort, H., 1980, A secreted glycoprotein induced by estrogen in human breast cancer cell lines, Cell, 20:352.

EFFECT OF RU 486 ON THE PITUITARY-ADRENAL AXIS IN THE DOG

Irving M. Spitz<sup>1</sup>, Charles E. Wade<sup>2</sup>, Dorothy T. Krieger<sup>3</sup>,  
Pekka Lahteenmaki<sup>4</sup>, and C. Wayne Bardin<sup>1</sup>,

<sup>1</sup>Center for Biomedical Research, Population Council  
New York, N.Y.

<sup>2</sup>Department of Clinical Investigation  
Letterman Army Medical Center  
San Francisco, California

<sup>3</sup>Division of Endocrinology and Metabolism  
Mount Sinai School of Medicine, New York, N.Y.

<sup>4</sup>Steroid Research Laboratory, Dept. of Medical Chemistry  
University of Helsinki, Helsinki, Finland

ABSTRACT

The aim of this study was to determine the effect of single and multiple doses of RU 486 on adrenal function in dogs. Single doses of RU 486 (10 mg/kg) by the oral or subcutaneous route produced no changes in cortisol levels. In the multiple dose study, low (5 mg/kg), intermediate (20 mg/kg) and high (50 mg/kg) doses of RU 486 were administered for 10 days to 7 female mongrel dogs at intervals of 4 weeks. There were no changes in ACTH or cortisol with the low dose. However, both ACTH and cortisol increased with the intermediate and high doses. The rise of cortisol preceded the increase in ACTH and cortisol levels remained persistently elevated for a few days following cessation of therapy. The cortisol increment observed with the intermediate and high dose of RU 486 was similar to that seen with insulin hypoglycemia.

There were no changes in plasma aldosterone with the low or intermediate doses but aldosterone levels did increase with the high dose of RU 486. During RU 486 administration there was a dose dependent increase in bodyweight associated with reduction in hematocrit and serum proteins. This was not accompanied by any change in serum sodium, potassium or osmolarity. These findings suggest the presence of fluid retention and dilutional hypervolemia. All dogs maintained the ability to excrete a water load implying intact adrenal action at the renal tubule.

Analysis of the response to the high dose of RU 486 revealed two distinct groups: The responders comprised three dogs who had increases in ACTH, cortisol and bodyweight with reduction in serum protein and minimum

change in aldosterone. In contrast, the remaining 4 dogs constituted the nonresponders and had minimal changes in ACTH, cortisol, serum proteins and hematocrit but a significant rise in aldosterone. Circulating drug levels were similar in the two groups. The same 3 responder dogs also had greater cortisol increments with the intermediate dose of RU 486 whereas the 4 nonresponders had lower cortisol rises. In contrast to RU 486, all dogs demonstrated a similar ACTH and cortisol response to insulin induced hypoglycemia. These findings suggest differential sensitivity of the dogs to the antiglucocorticoid action of RU 486. The observations also suggest that RU 486 may have an antimineralocorticoid effect especially in high doses.

## INTRODUCTION

The 19 norsteroid derivative, (17 $\beta$ -hydroxy-11 $\beta$ -(4-dimethylaminophenyl)-17a-(1-propynyl)estra-4,9 dien-3-one; RU 486) has potent antiprogestational and antiglucocorticoid activities (Philibert et al., 1981; Proulx-Ferland et al., 1982; Philibert et al., 1982a,b). Preliminary studies in humans show that this compound may have the potential of being an effective abortifacient (Herrmann et al., 1982). In view of its antiglucocorticoid properties, it was important to ascertain whether functional adrenal insufficiency was produced by this drug even though there is evidence suggesting that higher doses are required to produce antiglucocorticoid and antiprogestational effects (Gaillard et al., 1984).

The aim of this study was therefore to assess the effect of progressively increasing doses of RU 486 on adrenal function in conscious dogs. Dogs were selected since they can be trained to withstand repeated blood sampling without stress (Keller-Wood et al., 1982). RU 486 was administered for ten days to these animals since it has been shown that adrenalectomized dogs only demonstrate objective evidence of adrenal cortical insufficiency 5-9 days after withdrawal of dexamethasone (Boykin et al., 1978). The lowest dose administered has been shown to have antiprogestational activity in normal women (Herrmann et al., 1982; Gaillard et al., 1984).

### The Dog as Model for Studies of Adrenal Function

Seven female mongrel dogs weighing 21 to 30 kg were conditioned to stay in the laboratory in a Pavlov sling. Previous studies have shown that dogs trained in this way do not increase their ACTH or cortisol secretion at the time of venepuncture (Keller-Wood et al., 1982). Initially all seven dogs were given a single dose of RU 486 (10 mg/kg) by the oral route at 6 am. Twelve serial blood samples were taken up to 36 hours. Several months later, two dogs were given a subcutaneous injection of RU 486 in the same dose and 15 blood samples were taken over 24 hours.

In subsequent studies, RU 486 was given daily by the oral route for ten days: 5.0 mg/kg/day (low dose), 20 mg/kg/day (intermediate dose), and 50 mg/kg/day (high dose) in gelatin capsules in two divided doses at 6 am and 6 pm. The animals were also studied during a control period when they received placebo gelatin capsules twice daily for five days. All animals underwent all of the four experimental manipulations.

To assess pituitary adrenal responsiveness, each animal also underwent an insulin tolerance test in the fasting state. Following cannulation of saphenous vein, two control blood samples were taken. Insulin (0.5 U/kg) was then given intravenously and blood samples were taken serially for two hours. An interval of four weeks elapsed between the different tests.

## THE EFFECTS OF A SINGLE DOSE OF RU 486

The serum cortisol levels ranged from 1-2.5 ug/100 ml in the untreated dog and were not changed following either oral or subcutaneous administration of RU 486 at a dose of 10 mg/kg. This is at variance with observations in the ovariectomized monkey receiving estradiol and progesterone where the same dose given by the parenteral route produced transient 2-4 fold increases in ACTH, cortisol and ADH levels while reducing basal hyperprolactinemia (Healy et al., 1983). The reason for this discrepancy is unknown but is presumably related to differences in hormonal status, in absorption and/or metabolism of the compound in the two species. Following the oral dose, mean peak RU 486 levels in the dog were reached after four hours and the half disappearance time was approximately two hours (Fig. 1). For comparison, Figure 1 also depicts results in four normal women who received a similar total dose in the luteal phase. Circulating levels of the drug were higher and the half time for disappearance was approximately 24 hours. These results indicate that the drug may be metabolized differently in canine and primate species.

## THE EFFECT OF RU 486 ADMINISTRATION FOR 10 DAYS

### Changes in the Pituitary-Adrenal Axis

The results in Figure 2 show the ACTH and cortisol responses on the first, fifth, eighth and tenth day following treatment with the low, intermediate and high doses of RU 486. Control values were given for the first and fifth days only. No changes in ACTH, or cortisol were apparent in the control study and with the low dose of RU 486. The intermediate and high dose, however, produced significant increases in cortisol levels on the 5th, 8th and 10th day. ACTH levels were significantly elevated by the 8th and 10th day. For both ACTH and cortisol, there were no differences in response between the two higher doses of RU 486. These results show that these two doses of RU 486 increased cortisol levels at times when ACTH had apparently not changed. This suggests that the adrenal gland responds to minimal increases in ACTH which are not detected by the infrequent sampling intervals; alternatively the sensitivity of the ACTH assay might be too low to detect these changes. Similar observations have been made in the dog by others (Keller-Wood et al., 1981).

In order to ascertain the effect of RU 486 administration on the diurnal rhythm of ACTH and cortisol secretion, blood samples were taken at 6 am and 6 pm immediately prior to drug administration in dogs receiving the high and low dose of RU 486. No circadian rhythm of either hormone was present with either the high (Fig. 3) or the low dose.

The recovery of pituitary-adrenal function is illustrated in Figure 3 where ACTH and cortisol levels are shown for the week after cessation of therapy. ACTH but not cortisol levels had returned to baseline during this week of observation.

In order to relate the cortisol response to RU 486 to another well-defined stimulus for cortisol secretion, the dogs were challenged with insulin induced hypoglycemia. These results are shown in Figure 4. Following insulin administration, maximum hypoglycemia was attained by 15 mins and there was a brisk ACTH and cortisol response confirming previous observations (Keller-Wood et al., 1983). The maximum cortisol levels observed following administration of the intermediate and high dose of RU 486 were equivalent to those noted after insulin induced hypoglycemia (Keller-Wood et al., 1981). These cortisol values are similar to those attained during maximum adrenal stimulation with exogenous ACTH (Keller-Wood et al., 1981).

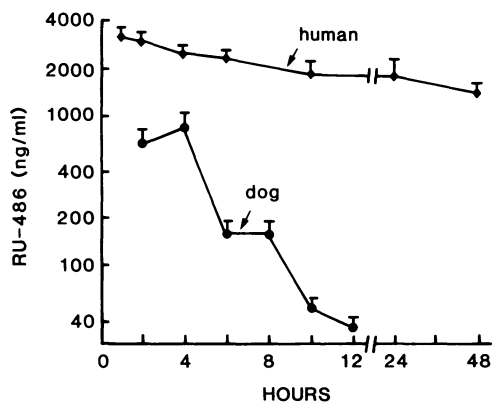


Fig. 1. The immunoassayable plasma RU 486 levels (drug + metabolites) following a single oral dose of RU 486 in seven dogs (10 mg/kg). Shown for comparison are the mean ( $\pm$  SEM) RU 486 plasma levels following a 600 mg total oral dose (approximately 10 mg/kg) in 4 normal women in the luteal phase. Values in ordinate are expressed on a logarithmic scale. The antiserum, cold standard and (6,  $^3\text{H}$ ) RU 486 were from Roussel Uclaf.

#### Metabolic Changes During Long-Term Therapy:

The results in Figure 5 show the serum sodium, potassium and osmolality in dogs receiving the high dose of RU 486. No changes in these parameters were apparent during and following drug administration. Similarly no changes occurred with the lower doses (data not shown).

The changes in body weight, hematocrit and serum protein during and one week after treatment with low and high doses of RU 486 are shown in figures 6a and 6b. During both treatment periods body weight increased but this was only significant with the high dose. Significant decreases in serum protein and hematocrit occurred with the high dose, although a downward trend was also evident with the low dose. Lowest values for hematocrit and serum protein concentrations were on days 12 to 14 i.e., a few days following cessation of therapy, and values gradually returned to baseline by day 17. Body weight was still increased during the entire week after cessation of therapy. All animals tolerated the high dose of RU 486 (50 mg/kg) without difficulty except dog number 22 (Fig. 6b) in which vomiting and diarrhea were associated with decreased body weight. This dog recovered following cessation of RU 486 administration.

The increase in body weight associated with the reduction in hematocrit and in serum protein suggests the presence of fluid retention with dilutional hypervolemia. Careful tolerance studies have shown that in untreated humans with cortisol deficiency fluid retention also occurs (Mendelsohn and Pearson, 1955). In the adrenalectomized human, hyponatremia is often evident (Lipsett and Pearson, 1956). In contrast dogs treated with large doses of RU 486 retained fluids isosmotically (Fig. 5). This latter observation suggests that the large dose of drug did not produce functional glucocorticoid deficiency. This possibility was nonetheless tested using a water load.

Free water excretion by the kidney is markedly reduced in humans and dogs with cortisol deficiency. In fact, impaired water excretion was used

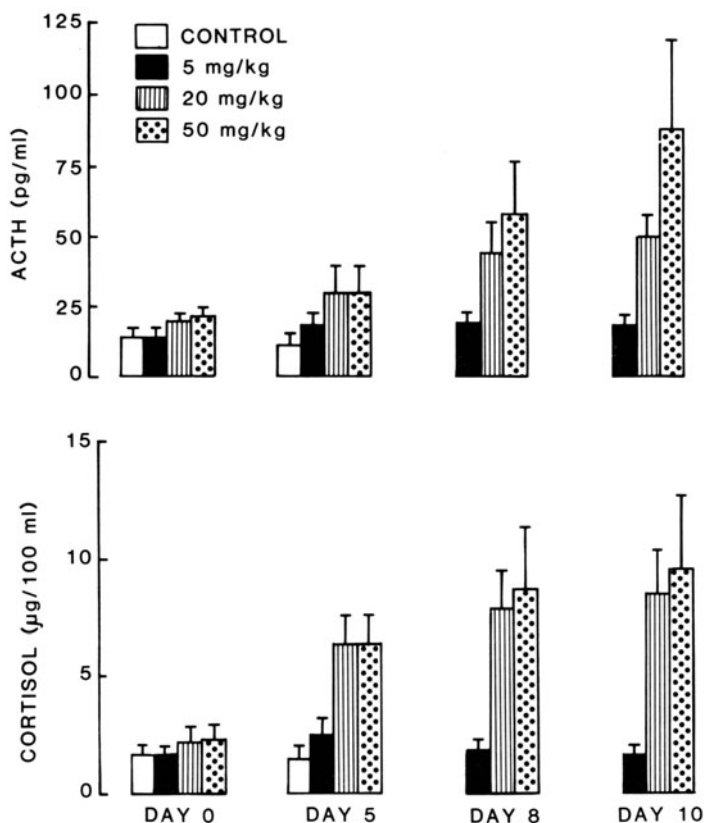


Fig. 2. The effect of 10 days of treatment with RU 486 on ACTH and cortisol levels in plasma. Values on day 0, 5, 8 and 10 are shown. Mean  $\pm$  SEM. A significant rise in ACTH and cortisol was only apparent with the 20 mg/kg and 50 mg/kg doses.

as a diagnostic test for Addisons disease before cortisol measurements were widely available. To ascertain whether the blockade of glucocorticoid receptors by RU 486 was sufficient to produce functional cortisol deficiency, water load tests were performed on the last day of RU 486 administration. The dogs were examined during all three RU 486 dose schedules as well as in the control experiment. Half normal saline was given in a volume of 40 ml/kg over twenty minutes by the intravenous route. As can be seen from Figure 7, the dogs maintained the ability to excrete a water load even with the highest dose of RU 486. In contrast adrenalectomized dogs fail to secrete a water load 5-9 days after cortisol withdrawal (Boykin et al., 1978). These results imply that there is no evidence for functional deficiency of cortisol at the level of the renal tubule. It is possible that the rise of cortisol associated with RU 486 administration can compensate in part for the blockade of the glucocorticoid receptors. Alternatively, RU 486 could have sufficient glucocorticoid agonistic activity at the doses used to retard the appearance of objective signs of hypocortisolemia.

#### Drug Levels

Figure 8 shows circulating drug levels of RU 486 expressed on a logarithmic scale. With the low dose of RU 486 mean circulatory levels

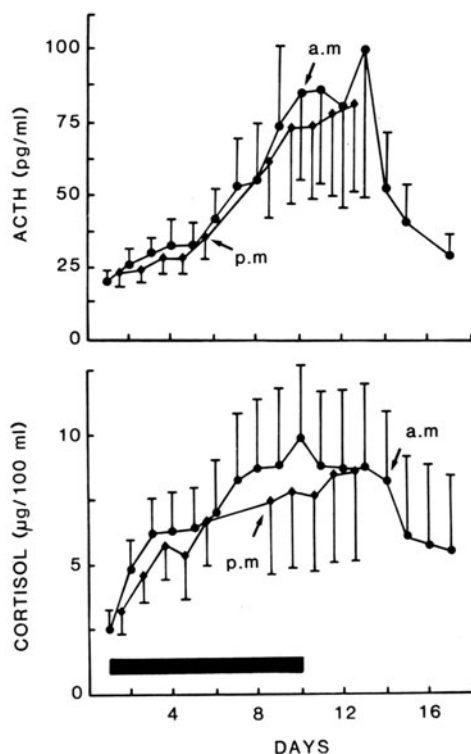


Fig. 3. The effect of 10 days of treatment with RU 486 on ACTH and cortisol response to the 50 mg/kg dose of RU 486. Values for both AM and PM samples are given. Note absence of diurnal rhythm. Note that the values shown in this figure are from the 3 responder dogs (see Figs. 10 and 11 for details).

ranged from 80 to 250 ng/ml. Following cessation of therapy, levels decreased with a half disappearance time of approximately twenty-four hours. In contrast, during administration of 50 mg/kg, plasma RU 486 levels were considerably higher, ranging from 2500 to 7500 ng/ml, and values remained persistently elevated during the subsequent week following cessation of therapy.

It should be noted that the antibody utilized in the immunoassay was not specific for the parent compound. Metabolic breakdown products are also measured in the radioimmunoassay (Philibert, 1984). Thus the high levels present in these dogs could in part represent immunologically active, but biologically inactive steroid. On the other hand ACTH, cortisol, body weight, serum protein, and hematocrit did not return to normal immediately after cessation of drug administration. This suggests that the persistently elevated levels of RU 486 represents to a certain extent a biologically active compound.

#### The Effect of RU 486 on Aldosterone

The aldosterone response during the three treatment schedules and the control period is shown in Figure 9. A significant rise was only seen with the highest dose of RU 486. There were no consistent trends with the other doses nor in the control study.

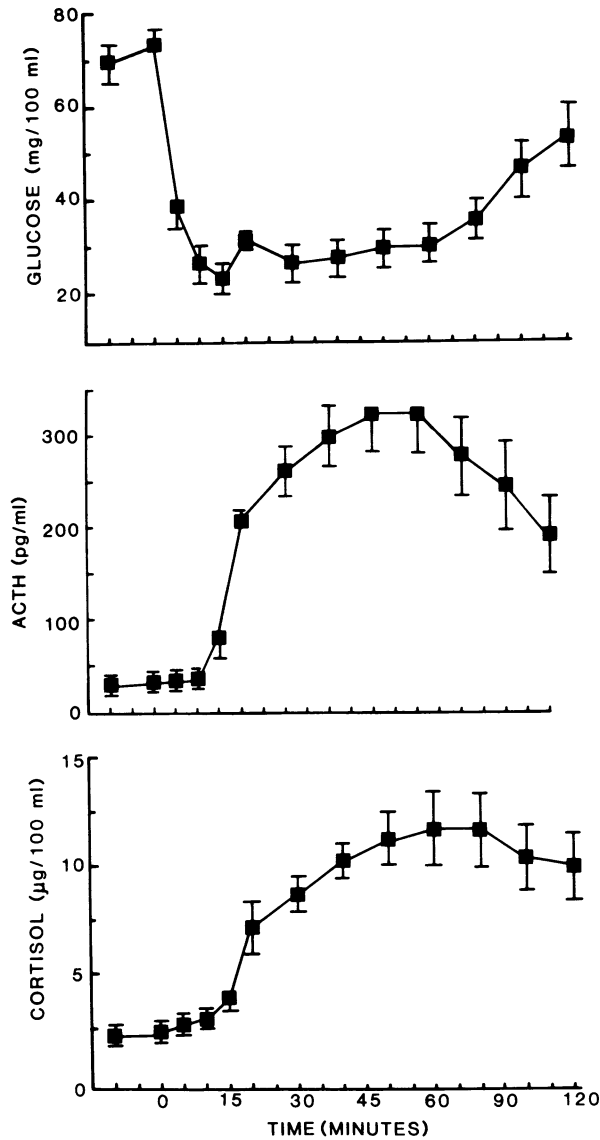


Fig. 4. The effect of insulin induced hypoglycemia on the ACTH and cortisol responses in seven dogs. Mean  $\pm$  SEM. Insulin was given at time 0.

Known stimuli for aldosterone secretion include the renin-angiotensin system, hyperkalemia, hyponatremia and ACTH itself. The major stimulus for the renin-angiotensin system is a reduction in circulatory volume. In these dogs the rise in body weight coupled with the reduction in protein and hematocrit is indicative of an expanded plasma volume. Thus although never measured, it would appear that the renin-angiotensin system was not responsible for the increase in aldosterone secretion. Neither were hyperkalemia nor hyponatremia evident.

#### Responder and Non Responder Groups

In an attempt to ascertain the relationship of cortisol to aldosterone secretion, we re-analysed the responses in dogs treated with the highest

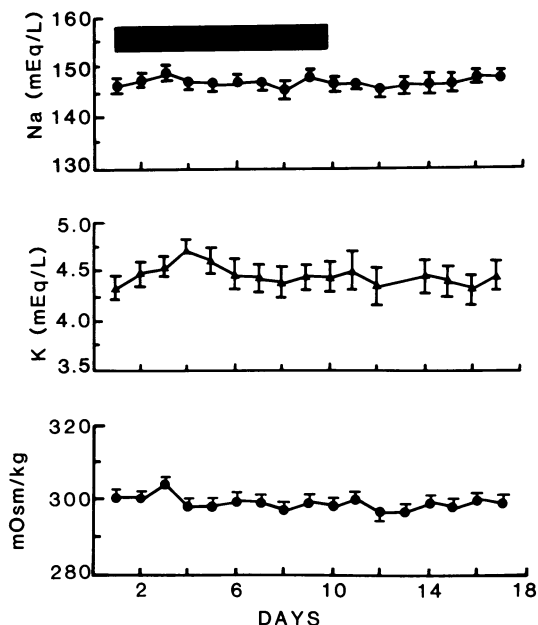


Fig. 5. The effect of RU 486 (50 mg/kg) on serum sodium, potassium and osmolality in 7 dogs. Mean  $\pm$  SEM.

dose of RU 486. This revealed the presence of two definite subgroups. With the highest dose of RU 486, three dogs had cortisol responses greater than 1 ug/100 ml which is at least four times greater than basal values, whereas in the other four animals peak levels were below this level. The former have been termed as "cortisol-responders" and the latter as "cortisol non-responders". The ACTH, cortisol and aldosterone levels in these two groups are shown in Fig. 10. The magnitude of the increases in ACTH correlated with the magnitude of the cortisol response in the two groups. On the other hand, the cortisol responders had a significantly lower aldosterone increment than did the non-responders. The weight change and serum protein profile in the responder and non-responder groups are shown in Fig. 11. The ACTH-cortisol responders had a greater gain in body weight and reduction in serum protein than did the non-responders.

To determine if the differences in responses in the two groups with the high dose of RU 486 could be due to variation in absorption of the drug, the mean drug levels of the two groups were calculated. Despite the distinctive hormonal patterns, circulating drug levels were similar in the two groups. However, since the assay used measures drug plus metabolites, the present study cannot exclude differential RU 486 metabolism in the two groups of responders.

Alternatively, the variable responses could also be due to differences in the sensitivity of each individual dog to the drug. To evaluate this possibility, an analysis of cortisol responses to the intermediate dose of RU 486 was performed. This revealed that the cortisol increases in the 3 "responder animals" were significantly greater than in the four non-responders (Fig. 12). There were, however, no differences in ACTH or aldosterone levels (data not shown). On the other hand, during insulin hypoglycemia, this differential effect was not evident and all seven dogs responded to a similar degree with regard to ACTH and cortisol. Since the identical responder-nonresponder pattern evolved in the same dogs, during

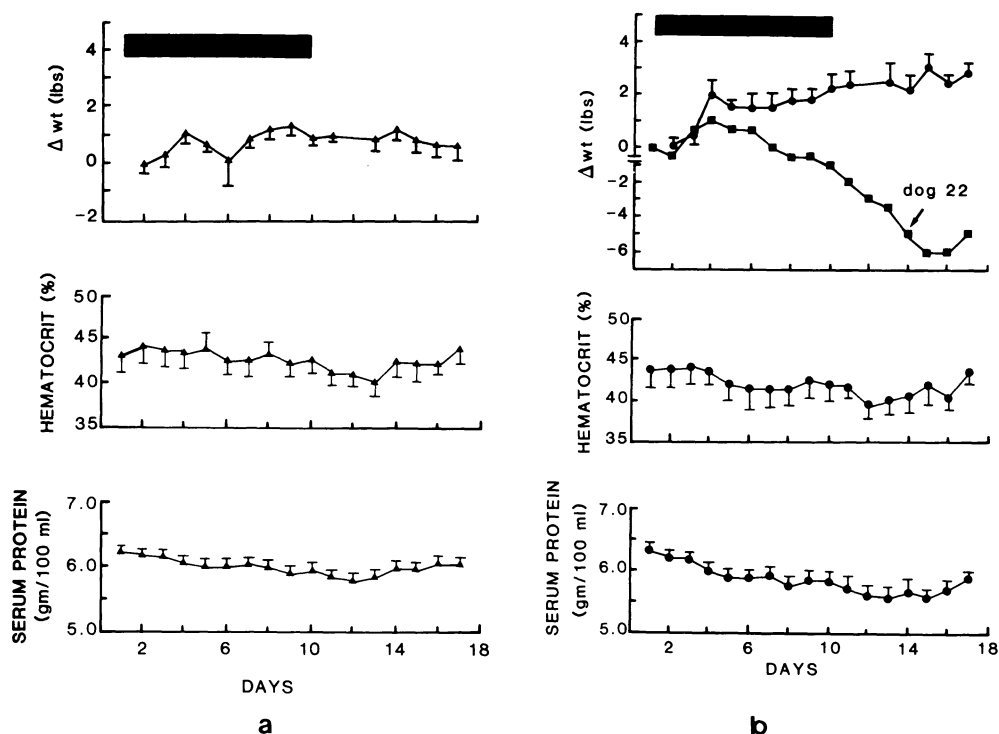


Fig. 6. The effects of Ru 486 on changes in body weight, serum protein and hematocrit in response to the low dose (6a) and to the high dose (6b) in the seven dogs. There was a significant rise in body weight and fall in serum protein and hematocrit with the high dose experiment. Dog 22 developed vomiting and diarrhea and as a consequence lost weight. The results for body weight for this dog have been graphed separately.

two different treatment periods with RU 486 but not with insulin induced hypoglycemia, some animals may be more susceptible to the glucocorticoid blocking activity of RU 486 than others.

The mechanism underlying the inverse relationship between cortisol and aldosterone is not known. Cortisol in itself has potent sodium retaining properties which could explain the isosmotic fluid retention and increase in bodyweight with reduction in serum protein. This fluid retention may in turn inhibit aldosterone secretion. In those four animals with the high aldosterone levels, ACTH, cortisol, and bodyweight rise and serum protein fall was not so dramatic implying less fluid retention. These results raise the possibility that in large doses, RU 486 might also be a mineralocorticoid antagonist. If this effect were uniform in all dogs this would account for a rise in aldosterone in animals without an increase in cortisol. Although *in vitro* studies have shown that RU 486 does not compete with aldosterone for mineralocorticoid receptors (Philibert et al., 1981), it is known that cortisol and dexamethasone may bind to mineralocorticoid receptors although they do with an affinity less than aldosterone (Swanek et al., 1970; Lan et al., 1982).

Preliminary studies in women have also demonstrated that some are susceptible to the antiprogesterational properties of RU 486 whereas others are not (Kovacs et al., 1984). This occurs despite apparent similarities in length of pregnancy, size of conceptus, circulating  $\beta$ hCG, and progesterone levels. It will be of interest to determine if individual susceptibility to any of the biological activities of RU 486 can be demonstrated in humans.

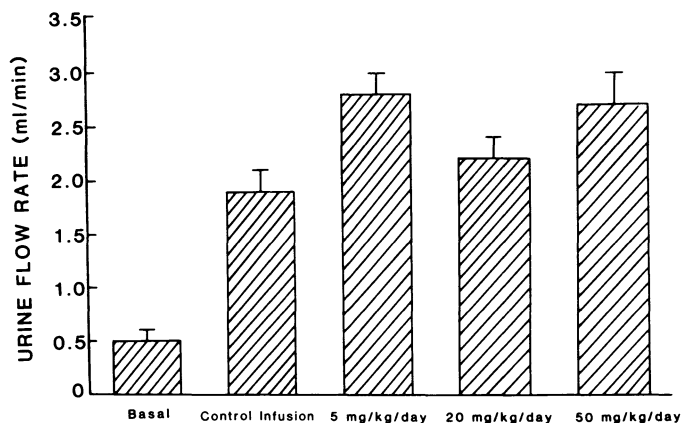


Fig. 7. The effect of RU 486 on water excretion as measured by urine flow. The urine flow in the basal state is shown in the left hand bar. The remaining four bars show the responses to the fluid load in the untreated state (control infusion) and following 10 days of treatment with the low, intermediate, and high dose of RU 486. Water excretion in the control period was similar to that in each treatment period (Mean  $\pm$  SEM).

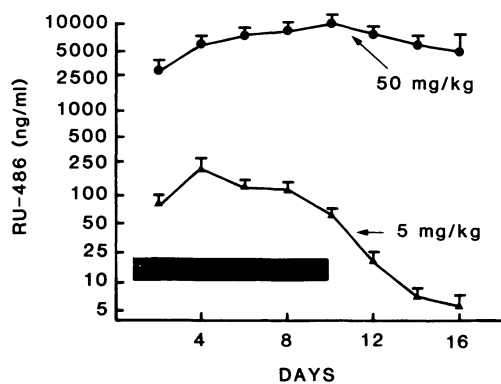


Fig. 8. Plasma RU 486 levels during and following the low and high dose of RU 486. Results have been expressed on a logarithmic scale. Treatment was stopped on day 10. Mean  $\pm$  SEM.

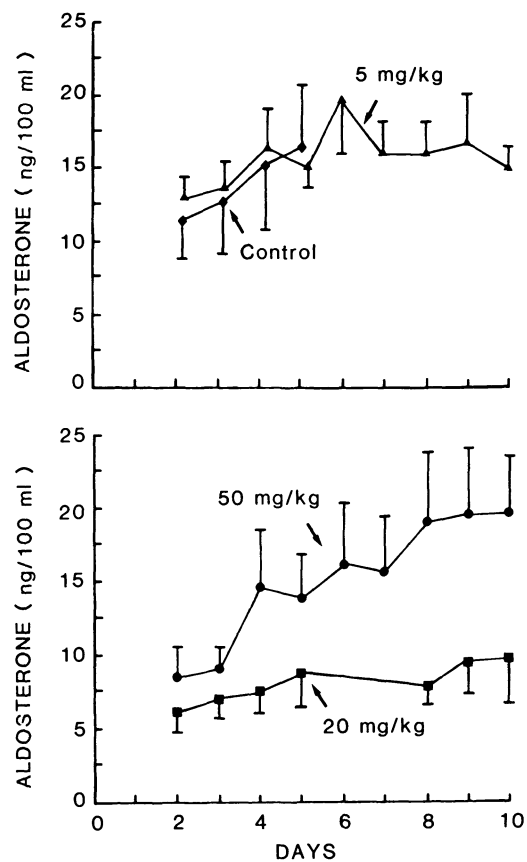


Fig. 9. The effect of RU 486 on plasma aldosterone levels during the three treatment periods. The control and low dose schedule is shown in the upper panel. The lower panel depicts the response to the intermediate and high dose. A significant aldosterone response was apparent only with the highest dose of RU 486 ( $p < 0.05$ ).

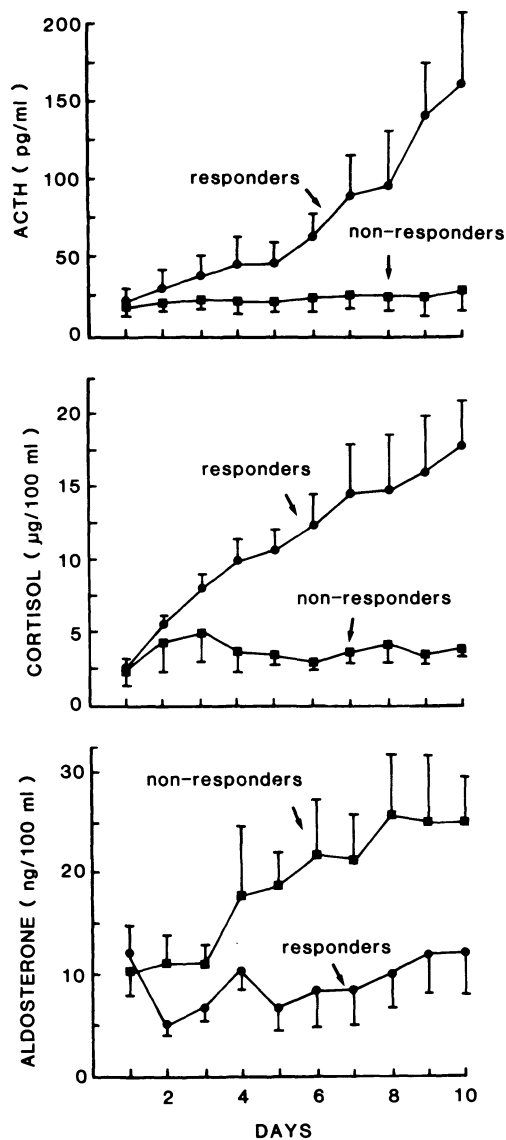


Fig. 10. The effect of RU 486 (50 mg/kg) on ACTH, cortisol, and aldosterone levels. The animals have been divided into two groups based on a cortisol response above or below 10 µg/100 ml (responders and nonresponders, respectively). See text for details. Mean  $\pm$  SEM.

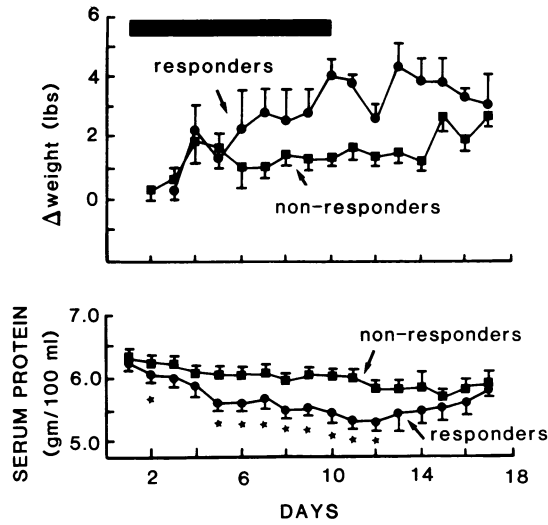


Fig. 11. The effect of RU 486 (50 mg/kg) on changes in body weight and serum protein in the responder and nonresponder groups. Over the entire period the responders had a greater increase in body weight than the nonresponders ( $p < 0.05$ ).

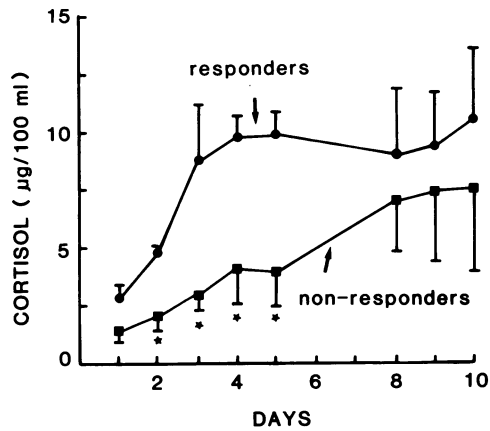


Fig. 12. The effect of RU 486 (20 mg/kg) on plasma cortisol levels in the responder and non-responder dogs. The responders had significantly greater cortisol increases from days 2 to 5 as compared to the non-responders ( $p < 0.05$ ).

## SUMMARY AND CONCLUSIONS

1. The administration of a single dose of RU 486 (10 mg/kg) to the dog by the oral or subcutaneous routes produced no change in cortisol levels. There was also no increase in ACTH or cortisol when 5 mg/kg was given daily for ten days.
2. ACTH and cortisol concentrations increased when RU 486 was given in an intermediate dose of 20 mg/kg/day or a high dose of 50 mg/kg/day for ten days. Cortisol was elevated by five days while ACTH only increased by day eight. There were no differences in cortisol and ACTH responses with these two doses. No diurnal rhythm of ACTH and cortisol was evident. One week after cessation of therapy, cortisol levels were still elevated. The cortisol increment observed with RU 486 was similar to that seen with insulin hypoglycemia. Aldosterone levels only increased with the high dose of RU 486.
3. There was an increase in body weight and reduction in hematocrit and serum proteins during drug treatment. This was not associated with a change in serum sodium, potassium and osmolality. These findings are compatible with isosmotic fluid retention and dilutional hypervolemia. All dogs maintained the ability to excrete a water load implying intact glucocorticosteroid action at the renal tubule.
4. Following the single oral dose, the apparent half time for disappearance of RU 486 was two hours. This is faster than that determined after a single oral dose in humans. When the drug was discontinued after ten days administration, the apparent half disappearance time was 24 hours with the low dose of 5 mg/kg/day. Circulatory levels did not show any decrease one week after cessation of the high dose (50 mg/kg/day).
5. Analysis of the response to ten days of treatment with the high dose of RU 486 revealed two distinct groups, responders and non-responders. The responder group comprised three dogs who had increases in ACTH, cortisol and bodyweight, with reduction in serum protein but no change in aldosterone. In contrast, the non-responders had minimum changes in ACTH, cortisol, serum protein, hematocrit but a significant rise in aldosterone levels. Circulating drug levels were similar in the two groups. The same three responder dogs had greater cortisol rises with the intermediate dose whereas the four nonresponders had a lower cortisol increment. In contrast to RU 486 all dogs demonstrated a similar ACTH and cortisol response to insulin hypoglycemia. Differential sensitivity of the dogs to the antiglucocorticoid action of RU 486 is proposed. An antimineralocorticoid effect is also suggested.

## ACKNOWLEDGMENT

This work was supported by grants from the Ford Foundation and the Mellon Foundation. The drug was supplied by Roussel Uclaf, Paris, France.

## REFERENCES

- Boykin, J., deTorrente, A., Erickson, A., Robertson, G., and Schrier, R. W., 1978, Role of plasma vasopressin in impaired water excretion of glucocorticoid deficiency., *J. Clin. Invest.*, 62:738-744.
- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., 1984, RU 486: A steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day., *Proc. Natl. Acad. Sci. USA*, 81:3879-3882.

- Healy, D. L., Chrousos, G. P., Schulte, H. M., Williams, R. F., Gold, P. W., Baulieu, E. E., and Hodgen, G. D., 1983, Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU 486 in primates., J. Clin. Endocrinol. Metab., 57:863-865.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effect d'un-steroide anti-progesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut., C. R. Acad. Sc. Paris, 294:933-938.
- Keller-Wood, M. E., Shinsako, J., Keil, L. C., and Dallman, M. F., 1981, Insulin-induced hypoglycemia in conscious dogs. I. Dose-related pituitary and adrenal responses., Endocrinology, 109:818-824.
- Keller-Wood, M. E., Wade, C. E., Shinsako, J., Keil, L. K., Van Loon, G. R., and Dallman, M. F., 1982, Insulin-induced hypoglycemia in conscious dogs: effect of maintaining carotid arterial glucose levels on the adrenocorticotropin, epinephrine and vasopressin responses., Endocrinology, 112:624-632.
- Keller-Wood, M. E., Shinsako, J., and Dallman, M. F., 1983, Integral as well as proportional adrenal responses to ACTH., Am. Physiol., 245:r53-r59.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - An anti-progestational compound., Contraception, 29:399-410.
- Lan, N. C., Graham, B., Bartter, F. C., and Baxter, J. D., 1982, Binding of steroids to mineralocorticoid receptors: implications for in vivo occupancy by glucocorticoids., J. Clin. Endocrinol. Metab., 54:332-342.
- Lipsett, M. B. and Pearson, O. H., 1956, Pathophysiology and treatment of adrenal crisis., N. Engl. J. Med., 254:511-514.
- Mendelsohn, M. L. and Pearson, O. H. 1955, Alterations in water and salt metabolism after bilateral adrenalectomy in man., J. Clin. Endocrinol. Metab., 15:409-423.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU38486, a potent antiglucocorticoid in vivo., 8th International Congress of Pharmacology, Tokyo, Abstract 1463.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C., and Sakiz, E., 1982a, RU38486 - a new lead for steroidal anti-hormones. Endocrine Society, 64th Annual Meeting, San Francisco, Abstract 668.
- Philibert, D., Deraedt, R., Tournemine, C., Mary, I., and Teutsch, G., 1982b, RU38486 - a potent antiprogestosterone., J. Ster. Biochem. Sixth International Congress on Hormonal Steroids, Jerusalem Abstract 104:17.
- Philibert, D., 1984, in: Proceedings on RU 486. (Segal, S. J. and Baulieu, E. E., eds., Bellagio, Italy.
- Proulx-Ferland, L., Cote, J., Philibert, D., and Deraedt, R., 1982, Potent anti-glucocorticoid activity of RU38486 on ACTH secretion in vitro and in vivo in the rat., J. Ster. Biochem. Sixth International Congress on Hormonal Steroids, Abstract 80:17.
- Swanek, G. E., Chu, L. L. H., and Edelman, I. S., 1970, Sterospecific binding of aldosterone to renal chromatin., J. Biol. Chem. 245:5382-5389.

## RU 486: STUDIES OF ITS ANTIGLUCOCORTICOSTEROID

### ACTIVITY IN MAN

R. C. Gaillard,<sup>1</sup> A. Riondel,<sup>2</sup> A. F. Muller,<sup>1</sup>  
W. Herrmann<sup>3</sup> and E. E. Baulieu<sup>4</sup>

<sup>1</sup>Clinique Médicale, <sup>2</sup>Division of Endocrinology and  
<sup>3</sup>Department of Gynecology, University Hospital  
Geneva, Switzerland, and <sup>4</sup>Laboratoire Hormone-Institut  
National de la Santé et de la Recherche Médicale U33  
F-94270 Bicetre, France

### INTRODUCTION

RU 486 was first used in humans to interrupt the luteal phase of the menstrual cycle and early pregnancy (Herrmann et al., 1982). An increase in blood cortisol was observed in women who received RU 486 to induce abortion during weeks 6-8 of pregnancy, whereas no changes occurred when RU 486 was used for menstrual cycle interruption. Because RU 486 possesses antiglucocorticosteroid activity in addition to its antiprogesterone property (Philibert et al., 1981), full investigation of this effect is essential in order to evaluate its importance and nature with respect to its use in human fertility control. The antiglucocorticosteroid activity may also be of interest to clinicians and to basic scientists. Clinical applications of RU 486 may include a new approach to the therapy of hypercortisolism (Cushing's syndrome) and possibly an alternative provocative test for ACTH reserve. The present chapter reports investigations on the antiglucocorticosteroid effect of RU 486 on the pituitary-adrenal axis in men and in pregnant and non-pregnant women.

### ANTIGLUCOCORTICOSTEROID ACTIVITY IN NORMAL YOUNG MEN

To study specifically the antiglucocorticosteroid properties of RU 486, particularly in the absence of known progesterone-dependent processes, we administered the compound to normal young male volunteers who gave their consent (Gaillard et al., 1984a). At a divided dose of 6 mg/kg given throughout the day, RU 486 showed an antiglucocorticosteroid activity. It amplified the normal circadian rhythm for two days, increasing specifically and significantly the morning plasma concentrations of ACTH,  $\beta$ -endorphin and cortisol (Fig. 1). These results strongly suggest that the disinhibition of the pituitary-adrenal system by RU 486 occurs at a specific time of day, i.e., during the morning hours. The influence of the circadian rhythm on the antiglucocorticosteroid effect was clearly demonstrated by the response to nocturnal versus diurnal RU 486 administration. The same subjects received 6 mg of RU 486 per kg in a single dose, once at midnight and once at 10 am one week later. Indeed, as shown in Figure 2, a significant rise in plasma levels of ACTH,  $\beta$ -endorphin and cortisol, occurred only in the

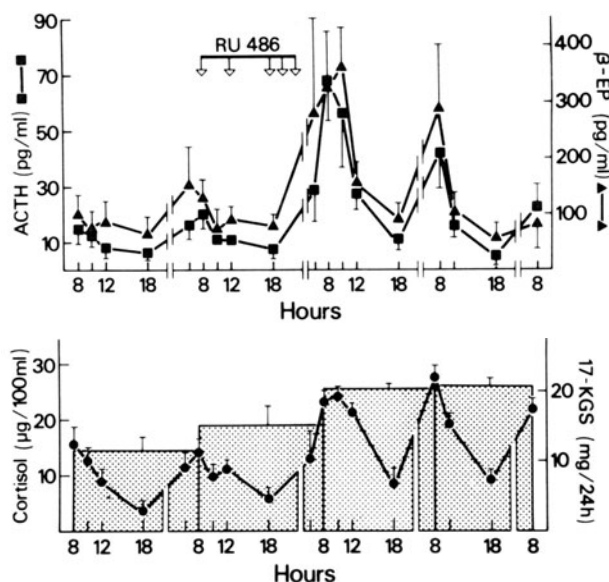


Fig. 1. Effect of RU 486 administered in five doses to 3 young men (6 mg/kg) on day 1 at 0800, 1200, 1800, 2200 and 2400 on plasma ACTH,  $\beta$ -endorphin ( $\beta$ -EP) and cortisol and on urinary 17-ketogenic steroids (17-KGS). Error bars show  $\pm$  SEM.

morning, whereas no effect was observed on afternoon and evening hormone levels. The fact that diurnal administration did not affect hormonal levels the same evening, but increased those observed the following morning, clearly confirmed the lack of effect during the afternoon and evening periods of the circadian cycle. It is conceivable that antagonizing the low level of plasma cortisol in the afternoon and evening periods has little effect on the negative control system. These results also show that antiglucocorticosteroid activity persisted up to 22 hours after drug administration, confirming the pharmacokinetic data showing that RU 486 has a half-life of at least 28 hours after oral administration. The disinhibiting effect of RU 486 on pituitary-adrenal function is dose-dependent. No changes in hormone levels were observed with a dose of 2.2 mg/kg, whereas doses of 4.5 and 6 mg/kg led to a significant rise (Fig. 3).

To confirm that the changes in hormone levels were in fact attributable to the antiglucocorticosteroid action of RU 486, we used the antihormone to challenge the inhibitory effect of dexamethasone on the pituitary-adrenal axis. When administered concomitantly with 1 mg of dexamethasone at midnight, 6 mg of RU 486 per kg completely suppressed the dexamethasone inhibitory effect on the pituitary-adrenal axis (Fig. 4). This confirms that the changes in hormone levels are attributable to the antiglucocorticosteroid action of RU 486. Conversely, it is interesting to note the anti-RU 486 effect of dexamethasone. Twenty four hours after RU 486 administration, 1 mg of dexamethasone was fully active, suppressing the pituitary adrenal axis despite the persistent antiglucocorticosteroid activity of RU 486 observed in the circadian rhythm study (Fig. 1). Furthermore when administered concomitantly with RU 486 at 4 mg/kg, 2 mg dexamethasone showed full glucocorticosteroid activity. These results are of pharmacological interest and suggest that any state of hypocortisolism that might be imputed to RU 486 could easily be reversed by corticosteroid administration.

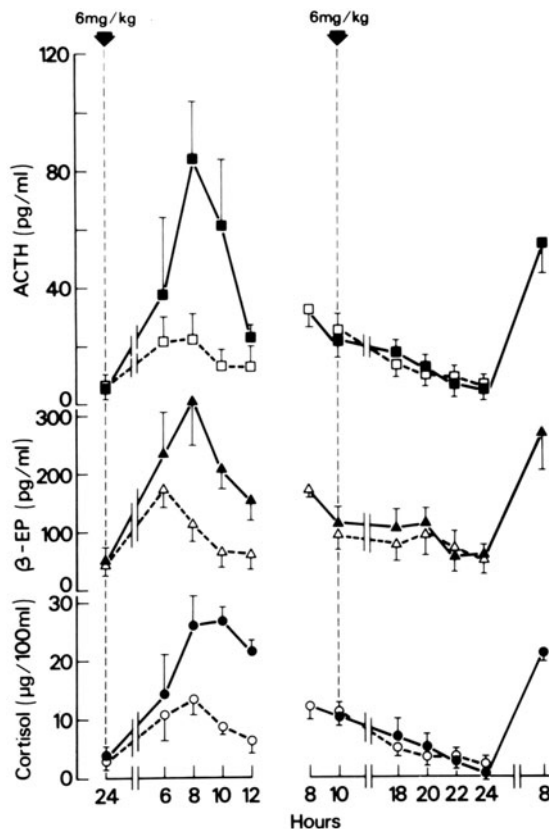


Fig. 2. Comparative effect of 6 mg of RU 486/kg, administered twice to the same four young men at 2400 and 1000 with a one-week interval, on plasma ACTH,  $\beta$ -endorphin ( $\beta$ -EP), and cortisol. Solid symbols = RU 486 administered; open symbols = controls.

RU 486 also antagonizes peripheral effects of glucocorticosteroids. It has been shown to reverse the peripheral effects of pharmacological doses of glucocorticosteroids on blood leukocytes (eosinophils, lymphocytes and neutrophils) (Rieu et al., 1984) and on steroid-induced vasoconstriction (Gaillard et al., 1984b). However, peripheral antiglucocorticosteroid effects were not observed after administration of RU 486 alone, because the homeostatic pituitary-adrenal reaction compensates the antiglucocorticosteroid action of RU 486. Thus, the water load test (Bertagna et al., 1983), the oral glucose tolerance test and white blood cell counts were not influenced by the administration of RU 486 alone (Rieu et al., 1984). In fact very large doses of RU 486 were required to produce signs of hypocortisolism, which could not be overcome by the endogenous homeostatic glucocorticosteroid response. This occurred in tolerance and toxicity studies in cynomolgus monkeys, receiving doses of 100 mg/kg per day for one month (Glomot, personal communication).

It is therefore obvious that RU 486 functions as an anti-glucocorticosteroid in humans, probably acting at the glucocorticosteroid receptor level. It antagonizes the negative pituitary feedback response to both the early morning endogenous cortisol rise and to exogenously administered dexamethasone. Similar results have been published recently by Bertagna et al. (1984).

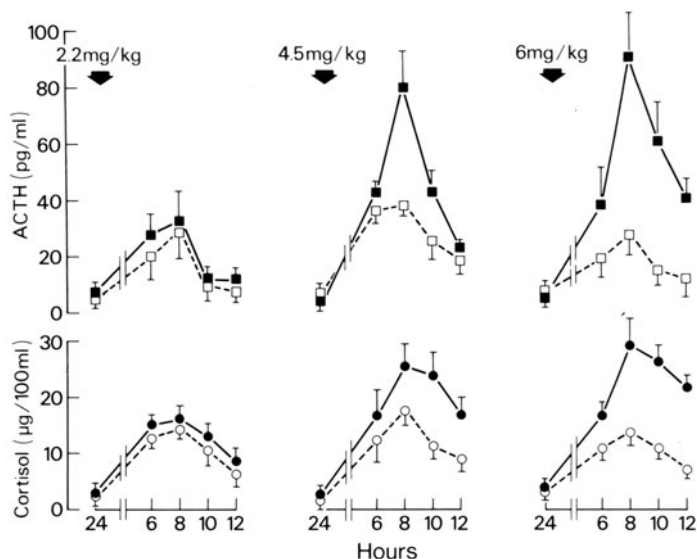


Fig. 3. Dose-dependent effect of RU 486 on ACTH and cortisol levels when administered at 2400 to three young men. Solid symbols = RU 486 administered. Open symbols = controls.

#### ANTI-GLUCOCORTICOSTEROID EFFECT IN WOMEN

##### Menstrual Cycle Interruption

In nonpregnant women, a dose of about 1 mg/kg per day of RU 486, administered from the 22nd day of the cycle for four days, demonstrated anti-progesterone activity indicated by the occurrence of bleeding within 48 hours, drop of basal body temperature, decline of plasma progesterone, and estradiol levels (Herrmann et al., 1982). However, in spite of the

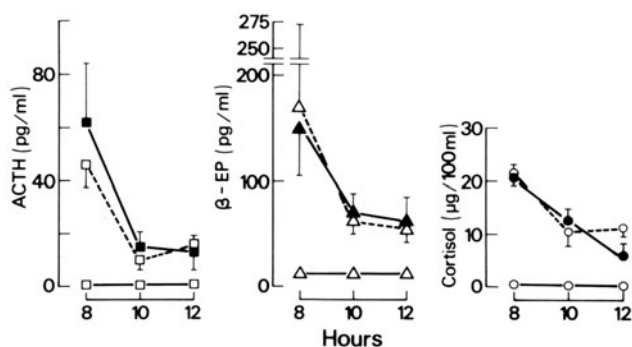


Fig. 4. Effect of RU 486 (6 mg/kg) and dexamethasone (1 mg) on plasma ACTH,  $\beta$ -EP and cortisol, when combined in an evening administration to three normal men (solid symbols). Dexamethasone was always given at 2400. Controls included no drug administration (open symbols with dotted line) and dexamethasone alone (open symbols with continuous line).

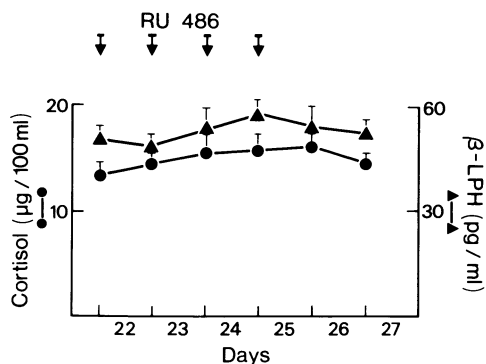


Fig. 5. Effect of 50 mg/d of RU 486, given from d 22-25 of the cycle, on plasma  $\beta$ -LPH and cortisol concentrations in three normal women during the luteal phase.

interruption of the luteal phase, no antiglucocorticosteroid activity was observed, as witnessed by the absence of any changes in plasma  $\beta$ -LPH and cortisol levels (Fig. 5). RU 486 always induced uterine bleeding when given earlier in the luteal phase at a dose of about 2 mg/kg/day for four days, starting on the 4th day after the basal body temperature shift (Schaison et al.). No antiglucocorticosteroid activity was observed, confirming the findings of the dose-response study in male subjects (Fig. 3). Therefore, it seems that the menstrual cycle can be interrupted without inducing antiglucocorticosteroid activity, and that this latter effect only occurs at doses exceeding those required for an antiprogesterone effect.

#### Pregnancy Interruption

The pituitary-adrenal system was manifestly disinhibited in pregnant women aborting after the administration of RU 486. Eleven women received 200 mg of RU 486 per day (approximately 4 mg/kg/day) during weeks 6-8 of pregnancy. Abortion occurred in 9 of the 11 patients, 3-8 days after starting RU 486 administration. Morning plasma ACTH,  $\beta$ -LPH and cortisol levels increased significantly in all patients after the first day of treatment, remained elevated during the 4 days of drug administration, and returned to control values one week after cessation of therapy (Fig. 6). This effect could have been due either to the antiglucocorticosteroid activity of RU 486 or could have been non-specifically related to the stress

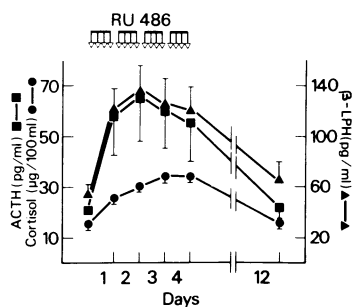


Fig. 6. Effect of 50 mg RU 486 administered 4 times/d on plasma ACTH,  $\beta$ -LPH and cortisol. RU 486 was administered on four successive days to eleven women in weeks 6-8 of pregnancy.

of the induced abortion. The latter possibility appears to be ruled out by the fact that the concentrations of blood cortisol were identical before and after abortion (Wyss et al.). However, Kovacs et al. (1984) recently showed that changes in cortisol values were similar to those observed during prostaglandin-induced abortion and that the relationship to stress cannot yet be ruled out. It should be emphasized that none of the pregnant women showed clinical signs or changes in biochemical parameters suggesting adrenal insufficiency (Wyss et al.).

#### CLINICAL USE OF THE ANTIGLUCOCORTICOSTEROID PROPERTY OF RU 486

RU 486 might be useful for correcting the clinical manifestations of those hypercortisolism states where no compensatory response to the antiglucocorticosteroid effect of RU 486 can be expected. It is therefore unlikely to be useful in pituitary Cushing's disease, where a compensatory mechanism is present, but it might have therapeutic potential in patients with Cushing's syndrome due to adrenal cancer or ectopic ACTH-secreting tumor. These patients often present gross hypercortisolism that is difficult to manage by available medical therapy. Their incurable cancer makes them poor candidates for surgery, and hypercortisolism is a serious problem leading to earlier death than might have occurred from the tumor alone. A patient presenting a metastatic carcinoid tumor with multiple lung and bone metastases and marked hypercortisolism was put on RU 486 therapy by Nieman et al. (1984). At a dose of 10-20 mg/kg/day, subjective and objective changes were apparent: somatic signs of Cushing's syndrome improved, depression resolved, libido was restored and blood pressure and fasting blood sugar fell. RU 486 may therefore be a useful therapeutic agent. It may also prove to be helpful in the preparation of patients with Cushing's syndrome requiring surgery.

RU 486 might also be used as a provocative test for exploring the hypothalamo-pituitary-adrenal axis. This would be very welcome because the classical test using metyrapone is often poorly tolerated. It could also be of great interest in the study of the corticotropin axis in pathological states such as depression and could be used to verify the pituitary response during corticotherapy. However, further investigation is required before proposing the replacement of metyrapone by RU 486 in the evaluation of pituitary ACTH reserve.

#### CONCLUSION

Antiglucocorticosteroid activity was the only side effect observed when RU 486 was used for human fertility control. It was therefore important to investigate its nature and extent. We have seen that the antiglucocorticosteroid effect is time dependent and is only apparent during the morning hours of the nycthemeron when cortisol levels are rapidly increasing, with RU 486 acting to disrupt the negative pituitary feedback mechanism. These findings permit a chronopharmacological dissociation of the two built-in antihormonal properties of the molecule. Furthermore, the antiglucocorticosteroid effect is only observed at doses exceeding those required for an antiprogesterone effect and can easily be reversed by administration of a corticosteroid such as dexamethasone. These findings concur to propose the possibility of optimizing both the antiprogestational effect of the compound and its potential use for human fertility control, by modifying the dose and the time of administration of the drug, thereby minimizing the antiglucocorticosteroid effect. Finally, this latter effect of RU 486 may be exploited both to challenge the pituitary-adrenal axis in various pathological conditions. However, the antiprogesterone property of RU 486 may limit its use in future testing or treatment of the

pituitary-adrenal axis in fertility women. Although it certainly would be interesting to possess a pure antiglucocorticosteroid compound, the antiglucocorticosteroid activity of RU 486 does not appear to compromise its safety as a human antifertility agent.

#### REFERENCES

- Bertagna, X., Bertagna, C., Girard, F., and Luton, J. P., 1983, Effect d'un antiglucocorticoïde (RU 38486) sur l'axe hypophyso-surrénalien et l'élimination d'une charge en eau chez l'homme, Annales d'Endocrinologie, 44:191, Abstr. 89.
- Bertagna, X., Bertagna, C., Luton, J. P., Husson, J. M., and Girard, F., 1984, The new steroid analog RU 486 inhibits glucocorticosteroid action in man, J. Clin. Endocrin. Metab., 59:25.
- Gaillard, R. C., Riondel, A., Muller, A. F., Herrmann, W., and Baulieu, E. E., 1984a, RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day, Proc. Natl. Acad. Sci. USA, 81:3879.
- Gaillard, R. C., Poffet, D., and Saurat, J. H., submitted.
- Glomot, R., Department of Toxicology, Roussel-Uclaf (unpublished results).
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E., and Baulieu, E. E., 1982, Effet d'un stéroïde anti-progestérone chez la femme: interruption du cycle menstruel et de la grossesse au début, C. R. Séances Acad. Sci (III), 294:933.
- Kovacs, L., Sas, M., Resch, B. A., Ugocsai, G., Swahn, M. L., Bygdeman, M., and Rowe, P. J., 1984, Termination of very early pregnancy by RU 486 - an antiprogesterone compound, Contraception, 29:399.
- Nieman, L. K., Chrousos, G. P., Spitz, J., Nisula, B. C., Cutler, G. B., Merriam, G. R., Bardin, C. W., and Loriaux, D. L., 1984, Successful treatment of Cushing's syndrome with glucocorticoid antagonist RU 38486, 7th International Congress of Endocrinology, Quebec, Excerpta Medica, Series 652:1119, Abstr. 1718.
- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 38486 a potent anti-glucocorticoid in vivo, 8th International Congress of Pharmacology, Tokyo, Japan, 668:1463 (Abstr.).
- Rieu, M., Bertagna, X., Basin, C., Hucher, M., Varet, B., Dugue, M. A., and Luton, J. P., 1984, RU 486 inhibits peripheral effects of exogenous glucocorticoids in man, 7th International Congress of Endocrinology, Quebec, Excerpta Medica, Series 652:1201 (Abstr. 1881).
- Schaison, G., George, M., Lestrat, N., Reinberg, A., and Baulieu, E. E., Effects of the antiprogesterone steroid RU 486 during mid-luteal phase in normal women, J. Clin. Endocrin. Metab., in press.
- Wyss, R. H., Bishof, P., Baulieu, E. E., and Herrmann, W. L., Hormonal and metabolic changes in first trimester abortions with an antiprogesterone (RU 486), submitted.

## USE OF THE GLUCOCORTICOID ANTAGONIST RU 486

### IN THE TREATMENT OF CUSHING'S SYNDROME

Lynnette K. Nieman,<sup>1</sup> George P. Chrousos,<sup>1</sup> Charles Kellner,<sup>2</sup> Irving M. Spitz,<sup>3</sup> Bruce C. Nisula,<sup>1</sup> Gordon B. Cutler, Jr.,<sup>1</sup> George R. Merriam,<sup>1</sup> C. Wayne Bardin,<sup>3</sup> and D. Lynn Loriaux<sup>1</sup>

<sup>1</sup>Developmental Endocrinology Branch, Natl. Institute of Child Health and Human Development

<sup>2</sup>The Biological Psychiatry Branch, Natl. Institute of Mental Health, Bethesda, Maryland

<sup>3</sup>The Center for Biomedical Research, The Population Council, New York, N.Y.

#### ABSTRACT

The recently described synthetic steroid RU 486 antagonizes cortisol action in vitro and in vivo. We assessed its therapeutic potential in Cushing's syndrome by giving increasing doses (5, 10, 15 and 20 mg/kg/day over a 9-week period) to a patient with Cushing's syndrome due to ectopic ACTH secretion. Treatment efficacy was monitored by evaluation of several glucocorticoid-sensitive parameters, including clinical status, fasting blood sugar, blood sugar at 120 min after oral glucose, and plasma concentrations of TSH, CBG, LH, TeBG, and total and free testosterone. With therapy, the somatic features of Cushing's syndrome (buffalo hump, central obesity, and moon facies) ameliorated, mean arterial blood pressure normalized, suicidal depression resolved, and libido returned. All biochemical glucocorticoid-sensitive parameters normalized. No side effects or drug toxicity were observed. Thus, RU 486 may provide a safe, well-tolerated, and effective medical treatment of hypercortisolism.

#### INTRODUCTION

A clinically applicable glucocorticoid antagonist is, in theory, an attractive alternative treatment for hypercortisolism and has been sought actively for many years (Chrousos et al., 1983). The recently discovered compound RU 486 is a 19-nor steroid with a high affinity for the rat glucocorticoid receptor. It has no agonist effects in vitro or in vivo but is a potent competitive glucocorticoid antagonist in rodents (Philibert et al., 1981), nonhuman primates (Healy et al., 1983, 1984) and man (Herrmann et al., 1981; Gaillard et al., 1983; Bertagna et al., 1984). We have reported the successful treatment with RU 486 of a 25 year old man with Cushing's syndrome caused by ectopic secretion of ACTH (Nieman et al., 1985). During therapy, the somatic features of Cushing's syndrome improved, suicidal depression cleared, and glucocorticoid-sensitive parameters, such as elevated fasting and post-absorptive blood sugar normalized. The drug

was well-tolerated and no side effects were noted during therapy or after its discontinuation.

#### THERAPEUTIC TRIAL WITH RU 486

The currently available treatments for Cushing's syndrome caused by metastatic ACTH-producing tumors or adrenal cancer are often unsatisfactory. Surgical resection of the tumor, when feasible, may be only partially or temporarily effective in controlling Cushing's syndrome. Medical therapy with adrenolytic agents (o,p'-DDD) or steroidogenic enzyme inhibitors (aminoglutethimide, metyrapone) is frequently associated with toxic side effects (Temple and Liddle, 1970; Carey et al., 1973; Gorden et al., 1968; Coll et al., 1968; Gold, 1979). RU 486 offers a new approach in the therapy of hypercortisolism that may be more effective and less toxic. However, the assessment of effectiveness of a glucocorticoid antagonist differs from that of the currently available treatments. Since the latter attempt to correct hypercortisolism, successful intervention can be assessed by measuring plasma cortisol concentrations or urinary cortisol or cortisol metabolite excretion. In contrast, glucocorticoid antagonists prevent the biologic expression of circulating cortisol by antagonizing it at the receptor level. Thus, successful treatment with a glucocorticoid antagonist can only be assessed indirectly by clinical improvement and by following glucocorticoid sensitive biochemical parameters, such as carbohydrate tolerance (Baxter and Tyrrell, 1981), thyroid function tests (Van Cauter et al., 1974), plasma gonadotropins (Luton et al., 1977), gonadal steroids (Luton et al., 1977; Smals et al., 1977) and plasma steroid transport proteins (Nieman et al., 1984).

Another issue in the use of a glucocorticoid antagonist is the estimation of the dose required for therapy. We attempted to define such a dose as follows. In man, a single oral dose of 6 mg/kg RU 486 given at midnight fully antagonizes the suppressive effect of 1 mg dexamethasone upon the morning plasma cortisol concentration (Gaillard et al., 1983). Assuming an average body weight of 70kg, RU 486 antagonizes dexamethasone at a ratio of 420:1. With a potency ratio of dexamethasone to cortisol of 40:1, a ratio of RU 486 to cortisol of 11:1 should be fully effective (Fauci et al., 1976). Since cortisol production rates of 4 mg/kg/day have been measured in patients with hypercortisolism due to adrenocortical carcinoma or the ectopic ACTH syndrome, up to 50 mg/kg/day of RU 486 may be necessary to reverse the effects of hypercortisolism. Thus, therapy of a single patient for one year could require a kilogram of the drug. Since the drug is produced in limited amounts and solely for experimental purposes, we could assess the efficacy of such a treatment in a single patient for a period of only two months.

The protocol for the therapeutic use of RU 486 was approved under an investigational exemption for new drugs by the National Center for Drugs and Biologics, DHHS and by the NICHD Clinical Research Committee. All tests were performed at the NIH Clinical Center. The initial oral daily dose of RU 486 was set at 5 mg/kg and increased by 5 mg/kg every one or two weeks to a maximum of 20 mg/kg per day. This incremental change in therapy was necessary to evaluate possible toxicity or adrenal insufficiency.

We treated a 25 year old man with a 2-year history of Cushing's syndrome secondary to an inoperable ACTH-producing carcinoid tumor. He was severely depressed and had a history of two suicide attempts. On admission to the National Institutes of Health he complained of disorientation, diminished memory and cognitive ability, impotence, 20 pound weight gain and long-standing muscle weakness. The patient had a ruddy, round face and was hypomimetic. His blood pressure was 180/120 mm Hg and his pulse was 90 beats per minute. He was anxious and mildly depressed. He performed

calculations slowly. A surgical scar was hyperpigmented. Computerized axial tomograms of the chest revealed multiple lung nodules. He had hypokalemic alkalosis (serum potassium was 1.9 mEq/L, bicarbonate 28 mEq/L, chloride 94 mEq/L, and sodium 147 mEq/L) and was frankly diabetic with elevated fasting plasma glucose.

At the conclusion of therapy the physical stigmata of Cushing's syndrome, including supraclavicular and dorsocervical fat pads and central obesity, showed appreciable regression. Maximum daily systolic and diastolic blood pressure decreased steadily during treatment with RU 486, from 200/120 prior to therapy, to 140/90 at its conclusion (Fig. 1, Panel A). The hypokalemic alkalosis resolved with serum potassium ranging between 3.9 and 4.6 mEq/L and serum bicarbonate between 25 and 29 mEq/L. Potassium supplements were discontinued after the sixth week of RU 486 therapy.

Both subjective and objective psychological measures improved during RU 486 therapy. The patient's depression subsided, and he reported increasing attention span, libido and sense of well-being. This subjective improvement was corroborated by self-rating questionnaires and psychiatric interviews.

Plasma glucose levels were initially 140 mg/dl in the fasting state (normal < 105 mg/dl) and 268 mg/dl two hours after ingestion of 100 mg of glucose (normal < 140 mg/dl) (Fig. 1, Panel A). The fasting blood sugar became normal while the patient was taking RU 486 at a dose of 10 mg/kg/d and the two hour post-OGTT blood sugar normalized when he was taking 20 mg/kg (Fig. 1, Panel A). Serum TSH concentration was initially subnormal (< 0.18 uU/ml), and rose progressively to 1.5 uU/ml during treatment (normal 0.5 - 4.5 uU/ml)(Fig. 1, Panel A). Cortisol binding globulin (CBG) binding capacity increased from 7.4 ug/dl (normal 12.2 - 20 ug/dl) to 16.8 ug/dl (Fig. 1, Panel A).

Plasma LH levels rose during treatment with RU 486 from 9.4 mIU/ml to 23.2 mIU/ml (normal 6 - 26 mIU/ml)(Fig. 1, Panel B). Similarly, plasma total and free testosterone concentrations and testosterone-estradiol binding globulin (TeBG) binding capacity increased from subnormal to normal levels during therapy with RU 486 (Fig. 1, Panel B). The total testosterone concentration was initially 73 ng/dl (normal 200 - 1000 ng/dl) and rose to 842 ng/dl when he was taking 20 mg/kg/d of RU 486. TeBG capacity increased from 0.063 ug/dl (normal 0.2 - 1.0 ug/dl) to 1.02 ug/dl at the conclusion of therapy. Free testosterone increased from 3.5 ng/dl (normal 5 - 30 ng/dl) to 17.4 ng/dl.

The 24 hour urinary nitrogen excretion fell from 22 g/d (normal 12 - 20 g/d) prior to treatment to 5 g/d at its conclusion. No abnormalities in serum chemistries (creatinine, BUN, SGOT, SGPT), urinalysis, EKG, or physical examination were found during or following therapy. No signs or symptoms of adrenal insufficiency or toxicity were seen at any of the dose levels.

In contrast to the marked improvement in these glucocorticoid sensitive parameters, urinary free cortisol, plasma cortisol, and ACTH levels remained significantly elevated throughout the treatment with RU 486. G50 gel chromatography revealed that 85% of ACTH immunoreactivity was at the same fraction as ACTH 1-39. Prior to initiation of RU 486 therapy, the mean plasma ACTH concentration was  $165 \pm 7.6$  pg/ml (mean  $\pm$  SE, n = 5); during treatment it was  $241 \pm 14$  pg/ml (mean  $\pm$  SE, n = 14; normal 8 - 15 pg/ml). The mean plasma cortisol concentration was  $43.5 \pm 3.3$  ug/dl (mean  $\pm$  SE, n = 7) before and  $31.8 \pm 2.0$  ug/dl (mean  $\pm$  SE, n = 27) during RU 486 administration (normal 8 - 18 ug/dl). Mean daily urinary free cortisol excretion rates were also elevated at  $4865 \pm 1159$  ug/24 hours (mean  $\pm$  SE, n = 11) prior to and  $1175 \pm 327$  ug/24 hours (mean  $\pm$  SE, n = 27) during therapy (normal 20 - 95 ug/24 hours).

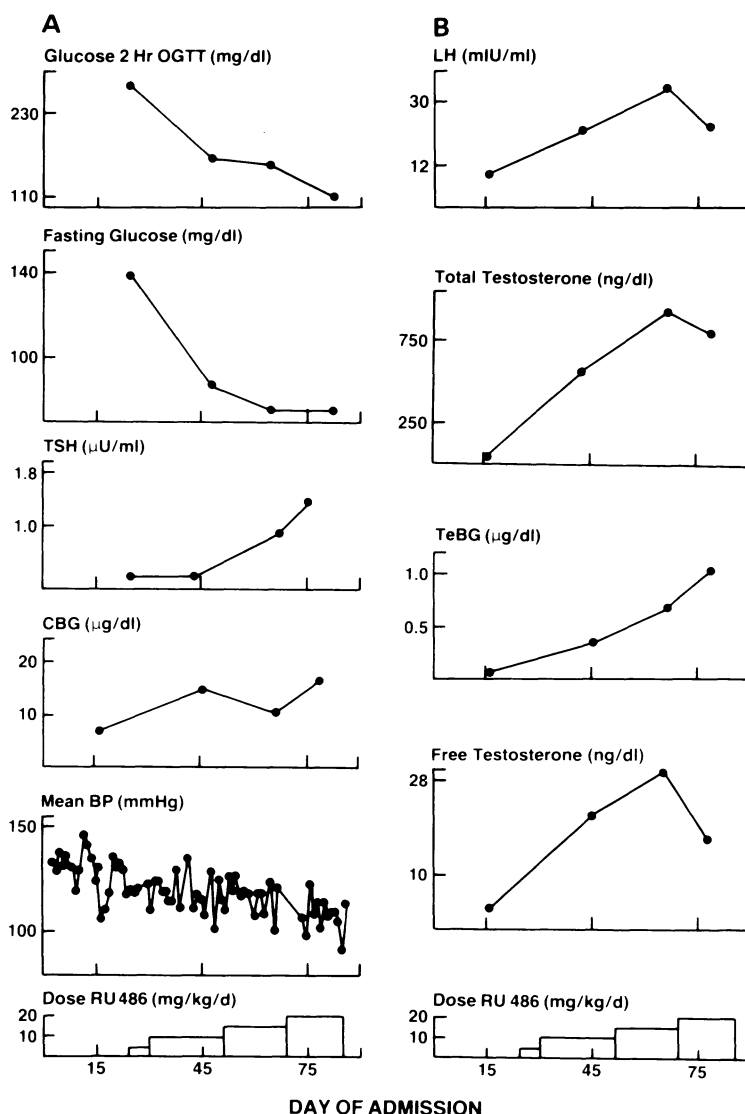


Figure 1. The effect of RU 486 treatment on glucocorticoid-sensitive parameters, cortisol and ACTH. (All ordinate scales are linear; for normal values see text). Panel A: Two hour post-OGTT and fasting blood sugar levels were elevated prior to RU 486 therapy and fell to normal levels during treatment. Serum TSH concentration was initially subnormal and rose progressively. CBG capacity, initially subnormal, normalized during therapy. Mean daily blood pressure decreased during RU 486 therapy. Panel B: Plasma concentration of LH, total testosterone and free testosterone were initially depressed; all normalized with RU 486 therapy. TeBG capacity showed similar increases. (From Nieman et al., 1985).

Plasma steroid precursor concentrations during therapy were within the normal range or mildly elevated. Pregnenolone was 124 ng/dl (normal <250), 17-hydroxyprogesterone was 706 ng/dl (normal <200), and 11-deoxycortisol was 431 ng/dl (normal <200). This suggests that no significant inhibition of 3 $\beta$ -hydrosteroid dehydrogenase, 21-hydroxylase or 11-hydroxylase had occurred during therapy with RU 486.

On the 10th week of therapy, because limited availability of RU 486 prevented further treatment, the patient underwent a bilateral adrenalectomy 48 h after discontinuation of therapy and under supplemental coverage with glucocorticoids. The tissue vascularity appeared normal at the time of surgery and his postoperative course and wound healing were satisfactory. There were no surgical complications.

#### COMMENT

The glucocorticoid antagonist RU 486 ameliorated the clinical and biochemical features of hypercortisolism in this patient. Treatment with RU 486 was associated with resolution of severe depression, hyperglycemia and hypertension, obviating the need for a variety of medications.

It is interesting that both the hypertension and the hypokalemic alkalosis normalized with RU 486 since presumably RU 486 has no antimineralocorticoid properties (Philibert et al., 1981). Probably, at the doses of RU 486 employed, such an activity may be present in addition to its known antiglucocorticoid potency.

No signs or symptoms of adrenal insufficiency were seen in our patient even at the highest dose of RU 486. However, this is a potential risk of RU 486 therapy if we assume that it has no agonist effects (Gaillard et al., 1983). Since glucocorticoid insufficiency cannot be assessed with measurement of adrenal steroids during RU 486 therapy, we suggest that patients be given RU 486 in gradually increasing doses in concert with careful evaluation for signs and symptoms of adrenal insufficiency.

Although RU 486 was effective therapy in our patient with Cushing's syndrome due to ectopic ACTH secretion, control may be more difficult to achieve in patients with hypercortisolism of pituitary origin (Cushing's disease). Previous studies in nonhuman primates and normal volunteers suggest that the dose of RU 486 necessary to achieve normal glucocorticoid status in Cushing's syndrome will depend on the free cortisol concentration and the presence of cortisol feedback. In nonhuman primates and normal men and women, doses of RU 486 greater than 5 mg/kg caused an increase in both cortisol and ACTH levels, presumably by antagonizing cortisol feedback at the pituitary or hypothalamus (Healy et al., 1983, 1985; Herrmann et al., 1981; Gaillard et al., 1983; Bertagna et al., 1984). In patients with Cushing's disease in whom cortisol feedback is present, ACTH levels may increase, perhaps in an exaggerated manner as is often the case with ACTH responses to CRF (Chrousos et al., 1984) or metyrapone (Tucci, 1975).

The lack of side effects or toxicity associated with RU 486 administration in our patient contrasts markedly with the morbidity that characterizes the other medical treatments for hypercortisolism. Although the incidence of side effects cannot be established until additional patients are studied, the present experience suggests that RU 486 therapy may be tolerated better than other currently available medical treatments of hypercortisolism. Currently, the major drawback of RU 486 therapy is that the drug is costly to synthesize and not available in quantities sufficient for extensive clinical studies. RU 486 holds promise as a safe, well-tolerated and effective medical therapy for hypercortisolism that merits further clinical evaluation.

## REFERENCES

- Baxter, J. D., and Tyrrell, J. B., 1981, The Adrenal Cortex in: "Endocrinology and Metabolism," P. Felig, J. D. Baxter, A. C. Broadus, and L. A. Frohman, eds., McGraw-Hill Book Co., New York.
- Bertagna, X., Bertagna, C., Luton, J. P., Hussen, J. M., and Girard, F., 1984, The new steroid analog RU 486 inhibits glucocorticoid action in man, J. Clin. Endocrinol. Metab., 59:25-28.
- Carey, R. M., Orth, D. N. and, Hartmann, W. H., 1973, Malignant melanoma with ectopic production of adrenocorticotrophic hormone: palliative treatment with inhibitors of adrenal steroid biosynthesis, J. Clin. Endocrinol. Metab., 36:482-487.
- Chrousos, G. P., Schulte, H. M., Oldfield, P. W., Cutler, G. B., and Loriaux, D. L., 1984, The corticotropin-releasing factor stimulation test: an aid in the evaluation of patients with Cushing's syndrome, N. Engl. J. Med., 310:622-626.
- Chrousos, G. P., Cutler, G. B. Jr., Sauer, M., Simons, S. S. Jr., and Loriaux, D. L., 1983, Development of Glucocorticoid Antagonists, Pharmacol. Ther., 20:263-281.
- Coll, R., Horner, Z., Kraiem, Z., and Gafni, J., 1968, Successful metyrapone therapy of the ectopic ACTH Syndrome, Arch. Intern. Med., 121:549-553
- Duick, D. S., and Wahner, W. H., 1979, Thyroid axis in patients with Cushing's syndrome, Arch. Intern. Med., 139:767-772.
- Fauci, A. S., Dale, D. G., and Balow, J. E., 1976, Glucocorticosteroid therapy: mechanism of action and clinical considerations (a combined clinical staff conference, Clinical Center, National Institutes of Health, Bethesda, Maryland), Ann. Intern. Med., 84:304.
- Gaillard, G. C., Riondel A., Herrmann, A. F., Muller, F., and Baulieu, E. E., 1983, The antifertility steroid RU 486 is an anticorticosteroid depressing the pituitary-adrenal system in the human, but only at a specific time during the day, Presented at the 65th Annual meeting of the Endocrine society, abstract 219.
- Gold, E. M., 1979, The Cushing syndrome: changing views of diagnosis and treatment, Ann. Int. Med., 90:829-844.
- Gorden, P., Becker, C. E., Levey, G. S., and Roth, J., 1968, Efficacy of amino-glutethimide in the ectopic ACTH syndrome, J. Clin. Endocrinol. Metab., 28:921-923.
- Healy, D. L., Chrousos, G. P., Schulte, H. M. Williams, R. F. Baulieu, E. E., Gold, P. W., and Hodgen, G.D., 1983, Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU 486 in primates, J. Clin. Endocrinol. Metab., 57:863-865.
- Healy, D. L., Chrousos, G. P., Schulte, H. M., Gold, P. W., and Hodgen, G. D., 1985, Increased adrenocorticotropin, cortisol and arginine vasopressin secretion in primates after the antiglucocorticoid steroid RU 486: dose response relationships, J. Clin. Endocrinol. Metab., 60:1-4.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch G., and Sakiz, E., 1981, Effet d'un stéroïde anti-progesterone chez la femme: interruption de la cycle menstruel et de la grossesse au debut, C. R. Acad. Sc. Paris., 294: 933-938.
- Luton, J-P., Thieblot, P., Valke, J-C., Mahoudeau, J. A., and Bricaire, H., 1977, Reversible gonadotropin deficiency in male Cushing's disease, J. Clin. Endocrinol. Metab., 45:488-495.
- Nieman, L. K., Chrousos, G. P., Schulte, H. M., Loriaux, D. L., and Nisula, B. C., 1984, Adrenal regulation of corticosteroid-binding globulin (CBG). 7th International Congress of Endocrinology, Quebec, Canada, Excerpta Medica, Elsevier Biomedical Press, New York, International Congress Series 652-1096 (1672A).
- Nieman, L., Chrousos, G. P., Kellner, C., Spitz, I., Nisula, B. C., Cutler, G. B., Merriam, G. R., Bardin, C. W., and Loriaux, D. L., 1985, Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU 486, (submitted).

- Philibert, D., Deraedt, R., and Teutsch, G., 1981, RU 38486 a potent antigluccorticoid in vivo, Intern. Congress of Pharmacol., Tokyo, abstract 668.
- Smals, A. G. H., Kloppenborg, P. W. C., and Benraad, T. J., 1977, Plasma testosterone profiles in Cushing's syndrome, J. Clin. Endocrinol. Metab., 45:240-245.
- Temple, T. E., and Liddle, G. W., 1970, Inhibitors of adrenal steroid biosynthesis, Ann. Rev. Pharmacol., 10:199-215.
- Tucci, J. R., Metyrapone test in Cushing's disease, 1975, J. Clin. Endocrinol. Metab., 40:520-523.
- Van Cauter, E., Leclercq, R., Vanhaelst L., and Golstein J., 1974, Simultaneous study of cortisol and TSH daily variations in normal subjects and patients with hyperadrenalcorticism, J. Clin. Endocrinol. Metab., 39:645-652.

RU 486 (MIFEPRISTONE): CLINICAL UPDATE, APRIL 1985

Etienne Baulieu and  
André Ulmann

In this brief note, we attempt to update clinical information on RU 486 which has become available since preparation of the manuscripts included in this volume. RU 486 now has been given the generic name "mifepristone."

Pooling available complete data obtained from Haspels, Bygdeman, Johansson, Mishell, Herrmann, Kovacs, Elia, Sitruk-Ware, and Cornec, we find that in approximately 200 cases of drug administration early in pregnancy between days 35 and 49 (6th and 7th weeks), RU 486 alone (doses of 60-200 mg/day, in 1-2 oral intakes per day for 3-7 days) resulted in about 70% complete success in inducing menstruation, approximately 20% partial success (requiring mechanical evacuation of the uterus), and approximately 10% failures. When a prostaglandin derivative (in most cases 0.25 mg of 16-phenoxy-tetranor-PGE<sub>2</sub>α methylsulfonylamide injected i.m. on the 4th day of RU 486 administration) was given to more than 50 patients, complete success rates were above 95% (Bygdeman). Neither excess bleeding nor any other significant side effect was encountered.

During the first week after the expected day of menses (5th week since first day of last menstrual period), RU 486 has been given orally 100 mg twice per day for four days, with 80% success in 35 patients. In a study still in progress, a group of ten women received 50 mg of RU 486 three times per day for four days, and success has been achieved in all cases (Schaison).

Further studies on early administration of RU 486 are in progress. They include co-administration with different types of prostaglandins (injected or oral), oxytocin, and methergin (oral or i.m.). Clinical trials are planned with injectable and vaginal forms of RU 486.

RU 486 is being tested as a post-coital contraceptive at the dose of 100 mg per day for four days. Preliminary results indicate that no pregnancy occurred in 29 women included in the study thus far (Haspels). When administered during the mid-luteal phase, a high dose of RU 486 (above or equal to 5 mg/kg) given once, always induced luteolysis (Loriaux).

## INDEX

- Abnormality
  - fetal, 2, 9
- Abortifacient, 179, 241, 243
- Abortion, 15, 16, 18, 226, 236, 243, 250, 331
- ACTH, 10, 15, 51, 339, 340, 341, 342, 343
  - ectopic, 339, 340, 343
- ACTH production, 213
- Administration
  - of RU 486, see RU 486, administration of
- Adrenal function, 19
  - of dogs, 315
- Agonist activity
  - of RU 3, 8, 87, 486, 296, 313
- Aldosterone
  - and RU 486, 11, 320
- Amenorrhea, 203, 215
- Analogues
  - of RU 486, 27-44
  - structure, 27-44
- Androgen receptor, 5, 49, 88, 94
- Antagonist, 34
- Antiandrogenic activity, see RU 486, antiandrogenic activity of
- Antifertility agent, 14
- Antiglucocorticoid, 19, 27, 55, 331, 335, 339, 340, 343
  - (see also RU 486, anti-glucocorticoid activity of)
- Antiglucocorticoid activity, see RU 486, anti-glucocorticoid activity of
- Antiglucocorticoid receptor, 5, 9-11
- Antiglucocorticosteroid, see Antiglucocorticoid
- Antigonadotropic activity
  - of RU 486, 276
- Antihormonal activity, 11, 34
- Antiinflammatory assay, 71
- Antiprogestational activity, see RU 486, antiprogestational activity of
- Antiprogesterin, 1-20, 40, 44, 50, 263, 279, 281, 282
- Atkinson, L., 2
- BBT, 13
- $\beta$ -Endorphin, 10
- Behavior, sexual, see Sexual behavior
- $\beta$ -hCG (see also Plasma hormone concentrations, Serum hormone concentrations) and RU 486, 16, 183, 216, 245
- Binding characteristics, 1, 49, 51, 55, 65, 88-90, 115, 298
- Binding site
  - progesterin, 3
- Bioavailability, 117, 155
- Bleeding, 1, 12, 180, 199, 218, 237, 244, 255
  - endometrial, 8
  - menstrual, 264, 266
    - amount of, 208, 218
    - effect of hCG on, 264
    - effect of RU 486 on, 264, 266, 286
  - uterine, 8, 199, 271, 273
- Breast cancer, 9, 307
- 11 $\beta$ -Substituted-19-norsteroids, 27, 44
- Cancer, 9, 307
- CBG, 340, 341, 342
- Cervix, 18, 71
- Clinical update on RU 486, 347
- Computer-generated formations, 27, 32-36
- Contraceptive, 1, 23, 279, 280, 282, 331, 347
- Contragestational agent, 2
- Contragestion, 1, 20, 27
- Corpus luteum, 2, 146, 147, 273, 282
- Corticosterone, 10
- Corticosterone biosynthesis, 49, 58
- Cortisol, 10, 246, 264 (see also Hormone levels)
  - in serum, see Serum hormone concentrations

- Cotton pellet, 72
- Crystal structure, 32-34
- Cushing's Syndrome, 19, 339, 340, 343
- Cytosolic receptor, 115
  
- Decidua, 17
- Decidual cells, 1, 261
- Deciduomata formation, 49, 51, 76, 82-83
  - assay for, 71, 76
- Depression, 19, 340, 343
- Dexamethasone, 136
- Diuresis, 58
- Djerassi, Carl, 2
- DNA, 4, 6, 9
- DNA polymerase alpha, 302
- Dosage, see RU 486, dose schedule of
  
- Early pregnancy
  - and RU 486, 179, 221
- Ectopic pregnancy, see Pregnancy, tubal
- Embryo, 82-83
- Embryotoxicity, 125
- End of the month pill, 193
- Endometrial dose response, 133
- Endometrial glands, 259
- Endometrial proliferation, 51
- Endometrial stroma, 259
- Endometrium, 1, 9, 259, 295, 300
- Epoxide opening, 28
- Estradiol, 3, 279, 280, 281 (see also Plasma hormone concentrations, Serum hormone concentrations)
  - and RU 486, 185, 216, 224, 246
- Estrodial benzoate, 295
- Estrodial dehydrogenase, 299, 302
- Estrogen, 2
- Estrogen receptor, 61, 88, 94
- Estrogenic activity, see RU 486, estrogenic activity of
- Excretion
  - of radioactivity, 117
- Extrauterine pregnancy, see Pregnancy, tubal
  
- Fertility control, 1, 2, 3, 27, 229, 249, 331
- Fetal abnormality, 9
- First-step reactions, 13
- Follicle maturation, 14
- Follicular growth, 14
- Follicular phase
  - and administration of RU 486, 190
  
- Genetic toxicity, 125
- Gestation
  - and RU 486, 155-157
  
- Giant mitochondria, 7, 79
- Glucocorticoid antagonist, 277, 339, 340
- Glucocorticoid receptor, 1, 5, 49, 55, 87-96, 285
- Glucocorticosteroid antagonist, see Glucocorticoid antagonist
- Glucocorticosteroid receptor, see Glucocorticoid receptor
- Glycerol
  - and RU 486 binding, 300
- Gonadotropins, 9, 264, 299
- Gonadotropin receptor, 149
- Gonadotropin secretion, 274
  - in vitro, 150-152
  - and RU 486, 271
- Granuloma
  - formation of, 72
- GTT
  - oral, 339, 340, 341, 342
  
- Half-life
  - of glucocorticoid agonists, 92
  - of RU 486, 7, 328
- HCG
  - administration, 9, 13, 149-150
  - effects of
    - on serum hormone levels, see Serum hormone concentrations
    - on menstrual cycle, see Bleeding, menstrual levels, 1, 237, 254 (see also Hormone levels)
    - production, 1, 16
- Hirsutism, 19
- Histopharmacology
  - of RU 486, 79
- Hormone levels, 16, 128, 129, 184-186, 212, 216, 237, 245 (see also Serum hormone concentrations, Plasma hormone concentrations)
- Hormone receptors, 88, 94
- Hormone secretion
  - inhibition of, 137
- Hydrogen bonding, 40, 41
- Hydrophobic pocket, 28, 41, 42, 44
- Hypertension, 19, 340, 342, 343
- Hypervolemia, 328
- Hypocorticosteroidism, 19
- Hypocortisolism, 288, 339, 341, 343
- Hypothalamic pituitary function, 8, 13, 127
- Hypothalamus, 13
  
- Implantation, 15, 16, 18, 75
- Incomplete abortion, 203
- Infertility, 179

- Interruption of pregnancy, see
  - Pregnancy, termination of
- Irregularity of menstrual cycle, see Menstrual cycle, irregularity of
- Late luteal phase, 12, 13, 197
- LH, 1, 8, 12; 340, 341, 342
  - decrease, 12, 13, 14, 15
  - increase, 8, 14
  - release, 11, 14
- LHRH
  - response in vitro, 151
- Luteal function
  - and RU 486, 271
- Luteal adenyl cyclase activity
  - human, 147
    - ethanol effects, 147
    - forskolin stimulation, 147, 148
    - hCG responsiveness, 147-149
    - RU 486 effects, 149, 150
- Luteal phase length, 2, 8, 12, 13, 14
- Luteal phase length
  - effect of RU 486 on, 9, 142-143, 285
- Luteolysis, 12, 281, 282
- Mammary cancer, see Breast cancer
- Mechanism of action, see RU 486, mechanism of action of
- Men
  - and RU 486, 331
- Menopause, 15, 295
- Menstrual cycle, 127, 128, 169, 279, 280, 282
  - effect of RU 486 on, 12, 149, 158, 170, 179-198, 195, 334
  - follicular phase, 190
  - interruption of, 12, 334
  - irregularity of, 169, 176, 195
  - late luteal phase, 197
  - luteal phase, 8, 12, 285
    - effect of hCG on, 264
  - midluteal phase, 163, 196, 271
    - and progesterone, 2, 3
  - secretory phase, 192
- Menstrual induction, 14, 127, 130, 160, 161, 279, 290, 347
- Menstrual regulation, 14, 15
- Menses, 279-282, 285
- Metabolic pathway, see RU 486, metabolic pathway of
- Microscopy
  - electron, 16, 79
  - light, 79
- Midluteal phase, 163, 196, 271
- Mifepristone, 347
- Mineralocorticoid receptor, 5, 88, 94
- Mitochondria, 52, 85
  - giant, see Giant mitochondria
- Myometrium, 17
- Mitosis, 84, 85
- Morning after pill, 199
- Myometrial contractions, 1, 17
- Necrosis, 82-83
- Neuroendocrine function, 19
- NMR, 32
- Non-responders
  - to RU 486, 321, 328
- Nuclear uptake, 93
- Ovariectomy, 82
- Ovulation, 2, 12, 14
- Oxytocin, 18
- Parturition, 18, 155
  - timing of in rats, 9, 69
- Pharmacokinetic studies, see RU 486, Pharmacokinetics of
- Pharmacokinetics, see RU 486, pharmacokinetics of
- Pharmacology
  - of RU 486, 49-65, 79
- Pinkus, Gregory, 2
- Pituitary cells, 1, 8, 10, 13, 55
  - in culture, 150-152
- Pituitary dose-response, 131, 133
- Pituitary function, 13, 14, 150, 275
- Pituitary-adrenal axis, 10, 15, 19, 271
  - of dog, 315
- Plasma hormone concentrations, 12, 13, 16, 134, 162, 165, 185, 199, 212, 223, 287, 292, 293, 299, 328, 331
  - ACTH, 10, 11, 15, 132, 224, 331
  - aldosterone, 11, 325
  - $\beta$ -hCG, 185, 199
  - cortisol, 224, 331
  - estradiol, 162, 224, 281, 299
  - gonadotropins, 264, 295, 299
  - hCG, 254
  - LH, 12, 13, 15
  - progesterone, 158, 162, 165, 185, 199, 254, 281, 299
  - prolactin, 132
- Plasma kinetics, 106
- Plasma protein, 6, 94
- Post-coital antifertility agent, 14, 204
- Post-coital contraceptive, 347
- Post-menopausal women, 15, 295
- Pregnancy, 3, 15, 71, 121
  - effect of RU 486 on, 179-198, 211
  - termination of, 1, 2, 9, 15, 71, 72, 74, 75, 199-209, 211-213, 221-233, 331, 335

Pregnancy (continued)  
 tubal, 18, 179, 187, 221  
 and RU 486, 179

Progestational activity, see RU 486, progestational activity of

Progesterone, 1, 2, 3, 5, 6, 9, 11, 13, 16, 18, 279-282(see also Plasma hormone concentrations, Serum hormone concentrations)  
 and RU 486, 185, 216, 246, 254, 261  
 role of, 1  
 secretion of during menstrual cycle, 133

Progesterone antagonist, 279

Progesterone receptor, 2, 5, 6, 27-44, 87-96, 261, 301, 307

Progestomimetic activity of RU 486, 77, 296

Prostaglandin F<sub>2</sub>-alpha, 187, 259

Prostaglandins, 16, 18, 83, 253  
 side effects of, 199

Pseudopregnancy, 7, 8, 71, 263, 267

Questionnaire  
 women's reaction to RU 486, 200-203

R 5020, 87

Radioimmunoassay, 99, 100, 103, 128, 249, 299(see also RU 486, radioimmunoassay of)

Reaction  
 personal to RU 486 treatment, see Questionnaire

Receptor activation, 90, 92, 93

Receptor binding, 1, 11, 298

Receptor complex activation, 44

Receptor mapping, 40-42

Releasing factor, 14

Responders  
 to RU 486, 11, 321, 328

RU 486, 1, 27, 37, 39, 41, 250, 259, 279, 280, 281, 282, 285, 316, 328, 339, 340, 343  
 administration of, 6, 9, 11, 49, 69, 71, 72, 73, 75, 82, 116, 123, 128, 156, 162, 169, 180, 199, 211, 222, 235, 249, 271, 286, 288, 291, 347  
 agonist activity of, 296, 313  
 antagonist activity of, 34  
 antiandrogenic activity of, 11, 49, 61, 62, 65  
 antiglucocorticoid activity of, 19, 55, 71, 73, 87, 213, 289, 335

RU 486 (continued)  
 antiprogestational activity of, 34, 37, 39, 50, 71, 73, 79, 85, 87, 156, 296  
 binding of, 300  
 bioavailability of, 155  
 biochemical profile of, 87-97  
 clinical effects of, 235  
 dose schedule of, 10, 11, 82, 69, 134, 158, 162, 221, 244  
 estrogenic activity of, 61  
 level of action of, 271  
 mechanism of action of, 4, 28, 44, 144  
 schematic representation, 44  
 metabolic pathway of, 8, 103, 113, 115  
 metabolism of, 6  
 pharmacokinetics of, 103-122, 250  
 plasma levels of, 116, 165, 287, 324  
 potency of  
 antiprogesterone, 73, 75  
 anticortisol, 73, 75  
 progestational activity of, 43  
 radioimmunoassay of, 99, 103  
 side effects of, see Side effects  
 synthesis of, 29, 30  
 toxicity of, 10, 123-126

Rubin test, 85

Sakiz, E., 3

SCRIPT program, 32, 33, 35-37, 41

Secretory phase, 192  
 and administration of RU 486, 193

Segal, S. J., 2, 14

Serum chemistry  
 effect of RU 486 on, 220

Serum hormone concentrations  
 effects of hCG on  
 cortisol, 264, 267, 268  
 estradiol, 264  
 FSH, 264, 265  
 LH, 49, 61, 63  
 progesterone, 264, 265  
 and RU 486 treatment, 185  
 cortisol, 246, 264, 267, 268  
 hCG, 243, 264, 266  
 LH, 142-146, 150  
 FSH, 142-146, 150  
 estradiol, 142-146, 150, 246, 264  
 progesterone, 49, 61, 63, 142-146, 150, 185, 246, 264, 266

Sexual behavior  
 effect of RU 486 on, 169-176

Side effects  
 of RU 486, 2, 11, 15, 143, 199, 211, 218, 219, 223, 235, 239, 241, 245, 336

Spontaneous abortion, 17, 179, 199

Steroid-receptor interaction  
kinetics, 27, 44  
Structure of RU 486, 1  
of RU 486 analogues, see  
Analogues, structure  
Structure-activity relationship,  
34-40

TEBG, 6, 340, 341, 342  
Testosterone, 5, 340, 341, 342  
Testosterone estradiol binding  
globulin, see TEBG  
Thymolytic assay, 71  
Thymocytes, 55  
Thymus involution, 73, 74  
Tietze, C., 2  
Toxicity  
acute, 123  
chronic, 123

Toxicity (continued)  
genetic, 125  
of RU 486, see RU 486,  
toxicity of  
Tubal pregnancy, see Pregnancy,  
tubal

Uterine bleeding, see Bleeding,  
uterine  
Uterine contractility, 251  
Uterine evacuation, 1, 17, 18  
Uterine pain, 199, 255  
Uterine sensitivity, 253  
Uterine weight, 11, 61  
Uteroglobin mRNA, 7  
Uterotrophy, 83

Vaginal bleeding, 142, 152  
early onset, 142-146, 150