HORMONE PRODUCTION
BY
THE HUMAN OVARY

A THESIS
SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
OF
THE UNIVERSITY OF GLASGOW
BY

DR. ABDUL SAMAD BIN ABDUL KADER

M.B., B.S. (S'PORE), M.R.C.O.G. (LONDON)

P.P.T.

DEP. OF OBSTETRICS & GYNAECOLOGY
GLASGOW ROYAL INFIRMARY

DEC. 1978.

CONTENTS

LIST OF ABBREVIATIONS

The abbreviations, symbols and conventions recommended by the IUPAC–IUB Commission on Biochemical Nomenclature and published in Biochem. J. (1973) 131, 1–20, have been used throughout this thesis.

In addition the following abbreviations have been used:–

OE_2	=	Oestradiol 17ß
OE_1	=	Oestrone
17OHP	=	17α hydroxy progesterone
A	=	Androstenedione
T	=	Testosterone
DHT	=	Dihydrotesterone
FSH	=	Follicle Stimulating Hormone
LH	=	Luteinising Hormone
PRL	=	Prolactin
P	=	Progesterone
DCC	=	Dextran Coated Charcoal
EP	=	Early Proliferative Phase
MP	=	Mid Proliferative Phase
LP	=	Late Proliferative Phase
ES	=	Early Secretory Phase
MS	=	Mid Secretory Phase
LS	=	Late Secretory Phase
P	=	Peripheral Plasma
O	=	Active Ovarian Vein
C	=	Contralateral Ovarian Vein

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACKNOWLEDGEMENT

I should like to thank Professor M.C. Macnaughton for his supervision of the work and for his perpetual guidance, in this project.

I am indebted to Dr. J.R.T.Coutts who throughout my study has been my mentor, my supervisor, my collaborator and above all my friend. He taught me the rudiments, the essentials and the best. His unselfish and ever accommodating ways will remain indelible in my mind. To you, Sir, my grateful thanks from the bottom of my heart.

My gratitude goes to the staff of the Gonadotrophin Laboratory, Glasgow Royal Maternity Hospital for carrying out the FSH and LH assays and to Dr. Alan Craig of Clinpath Services Ltd. for the PRL assays on the samples collected.

My sincere thanks go to all the staff of the department of the Gynaecological Research Laboratory, Glasgow Royal Infirmary who ensured that existing assays within the department were correctly applied to the study.

Mr. W. McNally of the Department of Obstetrics and Gynaecology, Glasgow University is due my grateful thanks for his assistance in data handling and presentation of figures.

To the Pathology Department of Glasgow Royal Infirmary, in particular Dr. Fred Lees, who performed endometrial and ovarian histology, I owe my thanks.

To the consultants and the nursing staff of the Gynaecology Department, Glasgow Royal Infirmary and Eastern District Hospital, Duke St., for allowing me access to the patients, I offer my sincere thanks.

I am also grateful to the State Government of Johore, Malaysia, for giving me a scholarship for this project.

Finally the ladies without whose participation as donors, this project would not have been made possible, to you I salute. I hope your contribution will serve to help your less fortunate sisters.

SUMMARY

Hormone Production by the Human Ovary

Sensitive, specific, precise and accurate radioimmunoassays have been developed for A, DHT, and T. These assays were applied to determine the profiles of these hormones in daily samples from normally menstruating women. Plasma samples were also obtained at hysterectomy from peripheral and ovarian veins; where practical samples were obtained from the veins draining both the active and the contralateral ovaries. In this study a larger number of such samples had been obtained than has been reported previously and in addition the stage of the menstrual cycle was better documented than in previous reports by using as many parameters as possible – menstrual history, ovarian and endometrial histology, and peripheral hormone levels.

Samples were obtained from 40 patients, of reproductive age, whose conditions did not affect ovarian function and in whom the stage of the menstrual cycle could be accurately assessed. Samples were also obtained from a number of patients with disorders which would affect their reproductive cycles. From the 40 patients with normal ovarian function a composite menstrual cycle was constructed. Using the newly developed radioimmunoassays and pre–established methods the levels of FSH, LH, PRL, P 17OHP, OE_2, A, DHT and T were determined in all plasma samples.

The hormone assays demonstrated that:–

(i) the peripheral levels from the"normally cycling" patients were within the normal ranges obtained from volunteers.

(ii) the Graafian Follicles produced P, 17OHP,A, DHT, T, and OE_2. The amount of these compounds produced varied with the stage of development of the Graafian Follicles.

(iii) the corpus luteum also produced all of the hormones which follicles produced [c.f. (ii) above]. The total production of steroid hormones was greatest during the MS stage of the cycle.

(iv) at certain stages of the cycle particularly the MS the contralateral ovary also produced small but significant quantities of hormones – A, DHT, T and OE_2.

(v) In the pathological and unphysiological conditions examined the ovarian production of hormones was unpredictable but allowed an insight into ovarian function in such patients.

On the basis of these results conclusions were drawn, as to the production of hormones by the human ovary, and the contributions made to this by the different ovarian structures, throughout the menstrual cycle.

INTRODUCTION

Steroid Biosynthetic Pathways

The two primary functions of the human ovary are considered to be the production of mature ova and the secretion of hormones. The hormones which are secreted by the ovaries are steroid hormones. The biosynthetic pathways to these hormones which are active in the human ovary appear to be the same pathways by which steroid hormones are synthesised in other steroid producing glands. Evidence suggests that both the Δ^4 pathway and the Δ^5 pathway to steroid hormones (Dorfman and Unger 1965) are functional (Figures 1.1, and 1.2) in the human ovary (Coutts 1976). These biosynthetic pathways involve the condensation of 15 molecules of acetyl Co A and cyclisation of the condensation product to produce squalene which is then converted to lanosterol (C.30) which in turn loses 3 molecules of carbon dioxide to give rise to cholesterol (C.27) which is the basic precursor of all steroid hormones. The cholesterol side chain is cleaved, iso–caprioc acid is removed and the Δ^5 3ß hydroxy steroid pregnenolone (C.21) is formed. Until this point the biogenesis is common to both Δ^4 and Δ^5 pathways. In the Δ^5 pathway (Figure 1.2) pregnenolone is sequentially converted to 17α hydroxypregnenolone (C.21) and dehydroisoandrosterone (C.19). Any of these Δ^5 steroids may be converted into the Δ^4 3 oxo equivalent and enter the Δ^4 pathway (Figure 1.1). In the latter pregnenolone is coverted to progesterone (Δ^4 3 oxo C.21; P) which is 17α hydroxylated and then converted to androstenedione (C.19;A). Where the Δ^5 persists as far as dehydroisoandrosterone, this compound is converted to A. Thereafter the two pathways are again common with androstenedione being interconvertible with its 17ß reduced isomer, testosterone (T) and these C.19

compounds being converted following oxidative attack at C.19, to C.18 oestrogens–oestrone (OE_1) being the product when A is the substrate and oestradiol (OE_2) where the substrate is T.

Steroidogenesis by the Ovary

The major biologically active hormones secreted by the ovary are P, the luteal hormone, and oestrogens, in particular OE_2. Since the biosynthetic pathways described above are active in the ovary the organ must have the capacity not only to synthesise these hormones but also all of the intermediates on the pathways. Many of these intermediates are important biological hormones in their own right and the rates of synthesis and interconversion of them is of paramount importance to the functional activity of the ovary.

The ovary is a complex organ containing a number of potential hormone secreting tissues including the Graafian follicle, the corpus luteum and the ovarian stroma or interstitial tissue. Initially secretory roles were assigned to different cell types at different stages of the menstrual cycle as a result of histological and histochemical examinations (Deane, Lobel and Romney 1962; Govan 1968). On the basis of these criteria it was concluded that during the follicular phase theca cells secreted steroids whilst granulosa cells acquired steroid synthesising characteristics just prior to ovulation (see Guraya 1971 for review). These initial histochemical studies have been complicated by in vitro biochemical studies. The original biochemical hypothesis, that oestrogens were produced by ovarian follicles, derived from the elegant animal experiments of Falck (1959) who showed that thecal cells and granulosa cells together caused vaginal cornification whilst granulosa cells alone did not. Short (1962) proposed a 'two cell type' theory of ovarian

steroid production during the cycle; the theca interna cells secreting oestrogen during the follicular phase whilst the other cell type, probably luteinised granulosa cells, produced P during the luteal phase. From in vitro incubations with cell preparations Ryan and his colleagues concluded that in the theca cells the Δ^5 pathway functioned producing OE_2 whilst the granulosa cells synthesised P and other steroids via the Δ^4 pathway (Ryan and Petro 1966; Ryan, Petro and Kaiser 1968). Synthesis of steroids by human corpora lutea was demonstrated by in vitro incubation studies largely performed by Savard and his colleagues (Hammerstein, Rice and Savard 1964; Savard, Marsh and Rice 1965). These authors showed synthesis by human corpora lutea, from acetate, of a number of steroids including P, 17α hydroxyprogesterone (17OHP), A, OE_2 and OE_1. This same group of workers (Rice and Savard 1966) and Lanthier and his colleagues (Patwardhan and Lanthier 1969; Somma, Sandor and Lanthier 1969) also showed the ability of the ovarian stroma to synthesise steroids, in particular androgens.

Ovarian Steroid Production

Attempts to confirm the secretion of these hormones by the ovary in vivo in the human have largely involved determination of the levels of these hormones in biological fluids. Prior to 1960 the fluid used was, of necessity, urine, since sensitive methods were not available for assay of the small levels of these hormones present in the plasma. The problems of extrapolation of the urinary metabolites to the active hormones made interpretation of such data difficult.

Since 1961 a number of methods have been described to measure submicrograms quantities of steroids in the peripheral plasma of females. The methods have included chronologically spectrophotometry, fluori-

metry, double isotope derivative techniques, gas liquid chromatography with hydrogen flame and/or electron capture detectors and finally saturation analyses – initially competitive protein binding assays and currently radioimmunoassay. The advent of radioimmunoassay, which allows the measurement of steroids in the picogram range has opened many doors for the determination of hormone levels in normal women and in women with apparently deficient ovarian function. As a result, a great deal of data has been accumulated from a number of laboratories describing the levels of hormones in peripheral plasma samples in women throughout the normal menstrual cycle.

Hormone Levels during the Menstrual Cycle

The biosynthetic activities of the ovaries cannot be isolated from their inter–relationships with other endocrine glands since activity of the ovary at any point in time is a direct consequence of the external stimuli it is receiving. The functioning of the normal menstrual cycle depends upon complex inter–relationships between the ovary, the hypothalamus and the anterior pituitary. Figure 1.3 shows a schematic diagram of these inter–relationships which include negative feedback effects of OE_2 and P on both the hypothalamus and the anterior pituitary; short–loop negative feedback effects of pituitary gonadotrophins on the hypothalamus; ultra–short–loop negative feedback effects of LHRH on the hypothalamus and the exceptional positive feedback effect of OE_2, on the hypothalamus or pituitary.

Data has been published previously from the Department of Obstetrics and Gynaecology showing the profiles of LH, FSH, OE_2, 17OHP and P in the peripheral plasma of normally menstruating women (Coutts 1976). Figures 1.4 – 1.8 depict the mean ± 1SD (n =

12) for FSH, LH, OE_2, 17OHP and P respectively in daily plasma samples throughout the normal menstrual cycle. To allow comparison of cycles of different length, all samples are plotted relative to the day of the LH peak which is designated day 0. Figure 1.9 depicts the mean values for all 5 of these hormones in the normal menstrual cycle. These levels of hormones in the normal cycle are similar to those published from other laboratories (Ross et al 1970; Mishell et al 1971; Abraham et al 1972; Shaaban and Klopper 1973). The basic sequence of events occurring in the normal cycle can be understood by reference to Figures 1.4–1.9. When the corpus luteum regresses the negative feedback inhibition of OE_2 and P is removed. Gonadotrophin releasing hormone is secreted and stimulates the pituitary release of FSH and LH. The peripheral levels of these gonadotrophins rise prior to and during menses (particularly FSH Figure 1.4). This FSH causes follicular development and production of OE_2 which reasserts the negative feedback inhibition on gonadotrophin release. One follicle which has bound FSH within it (McNatty et al 1975) continues to grow whilst in the other growing follicles the growth is arrested and they become atretic. The rising plasma level of OE_2 (Figure 1.6) eventually causes a positive feedback effect which produces the mid–cycle surge of LH (Figure 1.5). This LH causes luteinisation of the follicle and ovulation. Luteinisation begins prior to the LH peak in response to the initial rise in LH levels (see 17OHP levels Figure 1.7). Following ovulation the ruptured follicle develops into a corpus luteum which in the human secretes increasing amounts of steroid hormones including P (Figure 1.8), 17OHP (Figure 1.7) and OE_2 (Figure 1.6). The combined effects of OE_2 and P reimpose the negative feedback preventing gonadotrophin release until luteal regression causes a reduction in these steroids, menses occurs and the next cycle begins.

Ovarian Secretion of Hormones

Although the levels of steroid hormones in peripheral plasma have been of great value in understanding the events which occur in the normal human menstrual cycle and are of particular diagnostic value in certain cases of abnormal ovarian/hypothalamic/pituitary functions, they are not of great value in assessing the actual secretion of the hormones by the ovaries. Since most of the ovarian hormones are also synthesised by the adrenals it is impossible from peripheral plasma samples alone to determine ovarian secretion.

The ovaries are unlike other paired organs in the body in that usually only one of them is active whilst the contralateral ovary, although not inactive, may be described as quiescent and does not in that cycle produce a mature ovum.

Assessment of ovarian secretion of hormones can only be achieved by sampling the blood draining the ovary and in this situation both the active and the contralateral ovarian veins can be sampled. The limitations of the human as an experimental animal have resulted in only very few studies being performed in which levels of hormones have been determined in ovarian venous plasmas (e.g. Mikhail 1970).

Since initiation of the studies presented in this thesis, a few papers have been published giving levels of some hormones in small numbers of samples of ovarian veins (de Jong, Van der Molen and Baird 1974; Calabresi et al 1976; McNatty et al 1976; Guerrero et al 1976; Dell' Acqua et al 1976; Serio et al 1976; Kirschner, Zucker and Jespersen 1976).

Due to the inaccessibility of ovarian venous effluent except at the time of operation, when normal function might be disturbed by complicating factors such as stress or anaesthesia, determinations of secretion and production rates of ovarian hormones is difficult. However, a few such studies have been performed (Baird and Fraser 1974) and where hormone levels in ovarian venous plasma are determined, assessment of peripheral levels simultaneously can allow comparison with normal menstrual cycle samples obtained in the absence of such complications.

In recent years, guided ovarian vein catheterisation technique has enabled selected ovarian vein effluents to be sampled at will, with the subjects being in a state approximating normal physiological condition (Jacobs 1969; Kirschner and Jacobs 1971).

Aims of the Study

The aims of the study presented in this thesis were to assess the ovarian production of a number of hormones. As described previously, in addition to the hormones described in Figures 1.6 to 1.8 the ovary also secretes androgens. According to in vitro studies both A and T have been implicated and 5α dihydrotestosterone (DHT) has been shown to be an intracellular effector of T action (Baulieu 1973). Although peripheral plasma levels of these C.19 androgens had been published from other laboratories it was felt that the levels of A, T and DHT should be measured in normal cycle samples to compare these levels with published data and with peripheral levels in patients providing ovarian venous samples. Recently, prolactin (PRL) has been shown to be involved in the processes of the normal human menstrual cycle (McNatty et al 1975; Rolland et al 1976; Vekemans et al 1977) and in addition to the other hormones, the levels of PRL in peripheral

plasmas throughout the menstrual cycle and in ovarian venous samples were assessed.

The aims of the study presented in this thesis were –

(1) To develop specific, sensitive, precise and accurate radioimmunoassays for A, DHT and T.

(2) Using these assays to establish the profiles of each of these C.19 androgens in daily peripheral plasma samples throughout the menstrual cycle.

(3) To obtain blood samples from patients undergoing surgery for conditions which did not affect their reproductive function – peripheral and bilateral ovarian venous samples where possible.

(4) To determine in these patients which is the active ovary and to use all available material to assess the stage of the cycle.

(5) To obtain samples from peripheral and ovarian veins in patients undergoing surgery who do not have normal reproductive function e.g. post–menopausal patients, patients on oral contraceptives, patients with polycystic ovarian disease etc.

(6) To sample in the same patients (5 above) where present, ovarian cyst, luteal and follicular fluids.

(7) To assess in the samples from (3), (5) and (6), above the levels of A, DHT and T and using methods already available, LH, FSH, PRL, OE_2,

17OHP and P.

(8) To use the data obtained in the above to attempt to assess the ovarian secretion of the steroid hormones studied and where possible the ovarian structures responsible for steroid synthesis.

Figure 1.1

Biosynthetic pathways to steroid hormones – the Δ^4 pathway.

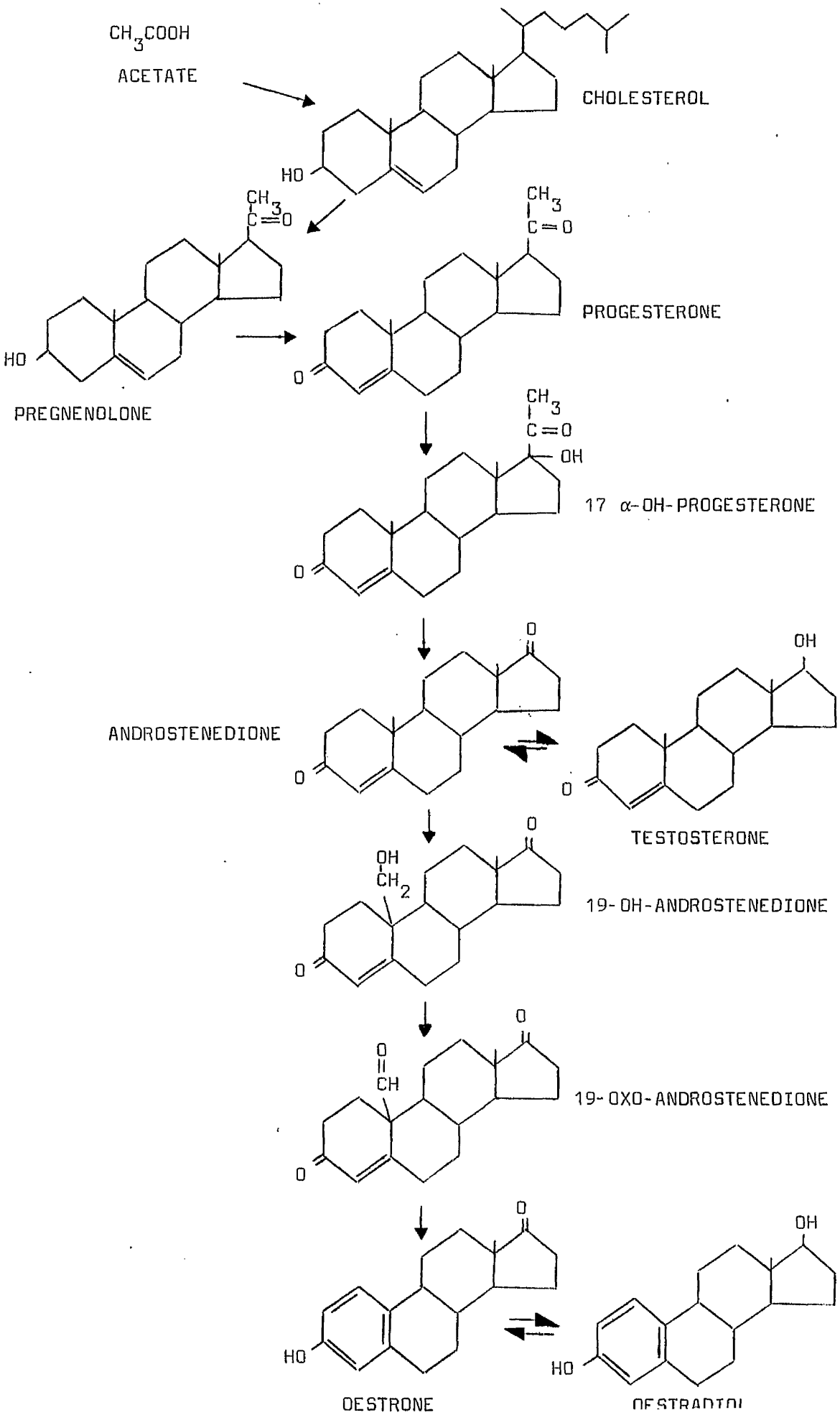


Figure 1.2

Biosynthetic pathways to steroid hormones – the Δ^5 pathway.

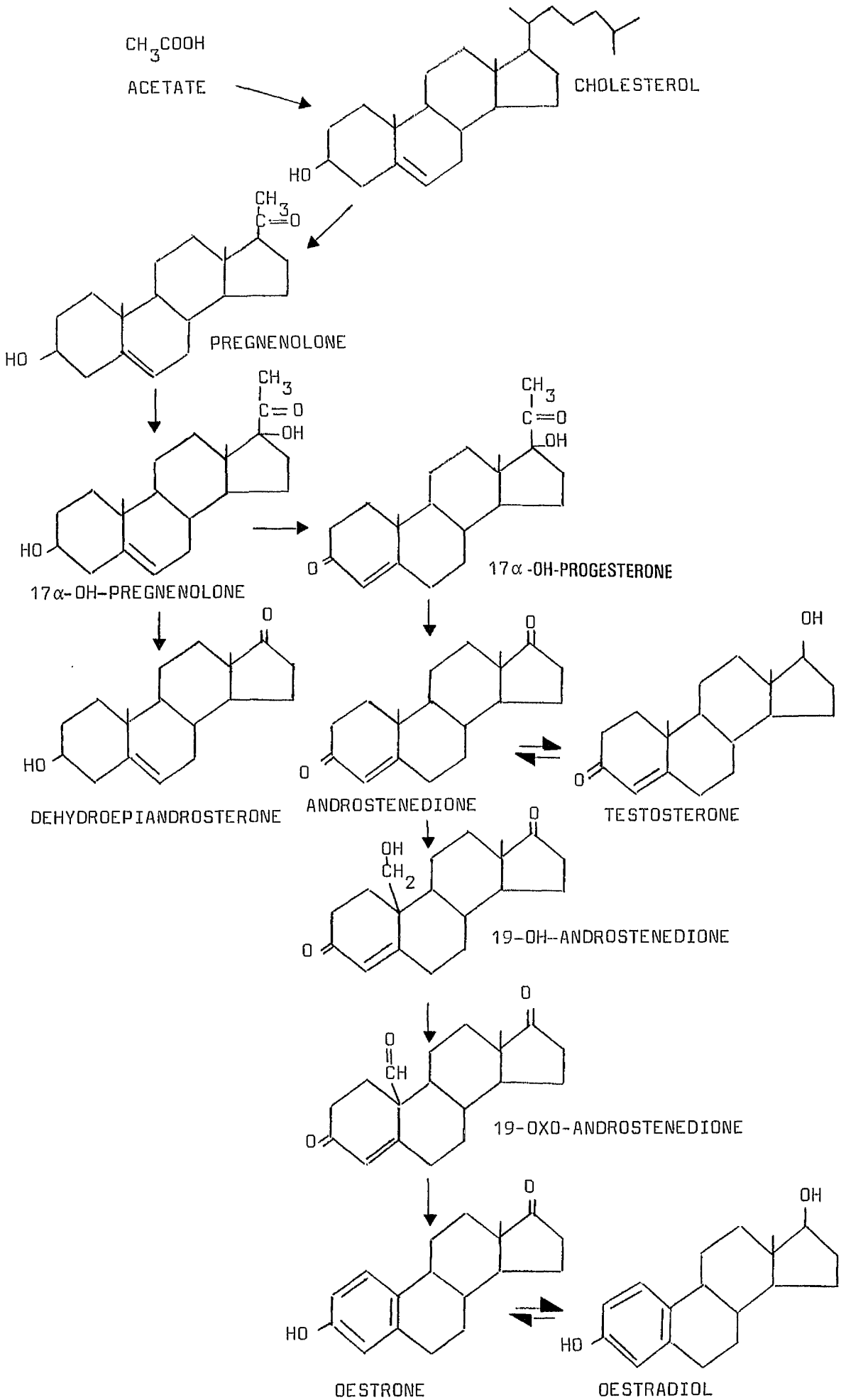


Figure 1.3

Schematic diagram showing the inter-relationships between the ovaries, the pituitary and the hypothalamus (– represents negative feedback and + positive feedback.)

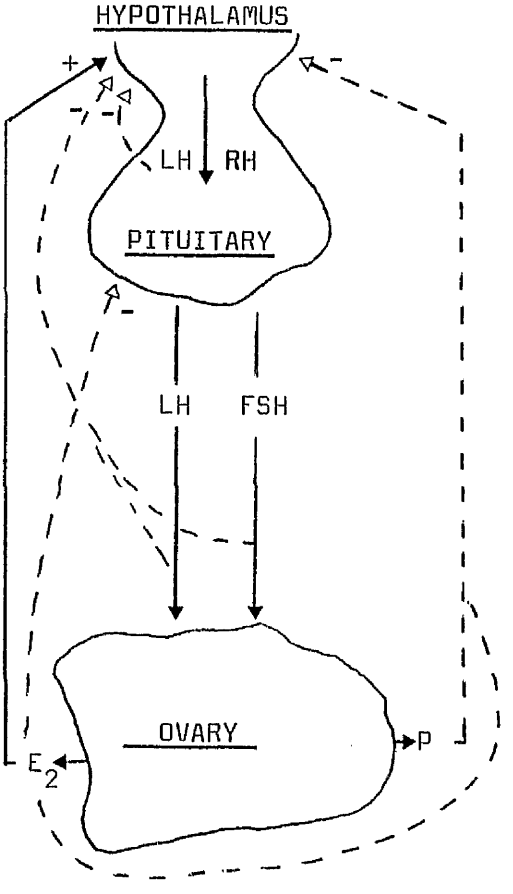


Figure 1.4

Levels of FSH (mean ± 1 I.S.D.) in daily plasma samples throughout the menstrual cycle in normally menstruating volunteers (n = 12).

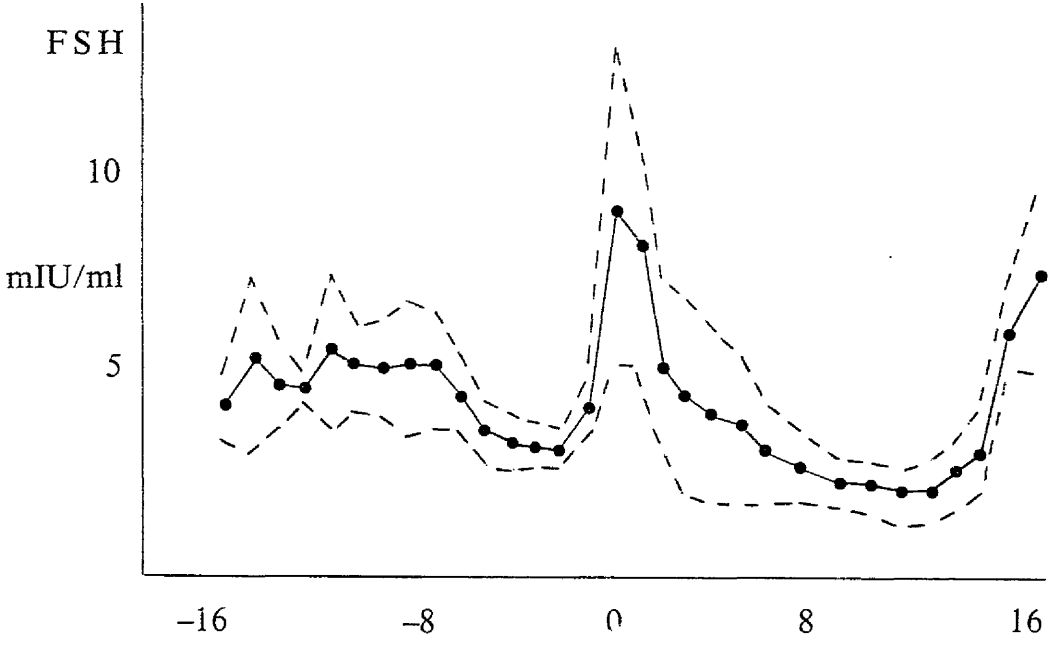


Figure 1.5

Levels of LH (mean ± I.S.D.) in daily plasma samples throughout the menstrual cycle in normally menstruating volunteers (n = 12)

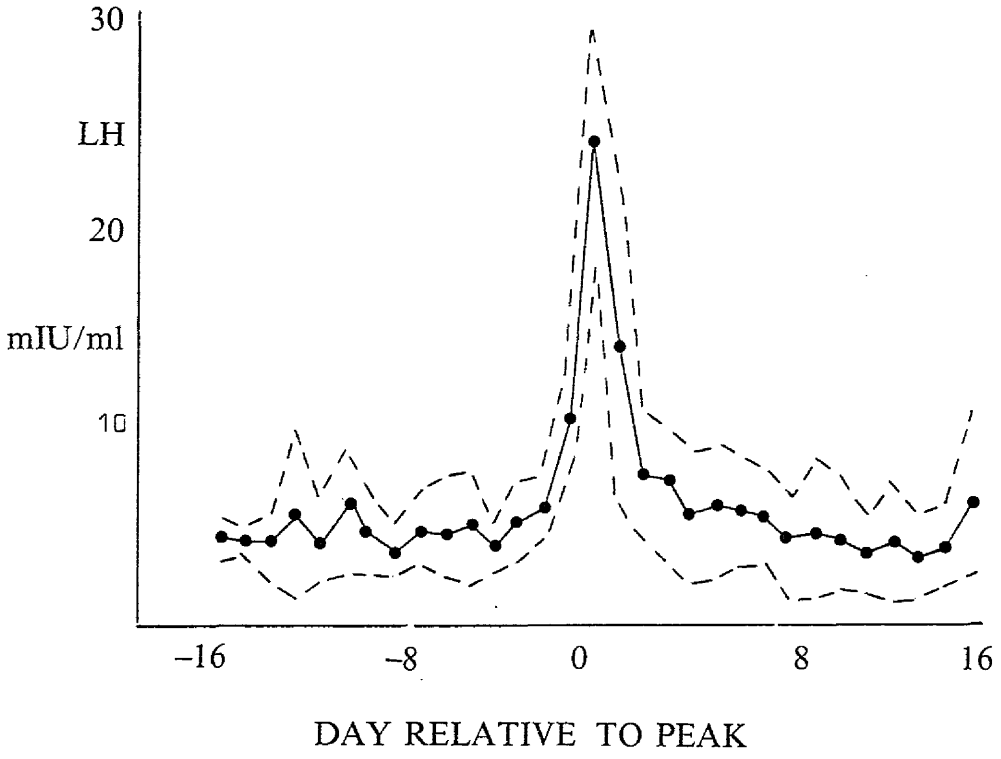


Figure 1.6

Levels of OE_2 (mean ± I.S.D.) in daily plasma samples throughout the menstrual cycle in normally menstruating volunteers (n = 12)

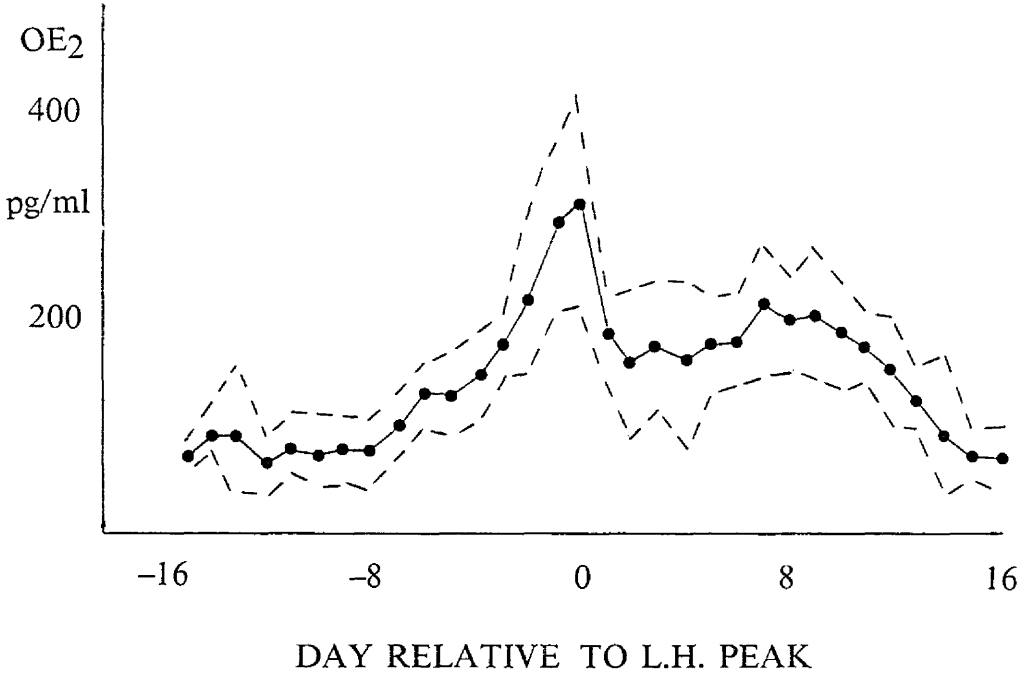


Figure 1.7

Levels of 170HP (mean ± I.S.D.) in daily plasma samples throughout the menstrual cycle in normally menstruating volunteers (n = 12)

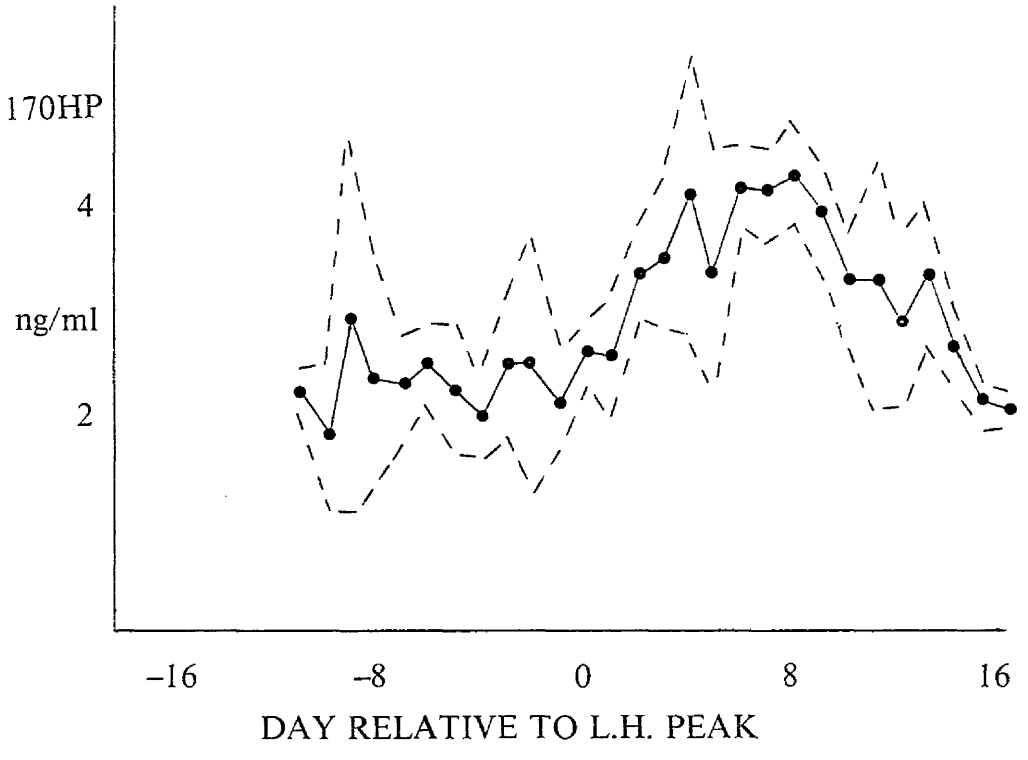


Figure 1.8

Levels of P (mean ± I.S.D.) in daily plasma samples throughout the menstrual cycle in normally menstruating volunteers (n = 12)

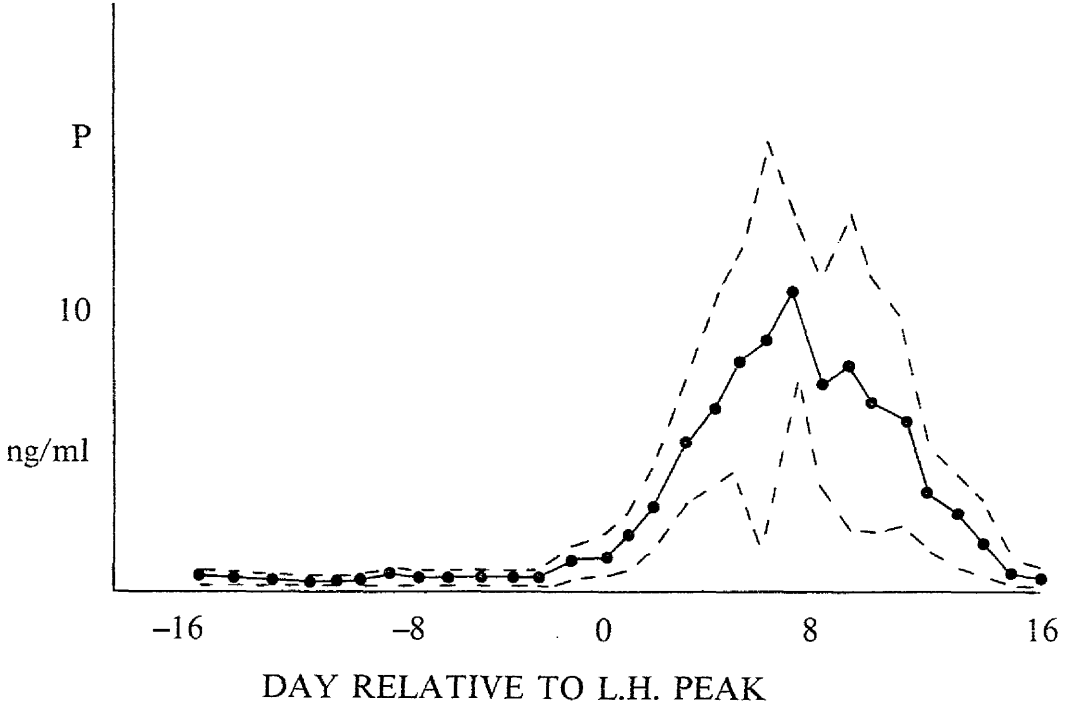
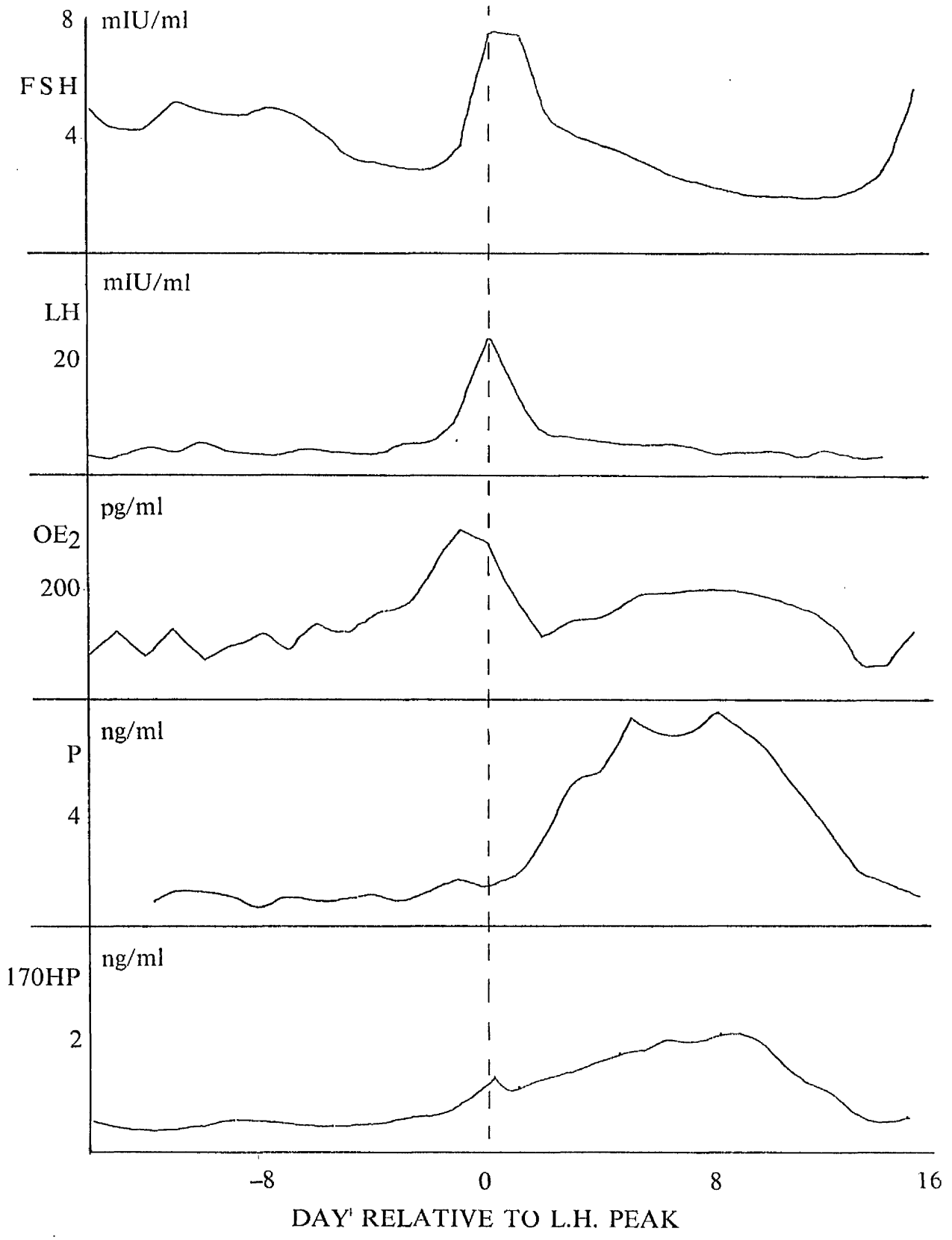


Figure 1.9

Composite graph of mean levels of FSH, LH, OE_2, P and 170HP during the normal human menstrual cycle.



MATERIALS AND METHODS

MATERIALS

Solvents

All solvents used were either 'Analar' grade obtained from BDH Limited, or 'Pronalys' grade purchased from May and Baker Limited. Where solvents were distilled, a 60 cm Vigreux Column was used (Quickfit Limited); the first and last 100 ml of each distillation being discarded.

Diethyl ether (BDH Limited), ethyl acetate (BDH Limited) and methanol (BDH Limited) were each distilled twice prior to use. Diethyl ether and both benzene (BDH Limited), and 2,2,4 trimethyl pentane (iso–octane) (BDH Limited), which were redistilled once, required to be distilled within 24 hours of use. Hexane (boiling point 67 – 70°; May and Baker Limited) and all other solvents were used as supplied by the manufacturers without purification.

Water used for rinsing glassware and to make up solutions for radioimmunoassay was de–ionised (Permutit Company Mark II portable de–ioniser) and then distilled in an all glass still (Quickfit from Jobling Limited).

Chemicals

Where possible 'Analar' grade chemicals were employed. Di-sodium hydrogen phosphate (anhydrous), sodium dihydrogenortho-phosphate and gelatin were obtained from BDH Limited and dextran

T70 from Pharmacia Fine Chemicals, Uppsala, Sweden. Charcoal (Norit NK) from Hopkins and Williams Limited was washed twice with methanol, the 'fines' being decanted each time, and dried thoroughly before use. Thimerosal, which was used as an antibacterial agent (0.01%), was obtained from Sigma Limited (London).

Steroids

Progesterone, 17OHP, T, A and DHT were purchased from Steraloids Limited. Oestradiol was purchased from Koch–Light Laboratories Limited.

These steroids were purified by paper chromatography and recrystallised prior to use as standards for radioimmunoassay. Stock solutions of these steroids (1mg/ml) were prepared in methanol and stored at 4°. Solutions of the concentration required for radioimmunoassay were prepared by dilution of these stock solutions.

Other authentic steroids which were used in cross reaction studies were generous gifts from Dr. Kirk of the MRC Steroid Reference Collection.

Radioactive Steroids

Progesterone–[1,2–H^3(N)] (33.5Ci/mmol), 17OHP– [1,2–H^3(N)] (49.2Ci/mmol), T–[1,2–H^3(N)] (42.3Ci/mmol), DHT–[1,2,4,5,6,7–H^3] (80Ci/mmol) and A [1,2–H^3] (40Ci/mmol), were purchased from New England Nuclear Corporation (Frankfurt, Germany).

Oestradiol–17ß–[6,7–H^3] (40Ci/mmol) and DHT–[4^{14}C](50mCi/-mmol) were purchased from the Radiochemical Centre (Amersham).

All radioactive steroids were stored in solutions, in methanol or methanol/benzene mixtures (100ml) at 4°. The purity of these standards was checked by paper chromatography on receipt from the manufacturers and at approximately 2 monthly intervals whilst in use.

Solutions for Radioimmunoassay

Phosphate Buffer Solutions

61.0ml 0.1M disodium hydrogen phosphate solution were thoroughly mixed with 39.0ml of sodium dihydrogen phosphate. An equal volume of this mixture when mixed with double distilled water gave 0.05M phoshate buffer solution of pH 7.0.

The pH was checked with a pH meter (E.I.L. Limited) prior to using this buffer solution to prepare reagents required for the radioimmunoassays.

Gelatin Solutions

0.1% gelatin solution was prepared by dissolving 100mg gelatin and 0.01mg thimerosal per 100ml of 0.05M phosphate buffer pH 7.0.

Dextran Coated Charcoal (DCC)

Dextran coated charcoal solutions were prepared by dissolving the required amount of charcoal, dextran T70 and gelatin in 0.05M phosphate buffer pH 7.0. The recipes for the DCC used in the individual assays are described under the methodological section for each assay.

Tritiated Steroid Solutions

Tritiated solutions of steroids which were used for radioimmunoassay were prepared by diluting the stock tritium labelled material to the desired concentration with 0.1% gelatin solution. The concentrations were adjusted to give the radioactivity described in the individual methods in 0.1ml of solution.

Antisera Solutions

Each antiserum was diluted to 1/100 with 0.1% gelatin solution and aliquots were stored at –20° until required. A fresh aliquot was diluted to the working dilution of the antiserum in 0.1% gelatin on the day of use.

Equipment

The following pieces of apparatus were used routinely in radioimmunoassays.

Whirlymixer

For the purposes of ensuring even mixing of the reagents in the tubes, each was placed on the whirlymixer (Fisons Scientific Apparatus Limited) and vortexed for a period of 4 seconds.

Magnetic Stirrer

A magnetic stirrer (Toyo, supplied by Chem Lab Instruments Limited) was used to stir solutions required for the radioimmunoassay to ensure homogeneity, particularly of the DCC suspension.

Repettes

A 10.0 ml repette (Jencons Limited) was used to deliver repetitive amounts of 1 ml DCC suspension.

Hamilton Syringes

For dispensing repetitive amounts of 10, 20, 100 and 200 ul Hamilton Gas Tight Syringes (V.A. Howe Limited) with repeating dispenser attachments (P.B. 600: P.B. 600–10) were used.

Oxford Samplers

Precise and reproducible sampling of plasmas was performed using mono–range Oxford samplers (Oxford Laboratories Limited; supplied by Boehringer Limited, London) with inert disposable, non–wetting fluoroplastic tips.

Vacuum Oven

Solvents were evaporated from extracts in a vacuum oven (Qualivac, LTE Limited from G.D. Searle and Company) at –24 inches of mercury and a temperature less than 40°.

Centrifuge

The free steroid fractions bound to DCC were spun down by centrifugation in a refrigerated centrifuge (MSE 6L with multiplace head) at 4° and 1,500 g.

Glassware

All tubes used for radioimmunoassay were used as disposable and discarded after use. Tubes (Excelo Limited) were rinsed copiously

before use with deionised distilled water, dried and washed with the extracting solvent.

Scintillation Counting

Samples for determination of radioactivity were counted at preset settings for H^3 in a Nuclear Chicago Isocap 300 Liquid Scintillation Spectrometer (G.D. Searle and Company) in 7ml of NE 250 liquid scintillation fluid. This scintillation fluid was obtained commercially from Nuclear Enterprises Limited, Edinburgh. It is a dioxan based scintillator similar to Bray's solution (Bray 1960). Using this scintillation fluid H^3 was counted in the aqueous samples from radioimmunoassays (1.2ml 0.05M phosphate buffer/7ml NE 250) with an efficiency of 34% and in the organic based samples from chromatography (0.25ml methanol/7ml NE 250) with an efficiency of 48%.

Scintillation Vials

Disposable polypropylene scintillation vials (Intertechnique Limited) were used in all cases. The scintillation fluid was placed in the vials and the aliquot for determination of radioactivity was added.

Blood Samples

Normal Cycle Samples

The determination of the levels of steroid and gonadotrophin hormones in the peripheral blood of normally menstruating volunteers presented a number of problems.

1. Definition of what is a 'normal cycle'.

2. Collection of all samples at the same time each day to ensure that any variability during the day did not complicate the results.

For the purposes of this study 'normal cycles' were defined as cycles which

a) had a luteal phase of at least 13 days (Rock, 1949; Vande Wiele and Turksoy, 1965) measured from the day of the maximum LH value until the onset of the following menses.

b) were obtained from subjects having no abnormalities or special peculiarities – either medical or menstrual.

Ideally in addition to the above criteria for inclusion in the normal series, the subjects should be of proven fertility.

It was not possible, however, to obtain volunteers who fulfilled the last criterion.

Serial daily plasma samples were collected throughout 7 cycles from 5 volunteers. These women were healthy, aged 18 to 26 years, nulliparous and unmarried. The criteria therefore used initially in selecting appropriate subjects were:–

a) a history of regular menstrual cycles of between 27 and 32 days,
b) no general medical or gynaecological abnormality and
c) no history of taking exogenous source of a hormone preparation.

In hindsight these volunteers were all shown to have luteal phases of at least 13 days.

Daily blood samples (10ml) were collected at 9.00 a.m. each morning from the antecubital veins into lithium heparin containers. Venesection was performed on alternate arms each day with the patient in a sedentary position. Plasmas were separated within 30 minutes by centrifugation on a bench centrifuge at 1500 r.p.m. Plasma aliquots were removed for protein hormones (LH, FSH and PRL) and the remainder of each plasma was stored at –20° until assay of the steroid hormones.

Ovarian Venous Samples

Informed consent was obtained from patients going for hysterectomy, to allow samples of ovarian venous blood to be taken by venepuncture under direct vision, once the abdomen was opened. Before excising the supporting structure of the uterus, straight Kochers clamps were applied on either side of the uterine body in such a manner that the fallopian tube, the round ligament, the ligament of the ovary, and the broad ligament right down to just above the utero–cervical junction, were clamped, all in one piece. This ensured that subsequent filling of the veins in the pampiniform plexuses could only have come from the ovary, because the utero–ovarian anastamoses had by now been excluded. The site of venepuncture for the ovarian venous sample was usually about 1 cm away from the hilus of the ovary, where the vessel begins to enter the infundibulo–pelvic ligament (see Figure 2.1).

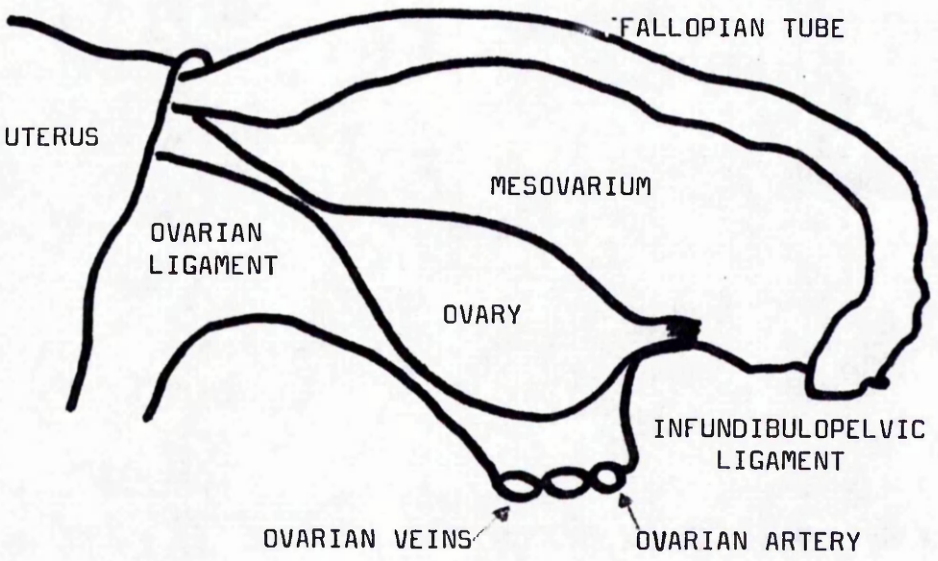
Using a sterile 5 ml syringe with a 25 guage needle the blood was drawn very slowly, with intermittent pressure, each momentary pause allowing the venous cavity to be refilled by fresh ovarian effluent. This procedure was performed initially on the ovary which on visualisation appeared more active, and then repeated on the contralateral ovary. A

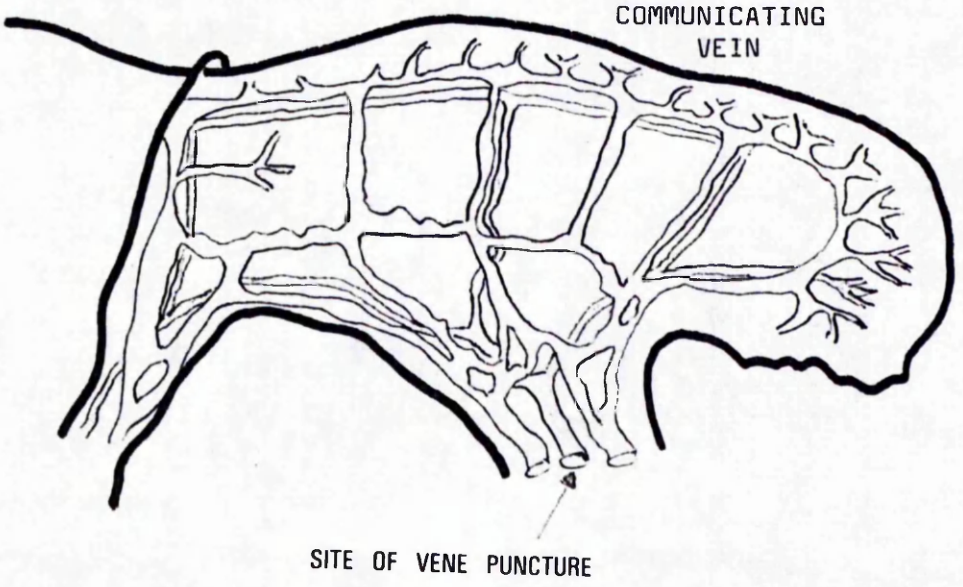
sample of peripheral blood was simultaneously taken from the antecubital vein. The blood was then transferred into lithium heparin containers.

The blood samples were centrifuged at 1500 r.p.m. in a bench centrifuge for ten minutes and each supernatant plasma was transferred into a sterile container. Aliquots for protein hormone assay were removed and the remainder of the plasma was stored at –20° until processed further.

Figure 2.1

Posterior view of the right uterine adnexa (top) with its vascular network (bottom) on which is illustrated the site of venepuncture for ovarian venous sampling.





METHODS

Dating of the stage of the menstrual cycle

A) Dating of the Endometrium according to Morphological Changes

The original observations of histological changes in the endometrium by Hitchman and Adler (1908), elaborated by Schroeder (1915), Novak (1915), O'Leary (1929), Bartelmez (1938) and correlated with coincident changes in the ovary by Frankel (1909), Meyer (1920) and Noyes, Hertig and Rock (1950) established the sequence of proliferative (pre–ovulatory) and secretory (post–ovulatory) morphological phases. Development of the biopsy method greatly assisted the correlation of the histology of the endometrium with the stage of the cycle. The usefulness of dating the endometrium lies in the fact that it gives a rough idea of quantitative progesterone effect, and also indirectly of the time of ovulation. Selye (1947) even goes further to say that 'endometrial biopsy is a superior form of hormone assay because of its freedom from a multitude of technical uncertainties.'

Determination of histological changes of the endometrium is based on a 28 day menstrual cycle with ovulation occurring on the 14th day. The cycle begins on the first day of menstruation. The menstrual phase is variable lasting on the average 4 days. From then until the 14th day is the proliferative phase and the two weeks following that the secretory phase. Whilst it is generally accepted that ovulation does not always occur on the 14th day of the cycle and that the proliferative phase is of variable duration, there is a constant relationship in that ovulation occurs 14 days $\pm$ 2 before the onset of the next menstrual cycle.

Morphological changes for dating the endometrium were evaluated from the most advanced area in the histology slide that was chosen. This was to overcome the possible sources of error resulting from the fact that there must exist a time lag in the responses to the hormone by the different parts of the endometrium. Even while menstrual breakdown is still taking place there is already regeneration of the endometrium in the adjacent portion, in readiness for the next cycle.

Criteria used in dating of the endometrium histologically (Noyes, Hertig and Rock, 1950).

Early Proliferative Phase (days 4 – 7)

The endometrium shows occasional glands left from the previous menstrual phase with its involutional changes (secretory exhaustion). The stroma is compact, spindle–shaped cells with anastamosing processes and relatively large nuclei undergoing mitosis are evident (Plate I). There is evidence of regenerating surface epithelium especially between glandular openings.

Mid Proliferative Phase (days 8 – 10)

In this phase the glands begin to curve and elongate into the stroma which is oedematous and laden with 'naked nucleus' type cells still showing mitosis (Plate II). The glandular epithelium is commencing to show pseudostratification.

Late Proliferative Phase (days 11 – 14)

The endometrial glands here are tortuous and show evidence of active growth (numerous mitotic nuclei). The stroma is again dense with cellular nuclei showing mitosis. The pseudostratification

mentioned earlier in glandular epithelium has become more marked (Plate III). The surface epithelium is somewhat undulating.

Early Secretory Phase (days 15 – 20)

Following ovulation the progestational effect on the endometrium becomes evident and a more critical appraisal of the dating of the endometrium can be made. After a 'silent period' in the endometrial changes (36 hours following ovulation) very occasional subnuclear vacuoles may be seen in the glandular epithelium of what is essentially proliferative endometrium (day 16).

The pseudostratification of the glandular epithelium becomes more marked (day 17) and basal vacuolation more evident whilst the mitotic figures in glandular and stromal cells persist.

By day 18 of the cycle, the nuclei of the glandular epithelial cells begin to line up in the centre of the cells, with the homogenous cytoplasm above and vacuoles below them. The glands themselves are becoming increasingly tortuous. Mitosis in the glandular and stromal cells are only occasionally seen (Plate IV).

As the basal vacuoles begin to decrease the mid secretory phase is reached.

Mid Secretory Phase (days 21 – 24)

Very occasional subnuclear vacuoles are present by now with the nuclei of the glandular epithelium being mainly basal. Acidophilic secretions in the glandular lumen are clearly seen (Plate V).

The stroma is maximal and oedematous with thin–walled spiral arteries in it (day 22). The prominence of spiral arteries and arterioles indicate a further development in the endometrium. Stromal cells begin to condense around the arterioles and acquire increasing amounts of cytoplasm. This is the earliest predecidual change detectable. The collection of predecidual cells round the arterioles become more marked; some still showing mitosis. The endometrium comes to the end of the mid secretory phase.

Late Secretory Phase (days 25 – 28)

On the commencement of the late secretory phase predecidual changes begin beneath the surface epithelium with still some stromal oedema existing except around the blood vessels. Lymophocytic infiltration begins to take place and soon this is associated with polymorphonuclear leucocytic infiltration indicating the prominence of predecidual changes. Focal necrosis of the decidua associated with haemorrhages and clumping of the stromal cells indicates early menstruation. The tortuous glands which, all through the secretory phase, have been secreting their acidophylic secretions into the glandular lumen are by now in a state of "secretory exhaustion" (Plate VI). The infiltration of polymorphonuclear leucocytes persists right through menstruation till day 3 or 4 of the cycle.

Menstruation (days 1 – 3)

Varying degrees of sloughing of the endometrium with haemorrhages are evident with concommitant early regeneration of epithelium starting usually near the 'mouths' of glandular tracts.

PLATE I

Histological preparation of human endometrium
in the EP phase.

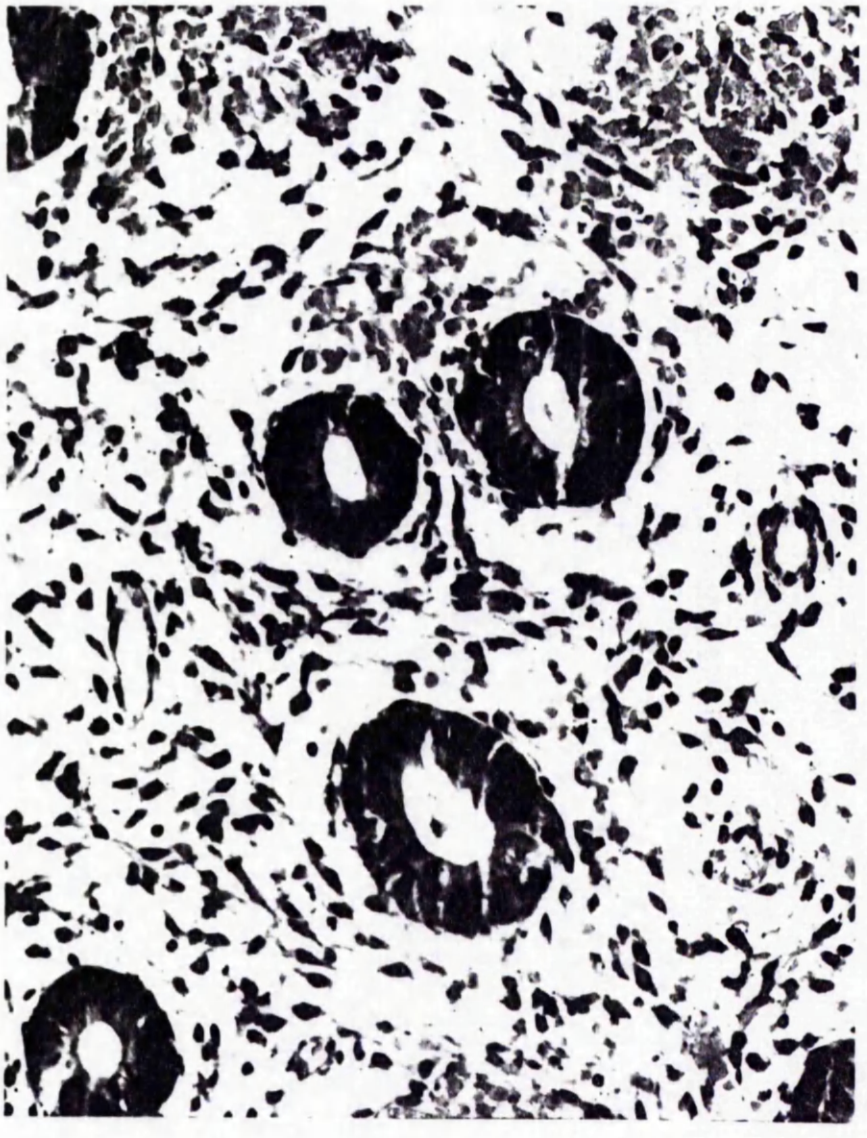


PLATE II

Histological preparation of human endometrium
in the MP phase.

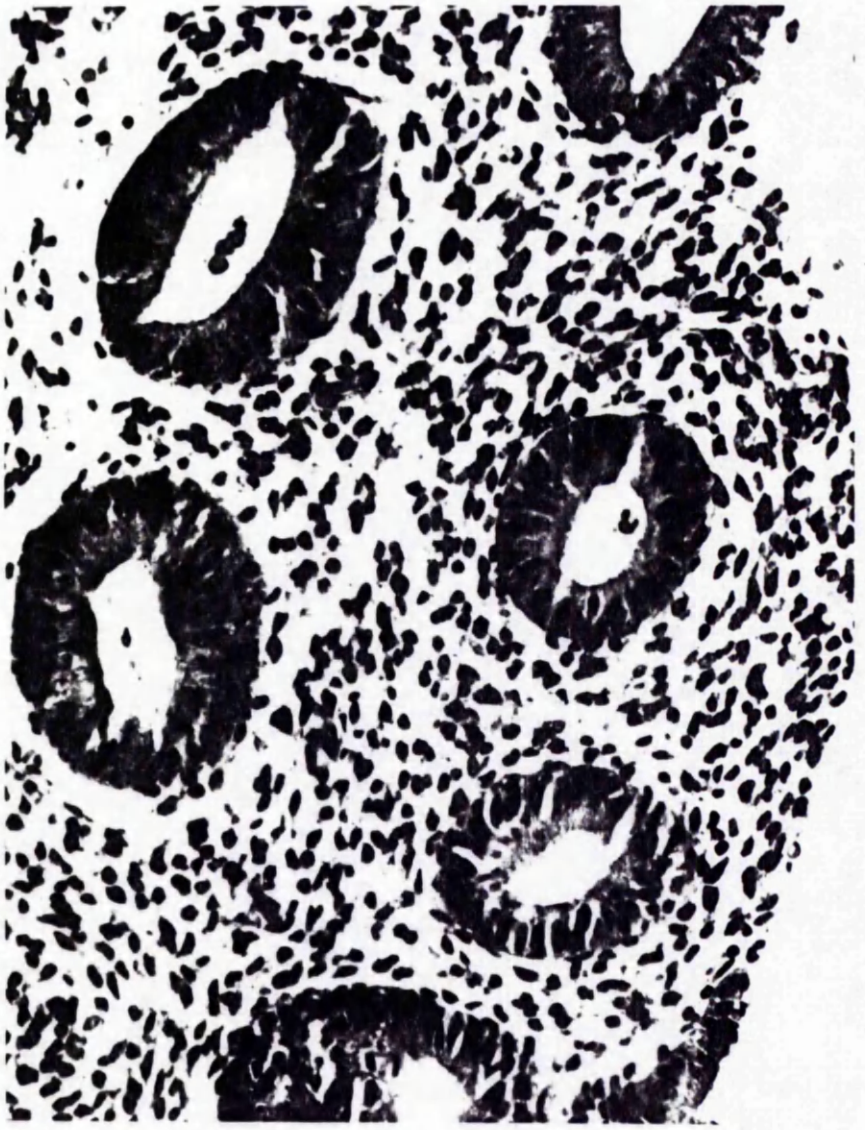


PLATE III

Histological preparation of human endometrium in the LP phase.

PLATE IV

Histological preparation of human endometrium
in the ES phase.

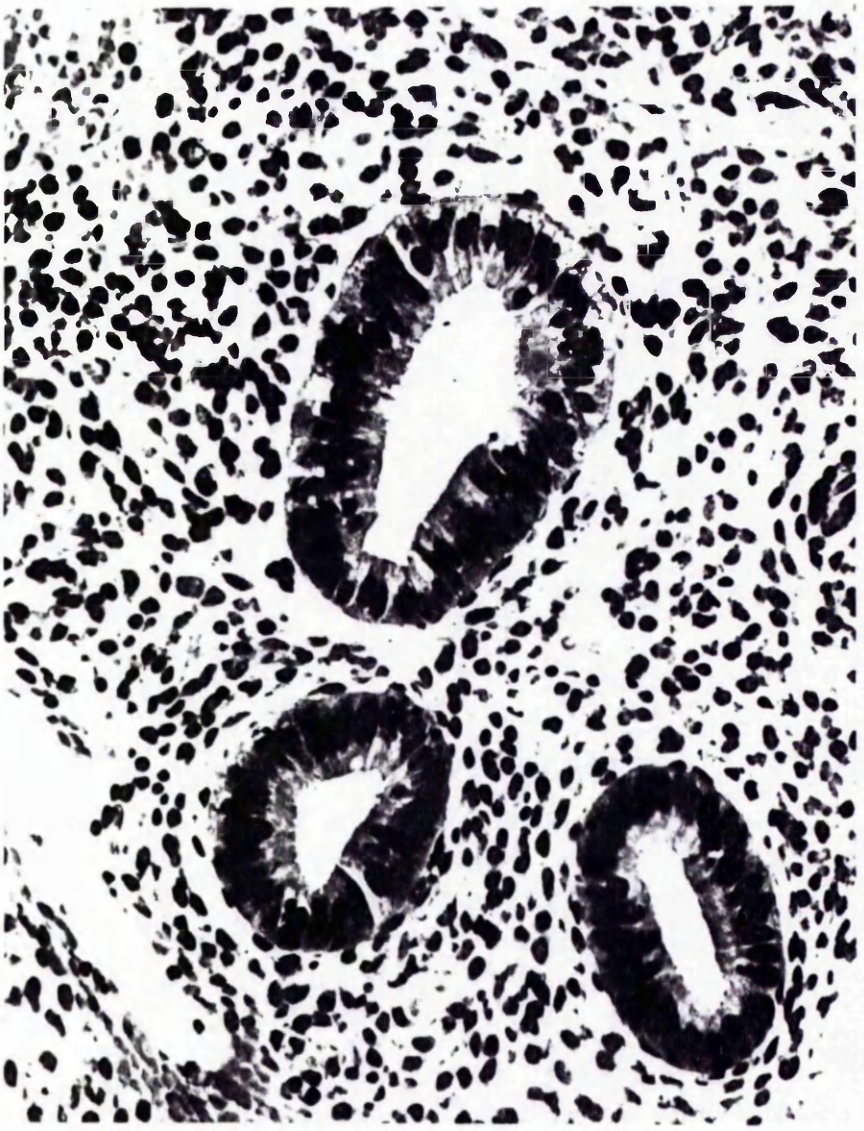


PLATE V

Histological preparation of human endometrium
in the MS phase.

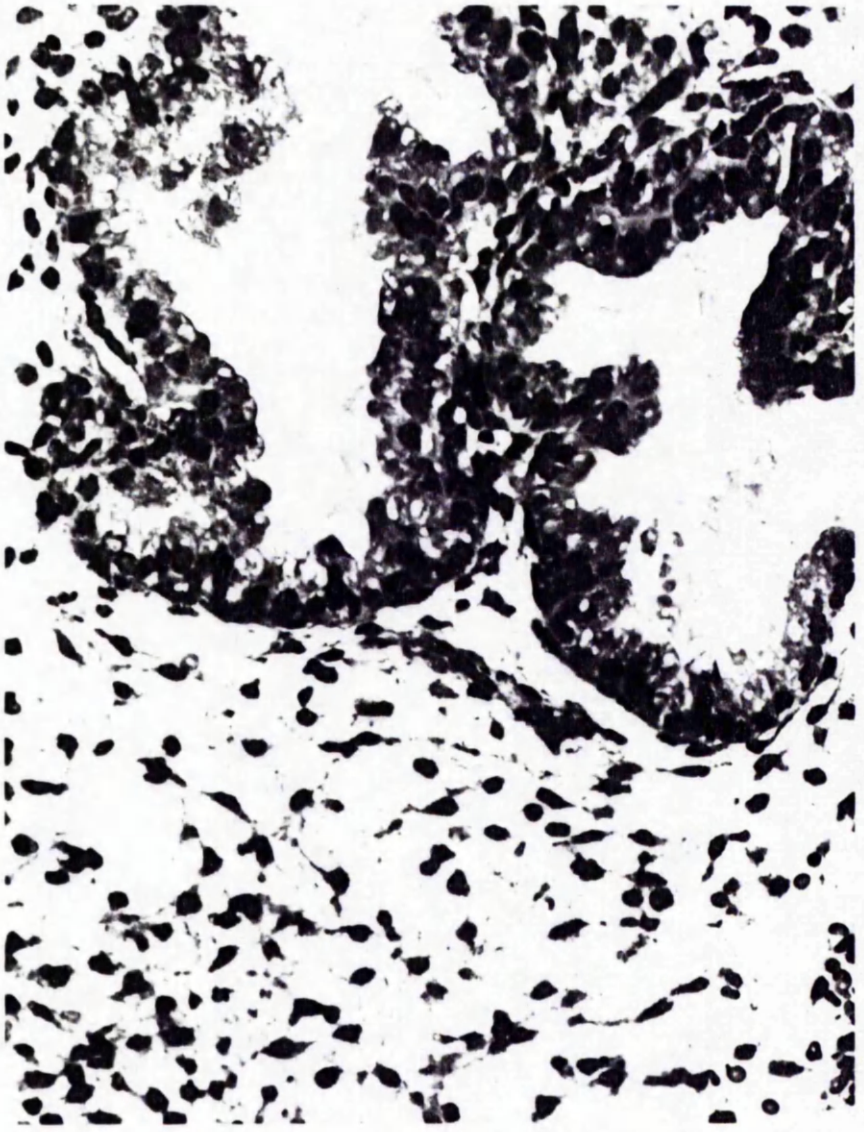
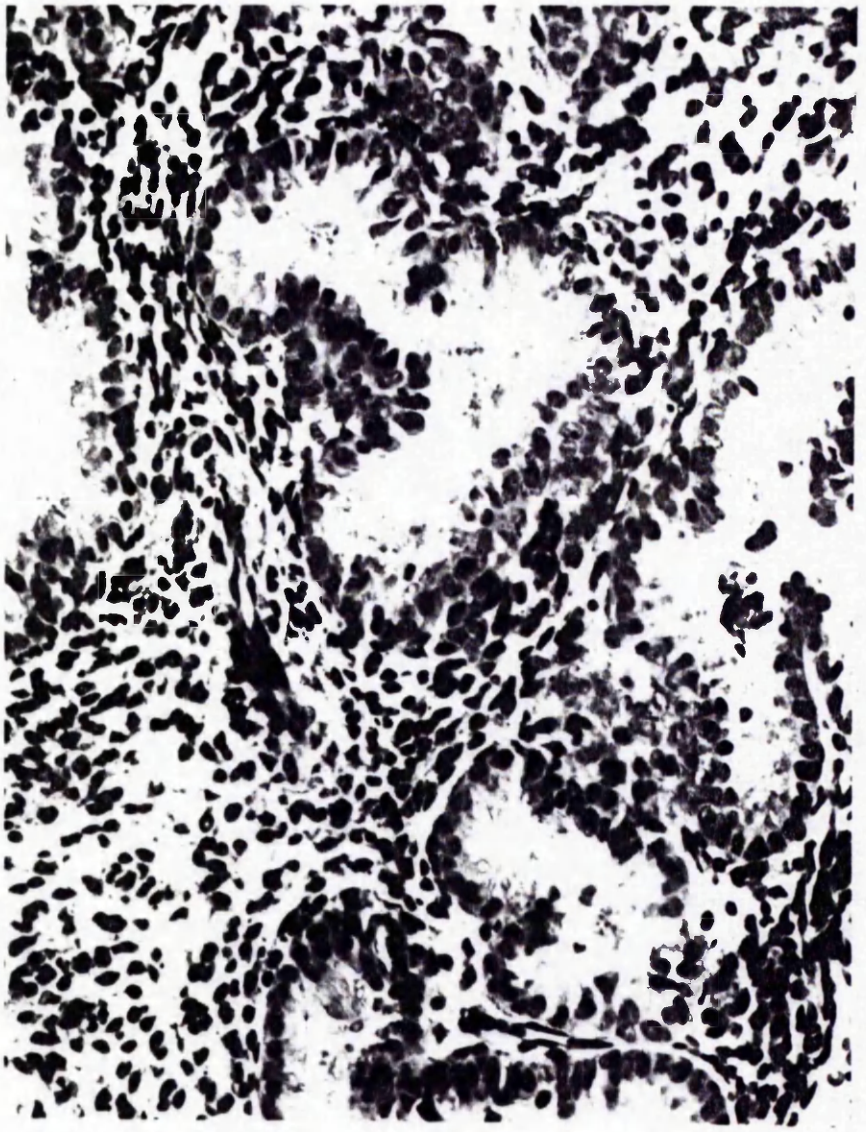


PLATE VI

Histological preparation of human endometrium
in the LS phase.



B) **Dating of the stage of the menstrual cycle using other criteria.**

1. **Visualisation of the ovaries**

On opening the abdomen the ovaries were visualised in detail for evidence of growing follicles (mid proliferative), large follicles with impending rupture (late proliferative), haemorrhages on the ovary (recent ovulation), corpora lutea (secretory phase) and 'silent' apparently inactive ovaries which might assist categorisation of it into one of the above mentioned stages. It must be pointed out that a negative finding macroscopically of the ovary was equally pertinent in assisting staging the phase of the menstrual cycle, especially in relation to other parameters.

2. **Histological appearance of the ovary**

Where ovaries were available for detailed histological study reports, on the serial sections performed, were obtained through the good offices of Dr. Lees, Senior Consultant Pathologist, Department of Pathology, Glasgow Royal Infirmary.

3. **Menstrual History**

The day of the cycle according to the date of the last menstrual period and the previous cyclical pattern of the patient, where available, were also used to categorise the patients in this study.

Extraction of Steroid Hormones from Plasma

Different volumes of plasma were required to be extracted for assay of the different hormones at different stages of the menstrual cycle and the following general scheme for extraction was employed.

The plasma (0.01 ml to 0.2ml diluted, where necessary, with deionised, distilled water to 0.05ml) was extracted with 3 mls of the

extracting solvents by shaking for 20 minutes, either horizontally in B 14 stoppered tubes (Jobling Limited) in an automatic shaker (Watson–Balgray Limited) or vertically in disposable tubes 16 x 100 mm (Excelo Limited) in a multivortex mixer (Baird and Tatlock Limited). After shaking, the tubes were allowed to stand for a few minutes to allow the two layers to separate and were then placed in a freezing mixture of solid carbon dioxide. After the lower aqueous phase had frozen the organic layers were decanted either into chromatography tubes or into assay tubes as required. The organic layers were then evaporated to dryness in the vacuum oven.

The extracting solvents used in the various assays were:–

OE_2, 17OHP, T and DHT — diethyl ether
A — Hexane (boiling point 67°–70°)
P — petroleum ether (boiling point 40°–60°)/ethanol (15/1 v/v)

In the cases of T and DHT, assays which employed chromatographic purification of the extracts, small amounts of tritiated hormones were added to the plasma prior to extraction to monitor methodological losses during chromatography.

Determination of the Extraction Efficiency

The efficiency of the extraction was checked by adding 0.01ml methanol,containing the tritiated hormones to be extracted, to each of 20 extraction tubes, evaporation of the solvent, addition of the appropriate volume of plasma from a mixed pool (dated plasmas obtained from the blood bank – a mixture of male and female plasmas), gentle mixing at room temperature for 1h and then extraction as described above. After freezing, the supernatant organic layers were decanted directly into scin-

tillation vials for radioactivity determination and percentage extraction for each was assessed by comparison with total vials prepared by adding to the scintillation fluid directly 0.01ml of methanol containing the hormone under test (H^3 labelled) plus 3ml of the extracting solvent.

Celite 545

Celite 545 (John's Manville Limited) was washed prior to use for chromatography. 400 g of celite were suspended in a solution of 1.5 x 10^{-2}M ethylenediaminotetra–acetic acid (E.D.T.A.) and the pH of the suspension was adjusted to between pH 2 and 3 by addition of conc. hydrochloric acid. An equal volume of distilled water was added and the mixture was heated at 70° for 45 minutes with constant stirring. After standing, the supernatant layer was decanted and the celite was washed with distilled water on a Buchner funnel until the washings were of neutral pH. The celite was further washed twice with methanol at 60° for 45 minutes with stirring and was then filtered on a large Buchner funnel under vacuum. The material remaining on the filter was washed sequentially with 500 ml each of iso–octane, benzene, hexane, dichlor-ethane and water – the solvents which were to be employed later in celite partition chromatography. Finally, the celite was washed with acetone to remove any remaining traces of water, air–dried in an open tray for several hours in a fume cupboard and oven–dried at 200° for 48h . The celite was then ready for use in chromatography.

Preparation of Celite Containing Stationary Phase for Partition Chromatography

Washed celite was suspended in iso–octane (100g/litre w/v) and ethylene glycol (50ml/100g celite v/w) was added drop–wise over a period of approximately 1h with constant stirring. After all of the

ethylene glycol had been added, the mixture was stirred for a futher 1h and the celite containing ethylene glycol as a stationary phase was now ready for use in chromatography columns.

Celite Partition Chromatography

Glass columns, 25 cm high and 0.75 cm in diameter, were used for celite partition chromatography. These columns were fitted with a tap to regulate flow rate and had a female B19 joint at the top to allow the fitting of a solvent reservoir (Figure 2.2). A plug of washed glass wool was placed in the column just above the tap and covered with a disc of fibreglass paper (0.75 cm in diameter) to prevent leakage of celite.

Washed celite 545 containing ethylene glycol (see above) was packed into these columns in iso–octane which was allowed to flow throughout the procedure thus preventing air bubbles collecting in the columns. The celite was packed under gravity ensuring that the solvent level was always above the top of the column. Columns were packed to a height of 19 cm, tightly enough to give a flow rate, when the tap was fully open, of 30 to 32 drops per minute.

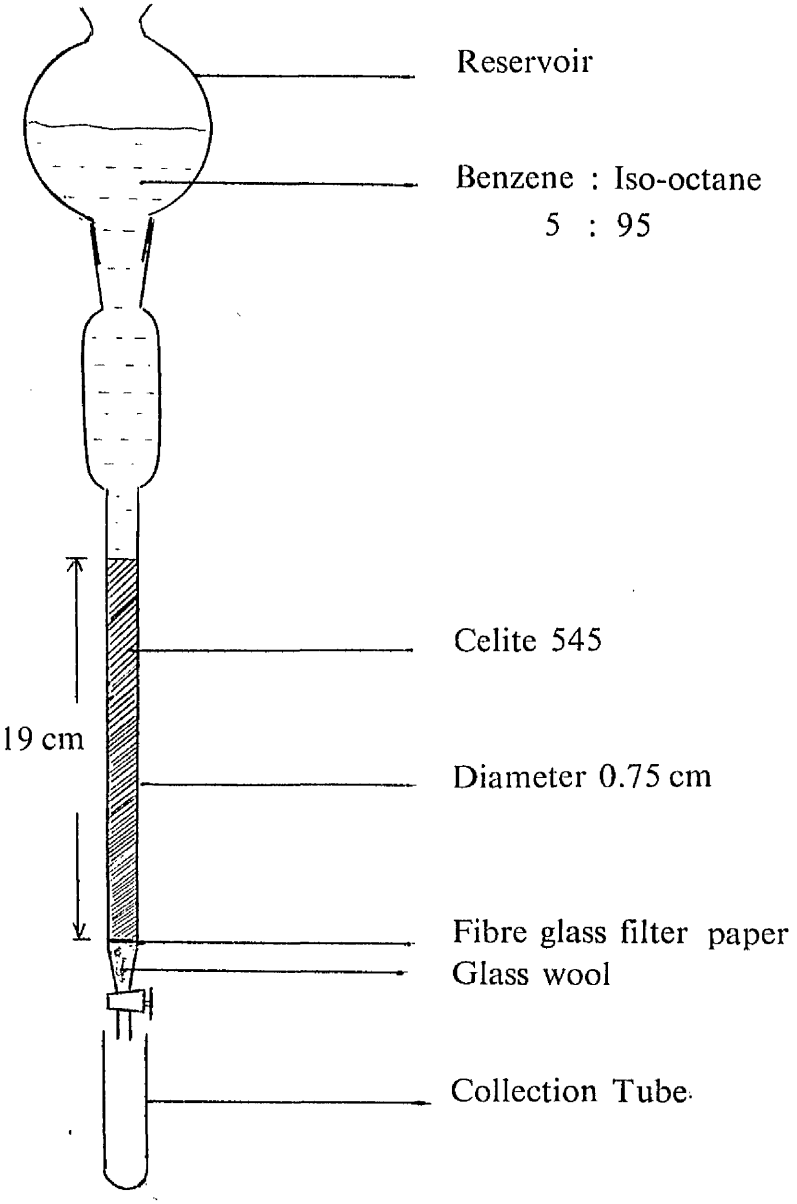
For chromatographic separation of DHT and T using these prepared celite columns the following mixtures of solvents were employed:–

Solvent I – benzene/iso–octane (5/95; v/v)
Solvent II – benzene/iso–octane (40/60; v/v)

Before application of the samples the iso–octane in which the column had been prepared was replaced with solvent I, and a total of 50mls were eluted through the column.

Figure 2.2

Diagram of the column used for celite partition chromatography.



Separation of Dihydrotestosterone and Testosterone from Plasma using Celite Partition Chromatography

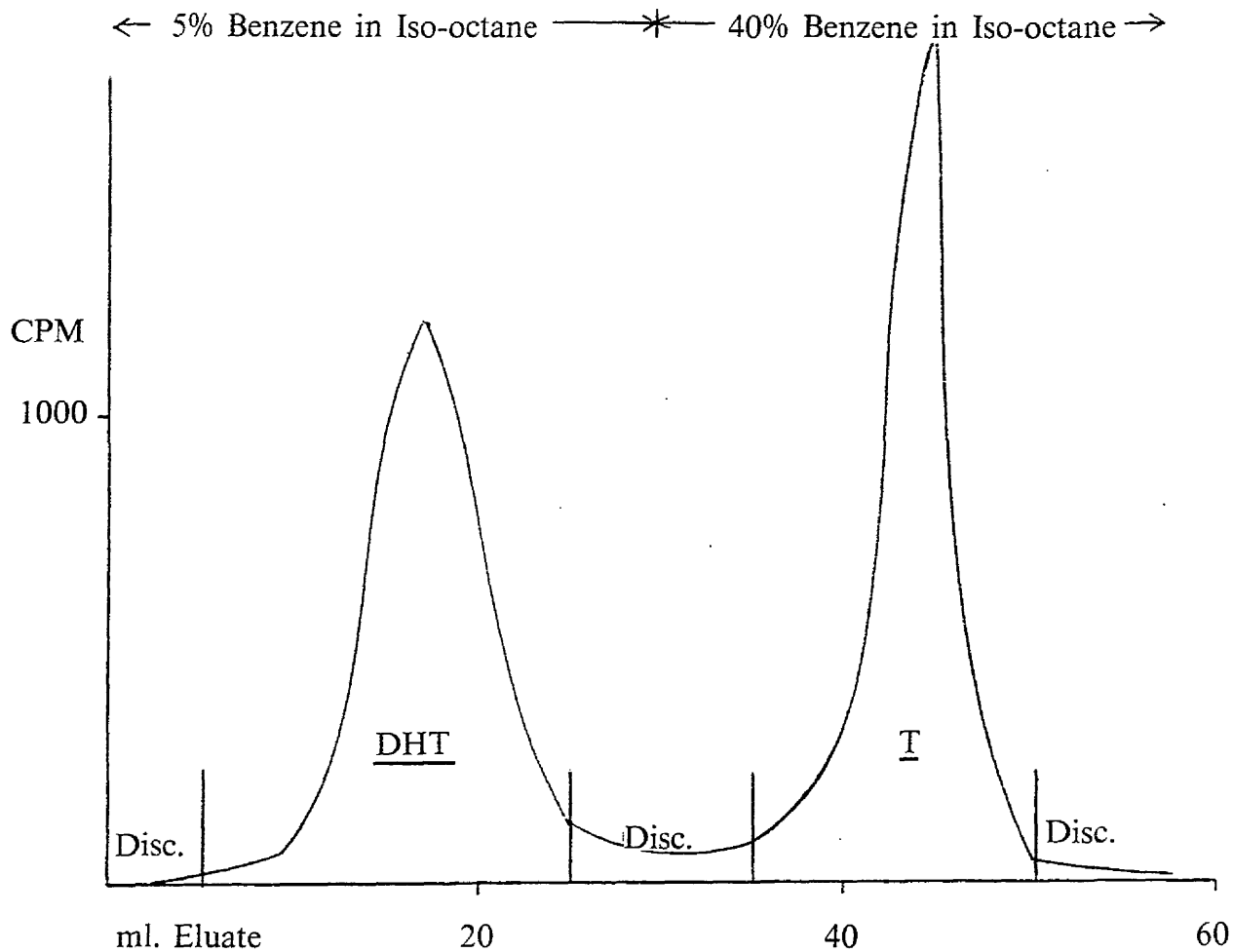
Tritiated T (0.75nCi;5pg) and tritiated DHT (1nCi;4pg) were added in 0.02ml methanol to 0.2ml of plasma and mixed. The plasma was then extracted as described above. The dried ether extract was redissolved in 1ml of solvent I and transferred to the column using a Pasteur pipette. This transfer procedure was carried out a further two times using 1ml of the solvent each time, whilst the first ml of the solvent was percolating into the column. When the three transfers had penetrated into the column, a further 27ml were added to the reservoir. Bulk fractions of the eluate were collected as desired.

The first 5ml were discarded, and the 6th to the 25th ml inclusive were collected in a disposable tube, (DHT fraction). The remaining 5ml of solvent were also discarded. When the miniscus of solvent I was level with the top of the celite column 30ml of solvent II were added to the reservoir. The first 5ml of this (31st to 35th ml) were discarded. The 36th to 50th ml inclusive (T fraction) were collected into a disposable tube and the remaining eluate was discarded. Figure 2.3 shows the profile obtained in such a separation. These columns were then eluted continuously with a further 50mls of solvent I and were ready for re–use.

Bulk fractions collected in disposable tubes were evaporated to dryness in the vacuum oven. Methanol (0.75ml) was added to each dried extract and the tube contents were dissolved by whirlymixing for 10 seconds. An aliquot (0.25ml) was transferred from each tube to a scintillation vial for determination of radioactivity and hence computation of recoveries and two further aliquots of 0.25ml were transferred to

Figure 2.3

Profile showing the chromatographic separation of DHT and T. (Disc. = Discard)



assay tubes for radioimmunoassay (see Figure 2.4).

Each batch of 5 columns contained 3 unknown samples. I blank to determine any methodological interfering factors and I internal quality control plasma for assay validation.

Values obtained for DHT and T following chromatography and radioimmunoassay were corrected for individual recoveries to give absolute values.

Radioimmunoassay of Dihydrotestosterone and Testosterone

The antiserum Y6/4, which was raised to T–3–oxime bovine serum albumin was used for radioimmunoassay of these C19 androgens. This antiserum was a generous gift from Dr. Brian Cook of the Department of Steroid Biochemistry, Glasgow University.

Standard curves in triplicate of 0, 10, 20, 50, 100, 200, 300, and 500pg were pipetted into assay tubes from a stock solution in methanol (1ng/ml of either DHT or T) and 0.25ml methanol was added and evaporated to dryness. This addition of methanol was to ensure that the standard curve was compatible with the unknown samples and to guard against any possible interference due to methanol.

1. **Dihydrotestosterone** : The antiserum (0.1ml; 1/6000 dilution) and [1, 2, 4, 5, 6, 7, (N) H^3] DHT (7.2nCi; 25pg;0.1ml) in buffer were added to the standard tubes and the sample aliquots obtained as described previously following chromatography. The mixtures were incubated at 4° for 1h, and the free and antiserum bound steroid fractions were separated by addition of 1ml of DCC (200mg of charcoal, 25mg of dextran T70

and 100mg gelatin/100ml).

The tubes were allowed to stand at 4° for a further 20 minutes (see stripping curve in Results Section) and then centrifuged at 1,500 g for 20 minutes at 4°. The supernatant fractions were decanted directly into 7ml NE 250 scintillation fluid in disposable vials and the radioactive content of each was determined.

In addition to the unknown samples and the standard curve, triplicate tubes which contained buffer in place of antiserum (no As tubes) and triplicate tubes to which were added buffer in place of charcoal (no C tubes) were processed with each assay. These tubes facilitated determination of the total amount of radioactivity added (no C tubes) and the non specific binding (no As. tubes).

A standard curve was constructed relating percentage radioactivity bound to the mass of DHT on a linear/log scale (Figure 2.5).

The percentage bound for unknown samples was calculated and the equivalent mass of DHT determined using the standard curve.

2. **Testosterone :** Antiserum (0.1ml;1/6000) and [1–2 (N) H^3] T (5.8 nCi;38pg;0.1ml) in phosphate buffer were added to each of the standard tubes (0–500pg) described previously, to the aliquots from chromatography and to triplicate of no C tube. Triplicate no As were also prepared. The mixtures were incubated at 4° for 1h. Free and antiserum bound fractions were separated following addition of 1ml of DCC to each tube using the same recipe as described for DHT assay. The tubes were allowed to stand at 4° for 10 minutes (see stripping curve in Results

Section), then centrifuged,(1500g;20 minutes), the supernatant bound fractions were decanted, the radioactive content of each determined and a standard curve constructed as described for DHT. A typical T standard curve is shown in Figure 2.6.

Tables 2.I and 2.II list the cross reactions obtained using Y6/4 antiserum with a variety of authentic steroids, for DHT and T respectively under the conditions employed in these assays.

Table 2 I

Table showing the cross reactions obtained using the antiserum Y6/4 to assay DHT

Steroids	% cross reaction
DHT	100
T	150
A	1.8
Dehydroisoandrosterone P OE_2 OE_1 17OHP Deoxycorticosterone	<0.2

Table 2 II

Table showing the cross reactions obtained using the antiserum Y6/4 to assay T

Steroids	% cross reactions
T	100.0
DHT	55.0
A	0.8
Dehydroisoandrosterone	<0.2
P	
OE_2	
OE_1	
17OHP	
Deoxycorticosterone	

For the purpose of determination of percentage cross reaction for all assays described in this thesis, the formula used was that of Abraham (1974):-

$$\% \text{ cross reaction} = \frac{\text{Mass of hormone of interest causing 50\% drop in binding}}{\text{Mass of cross reacting substance causing 50\% drop in binding}} \times 100$$

Figures 2.7 and 2.8 are flow sheets of the radioimmunoassays for DHT and T respectively.

Radioimmunoassay of Androstenedione

Androstenedione levels in plasmas were determined by radioimmunoassay using an antiserum raised to A–7–oxo–bovine serum albumin. The antiserum prepared using the technique described by Weinstein et al (1972) was purchased commercially from Miles–Yeda Limited. This antiserum was used for specific radioimmunoassay of A without chromatography.

The plasma (0.05ml or less diluted to 0.05ml with deionised distilled water) was extracted as described previously and the organic layers were decanted directly into assay tubes. A standard curve in triplicate of 0 to 500pg was pipetted into assay tubes from a 1ng/ml stock solution in methanol and no As and no C tubes in triplicate were also prepared. [1–2 (N) H^3] A, (6.3nCi; 40pg; 0.1ml) and antiserum (0.1ml) in buffer were added to each assay tube and the mixtures were incubated at 4° for 1h. The free and antiserum bound fractions were separated following addition of 1ml DCC (recipe as described for DHT). After standing at 4° for 10 minutes (see stripping curve Chapter 3) the tubes were centrifuged, the supernatant antiserum bound fractions decanted as described for DHT and the radioactivity present in each was determined.

A typical standard curve for A and a flow sheet of the A radioimmunoassay method are shown in Figures 2.9 and 2.10 respectively.

Figure 2.4

Flow diagram of extraction and chromatographic separation used prior to radioimmunoassays of DHT and T.

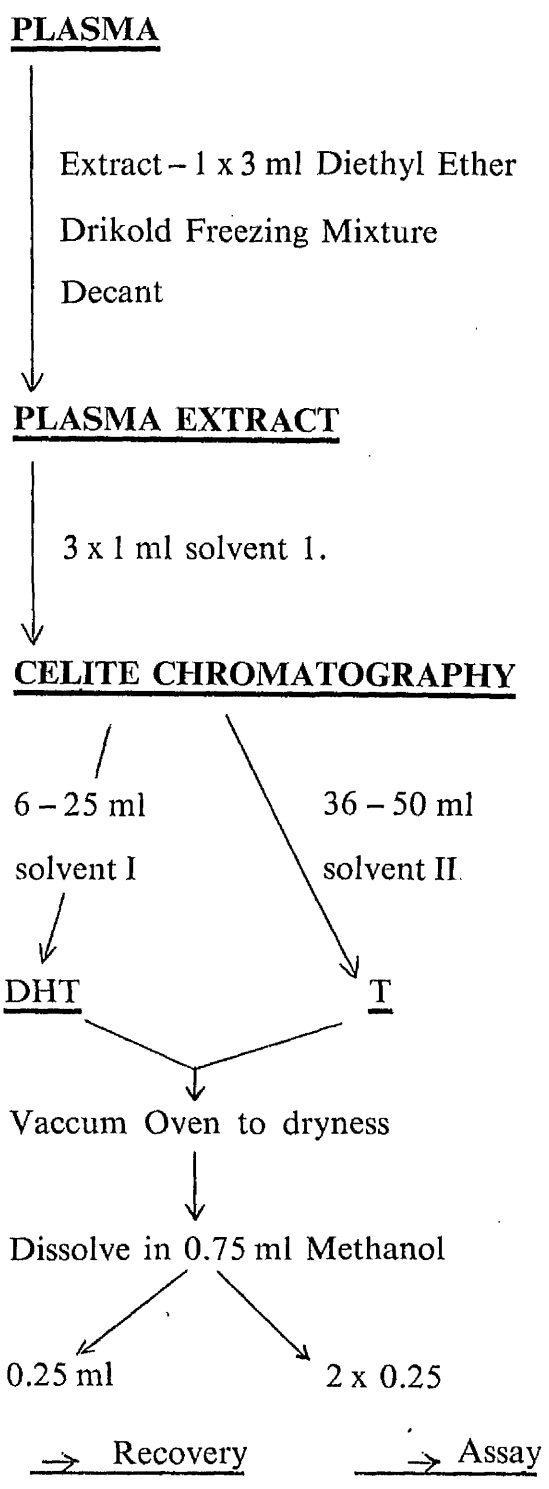


Figure 2.5
A typical standard curve for DHT radioimmunoassay.

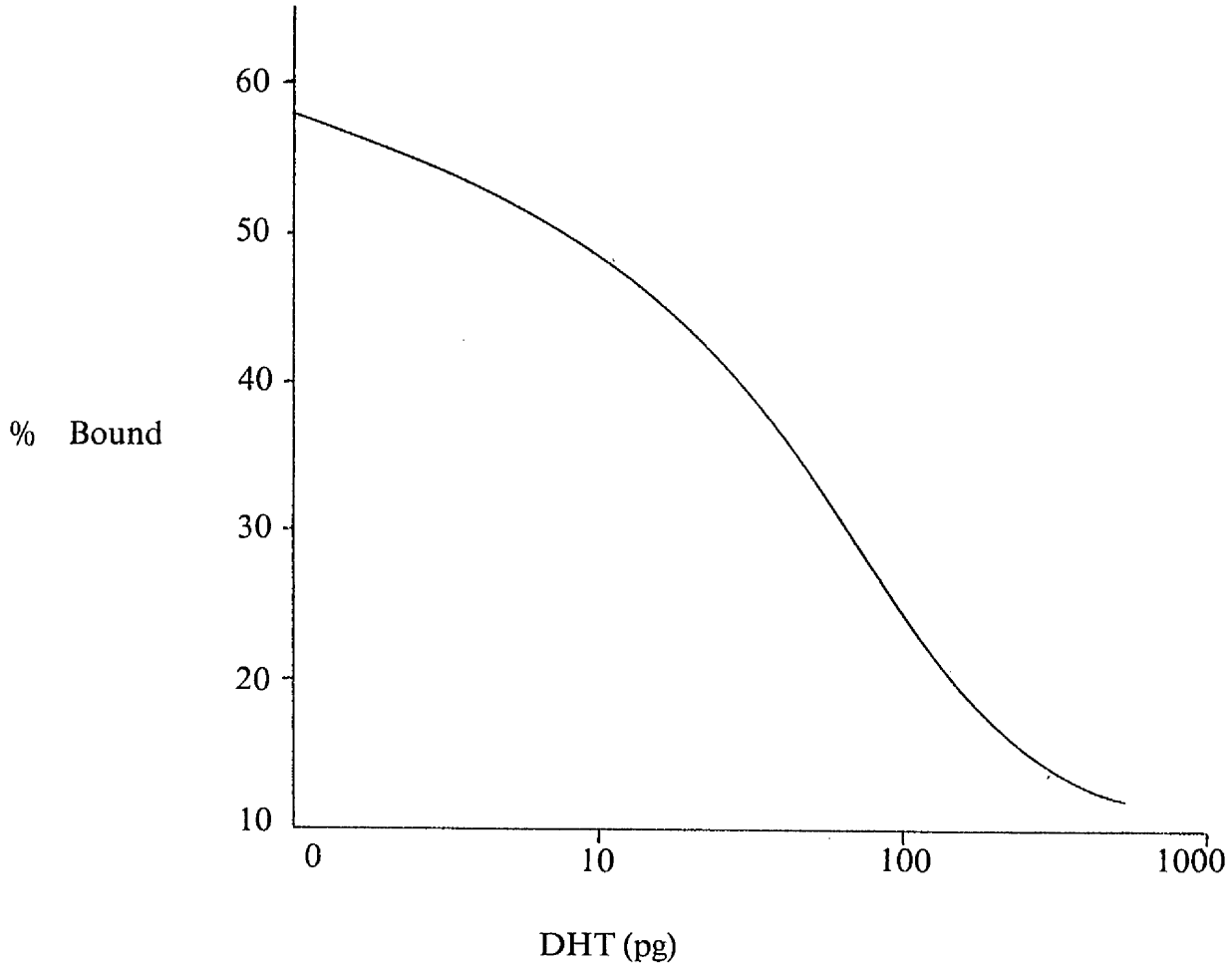


Figure 2.6
A typical standard curve for T radioimmunoassay.

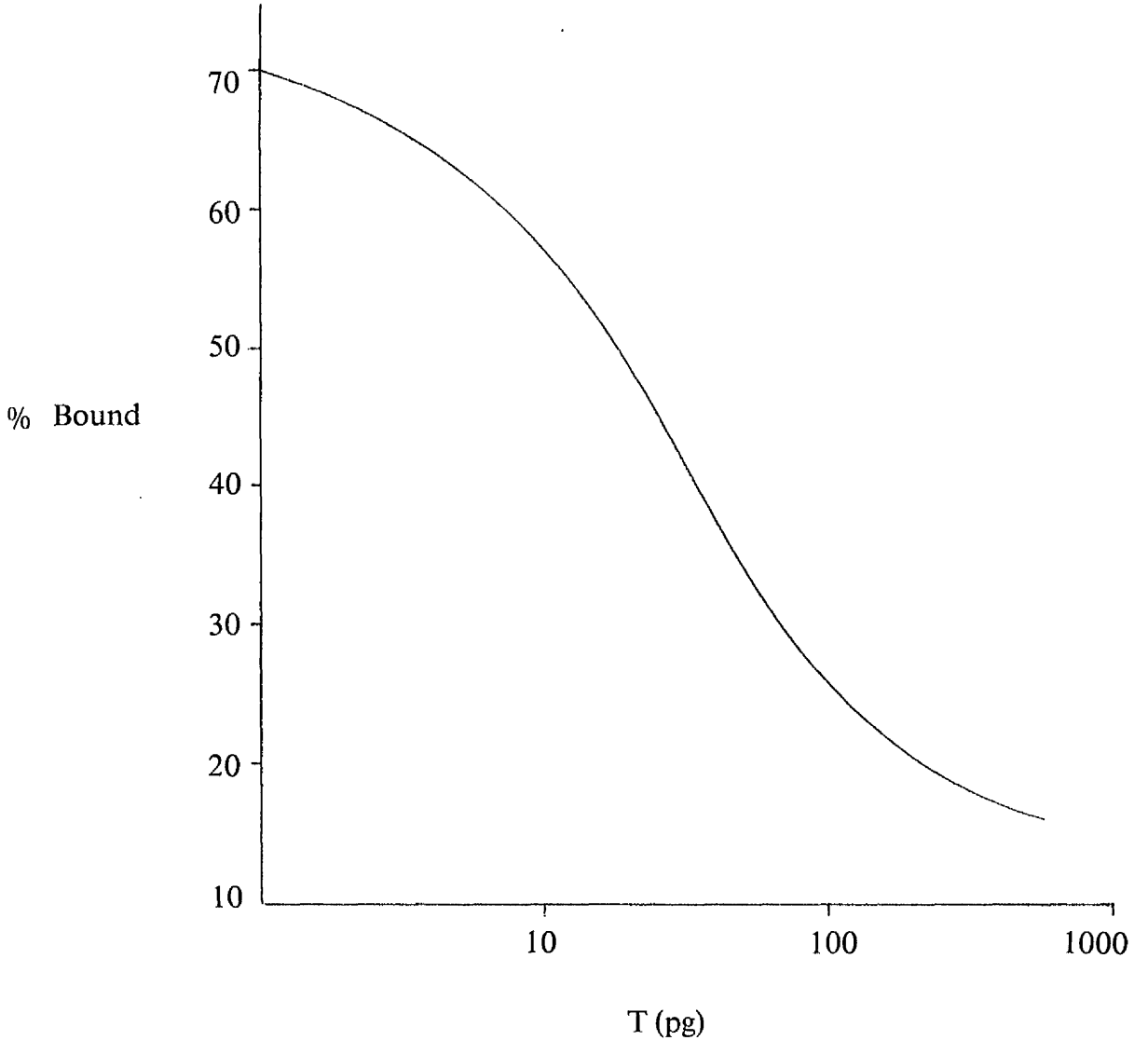


Figure 2.7

Flow-diagram of DHT radioimmunoassay.

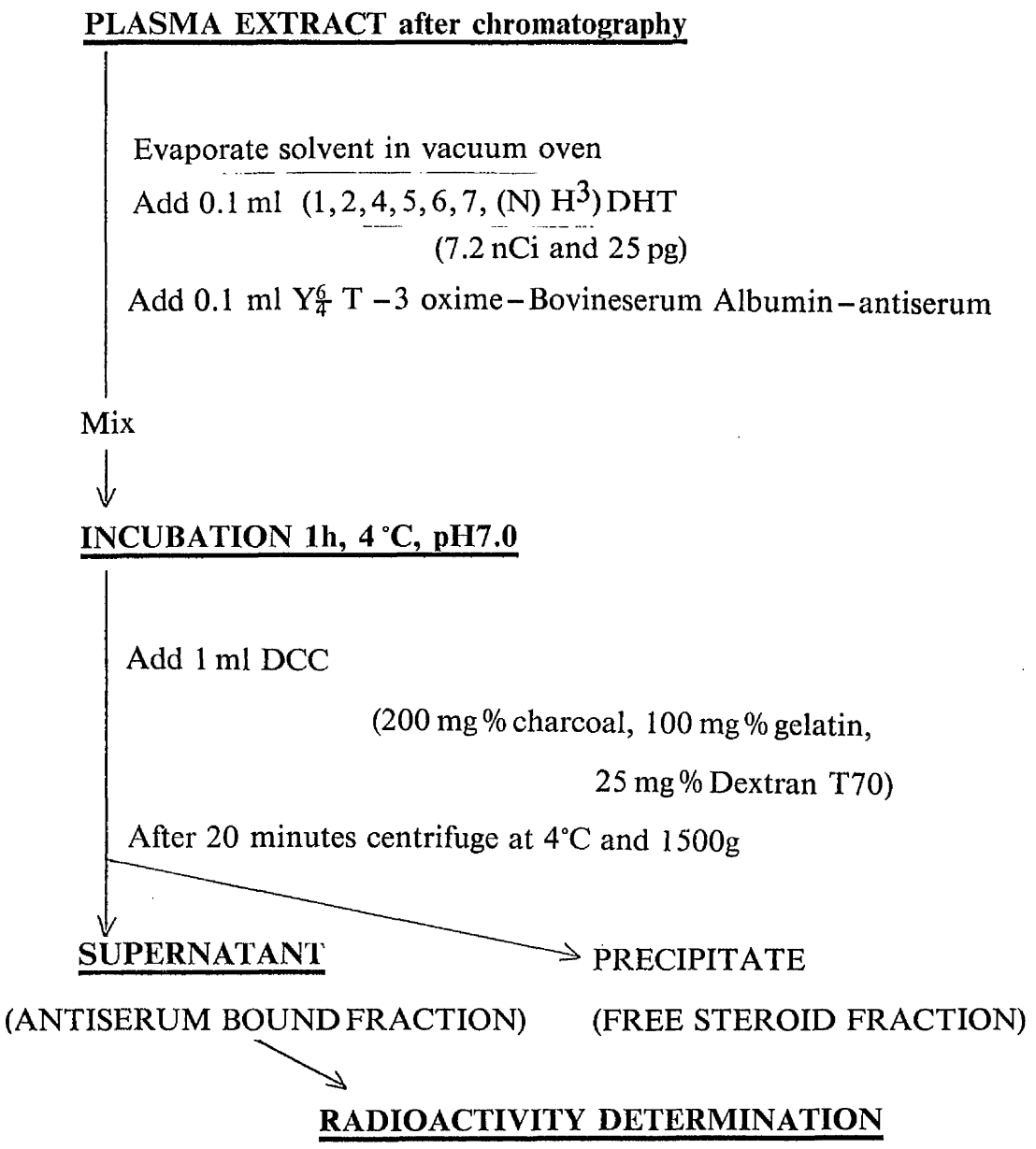


Figure 2.8

Flow diagram of T radioimmunoassay.

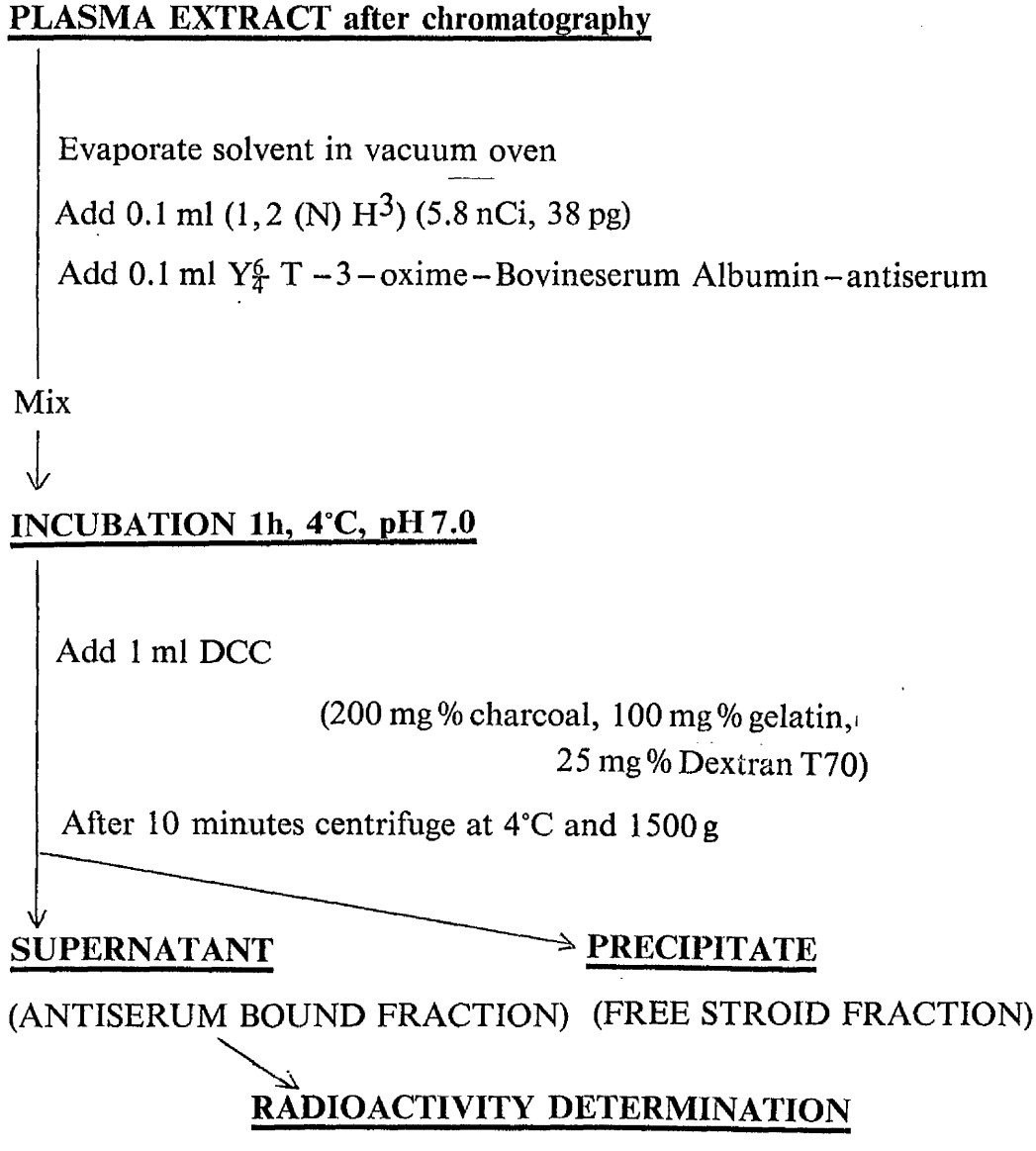


Figure 2.9
A typical standard curve for A radioimmunoassay

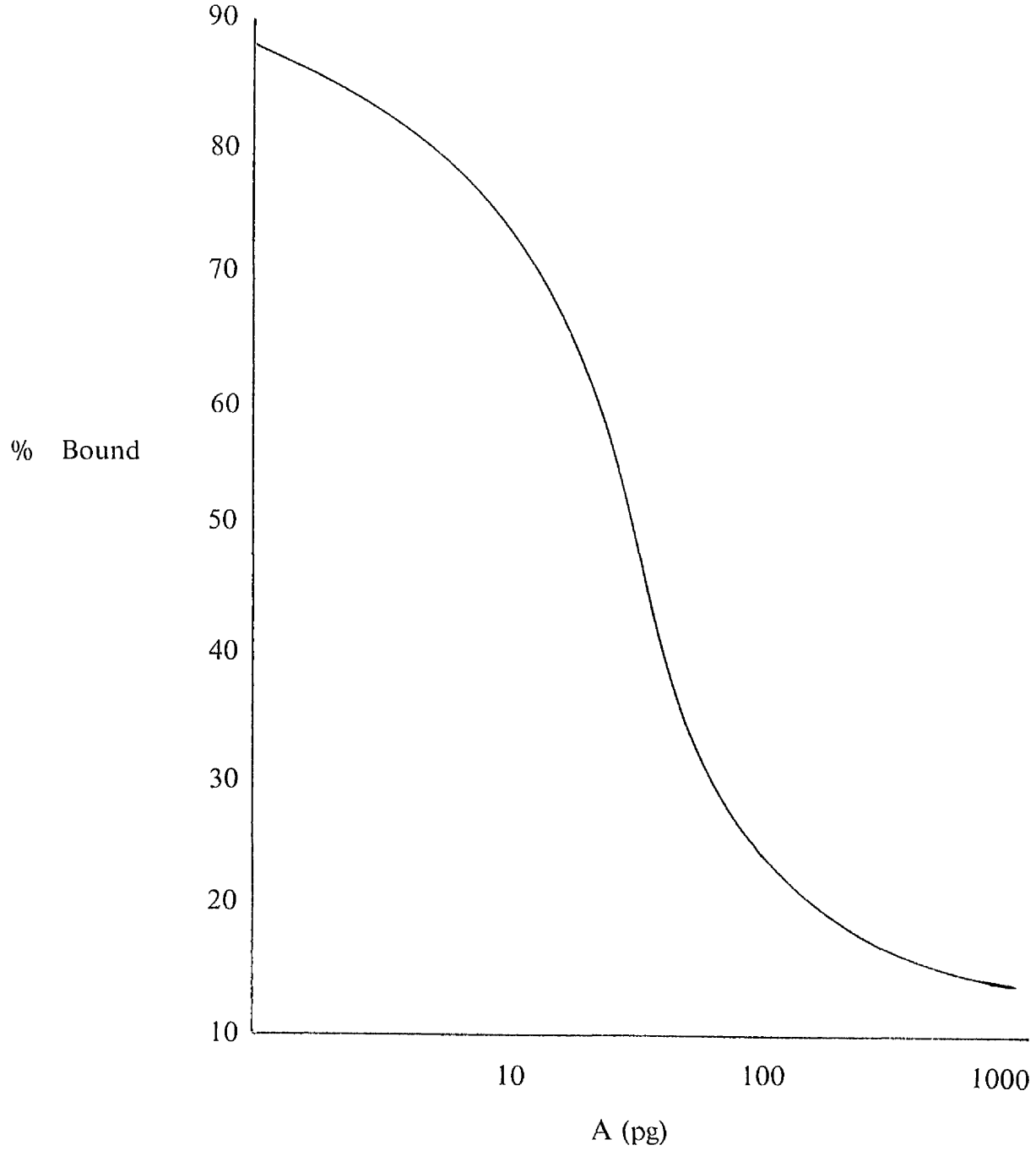
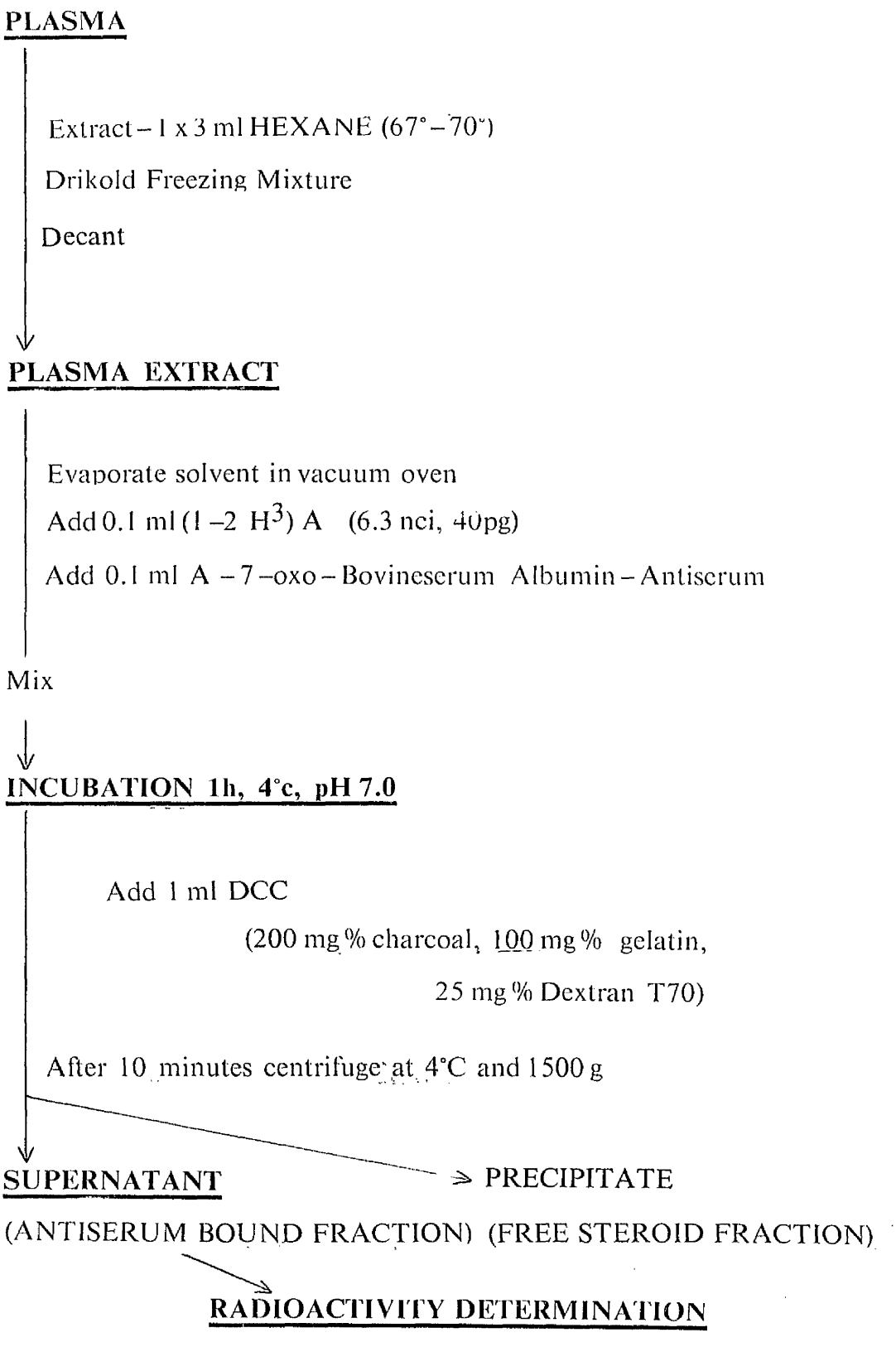


Figure 2.10

Flow diagram of A radioimmunoassay.



RESULTS

1. Extraction of Steroids from Plasma

The efficiency of the extraction procedures employed prior to steroid radioimmunoassay was checked using tritium labelled hormones as described in Chapter 2. Table 3.I lists the mean percentage extraction plus the standard deviations for A, T, DHT, P, 17OHP and OE_2. The extractions of OE_2, 17OHP and P were taken from these figures as being quantitative and no corrections were applied.

Table 3.I

Mean % extractions (± SD) of steroid hormones from plasma

STEROID	Mean % Extraction*	S.D
A	80.0	± 1.2
DHT	97.2	± 5.1
T	92.0	± 6.1
P	96.6	± 5.7
17OHP	99.7	± 5.9
OE_2	94.6	± 3.8

* in each case replicates (n=20) of H^3 labelled hormone were extracted from mixed plasma (0.2ml).

Dihydrotestosterone and T were also extracted quantitatively but as described in Methods section internal tritiated standards of each were added to individual samples to determine recoveries throughout the method which involved a chromatographic step. The recoveries of

DHT and T are described under the chromatography section later in this chapter. Androstenedione, as shown in Table 3.1, was extracted with an efficiency of 80% ± 1.2%. All A assays were routinely corrected for a 20% extraction loss.

2. Radioimmunoassay of C19 Androgens

a) ANDROSTENEDIONE

i) Separation of free and antibody bound steroid hormones.

In steroid radioimmunoassay one of the most critical steps is the separation of the free and antiserum bound steroid fractions following equilibration of antigen and antibody. Figure 3. 1 shows the standard curves obtained for A with different charcoal contact times (0, 10 minutes and 20 minutes). Significant stripping occurred prior to 10 minutes. Thereafter, a further significant reduction in the % bound did not occur. As a result 10 minutes contact time with DCC was employed in the A radioimmunoassay.

ii) Comparison of A radioimmunoassay with and without chromatography.

To confirm that the A antiserum was specific, aliquots from a pool of mixed plasma were assayed for A by the method as described in Methods Section, and compared with aliquots of the same plasma pool in which A was assayed using the same antiserum and conditions after purification of the plasma extract using column chromatography.

10 x 0.025ml aliquots of the plasma pool were extracted (after addition of an internal standard of tritium labelled A) with diethyl ether as described in Methods. These extracts were chromatographed individually on columns (10 cm x 0.75 cm) of "Lipidex R" (Packard–Becker Limited) with petroleum ether (boiling point 40° – 60°)/cyclohexane (95:5; v/v) as the eluting solvent. Under these conditions A is eluted

between 12 and 15ml inclusive and is separated from all other C19 androgens. The fractions 12ml – 18ml were collected from the chromatogram of each aliquot, the solvent evaporated, an aliquot (25%) removed to determine chromatographic losses and the remainder was subjected to radioimmunoassay. Results were corrected for individual recoveries.

A further 10 x 0.025ml aliquots of the same plasma pool were extracted with hexane and subjected to direct radioimmunoassay as described in Methods. In this case each result was corrected for 80% recovery (Table 3.I). Table 3.II shows the mean and S.D. values obtained for the level of A in the mixed plasma pool when assayed by the two different methods.

Table 3.II

Androstenedione levels in Mixed Plasma Pool (pg/0.025ml)

Direct Assay	With Chromatography
64	65
58	56
59	68
53	58
48	75
51	54
50	67
56	85
58	52
61	57
Mean 55.8	63.7
S.D. 5.16	10.44

No significant difference was observed between the two sets of values and as a result the method without chromatography was used in this study.

iii) Statistical Assessment of the A Radioimmunoassay

The data obtained for the radioimmunoassay of A are listed below.

1. Blank:– 1.4pg ±2.1pg (S.D.) n=72 / 0.05ml of ovariectomised, adrenalectomised plasma.

2. Sensitivity:– 10pg of cold A caused a significant drop in the % bound and was distinguishable from the blank.

3. Intra–assay precision and accuracy:–
Twenty replicate standards of both 30pg and 70pg were added to 0.05ml of ovariectomised, adrenalectomised plasma and assayed within a single assay to give values of 32.3pg ± 5.3pg (S.D.) and 72.5pg ± 6.5pg (S.D.) respectively.

These results gave values for accuracy and intra–assay precision of:–

	30pg	70pg
Accuracy	107.7% ± 17.7% (S.D.)	103.6% ± 9.3% (S.D.)
Precision	16.4%	9.0%

4. Inter–assay precision
Four x 0.05ml aliquots from a mixed plasma pool were analysed with each assay and gave a cumulative value of 813pg/ml ± 71pg (S.D.)
Precision = 8.7%

b) DIHYDROTESTOSTERONE AND c) TESTOSTERONE

i) Chromatography Column Recoveries

The recoveries of DHT and T achieved through the celite column partition chromatography were assessed by simultaneous chromatography of labelled standards of [$4^{14}C$] DHT and [$1-2H^3$] T. When the DHT and T fractions were collected (see Figure 2.3) and their radioactive content determined the results shown in Table 3.III were obtained.

Table 3.III

Percentage recovery of [^{14}C] DHT and [$1-2H^3$] T after celite column partition chromatography.

	[$4^{14}C$] DHT	[$1-2H^3$] T
	Mean % recovery ± SD (n=5)	
DHT Fraction (6–25ml)	79 ± 5.3	0
T Fraction (36–50ml)	0	90 ± 7.3

ii) Method Recoveries

Internal standards of [$1,2,4,5,6,7H^3$] DHT and [$1-2H^3$] T were added to each

sample prior to extraction. Immediately before radioimmunoassay aliquots were removed from both the DHT and T fractions for determination of radioactivity to compute recoveries up to that point – i.e. to assess all methodological losses prior to radioimmunoassy. Table 3.IV shows the mean ± S.D. % recoveries obtained.

Table 3.IV

Percentage recoveries of DHT and T prior to assay.

	% Recovery
DHT	69.3 ± 7.5 (S.D.; n=90)
T	75.3 ± 5.0 (S.D.; n=90)

Each individual DHT and T fraction was corrected for its own recovery determined on an aliquot equal to that used for radioimmunoassay.

iii) Separation of free and antibody bound steroid hormones.

This separation was again achieved using DCC. Figures 3.2 and 3.3 depict the effect of different charcoal contact time (0, 10 minutes, 20 minutes and 30 minutes) on the per cent bound radioactivity in the presence of 0, 50pg, 100pg and 500pg of DHT (Figure 3.2) and 0, 20pg, 100pg and 500pg of T(Figure 3.3).

In the case of T stripping occurred prior to 10 minutes whilst for DHT reduction of the per cent bound continued until 20 minutes. As a result of these findings the DCC contact times used in the assays were 10 minutes for T and 20 minutes for DHT.

iv) Statistical Assessment of DHT Assay

1. Blank:– 1.97pg ± 0.36pg (S.D.) n=30/0.05ml of ovariecto-mised, adrenalectomised plasma.

2. Sensitivity:– The sensitivity limit for DHT was taken as that value which caused a significant displacement of the zero binding (Bo) and was distinguishable from the blank. For DHT this value was 8pg (90% of Bo).

3. Intra–assay precision and accuracy:–
Ten replicate standards containing 50pg and 100pg of both T and DHT were added to 0.2ml of ovariectomised adrenalectomised plasma and assayed within a single assay. The values obtained for these plasmas and the resultant values for accuracy and precision at these two levels are shown in Table 3.V.

Table 3.V

	pg of DHT	
Amount added	50	100
Amount determined by assay	51.0	100.5
S.D.	± 6.1	± 11.6
n	10	10
Accuracy	102% ± 12.2%	100.5% ± 11.6%
Intra Assay Precision	12.0%	11.5%

4. Inter–assay Precision:–

Eight x 0.2ml aliquots from a mixed plasma pool were analysed with each assay giving a cumulative value of 82.9pg ± 9.9pg (S.D.; n=104) and an inter–assay precision of 12.04%.

v) Statistical Assessment of T Assay

1. Blank:– 4.3pg ± 3.1pg (S.D.; n=30)/0.05ml of ovariectomised, adrenalectomised plasma.

2. Sensitivity:– 10pg cold T caused a significant drop in the binding of tritium labelled hormone (82% of Bo – see Figure 3.2) and was distinguishable from the blank. This level was taken as the limit of sensitivity.

3. Intra–assay precision and accuracy:–

This was determined in the same assay as that for DHT above and the levels observed in spiked plasmas shown in Table 3.VI. gave the noted results for accuracy and precision.

Table 3.VI

	pg of T	
Amount added	50	100
Amount determined by assay	56.3	101.3
S.D.	± 3.7	± 8.4
n	10	10
Accuracy	112.6% ± 7.4%	101.3% ± 8.4%
Intra Assay Precision	6.6%	8.2%

4. Inter–assay Precision:–

The cumulative value obtained for assay of a mixed plasma pool (8 x 0.2ml/assay) was 2356pg ± 346pg (S.D.; n=104) which gives an inter–assay precision of ± 14.68%

3. Radioimmunoassay of P, 17OHP and OE_2.

These steroid hormones were assayed as described in Methods, using methods already in use in the Department of Obstetrics and Gynaecology. A summary of the precision, accuracy and sensitivity data from these assays is shown in Table 3.VII.

Precision was determined in each assay at a point on the standard curve in the middle of the working range.

Table 3.VII

Statistical Assessment of Steroid Radioimmunoassays

	P	17OHP	OE_2
Blank Value	17 ± 2.3pg (S.D.; n=52)	12 ± 1.3pg (S.D.; n=51)	3.0 ± 2.0pg (S.D.; n=50)
Lowest limits of detection	20pg	10pg	10pg
Accuracy %	100 ± 9.1 (n=51)	98.8 ± 8.6 (n=50)	108.9 ± 11.12 (n=20)
Intra–assay precision	± 9.2 (n=51)	± 8.7 (n=50)	± 10.2 (n=20)
Inter–assay precision	± 11.8 (n=53)	± 12.7 (n=53)	± 11.3 (n=50)

3a) **Radioimmunoassay of FSH, LH and PRL.**

The protein hormones were kindly assayed by the Research Department, Glasgow Royal Maternity Hospital (FSH and LH – Dr. Philip England) and Clinpath Services Ltd., High Wycombe (PRL – Dr. Alan Craig). Full details of the validation data for these assays are not included but all showed acceptable inter and intra assay precisions.

4. Androgen Profiles in the Normal Human Menstrual Cycle

Levels of A, DHT and T were determined in serial daily plasma samples obtained throughout seven cycles from volunteers. All of the samples from one cycle were determined in a single assay, for each hormone, to ensure that only intra–assay variation affected the results. 0.05ml of each plasma sample was assayed for A, whilst DHT and T were assayed in 0.2ml of each plasma sample.

Figures 3.4, 3.5 and 3.6 show the mean levels ± SEM in the seven normal menstrual cycles for A, DHT and T respectively.

These results are plotted relative to the day of the LH peak which was designated day 0. Days prior to this peak are signified as negative and days following it as positive.

The A levels (Figure 3.4) are fairly constant throughout the menstrual cycle showing levels between 1 and 2ng/ml. A rise in the level occurred at around mid cycle; the levels from day 0 to day 4 being elevated compared to those of previous and succeeding days. This early luteal peak was observed in all patients, but in this small series was not statistically significant. The highest mean value obtained was on day

+2 and was 2.32 ± 0.4 (SEM) ng/ml. The levels in the late luteal phase and the early follicular phase were similar to each other.

Mean DHT levels (Figure 3.5) ranged from 100 to 173 pg/ml. The levels rose throughout the follicular phase from a base-line of 100pg/ml to reach peak values of 173pg/ml on day –2. Following a slight drop the levels rose again to reach an identical peak on day +2. Thereafter the levels declined slowly to basal values during the remainder of the luteal phase.

The mean levels of T (Figure 3.6) ranged from 172 pg/ml to 579 pg/ml. The pattern of T, particulary in the follicular phase, was similar to that described for DHT. T levels rose during the follicular phase from basal levels on day –8 to reach peak values at around mid cycle (day –1, 527 ± 176 (SEM) pg/ml, day +1, 579 ±97 (SEM) pg/ml). After day +1 levels declined until day +7 (296 ± 75 (SEM) pg/ml). The levels between days –2 and +4 being significantly higher than those between days –8 and –4 and days +5 and +7 respectively ($p<0.01$). Levels rose again in the late luteal phase to peak on day +14 (531 ± 85 (SEM) pg/ml). Following menstruation the levels declined until day –8 when the follicular rise described above began.

5. Levels of FSH, LH, 17OHP, P and OE_2 in daily Plasma Samples throughout the Normal Human Menstrual Cycle

The levels of FSH, LH, 17OHP, P and OE_2 were also determined in the samples from the seven volunteers. The levels for all of these hormones were not significantly different from their previously published profiles (Coutts 1976) which were shown in Introduction (Figures 1.4, 1.5, 1.6, 1.7, 1.8).

6. Prolactin Levels in the Normal Human Menstrual Cycle

Levels of human PRL were also determined throughout the normal cycles from the seven volunteers and the mean levels ± SEM are shown in Figure 3.7. The levels ranged from approximately 280 to 450 mIU/ml showing a small peak at mid cycle and a secondary rise in the late secretory phase. Despite these rises assessment of single samples showed no significant differences throughout the cycle.

7. Dating of Stage of Menstrual Cycle in Patients Sampled at Hysterectomy

The stage of the menstrual cycle was assessed, in patients undergoing surgery, from the menstrual history, the macroscopic and microscopic examination of the ovaries and the histological changes of the endometrium. Table 3.VIII lists the stage of the menstrual cycle assessed by each of these parameters in patients undergoing hysterectomy. This assessment of the stage of the menstrual cycle was confirmed from the levels in peripheral venous plasmas of LH, FSH, OE_2 and P. Table 3VIII also lists the conditions indicating surgery. In a few patients (Table 3.VIIIa) the information from the menstrual history, the ovaries and the endometrium was insufficient to achieve accurate assessment of the stage of the menstrual cycle. In these patients the assessment was based on what information was available regarding the above parameters and the levels of LH, FSH, P and OE_2.

Using the information in Tables 3.VIII and 3.VIIIa the patients were designated as being in one of the six stages of the cycle – early proliferative (EP), mid proliferative (MP), late proliferative (LP), early secretory (ES), mid secretory (MS) or late secretory (LS). Figure 3.8 is a schematic diagram of the normal menstrual cycle showing follicular and luteal phases and the division of these two phases into early, mid and late stages.

8. Levels of Hormones in Peripheral and Ovarian Venous Samples obtained from Patients at Hysterectomy who had Normal Reproductive Function.

As described above in Tables 3.VIII and 3.VIIIa, patients were grouped into EP, MP, LP, ES, MS and LS. The levels of the hormones in the peripheral and the ovarian venous samples are presented under these stages of the menstrual cycle for each hormone. Each hormone is described in two separate tables, one showing the levels in patients in the proliferative phase and the second those in patients in the secretory phase. The tables list the results in peripheral samples and in ovarian venous samples draining the active and/or the contralateral ovary. Where sufficient samples were available mean ± SEM values are listed for each stage of the cycle in the three different sites (*P*= peripheral vein; *O*=active ovarian vein; *C*= contralateral ovarian vein).

For the individual hormones the levels are described in the tables as follows:–

A.	FSH	Table 3.IX and 3.X
B.	LH	Table 3.XI and 3.XII
C.	PRL	Table 3.XIII and 3.XIV
D.	P	Table 3.XV and 3.XVI
E.	17OHP	Table 3.XVII and 3.XVIII
F.	OE_2	Table 3.XIX and 3.XX
G.	A	Table 3.XXI and 3.XXII
H.	DHT	Table 3.XXIII and 3.XXIV
I.	T	Table 3.XXV and 3.XXVI

The results shown in Tables 3.IX to 3.XXVI are also depicted graphically, as histograms of the mean values ± SEM for each hormone at the six different stages of the menstrual cycle, in Figures 3.10 to 3.18. Each Figure describes the results for one hormone in the same order as described for the tables. Figure 3.9 represents a key describing the symbols used in these histograms showing the designation used for peripheral vein (*P*), active ovarian vein (*O*) and contralateral ovarian vein (*C*), and also the abbreviations used to describe the six stages of the menstrual cycle. Where single patient results showed wide variations from the results of other patients in the same group they have not been included in the histograms to prevent unnecessary skewing of the data. This explains the occurrence of a few minor discrepancies between the tabulated and diagrammatic data.

Figures 3.19 to 3.27 show the same data with the hormone levels in peripheral veins contrasted only with those in veins draining the active ovaries at different stages of the cycle.

Table 3.VIII

Dating of the Stage of the Menstrual Cycle using Clinical/Pathological Criteria

Patient	Age	Day of Cycle	Endometrial Biopsy Dating	Ovarian Features *			Stage ** Assessed	Indication for Operation
				Macro	Micro	Active Side		
MF	38	5	P	SF	–	(L)	EP	Dysmenorrhea
CM	33	5	EP	SF	SF	(R)	EP	Cancer in Situ
CA	35	9	EP	LF	–	(L)	EP	Menorrhagia
KL	48	11	EP	FC	–	(L)	EP	Fibroid With Endometriosis
JS	35	10	P	–	LF	(R)	MP	Fibroid
AK	39	–	P	–	LF	(R)	MP	Menorrhagia
AMcG	44	8	MP	LF	–	(L)	MP	Fibroid
SK	47	11	MP	LF	CA	(L)	MP	Menorrhagia
MM	54	14	LP	–	–	(R)	LP	Cancer Corpus
VB	35	14	LP	LF	–	(L)	LP	Menorrhagia
ED	40	11	LP	LF	–	(L)	LP	Menorrhagia
JA	28	14	LP	LF	–	(L)	LP	Menorrhagia
HB	40	15	ES	–	CL	(L)	ES	Irregular Period
EP	46	16	ES	–	CL	(L)	ES	Ca in situ

* SF – Follicles up to 5 mm in diameter
LF – Follicles > 5 mm in diameter
FC – Follicular cyst
CL – Corpus luteum
CA – Corpus albicans
HF – Haemorrhagic follicle
(L) – Left Ovary
(R) – Right Ovary

** These stages were confirmed by peripheral hormone estimations.

Patient	Age	Day of Cycle	Endometrial Biopsy Dating	Ovarian Features *			Stage **	Indication for Operation
				Macro	Micro	Active Side	Assessed	
AB	44	18	ES	FC	–	(L)	ES	Menorrhagia
FH	30	–	ES	–	CL	(L)	ES	Menorrhagia
ID	45	–	ES	HF	–	(L)	ES	Dysmenorrhea
EM	33	–	S	CL	CL	(R)	MS	Fibroid
AH	45	7	S	CL	–	(R)	MS	Fibroid
MH	36	27	LS	–	FC	(L)	MS	Menorrhagia
EMcA	27	20	S	–	FC	(L)	MS	Menorrhagia
MR	34	28	S	–	CL	(L)	MS	Endometriosis
MF	42	17	MS	CL	–	(R)	MS	Irreg. Bleeding
MM	34	21	S	CL	–	(L)	MS	Irreg. Bleeding
CN	34	30	S	CL	–	(L)	MS	Fibroid
AB	44	18	S	CL	CL	(L)	MS	Ovarian Fibroma
DC	36	14	MS	LF	CL	(R)	MS	Irreg. Bleeding
MT	40	7	LS	CL	CL	(R)	LS	Irreg. Bleeding
SL	38	22	LS	CA	–	(L)	LS	Irreg. Bleeding
MW	39	32	LS	CL	CL	(R)	LS	Fibroid
EMcI	25	30	LS	CL	–	(R)	LS	Fibroid

* SF – Follicles up to 5 mm in diameter
LF – Follicles > 5 mm in diameter
FC – Follicular cyst
CL – Corpus luteum
CA – Corpus albicans
HF – Haemorrhagic follicle
(L) – Left Ovary
(R) – Right Ovary

** These stages were confirmed by peripheral hormone estimations.

Table 3.VIIIa

Dating of the stage of the Menstrual Cycle using
Clinical/Pathological Criteria and Steroid Hormone Levels

Patient	Age	Day of Cycle	Endometrial Biopsy Dating	Ovarian Features *			Δ^4P in ng/ml	$OE_2$17ß pg/ml	Stage Assessed	Indication for Operation
				Macro	Micro	Active Side				
Mgt.W	42	26	–	CL	–	(R)	2.3	70	LS	Cancer in Situ
MMcG	35	–	LP	–	–	(R)	1.4	120	LP	Endometriosis
AM	33	10	P	FC	–	(R)	1.2	50	LP	Menorrhagia
SM	39	–	–	SF	LF	(L)	0.8	105	MP	Menorrhagia
JG	28	8	MP	CL	–	(R)	0.4	110	MP	Cancer in situ
EH	43	21	S	CL	CL	(R)	1.1	250	MS	Metropathia Haemorrhagia
JH	45	24	EP	CL	CL	(L)	LS based on ovarian vein levels		LS	Fibroids
CS	33	13	LP	FC	–	(L)	0.6	160	LP	Fibroids

* SF – Follicles up tp 5 mm in diameter
LF – Follicles > 5 mm in diameter
FC – Follicular cyst
CL – Corpus luteum
CA – Corpus albicans
HF – Haemorrhagic Follicle.

(L) – Left Ovary
(R) – Right Ovary

Table 3.IX

Levels of FSH (mIU/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		> 50.0	> 50.0	
	C.M.		6.6	7.4	
	C.A.		4.4	4.6	
	K.L.		9.2	8.0	10.3
		Mean	17.68	17.67	10.3
		SEM	10.99	10.99	–
M.P.*					
	J.S.		4.1		4.1
	A.K.		6.0	8.4	
	A.McG.		10.3	10.1	
	S.K.		> 50.0		> 50.0
	S.M.		4.0	3.1	
	J.G.		5.3	4.6	
		Mean	13.37	6.55	27.5
		SEM	7.49	1.63	–
L.P.*					
	M.M.		> 50.0	> 50.0	
	V.B.		3.8		
	E.D.		6.7	6.3	
	A.M.		2.6	2.5	
	J.A.		4.7	4.1	5.8
	C.S.		7.7	6.2	
	M.McG.		8.2	10.0	
		Mean	12.03	13.27	5.8
		SEM	6.46	7.52	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.X

Levels of FSH (mIU/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		5.8	10.2	
	E.P.		> 50.0		> 50.0
	A.B.		73.0	9.0	15.1
	F.H.		3.8	4.7	
	I.D.		5.6	5.3	
		Mean	27.74	7.3	15.1
		SEM	14.34	–	–
M.S.*					
	E.M.		6.0	5.4	
	A.H.		4.8	4.5	
	M.H.		4.8	4.5	
	E.McA.		7.6	6.9	
	E.H.		6.3		6.5
	M.R.		3.8	3.6	
	M.F.		4.8	5.3	
	M.M.		4.8	4.0	3.7
	C.N.		7.8		
	A.B.		2.7	2.7	2.5
	D.C.		11.6	15.6	
		Mean	5.91	5.86	4.2
		SEM	0.73	1.3	1.2
L.S.*					
	M.T.		7.0	6.9	
	S.L.		3.9	2.0	
	Mgt.W.		4.1	4.3	
	J.H.		> 50.0	> 50.0	> 50.0
	E.McJ.		3.5	29.0	2.8
	M.W.		4.2	8.7	
		Mean***	4.5	5.5	2.8
		SEM	0.6	1.2	–

* See Figure 3.9.

**_P_=peripheral plasma; _O_=active ovarian vein; _C_=contralateral ovarian vein.

*** Omitting JH results and in case of LS, O–EMcJ as well.

Table 3.XI

Levels of LH (mIU/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		11.6	6.5	
	C.M.		7.5	7.3	
	C.A.		5.5	7.2	
	K.L.		6.0	8.5	7.6
		Mean	7.65	7.38	7.6
		SEM	1.38	0.42	–
M.P.*					
	J.S.		3.1		6.0
	A.K.		15.2	19.0	
	A.McG.		10.0	13.7	
	S.K.		52.0		34.3
	S.M.		7.9	7.0	
	J.G.		4.5	4.9	
		Mean	15.45	11.15	20.15
		SEM	7.52	3.22	–
L.P.*					
	M.M.		61.4	54.0	
	V.B.		2.0		
	E.D.		10.0	9.2	
	A.M.		2.0	2.0	
	J.A.		7.1	7.1	8.2
	C.S.		51.8	47.5	
	M.McG.		40.5	34.0	
		Mean	24.97	25.63	8.2
		SEM	9.60	9.17	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XII

Levels of LH (mIU/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		13.8	15.5	
	E.P.		41.5		41.0
	A.B.		< 2.0	4.6	2.6
	F.H.		4.1	4.7	
	I.D.		9.4	12.5	
		Mean	14.16	9.33	21.8
		SEM	7.39	2.77	–
M.S.*					
	E.M.		11.7	9.4	
	A.H.		2.8	4.6	
	M.H.		6.4	3.5	
	E.McA.		21.7	21.0	
	E.H.		2.1		2.0
	M.R.		7.1	10.5	
	M.F.		10.4	10.6	
	M.M.		5.9	7.5	8.9
	C.N.		5.1		
	A.B.		3.4	3.0	4.3
	D.C.		38.3	43.5	49.5
		Mean	10.45	12.62	16.2
		SEM	3.25	4.26	10.43
L.S.*					
	M.T.		28.3	5.5	
	S.L.		10.7	2.0	
	Mgt.W.		7.0	7.2	
	J.H.		8.6	10.4	9.8
	E.McJ.		5.5	3.5	2.1
	M.W.		3.6	4.2	
		Mean	10.6	5.5	6.0
		SEM	3.67	1.2	–

* See Figure 3.9.

**P=peripheral plasma; O=active ovarian vein; C=contralateral ovarian vein.

Table 3.XIII

Levels of PRL (mIU/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		4290	4600	
	C.M.		4100	3800	
	C.A.		5600		
	K.L.		3000		
		Mean	4248	4200	
		SEM	534	–	–
M.P.*					
	J.S.		3860		370
	A.K.		4000	6200	
	A.McG.		1100		
	S.K.		>8000		3500
	S.M.		2400	3400	
	J.G.		1580	1640	
		Mean	3490	3750	1935
		SEM	1021	1328	–
L.P.*					
	M.M.		2480		
	V.B.		2400		
	E.D.		3800	3000	
	A.M.		2600	5000	
	J.A.		7920	3100	
	C.S.		8000		
	M.McG.		2700	2200	
		Mean	4271	3325	–
		SEM	969	594	–

* See Figure 3.9.

**_P_=peripheral plasma; _O_=active ovarian vein; _C_=contralateral ovarian vein.

Table 3.XIV

Levels of PRL (mIU/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		4140	5600	
	E.P.		4400		
	A.B.		4520	6900	4600
	F.H.		3060	3800	
	I.D.		2500	3000	
		Mean	3324	4824	4600
		SEM	752	880	
M.S.*					
	E.M.		1000		
	A.H.		7420	7000	
	M.H.		2620	3100	
	E.McA.		5440	3400	
	E.H.		1720		3200
	M.R.		4100		
	M.F.		2300		
	M.M.		6400		
	C.N.		1920		2040
	A.B.		1800	2300	
	D.C.		1500	1720	3200
		Mean	3300	3504	3080
		SEM	660	922	180
L.S.*					
	M.T.		2300	2880	
	S.L.		8000	7000	
	Mgt.W.		4400	4300	
	J.H.		3400	4600	
	E.McI.		2400	1660	1660
	M.W.		1200	1220	
		Mean	3600	3610	1660
		SEM	982	874	–

* See Figure 3.9.

**P=peripheral plasma; O=active ovarian vein; C=contralateral ovarian vein.

Table 3.XV

Levels of P (ng/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		0.6	1.8	
	C.M.		0.3	2.2	
	C.A.		1.0	0.6	
	K.L.		0.7	0.3	0.3
		Mean	0.65	1.2	0.3
		SEM	0.14	0.46	–
M.P.*					
	J.S.		0.85		1.3
	A.K.		0.75	10.0	
	A.McG.		2.0	5.0	
	S.K.		2.5		1.6
	S.M.		2.8	5.8	
	J.G.		0.4	1.5	
		Mean	1.56	5.58	1.45
		SEM	0.41	1.75	
L.P.*					
	M.M.		0.52	1.3	
	V.B.		0.22	2.4	
	E.D.		3.5	5.0	
	A.M.			0.8	
	J.A:		5.0	8.5	0.5
	C.S.		0.6	4.3	
	M.McG.		1.4	1.25	
		Mean	1.87	3.36	0.5
		SEM	0.79	1.05	

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XVI

Levels of P (ng/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		3.5	14.0	
	E.P.		3.5		1.3
	A.B.		3.5	14.0	3.75
	F.H.		1.1	6.6	
	I.D.		5.3	21.0	
		Mean	3.38	13.9	2.5
		SEM	0.67	2.9	–
M.S.*					
	E.M.		2.9	13.2	
	A.H.		6.0	300.0	
	M.H.		11.5	19.0	
	E.McA.		1.5	13.0	
	E.H.		1.1		0.6
	M.R.		11.0	24.0	
	M.F.		2.1	140.0	
	M.M.		14.5	140.0	11.0
	C.N.		1.7	15.0	
	A.B.		2.2	80.0	2.3
	D.C.		2.8	140.0	
		Mean	5.2	88.4	4.6
		SEM	1.45	29.5	3.2
L.S.*					
	M.T.		6.0	8.5	
	S.L.		1.8	12.0	
	Mgt.W.		2.6	60.0	
	J.H.			8.0	2.8
	E.McI.		2.3	26.0	
	M.W.		2.25	9.6	
		Mean	2.99	20.7	2.8
		SEM	0.76	8.33	–

* See Figure 3.9.

**P=peripheral plasma; O=active ovarian vein; C=contralateral ovarian vein.

Table 3.XVII

Levels of 17OHP (ng/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		1.60	1.92	
	C.M.		1.90	2.92	
	C.A.		2.72	3.44	
	K.L.		3.30	4.40	3.64
		Mean	2.38	3.18	3.64
		SEM	0.39	0.52	–
M.P.*					
	J.S.		3.4		3.36
	A.K.		1.68		
	A.McG.		0.86	9.3	
	S.K.		20.6		4.0
	S.M.		1.54	4.9	
	J.G.		2.2	18.0	
		Mean	5.05	10.7	3.68
		SEM	3.13	3.84	–
L.P.*					
	M.M.		1.3	4.06	
	V.B.		4.56	5.50	
	E.D.		2.88	6.60	
	A.M.		2.02		
	J.A.		5.40	12.60	5.50
	C.S.		4.8	7.20	
	M.McG.		3.92		
		Mean	3.56	7.2	5.5
		SEM	0.58	1.5	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XVIII

Levels of 17OHP (ng/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		2.32	4.5	
	E.P.		3.32		2.52
	A.B.		0.86	2.28	1.20
	F.H.		1.58	5.6	
	I.D.		3.52	3.88	
		Mean	2.32	4.07	1.86
		SEM	0.5	0.69	–
M.S.*					
	E.M.		2.6	7.4	
	A.H.		3.04	> 100.0	
	M.H.		2.92	18.2	
	E.McA.		1.60	5.0	
	E.H.		5.6		4.2
	M.R.		2.02	5.4	
	M.F.		2.14	40.0	
	M.M.		3.48	40.0	3.92
	C.N		1.48	12.0	
	A.B.		3.36	> 100.0	3.84
	D.C.		1.82	> 100.0	
		Mean	2.73	42.8	3.99
		SEM	0.35	13.1	0.11
L.S.*					
	M.T.		4.12		
	S.L.		2.08	3.76	
	Mgt.W.		2.44	11.40	
	J.H.		2.16	3.44	3.48
	E.McI.		2.20	3.24	2.28
	M.W.		3.44	4.3	
		Mean	2.74	5.23	2.88
		SEM	0.34	1.55	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XIX

Levels of OE_2 (pg/ml) in Plasma Samples from Patients in the Proliferative Phase

	PATIENT		*P.***	*O.***	*C.***
E.P.*	M.F.		58	410	
	C.M.		58	1550	
	C.A.		50	700	
	K.L.		118	480	450
		Mean	71.0	785.0	450.0
		SEM	15.78	262.0	–
M.P.*					
	J.S.		125		490
	A.K.		80	1030	
	A.McG.		60	3880	
	S.K.		73		500
	S.M.		105	1200	
	J.G.		110	3030	
		Mean	92.2	2285.0	495.0
		SEM	10.2	698.0	–
L.P.*					
	M.M.		66	300	
	V.B.		112	400	
	E.D.		140	500	
	A.M.		50	300	
	J.A.		110	420	200
	C.S.		160	990	
	M.McG.		120	650	
		Mean	108.3	508.6	200.0
		SEM	14.7	92.4	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XX

Levels of OE_2 (pg/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		115	1280	
	E.P.		45		430
	A.B.		70	780	1100
	F.H.		75	500	
	I.D.		70	600	
		Mean	75.0	790.0	765.0
		SEM	11.6	173.0	–
M.S.*					
	E.M.		86	1680	
	A.H.		86	2550	
	M.H.		93	900	
	E.McA.		58	850	
	E.H.		250		1350
	M.R.		183	780	
	M.F.		170	25000	
	M.M.		120	1180	430
	C.N.		69	1250	
	A.B.		106	4350	480
	D.C.		183	5000	
		Mean	128.0	2060.0***	753.0
		SEM	18.0	529.0	299.0
L.S.*					
	M.T.		120	690	
	S.L.		35	585	
	Mgt.W.		68	980	
	J.H.			600	500
	E.McI.		115	1580	350
	M.W.		70	430	
		Mean	81.6	810.8	425.0
		SEM	15.9	170.8	–

* See Figure 3.9.

**P=peripheral plasma; O=active ovarian vein; C=contralateral ovarian vein.

*** Value from MF is not included in this calculation

Table 3.XXI

Levels of A (ng/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		1.95	2.5	
	C.M.		1.85	7.5	
	C.A.		2.47	4.2	
	K.L.		2.86	3.75	2.98
		Mean	2.28	4.49	2.98
		SEM	0.23	1.07	–
M.P.*					
	J.S.		2.05		3.50
	A.K.		2.2	8.26	
	A.McG.		1.5	7.76	
	S.K.		2.5		3.5
	S.M.		1.06	3.80	
	J.G.		3.83	13.26	
		Mean	2.19	8.3	3.5
		SEM	0.39	1.9	–
L.P.*					
	M.M.		1.4	7.66	
	V.B.		2.25	12.01	
	E.D.		2.5	4.72	
	A.M.		1.38	6.66	
	J.A.		2.35	7.25	2.25
	C.S.		4.0	9.5	
	M.McG.		3.2	7.0	
		Mean	2.44	7.8	2.25
		SEM	0.35	0.88	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XXII

Levels of A (ng/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		3.1	6.9	
	E.P.		2.9		3.0
	A.B.		1.01	6.8	1.18
	F.H.		2.95	10.88	
	I.D.		3.03	7.26	
		Mean	2.6	7.96	2.09
		SEM	0.40	0.98	–
M.S.*					
	E.M.		2.45	20.1	
	A.H.		2.28	49.1	
	M.H.		2.95	31.2	
	E.McA.		2.78	30.5	
	E.H.		1.70		3.43
	M.R.		2.6	12.0	
	M.F.		1.93	195.1	
	M.M.		1.51	18.0	3.8
	C.N.		3.10	32.0	
	A.B.		2.61	50.0	2.86
	D.C.		2.51	29.3	13.24
		Mean	2.40	46.7	5.83
		SEM	0.15	16.9	2.00
L.S.*					
	M.T.		1.61	3.1	
	S.L.		2.25	3.28	
	Mgt.W.		4.5	8.0	
	J.H.		1.56	7.3	2.0
	E.McI.		3.45	10.71	
	M.W.		2.93	5.5	
		Mean	2.72	6.3	2.0
		SEM	0.5	1.2	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XXIII

Levels of DHT (pg/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		141	193	
	C.M.		141	276	
	C.A.		140	187	
	K.L.		113	377	268
		Mean	133.8	258.0	268.0
		SEM	6.9	44.0	–
M.P.*					
	J.S.		122		366
	A.K.		145	355	
	A.McG.		119	367	
	S.K.		90		173
	S.M.		93	198	
	J.G.		231	1350	
		Mean	133.0	568.0	270.0
		SEM	21.0	264.0	–
L.P.*					
	M.M.		93	235	
	V.B.		106	581	
	E.D.		168	192	
	A.M.		146	220	
	J.A.		219	394	239
	C.S.		194	302	
	M.McG.		108	424	
		Mean	148.0	335.0	239.0
		SEM	18.0	53.0	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XXIV

Levels of DHT (pg/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		141	499	
	E.P.		86		133
	A.B.		103	471	127
	F.H.		162	578	
	I.D.		93	439	
		Mean	117.0	497.0	130.0
		SEM	14.7	30.0	–
M.S.*					
	E.M.		179	944	
	A.H.		182	1433	
	M.H.		131	796	
	E.McA.		138	730	
	E.H.		159		199
	M.R.		164	473	
	M.F.		121	2549	
	M.M.		160	677	245
	C.N.		182	514	
	A.B.		132	741	335
	D.C.		134	1306	
		Mean	153.0	1016.3	260.0
		SEM	7.0	196.5	97.5
L.S.*					
	A.T.		140	392	
	S.L.		118	225	
	Mgt.W.		125	484	
	J.H.		146	250	261
	E.McI.		220	614	
	M.W.		196	269	
		Mean	158.0	372.0	261.0
		SEM	67.0	63.0	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XXV

Levels of T (pg/ml) in Plasma Samples from Patients in the Proliferative Phase

E.P.*	PATIENT		*P.***	*O.***	*C.***
	M.F.		314	255	
	C.M.		316	562	
	C.A.		171	357	
	K.L.		184	400	
		Mean	246.0	394.0	–
		SEM	40.0	64.0	–
M.P.*					
	J.S.		279		276
	A.K.		363	479	
	A.McG.		151	585	
	S.K.		389		1220
	S.M.		233	285	
	J.G.		577	935	
		Mean	332.0	571.0	748.0
		SEM	60.4	136.0	–
L.P.*					
	M.M.		586	3723	
	V.B.		636	451	
	E.D.		324	472	
	A.M.		574	419	
	J.A.		428	1938	414
	C.S.		595	723	
	M.McG.		416	1797	
		Mean	508.0	1360.0	414.0
		SEM	44.0	463.0	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3.XXVI

Levels of T (pg/ml) in Plasma Samples from Patients in the Secretory Phase

E.S.*	PATIENT		*P.***	*O.***	*C.***
	H.B.		631	367	
	E.P.		334		300
	A.B.		182	243	200
	F.H.		482	1285	
	I.D.		184	1562	
		Mean	363.0	864.0	250.0
		SEM	87.0	329.0	–
M.S.*					
	E.M.		171	1467	
	A.H.		393	2215	
	M.H.		464	2809	
	E.McA.		404	1146	
	F.H.		489		1257
	M.R.		624	983	
	M.F.		369	3225	
	M.M.		239	590	664
	C.N.		232	1100	
	A.B.		205	1657	596
	D.C.		205	5160	
		Mean	345.0	2041.0	839.0
		SEM	44.0	435.0	210.0
L.S.*					
	M.T.		314	428	
	S.L.		331	388	
	Mgt.W.		319	576	
	J.H.		381	273	488
	E.McI.		294	434	422
	M.W.		236	446	
		Mean	313.0	422.0	455.0
		SEM	19.0	40.0	–

* See Figure 3.9.

***P*=peripheral plasma; *O*=active ovarian vein; *C*=contralateral ovarian vein.

Table 3XXVII

Levels of Steroid Hormones in Patients
in Whom Samples were obtained from Both
Ovarian Veins and the Peripheral Plasma

PATIENT	STAGE OF CYCLE	P			17OHP			OE_2			A			DHT			T		
		P	*O*	*C*	*P*	*O*	*C*	*P*	*O*	*C*	*P*	*O*	*C*	*P*	*O*	*C*	*P*	*O*	*C*
KL	EP	0.7	0.3	0.3	3.3	4.4	3.6	118	480	450	2.8	3.75	2.98	113	377	268	184	400	–
JA	LP	5.0	8.5	0.5	5.4	12.6	5.5	110	420	200	2.35	7.25	2.25	219	394	239	428	1938	414
AB	ES	3.5	14.0	3.8	0.9	2.3	1.2	70	780	1100	1.01	6.80	1.18	103	471	127	182	243	200
MM	MS	14.5	140.0	11.0	3.5	40.0	3.9	120	1180	430	1.51	18.00	3.80	160	677	245	239	590	664
JH	LS	–	8.0	2.8	2.2	3.4	3.5	–	600	500	1.56	7.30	2.00	146	250	261	381	273	488
EM		2.3	26.0	–	2.2	3.2	2.3	115	1580	350	3.45	10.71	–	220	614	–	294	434	422
	Mean	5.2	32.8	3.7	2.9	11.0	3.3	107	840	340	2.12	8.97	2.4	160	464	228	285	646	438
	SEM	2.4	21.7	1.9	0.6	6.0	0.6	9	163	63	0.38	2.02	0.4	21	65	26	42	263	75

From these results (Tables 3.IX to 3.XXVI and Figures 3.10 to 3.27) a number of points may be drawn with respect to the relative levels of each hormone in peripheral venous plasma, plasma from veins draining the active ovary and plasma from contralateral ovarian veins. These points are made below for each hormone using the same alphabetical key A to I as used previously.

A. FSH

B. LH

C. PRL

D. P

E. 17OHP

F. OE_2

G. A

H. DHT

I. T

A. FSH

The levels of FSH found in the peripheral plasmas of the patients in the six stages of the menstrual cycle (Figures 3.10 and 3.19) are consistent with those found in peripheral plasma from normally menstruating volunteers (Figure 1.4) showing highest levels in the early proliferative phase followed by a reduction as the cycle progresses. At all six stages no significant differences were observed between the levels in plasmas draining active ovaries and those in peripheral plasmas. Where contralateral ovarian venous plasmas were available, their levels were again not significantly different from the peripheral values.

B. LH

In samples from normally menstruating volunteers LH levels peak around mid cycle (Figure 1.5). Maximum levels of LH were observed in these patients in the late proliferative stage of the cycle (Figures 3.11 and 3.20). No significant difference was observed in any case between the LH values in plasmas draining active ovaries and those in peripheral plasmas. In the small number of contralateral ovarian venous plasma samples studied levels were also not significantly different from these in peripheral plasma.

C. PROLACTIN

Levels of PRL observed in the samples obtained from these patients at surgery were considerably elevated compared to normal cycle plasma levels obtained from volunteers (Figure 3.7); the levels at surgery being some four to five times higher. This was presumably a response to anaesthetics and will be discussed in the Discussion. Despite these elevation it can be seen (Figures 3.12 and 3.21) that the levels were constant throughout the cycle and no significant difference was observed

between the levels in plasma from the active ovarian vein, the peripheral vein or the contralateral ovarian vein.

D. PROGESTERONE

As found in sampes from normally menstruating volunteers (Figure 1.8), plasma obtained during the secretory phase of the cycle contained much higher levels of P than that obtained in the proliferative phase. The levels in the peripheral plasmas were consistent with normal menstrual cycle data showing peak levels of greater than 5ng/ml in the MS phase (Figures 3.13 and 3.22). There was also evidence of increased P levels in the MP and LP phases prior to ovulation. In the plasma samples obtained from veins draining active ovaries a similar picture was obtained but levels of P of up to seventeen times the peripheral levels were found during the secretory phase. In the MP and LP phases the levels of P were also greater in active ovarian venous plasmas than in the peripheral venous samples.

Levels in contralateral ovarian venous plasmas, where sampled, were not at any stage of the cycle significantly different from the levels in the peripheral plasma (Figure 3.13). The levels of P in plasma from veins draining active ovaries were significantly higher than those in plasmas from peripheral or contralateral ovarian veins in the MP phase ($p < 0.05$) and in the MS phase ($p < 0.02$).

E. 17α HYDROXYPROGESTERONE

Levels of 17OHP in peripheral venous samples obtained from patients at surgery showed a rise in the LP phase (mean 3.56ng/ml) followed by a fall in the ES phase and a secondary rise in the MS and LS phases (ca 2.7ng/ml) (Figures 3.14 and 3.23). This pattern is in agreement with the findings in normally menstruating volunteers for 17OHP

(Figure 1.7). At all stages of the cycle studied, apart from the EP, the levels of 17OHP in plasmas from veins draining active ovaries were higher than those in peripheral plasmas – maximum levels were found in the MS stage, where the mean level in the active ovarian vein was 42.8 ng/ml, a value sixteen times that obtained in corresponding peripheral plasma samples.

The levels from the active ovary were significantly higher than those in peripheral plasmas in MP and LP ($p<0.05$) and MS ($p<0.01$) phases. Contralateral ovarian venous levels, where examined, were higher than the corresponding peripheral plasma levels in the MP and MS stages of the cycle. These increases in the contralateral ovarian veins were not significant ($p<0.1$).

F. OESTRADIOL

As observed in samples obtained from normally menstruating volunteers, OE_2 levels rose during the proliferative phase to peak at mid cycle. Following a nadir, peak levels were again observed in the MS phase (Figures 3.15 and 3.24). The mean levels assayed in the peripheral samples from the patients studied at surgery in the LP (108pg/ml) and MS phases (128pg/ml) were considerably lower than equivalent levels measured in normally cycling volunteers (Figure 1.6).

The OE_2 levels in venous plasmas draining active ovaries were higher than those in peripheral plasmas at all six stages of the menstrual cycle; the pattern of levels being similar to that observed in the normal menstrual cycle with the exception that the proliferative phase peak was found in the mid rather than the late stage (Figure 3.15). Oestradiol levels in the veins draining the active ovaries were significantly higher at all stages of the cycle except EP than corresponding peripheral plasma levels (MP, ES and LS – $p<0.01$; LP, MS – $p<0.005$).

Levels of OE_2 in contralateral ovarian veins were also higher than the corresponding peripheral venous levels. Where sufficient contralateral ovarian venous samples were available to allow statistical assessment, the levels in the contralateral ovarian veins were significantly higher than those in peripheral plasmas ($p<0.05$). The mean OE_2 levels from veins draining active ovaries were up to twenty five times higher than the corresponding peripheral levels (MP) whilst the contralateral ovarian venous levels were greater than the peripheral levels by up to nine times (ES).

G. ANDROSTENEDIONE

Androstenedione levels in the samples from normally menstruating volunteers (Figure 3.4) were fairly constant throughout the cycle with a minor rise in the ES phase. The A levels observed in samples of peripheral plasma obtained from patients at surgery were consistent with those findings; similar levels being observed at all six stages of the cycle, with the secretory phase levels slightly higher than those in the proliferative phase (Figures 3.16 and 3.25).

At all six stages of the cycle levels of A in venous plasmas draining active ovaries were higher than the corresponding peripheral levels; this difference was highly significant at all stages except in the EP (MP $-p<0.02$; LP and MS – $p<0.001$; ES – $p<0.005$; LS – $p<0.05$). Levels of A in active ovarian venous plasmas were highest during the secretory phase, the levels in the MS phase being more than twelve times the corresponding peripheral venous levels. Active ovarian venous levels of A were also elevated during the proliferative phase being 3–4 times the corresponding peripheral levels in the MP and LP phases.

Contralateral ovarian vein levels of A, where available, were identical to peripheral levels except in the MS stage where a small, but significant, elevation was observed ($p<0.02$). Although A levels in contralateral ovarian veins were increased at this stage of the cycle they were insignificant compared to the levels at the same stage in active ovarian venous plasmas ($p<0.01$).

H. DIHYDROTESTOSTERONE

Levels of DHT in peripheral venous plasma samples, obtained from patients at surgery, were constant throughout the EP and MP phases, rose slightly in the LP stage, decreased during the ES phase and rose again in the MS and LS stages (Figures 3.17 and 3.26). This pattern was consistent with that described earlier for peripheral samples from normally menstruating volunteers (Figure 3.5) and levels assayed (100pg/ml – 200pg/ml) were similar.

Dihydrotestosterone levels in plasmas from veins draining active ovaries were higher than corresponding peripheral plasma levels at all six stages of the cycle. This increase, as in the case of A, was significant at all stages of the cycle except the EP(MP and LS – $p<0.02$; LP – $p<0.01$; ES and MS –$p<0.001$). The highest DHT levels in active ovarian veins were observed in the MS phase where they were more than six times the corresponding peripheral levels. Levels of DHT in contralateral ovarian venous plasmas, where sampled, were identical to or slightly higher than those in corresponding peripheral plasmas. The level in contralateral ovarian veins compared to corresponding peripheral veins was significantly higher in the MS phase ($p<0.005$).

I. TESTOSTERONE

The mean levels of T in the peripheral venous samples from patients at surgery rose from the beginning of the cycle to peak in the LP phase. Levels during the secretory phase were lower than those at mid cycle but were still elevated compared to those in the EP stage (Figures 3.18 and 3.27). This pattern of T in peripheral venous samples was similar to that described previously (Figure 3.6) for peripheral venous plasma from normally menstruating volunteers.

The levels of T in plasmas from veins draining active ovaries were higher than those in corresponding peripheral venous plasmas at all six stages of the cycle (Figure 3.27) being significantly so in the MP ($p<0.02$) the MS ($p<0.005$) and the LS ($p<0.05$) stages. The values for p at the other stages of the cycle where the increase in T was not statistically significant were all less than 0.2.

Testosterone levels in plasmas from veins draining contralateral ovaries, where sampled, were similar to their corresponding peripheral plasma levels except in the MS and LS phases where they were significantly higher than the peripheral values (MS – $p<0.01$; LS –$p<0.02$). In the LS and MP stages the contralateral ovarian venous plasma levels of T were higher than those in plasmas from veins draining the active ovaries but the differences were not statistically significant.

8. a) Hormone Levels in plasmas from veins draining both ovaries and in peripheral plasma from the same patient.

Tables 3.IX to 3.XXVI show levels of the various hormones in peripheral plasmas and in plasmas from veins draining active and contralateral ovaries. However, in many of the patients only one

ovarian vein was sampled. The use of such data to draw conclusions with regard to the relative hormone concentrations in the two ovarian venous effluents might be subject to criticism. In a number of patients samples from veins draining both ovaries were obtained. Table 3.XXVII shows the levels of the six steroid hormones assayed in peripheral venous plasmas and plasmas from ovarian veins in six patients in whom bilateral ovarian samples were obtained. These patients represent the entire menstrual cycle with patients in all stages except MP. The Table also shows Means ± SEM for each hormone *P*,*O* and *C* samples from all of these patients. The levels of P and 17OHP were not significantly different between peripheral and contralateral venous plasmas. In the active ovarian venous effluents the levels of P and 17OHP were higher than those from the other two plasmas in all patients except the one in the EP stage. The levels of P and 17OHP in active ovarian venous plasmas were significantly higher in the other five patients ($p<0.05$ for both hormones). As was expected for progestogens the increases were most marked during the secretory phase. Oestradiol levels in venous plasmas draining active ovaries were significantly higher than those in both peripheral and contralateral venous plasmas at all stages ($p<0.005$). In addition, however, although significantly lower than active ovarian venous levels, the concentrations in contralateral ovarian venous plasmas were significantly higher than the peripheral venous levels at all stages of the cycle ($p<0.02$).

Androstenedione levels in peripheral plasma were not significantly different from those in contralateral ovarian venous plasmas at any stage of the cycle. The active ovarian vein levels of A were higher than both the peripheral and contralateral vein levels at all stages. The active ovarian vein increase in A concentrations was most marked at the MS stage and was significant throughout the cycle ($p<0.02$).

Active ovarian venous plasma levels of DHT were significantly higher than those in either contralateral or peripheral venous samples at all stages of the cycle (peripheral $p<0.05$; contralateral $p<0.02$). The levels in contralateral ovarian veins were higher than those in peripheral plasmas at all stages but in the small series studied this difference was not significant ($p<0.1$).

Testosterone levels were variable throughout the cycle. The only increase associated with the active ovarian venous effluent was in the patient in the LP stage when maximal levels of T were observed in normally menstruating volunteers (Fig. 3.6). The levels of T in contralateral ovarian veins were higher than those in peripheral veins in most of the samples, particularly late in the cycle when contralateral ovarian venous levels were at least as high as those in active ovarian venous effluents. None of these T differences were significant in the series described in Table 3.XXVII.

9) Hormone Levels in the Ovarian Artery

To determine whether the levels of the steroid hormones examined in this study increased during passage through the ovary plasmas were obtained from the peripheral vein and also the ovarian artery and ovarian vein, supplying and draining the active ovary, in one patient. The levels of hormones in the peripheral and ovarian veins and the ovarian artery in this patient who was in the LP stage of the cycle are shown in Table 3.XXVIII. The levels of all of the hormones were identical in the peripheral vein and the ovarian artery whereas in the ovarian vein, the levels of OE_2, A and P were considerably elevated whilst those of DHT and 17OHP were slightly elevated.

Table 3.XXVIII

Hormone levels in peripheral and ovarian veins and in ipsilateral ovarian artery

Hormone	Peripheral Vein	Ovarian Artery	Ovarian Vein
P (ng/ml)	0.22	0.25	2.4
OE_2 (pg/ml)	113	130	400
17OHP (ng/ml)	4.56	4.38	5.50
A (ng/ml)	2.25	2.31	12.01
DHT (pg/ml)	106	144	381
T (pg/ml)	436	424	451

10. Hormone Levels in Peripheral Plasma Samples Before and After Ovariectomy

In one patient, in the EP phase, the contribution of the ovaries to the levels of steroid hormones in peripheral plasma was assessed by assaying the hormone levels in peripheral plasma obtained at surgery, prehysterectomy and for six days after bilateral ovariectomy. The levels of the six steroid hormones in these plasma samples are shown in Table 3.XXIX.

Table 3.XXIX

Levels of steroid hormones in peripheral plasmas
pre and post ovariectomy
in a patient in the early proliferative stage

Hormone	P (ng/ml)	17OHP (ng/ml)	OE_2 (pg/ml)	A (ng/ml)	DHT (pg/ml)	T (pg/ml)
Sample						
Before removal of ovaries	0.70	3.30	110	2.98	200	526
Post Ovariectomy						
Day 1	0.30	0.62	58	0.98	119	190
Day 2	0.20	0.56	68	0.78	141	207
Day 3	0.25	0.48	60	0.80	132	257
Day 4	0.35	0.64	55	1.23	149	259
Day 5	0.25	0.48	56	1.10	151	215
Day 6	0.35	0.50	64	1.13	123	255

These results show that the levels, of all six steroid hormones measured, were reduced in the post ovariectomy samples even although this patient was in the least active stage of the cycle. The levels of P, OE_2, DHT and T, were about 50% of their pre-ovariectomy values, those of 17OHP were 20% of the pre-ovariectomy level and A values were 33% of their pre-ovariectomy level following surgical removal of the ovaries.

11. Hormone Levels in Fluid from a Corpus Luteum

In a patient at hysterectomy, who was categorised as being in the late secretory phase, difficulties were experienced in obtaining ovarian venous samples from either ovary. However, the corpus luteum was clearly visible in the right ovary and the luteal fluid from this was aspirated at the same time as a sample of peripheral venous blood was collected. Figure 3.28 shows histograms comparing the levels of the nine hormones in the luteal fluid with those in the peripheral venous plasma. The luteal fluid contained small amounts of the three protein hormones but at considerably lower concentrations than were present in the peripheral plasma. However, in the case of all six steroid hormones much higher concentrations were observed in the luteal fluid than in the peripheral plasma; luteal fluid levels being of the order of 4.5 times higher for all steroid hormones even although this corpus luteum was presumably regressing.

12. Levels of Hormones in Peripheral Plasmas and Ovarian Fluids obtained at Hysterectomy from Patients who had Apparently Abnormal Cycles

i) POLYCYSTIC OVARIAN DISEASE

Two patients were sampled in whom both of the ovaries on macroscopic and microscopic examination were found to contain multiple ovarian cysts. Unlike many patients with polycystic ovarian disease (McConway et al 1977) they both had ovulatory cycles and were categorised as being in the secretory phase – DC (MS), EMcN (LS). The presence of a corpus luteum in both of these patients allowed the active ovary to be identified although both ovaries were cystic. Figures 3.29 and 3.30 show the levels of the nine hormones in plasma samples from peripheral and both ovarian veins in these two patients. As shown

previously, for patients in the MS and LS stages of the normal cycle, levels of all six of the steroid hormones assayed were significantly elevated in the plasmas from the veins draining the active ovaries compared to the peripheral plasma levels. However, in these two patients with polycystic ovarian disease, in contrast to the normal patients, elevated levels of all steroid hormones were also found in the plasmas from the veins draining the contralateral ovaries. The levels in the contralateral ovarian veins were less than those in the active ovarian veins but were higher than those in the peripheral plasma; much higher in the patient in the MS phase than in the one in the LS phase.

ii) PATIENT WITH PRIMARY INFERTILITY AND HIRSUTISM

Patient E.W. aged 30, with a history of primary infertility and clinically hirsute, had a laparotomy performed for an ovarian mass, detected on examination under anaesthesia. At operation an ovarian tumour was found on the right ovary. The origin of the tumour which was fluid-filled was indeterminate but Pathology stated that it was "not a granulosa cell tumour". A sample of the tumour fluid, plasma from the ovarian venous effluent draining the same ovary and a peripheral venous plasma sample were collected.

Hormone levels found in these samples are shown in Figure 3.31. On the basis of the patient's menstrual history, the macroscopic and microscopic appearance of the ovaries and the histology of the endometrium this patient was classified as being in the LP of the cycle. The levels of P, 17OHP, LH and FSH in the peripheral and ovarian venous samples were consistent with the LP phase of the cycle. Oestradiol and A levels were considerably higher, in both peripheral and

ovarian venous samples, than the levels observed in normal patients at the same stage of the cycle (Figures 3.15 and 3.16).

High levels of steroid hormones were present in the tumour fluid – especially OE_2 and the three androgens. These levels were much higher than those observed in the ovarian venous plasma.

13. Hormone Levels in Samples obtained at Hysterectomy from Patients who were not Cycling Normally.

i. PATIENTS WHO HAD BEEN TAKING ORAL CONTRACEPTIVES.

Three patients who were taking oral contraceptives (variable; of the mixed oestrogen/progestogen type) until four days pre-operation were studied. These patients were (1) A.C. who was operated upon on day five of the cycle and had been on the 'Pill' for seven years. Being on day five she was at the stage of the cycle when she would not normally have been taking the 'Pill'. (2) A.W. who was operated upon on day fourteen of the cycle and had stopped taking the 'Pill' four days prior to operation. She had been on the 'Pill' for six months. (3) C.E. who was operated upon on day twenty four of the cycle and had been on the 'Pill' for an indeterminate period until stopping four days prior to operation. From each of the latter two patients a retention cyst greater than 2cm in diameter was found in one of the ovaries. [It was of interest that in another patient, who was included in the normal cycle late secretory group (J.H.), who had been on the 'Pill' for more than ten years until stopping four months before the operation a similar sized retention cyst was found.]

Samples were obtained at hysterectomy in these patients from peripheral and at least one ovarian veins. Where a retention cyst was

found, the cyst fluid therein was also collected. (Cyst fluid was also obtained from J.H.).

The hormone levels found in patient A.C. – in peripheral and ovarian venous samples are shown in Figure 3.32. The levels of the hormones found in the peripheral plasma sample from this patient were consistent with the EP phase of the menstrual cycle, showing high gonadotrophin levels at a time when the steroid hormone levels were basal. The steroid hormone levels in the ovarian venous effluent were considerably higher than those found in normal cycle patient at the same stage of the cycle.

Figure 3.33 shows the hormone levels in the plasma samples from the peripheral and both ovarian veins in patient A.W. The levels of hormones observed in the samples from this patient were consistent with her being in the MS phase of the cycle. This was confirmed by the finding of a small corpus luteum on both macroscopic and microscopic examination of the ovaries. This corpus luteum was present in the same ovary as that containing the large retention cyst, described earlier. It is of interest that the levels of 17OHP, T and DHT in A.W. were greater in the vein draining the contralateral ovary than in the vein draining the active ovary.

The levels of hormones in peripheral and ovarian venous samples in patient C.E. are shown in Figure 3.34. Although this patient was sampled on day twenty four of the cycle the levels of progesterone show that this was an anovulatory cycle. Macroscopic examination of the ovaries of this patient showed the presence of numerous atretic follicles

particularly in the left ovary. Presumably the atretic follicles were responsible for the increased levels of A, OE_2 and 17OHP found in the venous plasma draining the left ovary. The right ovary of this patient contained the retention cyst described earlier.

The levels of steroid hormones in the retention cyst fluids obtained from the three patients – (i.e. patients A.W. and C.E. who were on current contraceptive therapy and patient J.H. who had previously been on long term contraceptive therapy) are shown in Figure 3.35. Steroid hormone levels in the fluids from these cysts were high. In the case of patient J.H. who had ceased contraceptive therapy some months prior to sampling, the levels of androgens in the cyst fluid were much less than those observed in the other two patients.

ii) EARLY PREGNANCY

Patient M.R. was admitted for hysterectomy because of a history of irregular bleeding. At laparotomy she was found to be pregnant, the uterus being of six weeks size. Following hysterectomy a fetus of six weeks size was observed. At laparotomy blood samples were obtained from a peripheral vein and the ovarian vein draining the ovary containing the corpus luteum of pregnancy. Figure 3.36 shows the levels of hormones found in the peripheral and ovarian venous plasmas in this patient. The assay used for LH in this study did not distinguish between LH and HCG and the high levels measured in this patient presumably represent the HCG rise associated with early human pregnancy. In the peripheral plasma levels of OE_2, P, 17OHP and prolactin, as well as HCG were elevated in this patient compared to levels in patients in the MS phase of the cycle. Levels of the same hormones were also elevated in plasma from the ovarian vein draining the ovary containing the

corpus luteum, confirming, that at this stage of pregnancy the ovary is very active in producing steroid hormones. The levels of OE_2, P and 17OHP in the ovarian vein were not, however, elevated in comparison with levels found in venous plasmas draining active ovaries in the MS stage of the cycle (cf Figures 3.13, 3.14 and 3.15). Androgen levels (A, DHT and T) were neither elevated in the peripheral plasma sample nor in the ovarian vein sample in comparison with the levels in similar samples from the MS phase (cf Figures 3.16, 3.17 and 3.18); in fact the A level in the ovarian vein sample in M.R. was lower than that found in similar samples from the MS phase (cf Figure 3.25).

iii) POST–MENOPAUSAL PATIENTS

Two patients who presented for hysterectomy were post–menopausal. 1) I.McV. aged 55, whose last menstrual period was three years before the operation and who was having surgery for post–menopausal bleeding. Following hysterectomy the ovaries in this patient showed the typical picture of post–menopausal small atrophic white ovaries. 2) M.McL aged 69 years, who last menstruated twelve years previously and was operated on because of swelling of the abdomen. At surgery the left ovary contained a 60cm cyst which on histopathological examination was shown to be a benign mucus cyst of the ovary. The right ovary was a typical small white atrophic post–menopausal ovary.

Figure 3.37 shows the hormone levels in peripheral venous plasma and one ovarian vein plasma from I.McV. As expected in a post–menopausal patient the gonadotrophin levels were very high whilst the levels of all of the steroid hormones were low; the levels of the steroids in the ovarian venous plasma samples being less than or equal to the peripheral plasma levels for all six steroid hormones.

The hormone levels in peripheral venous plasma and in ovarian venous plasma draining the ovary containing the large cyst in patient MMcL. are shown in Figure 3.38. As in the previous post–menopausal patient (Figure 3.37) the levels of gonadotrophins were very high. The steroid hormone levels in peripheral plasma in M.McL. were low and consistent with post–menopause but were in some instances (P, 17OHP and T) higher than the equivalent values obtained in patient I.McV. In patient M.McL., in contrast to the findings in I.McV., the levels of all six steroid hormones assayed were higher in the ovarian venous plasma sample than in the peripheral vein sample.

Figure 3.1
Effects of different DCC Contact Times
on A standard curve

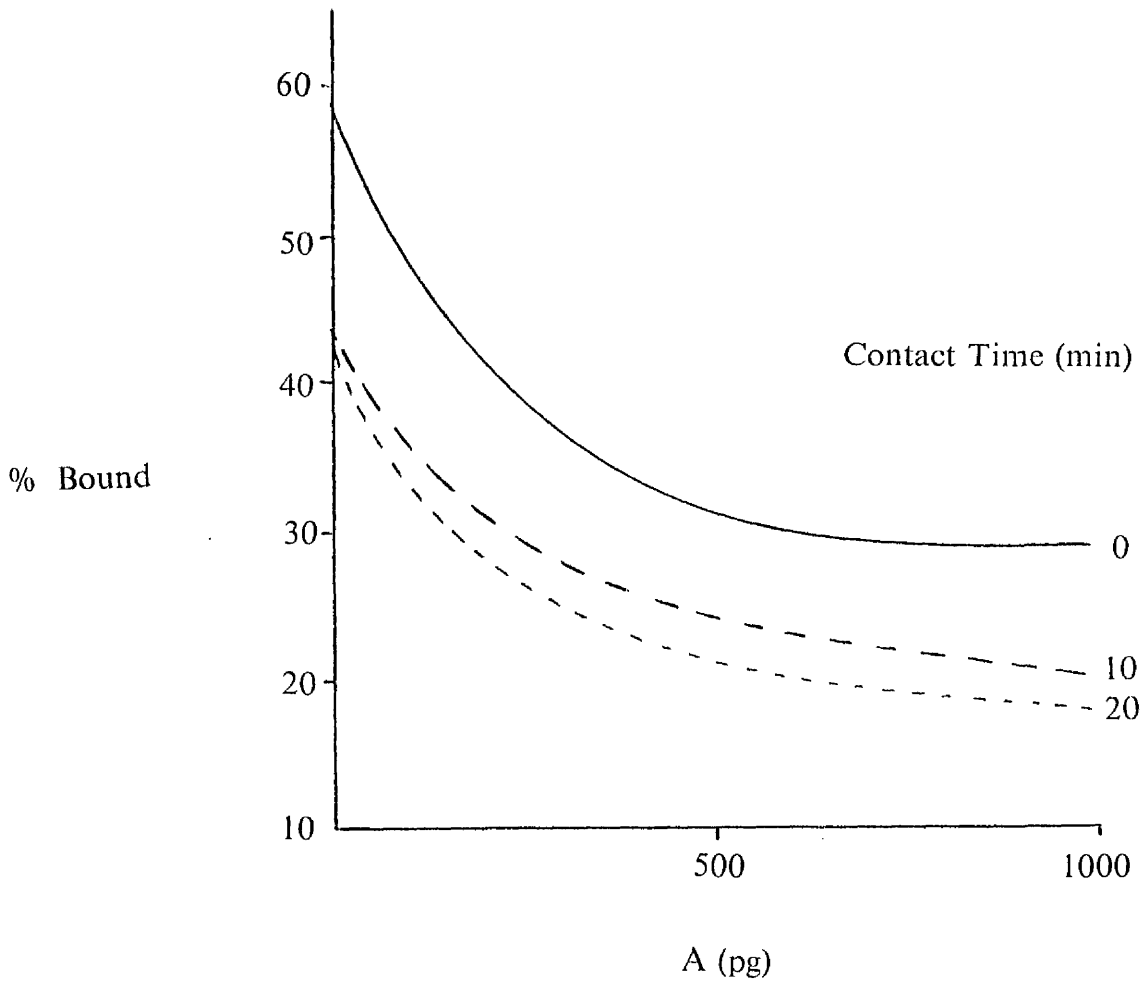


Figure 3.2
Effects of different DCC Contact Times
on DHT standard curve

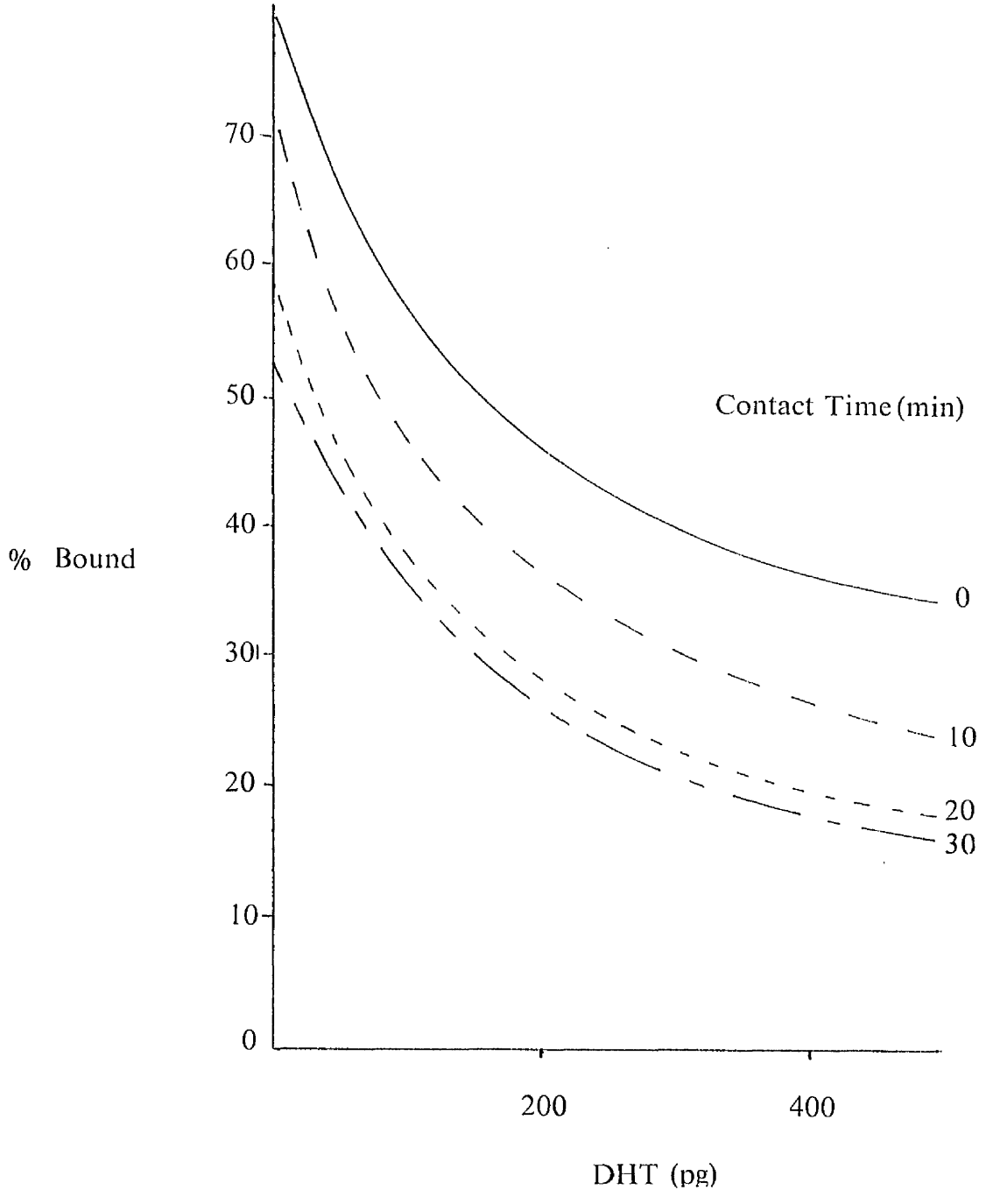


Figure 3.3
Effects of different DCC Contact Times
on T standard curve.

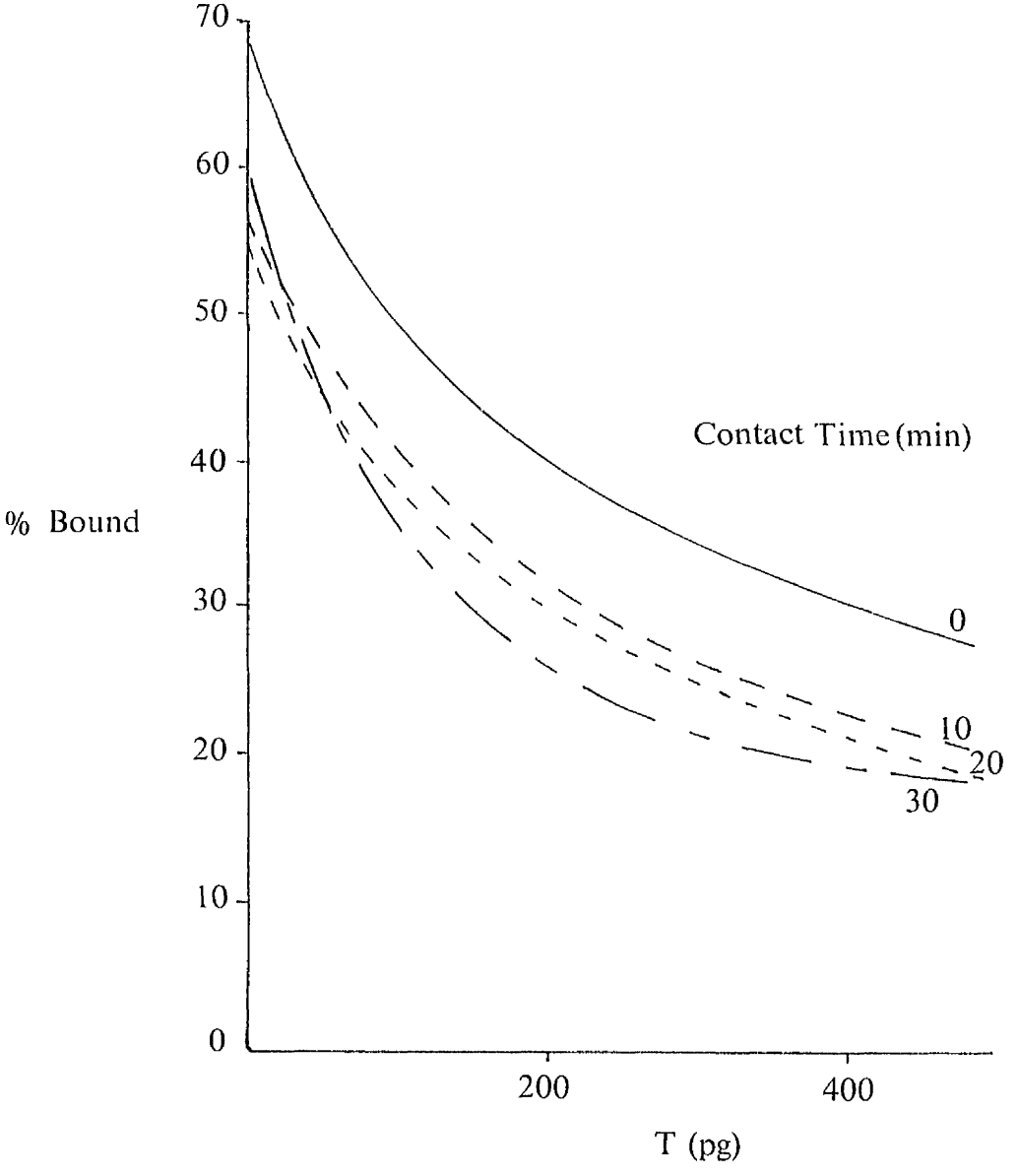


Figure 3.4

Levels of A (mean±SEM) in daily samples throughout the normal menstrual cycle (n=7)

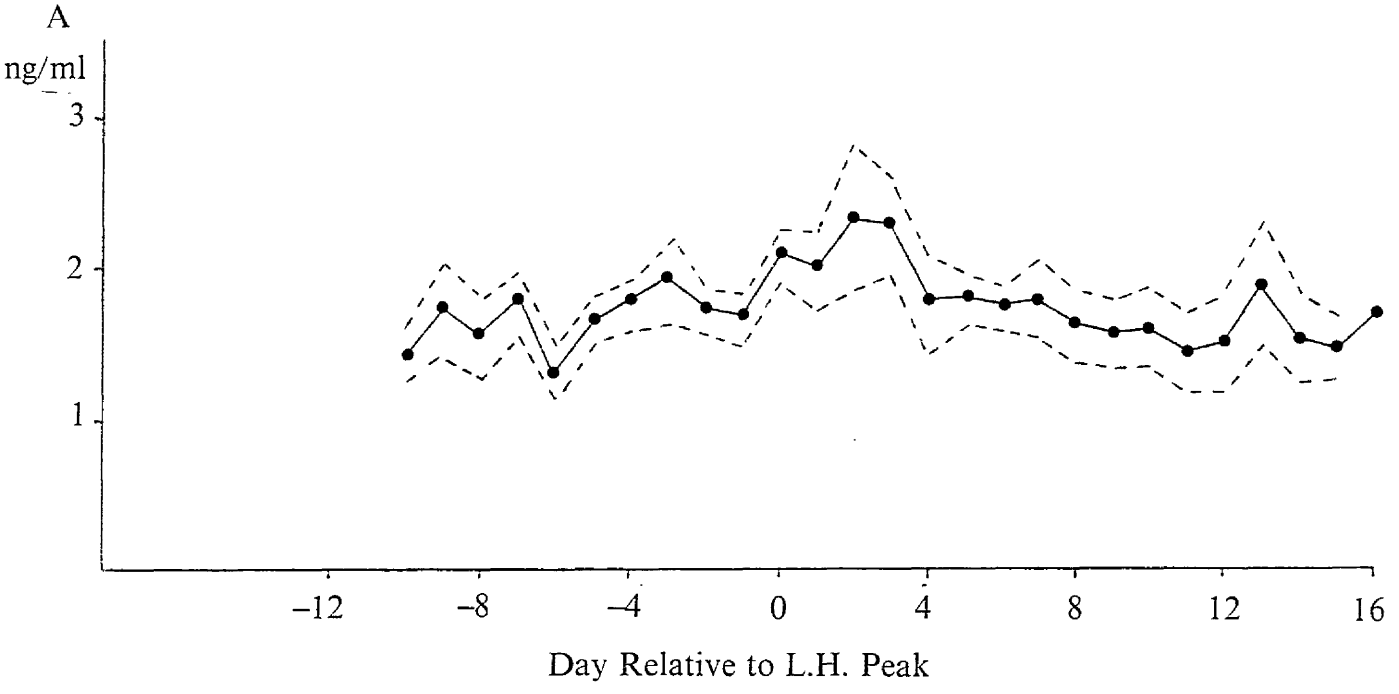


Figure 3.5

Levels of DHT (mean±SEM) in daily plasma samples throughout the normal menstrual cycle (n=7)

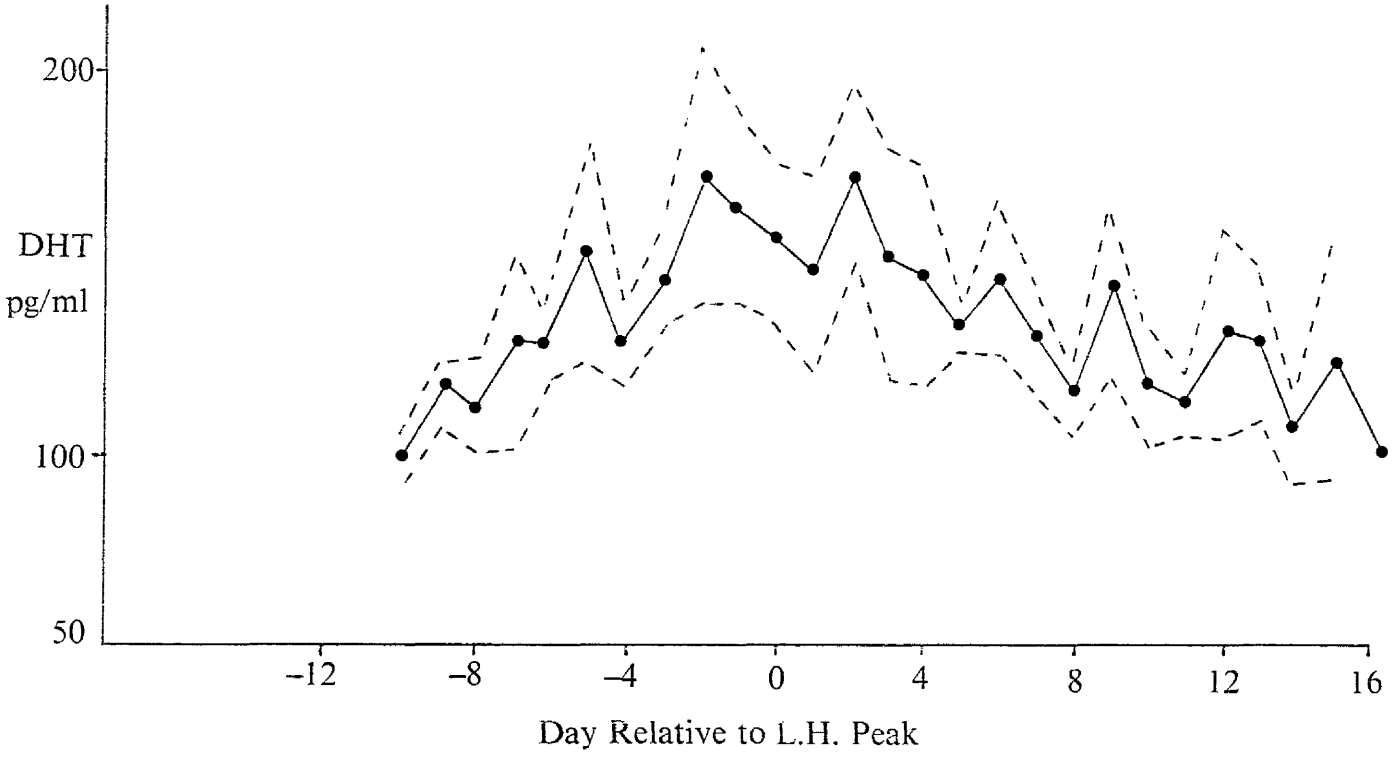


Figure 3.6

Levels of T (mean±SEM) in daily plasma samples throughout the normal menstrual cycle (n=7)

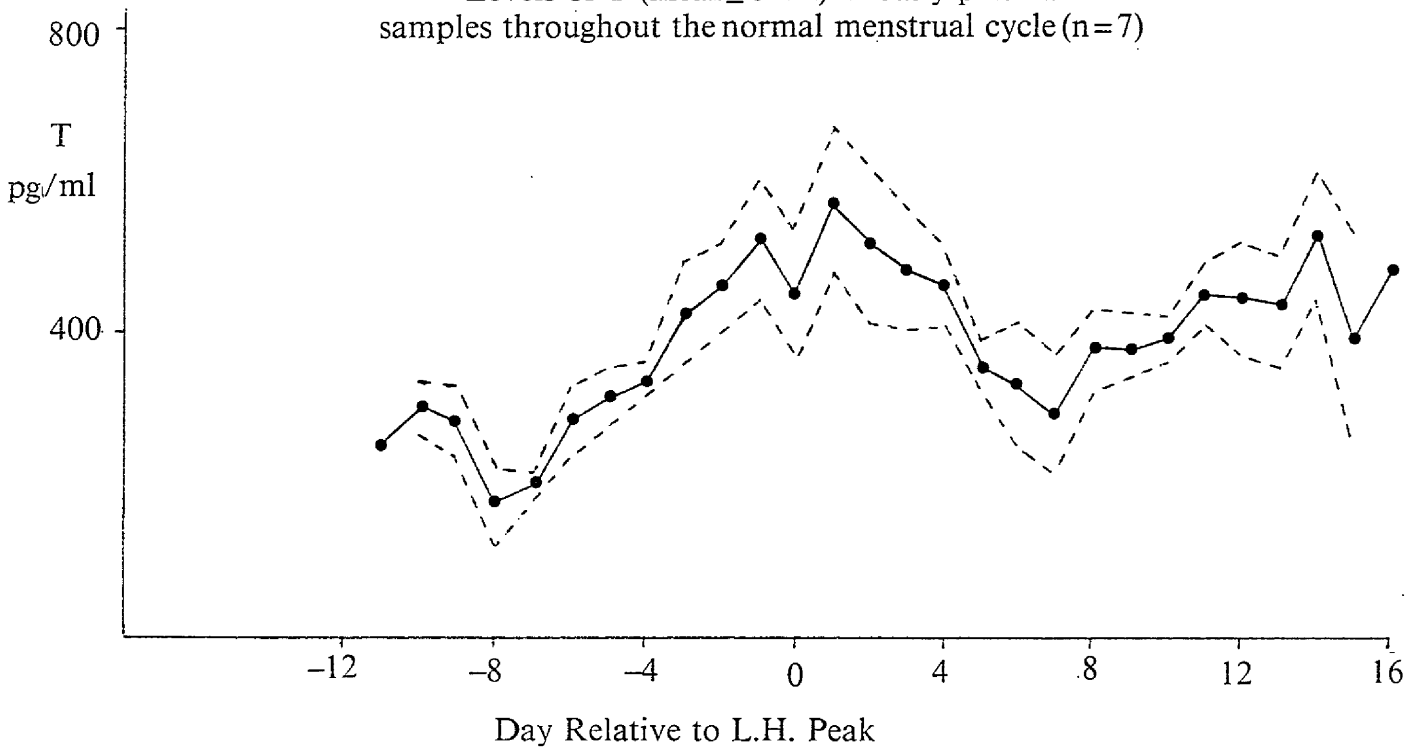


Figure 3.7

Levels of PRL (mean±SEM) in daily plasma samples throughout the normal menstrual cycle (n=7)

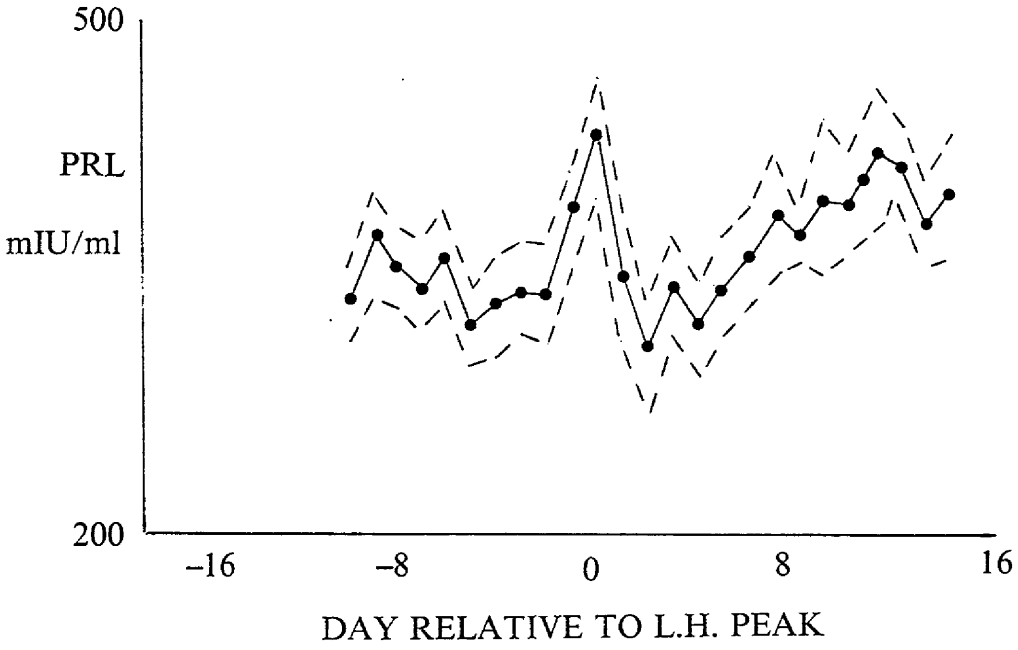


Figure 3.8

Diagrammatic representative of the idealised human menstrual cycle

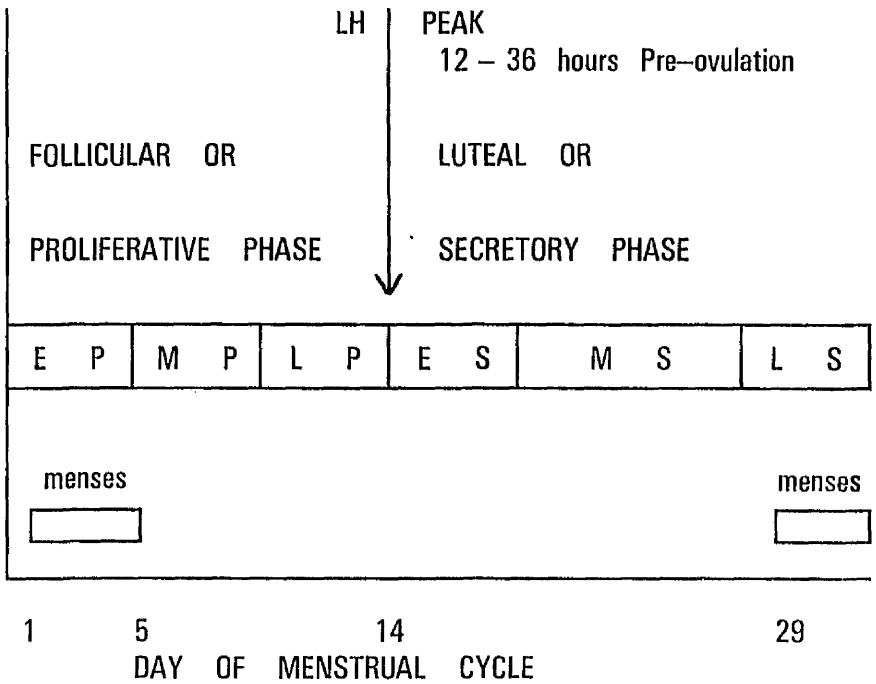
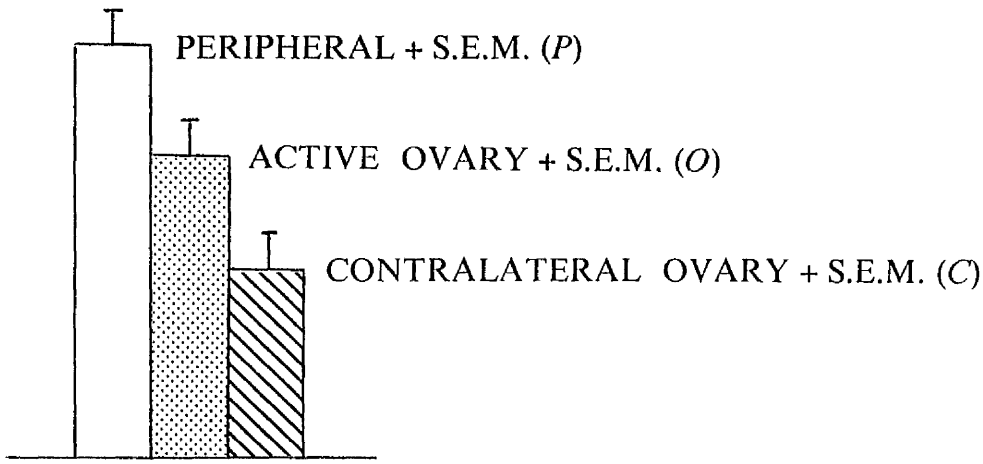


Figure 3.9

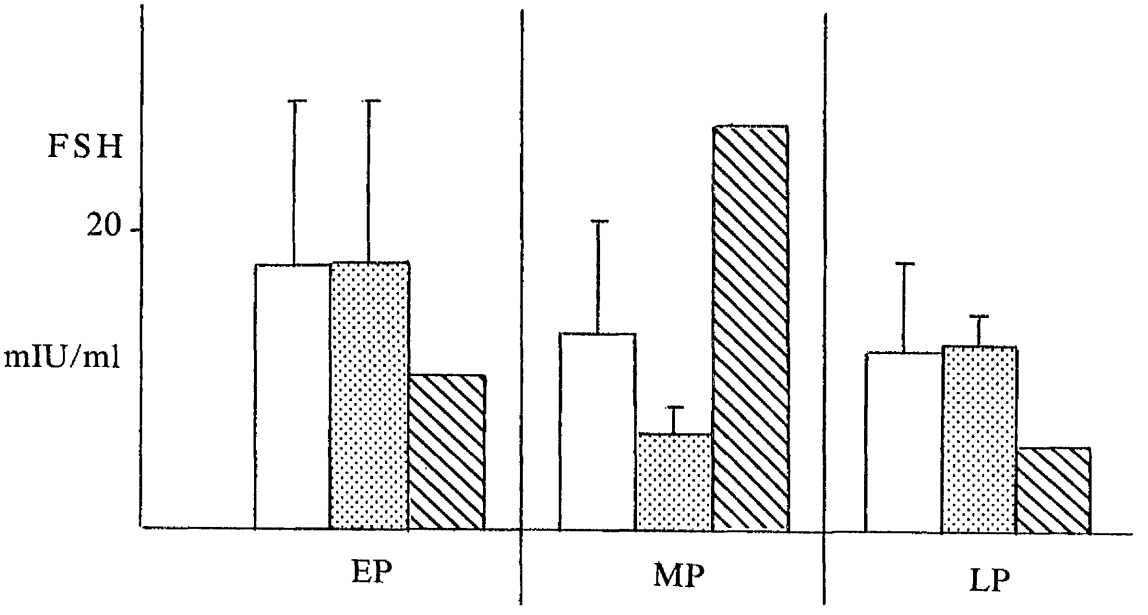
Key used to depict plasmas from peripheral, active ovarian and contralateral ovarian veins and abbreviations to denote the six stages of the menstrual cycle
(The same key and abbreviations are used for the remainder of the Figures in this thesis)



EP – Early
MP – Mid } Proliferative
LP – Late

ES – Early
MS – Mid } Secretory
LS – Late

Figure 3.10
Histograms showing levels (mean + SEM) of FSH in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle



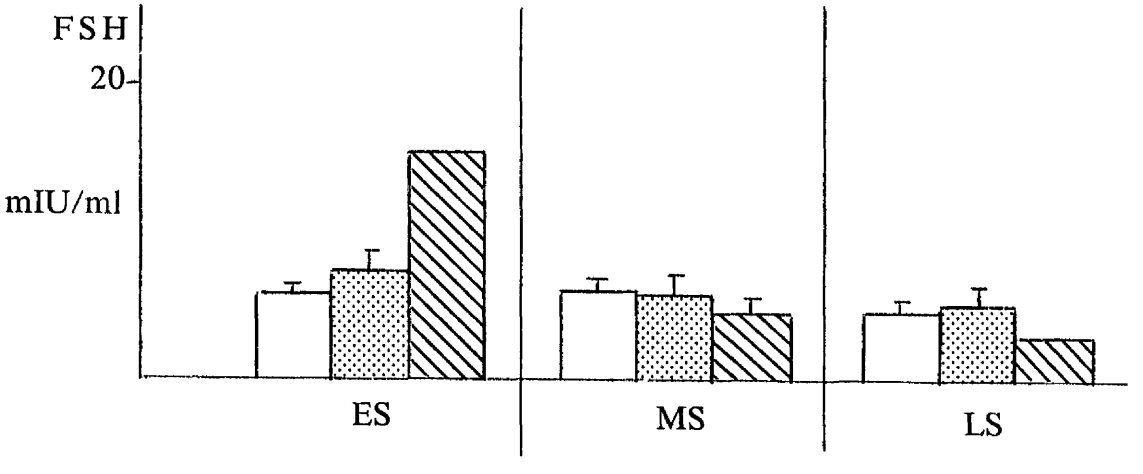


Figure 3.11

Histograms showing levels (mean + SEM) of LH in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

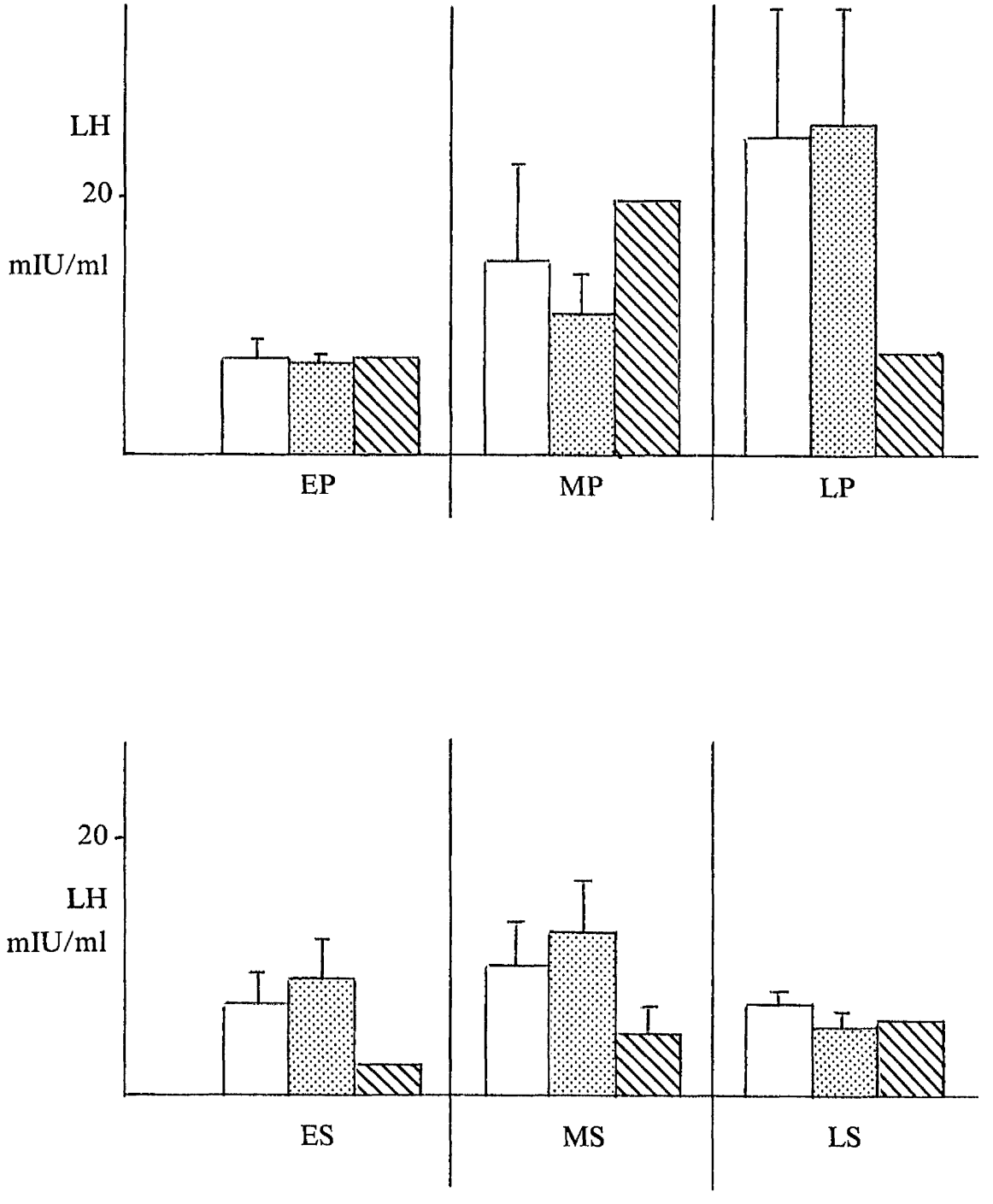


Figure 3.12

Histograms showing levels (mean + SEM) of PRL in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

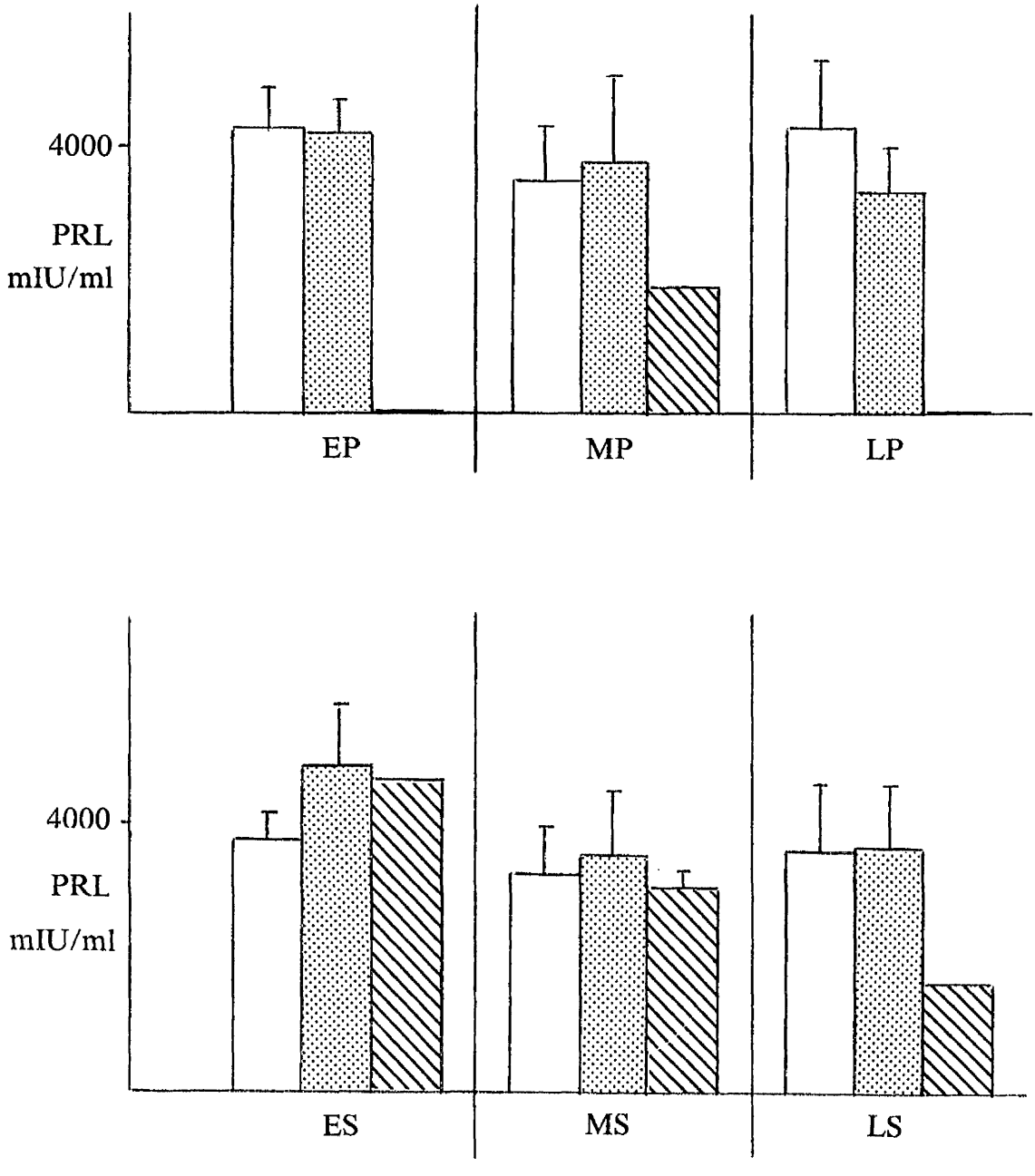


Figure 3.13

Histograms showing levels (mean + SEM) of P in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

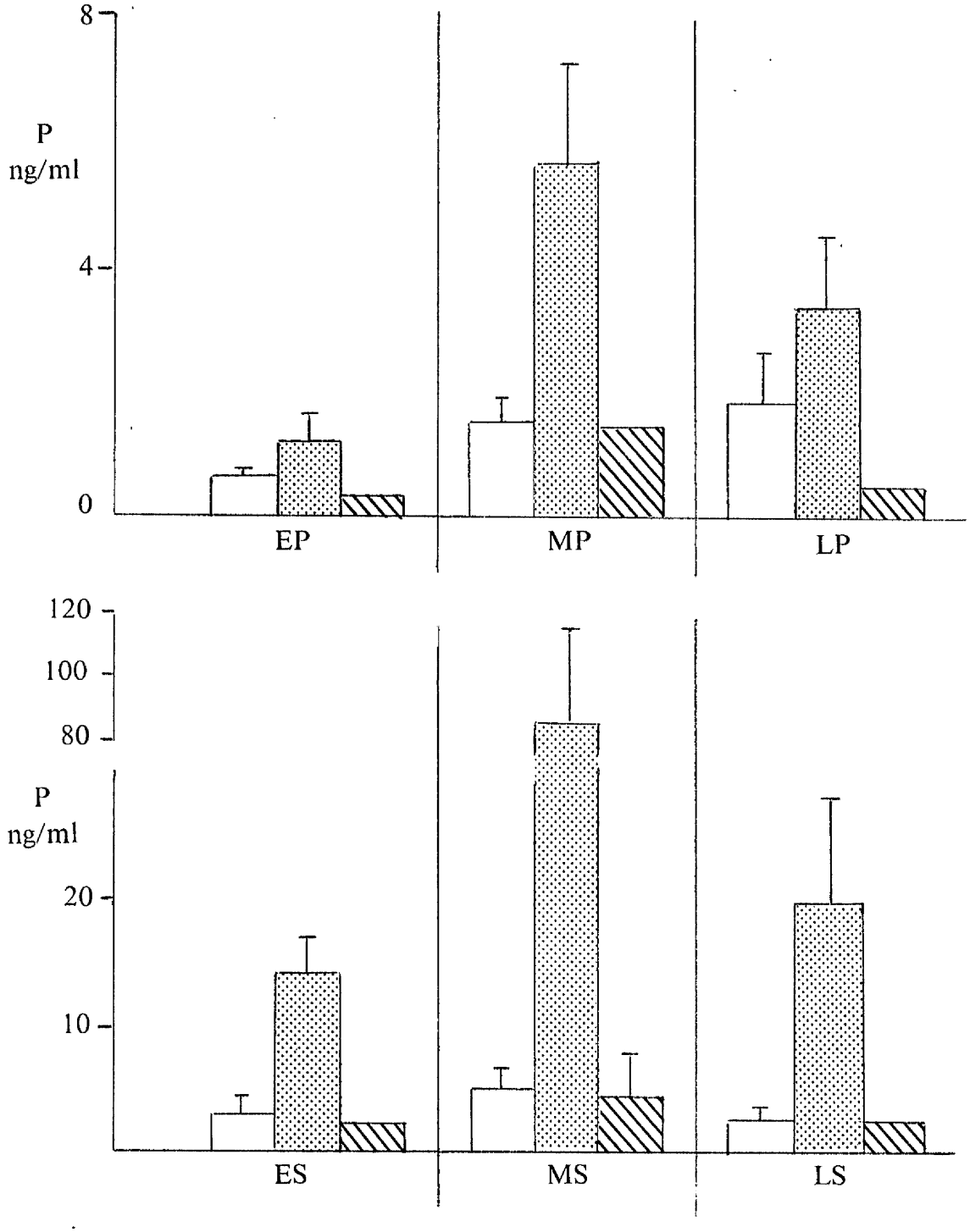


Figure 3.14

Histograms showing levels (mean + SEM) of 17OHP in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

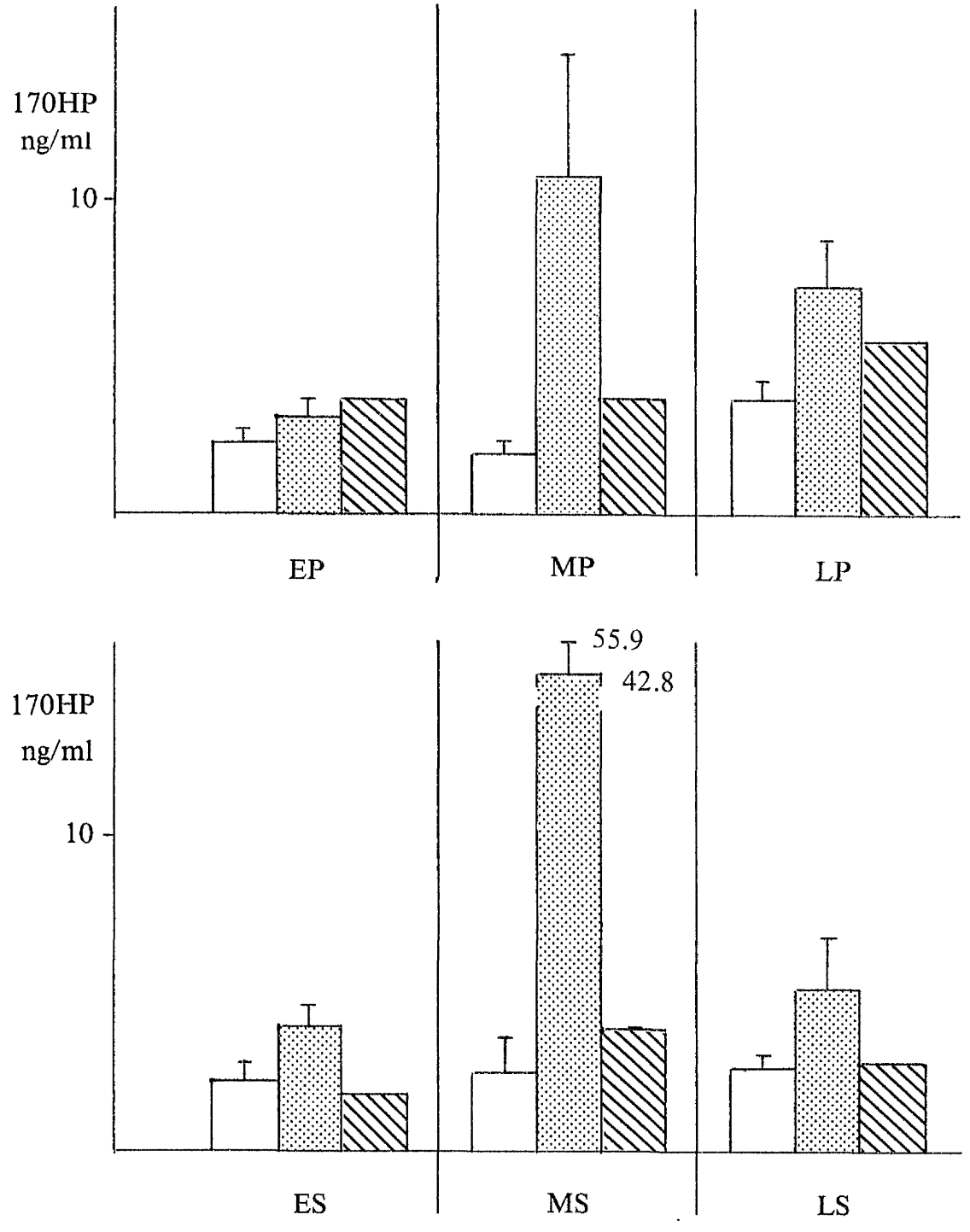
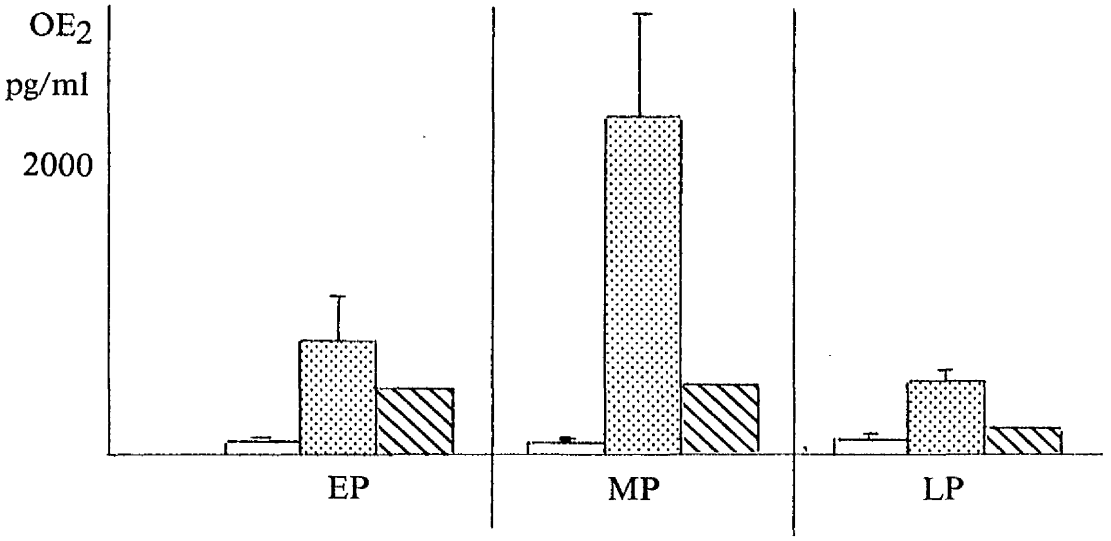


Figure 3.15

Histograms showing levels (mean + SEM) of OE_2 in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle



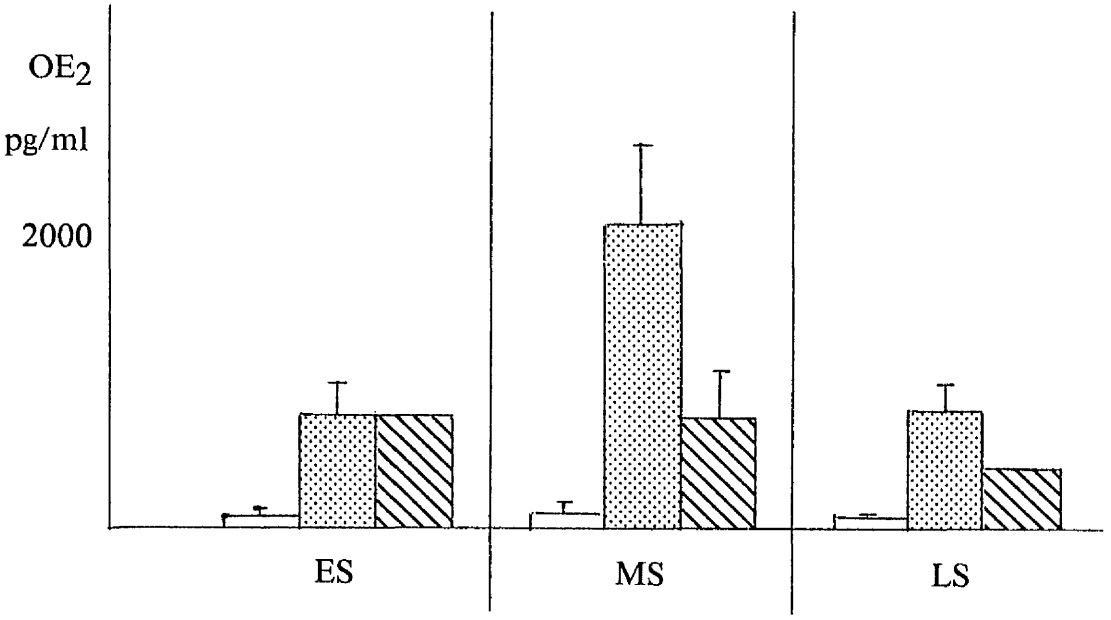


Figure 3.16

Histograms showing levels (mean + SEM) of A in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

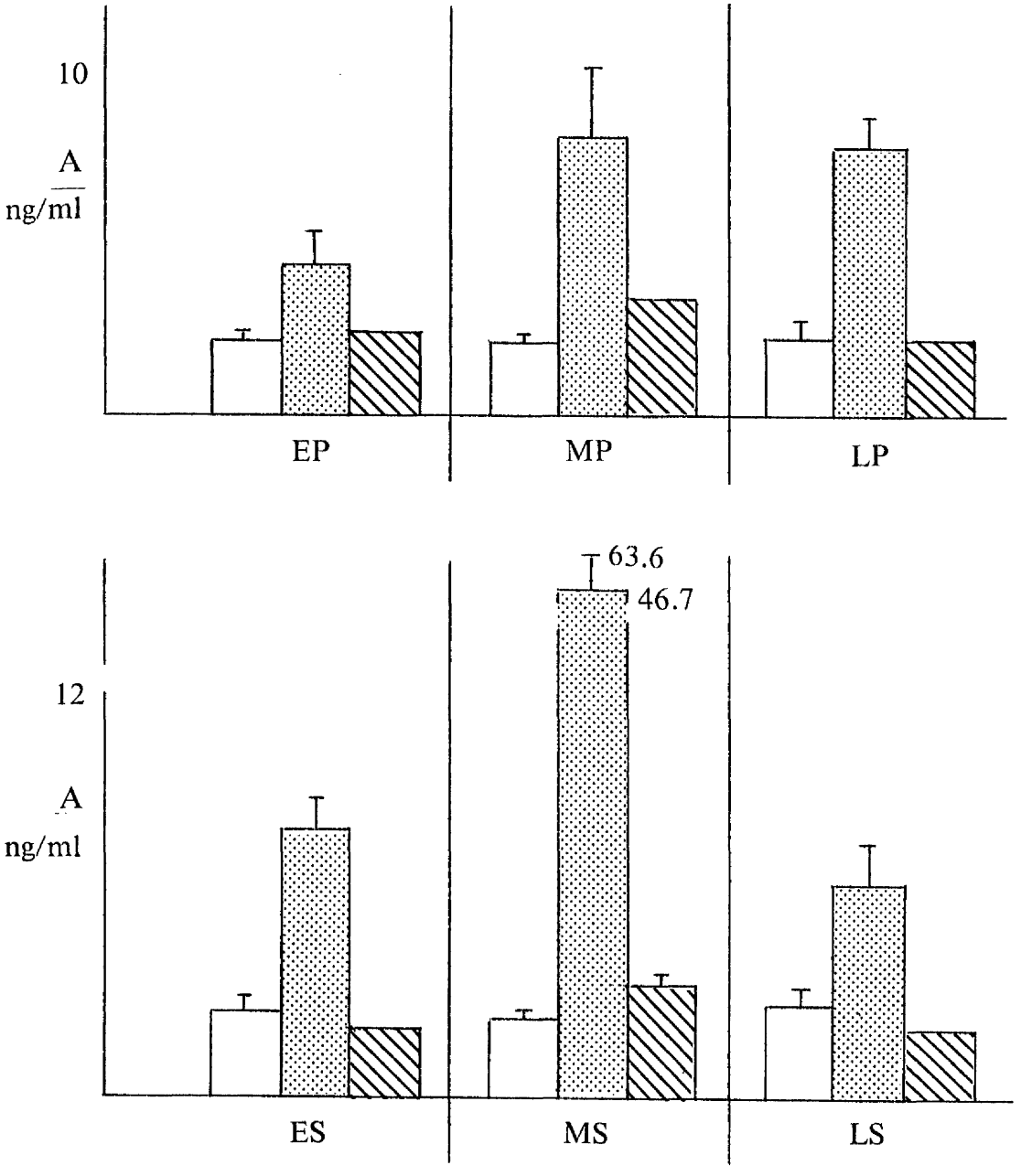


Figure 3.17

Histograms showing levels (mean + SEM) of DHT in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

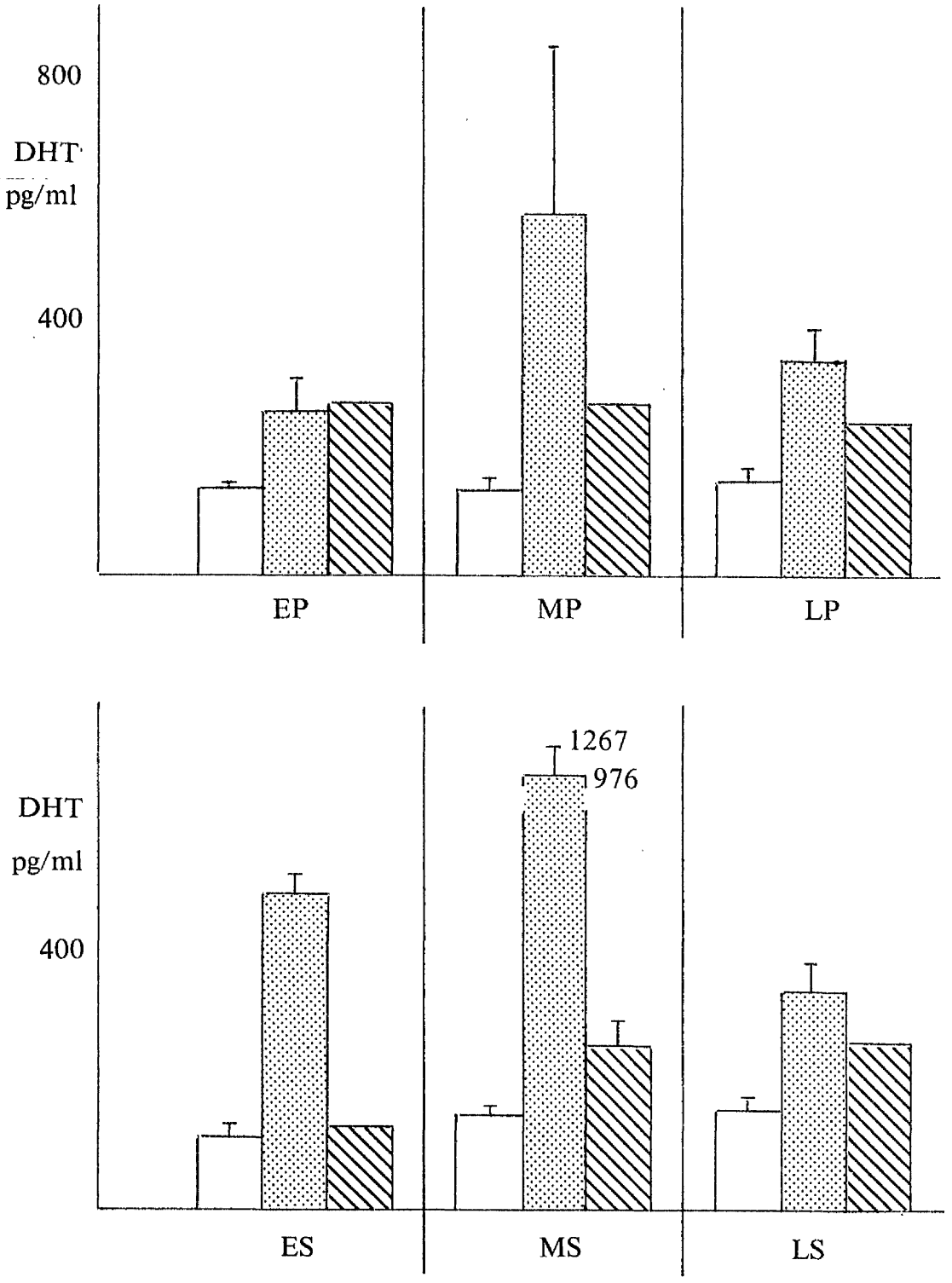


Figure 3.18
Histograms showing levels (mean + SEM) of T in *P*, *O* and *C* venous plasmas throughout the normal menstrual cycle

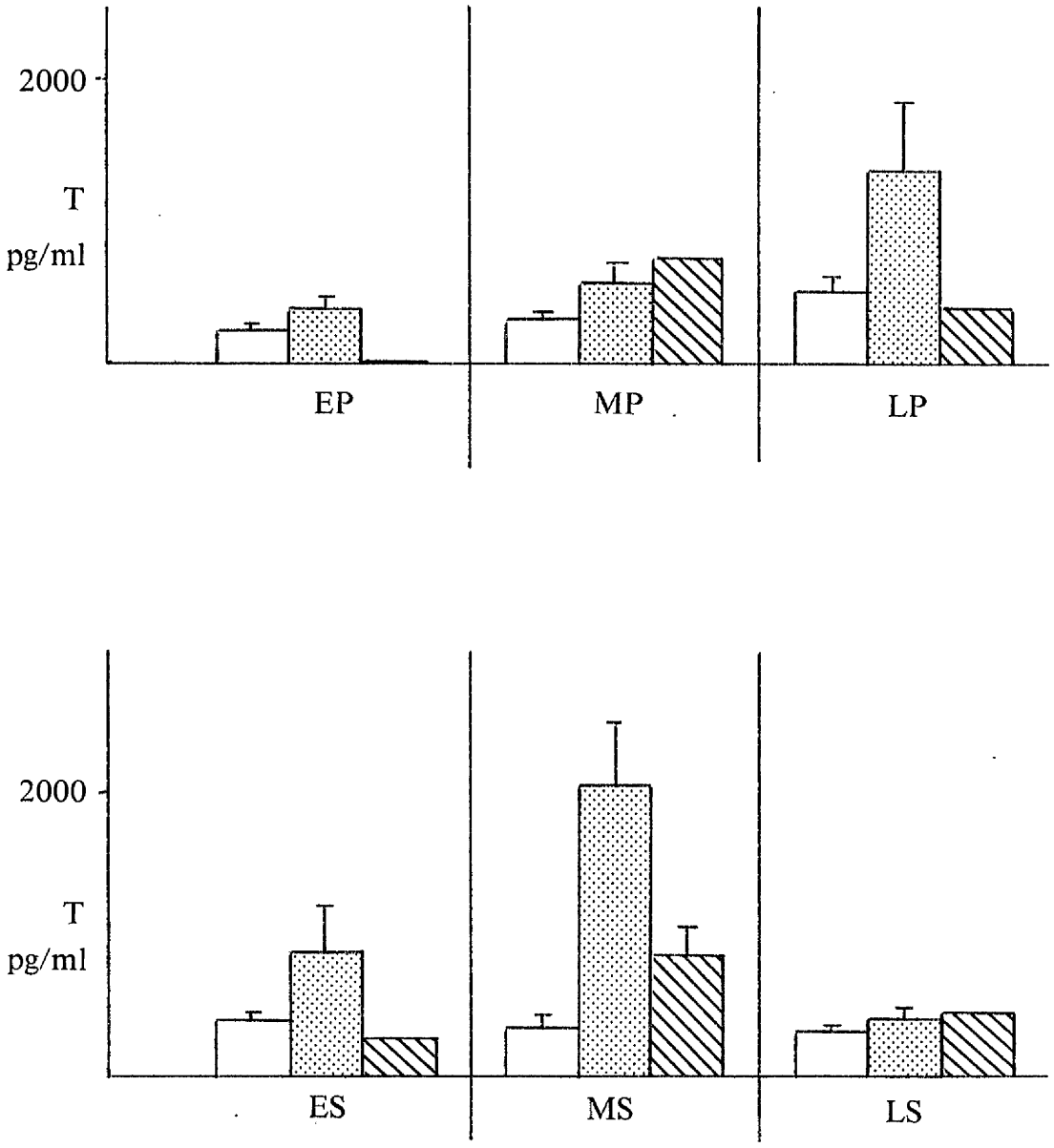


Figure 3.19

Comparision of FSH levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

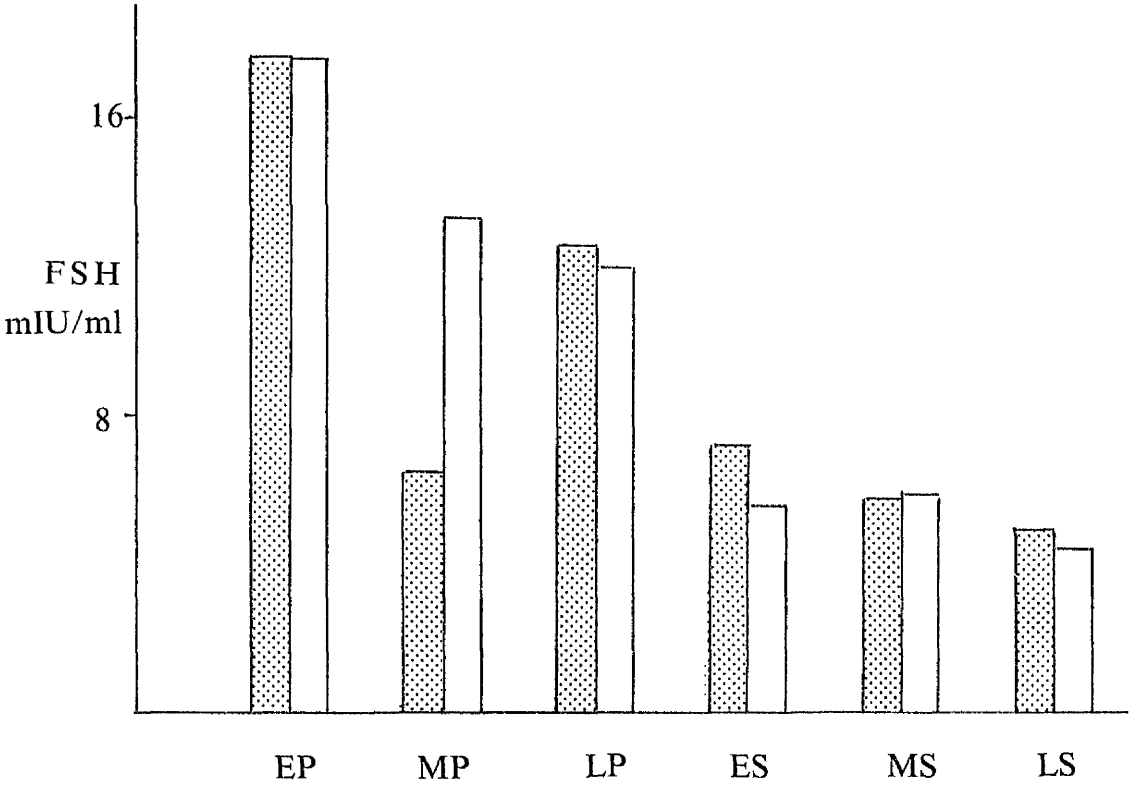


Figure 3.20

Comparision of LH levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

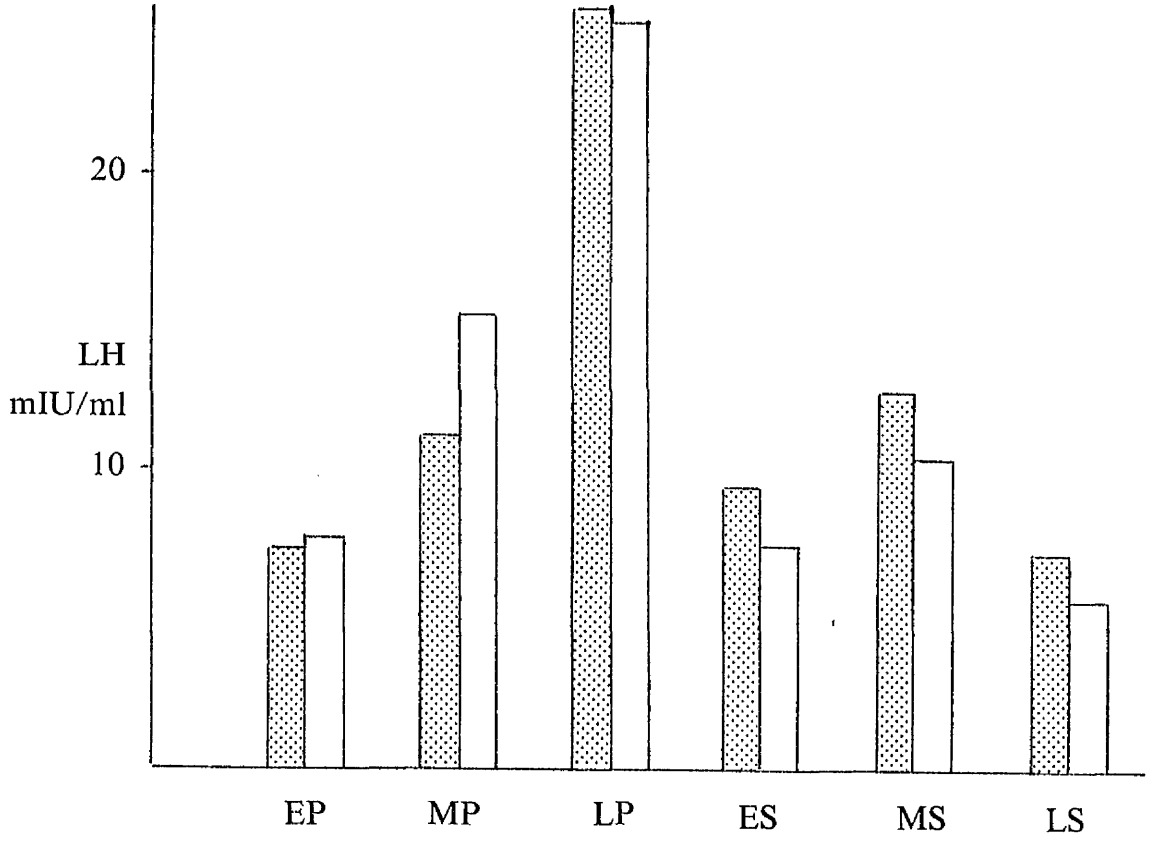


Figure 3.21
Comparision of PRL levels in *P* and *O* venous plasmas
at the six stages of the menstrual cycle

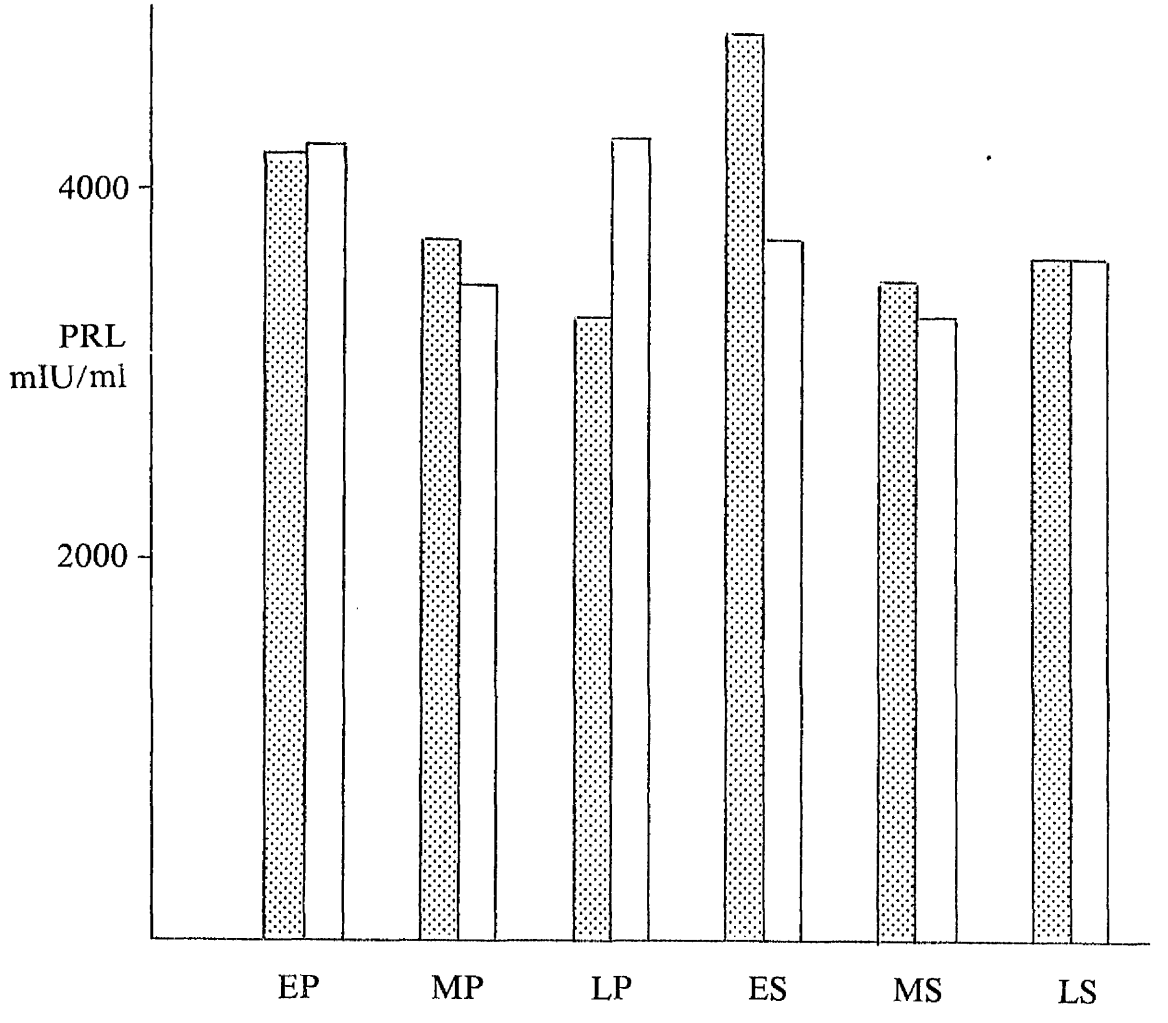


Figure 3.22

Comparision of P levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

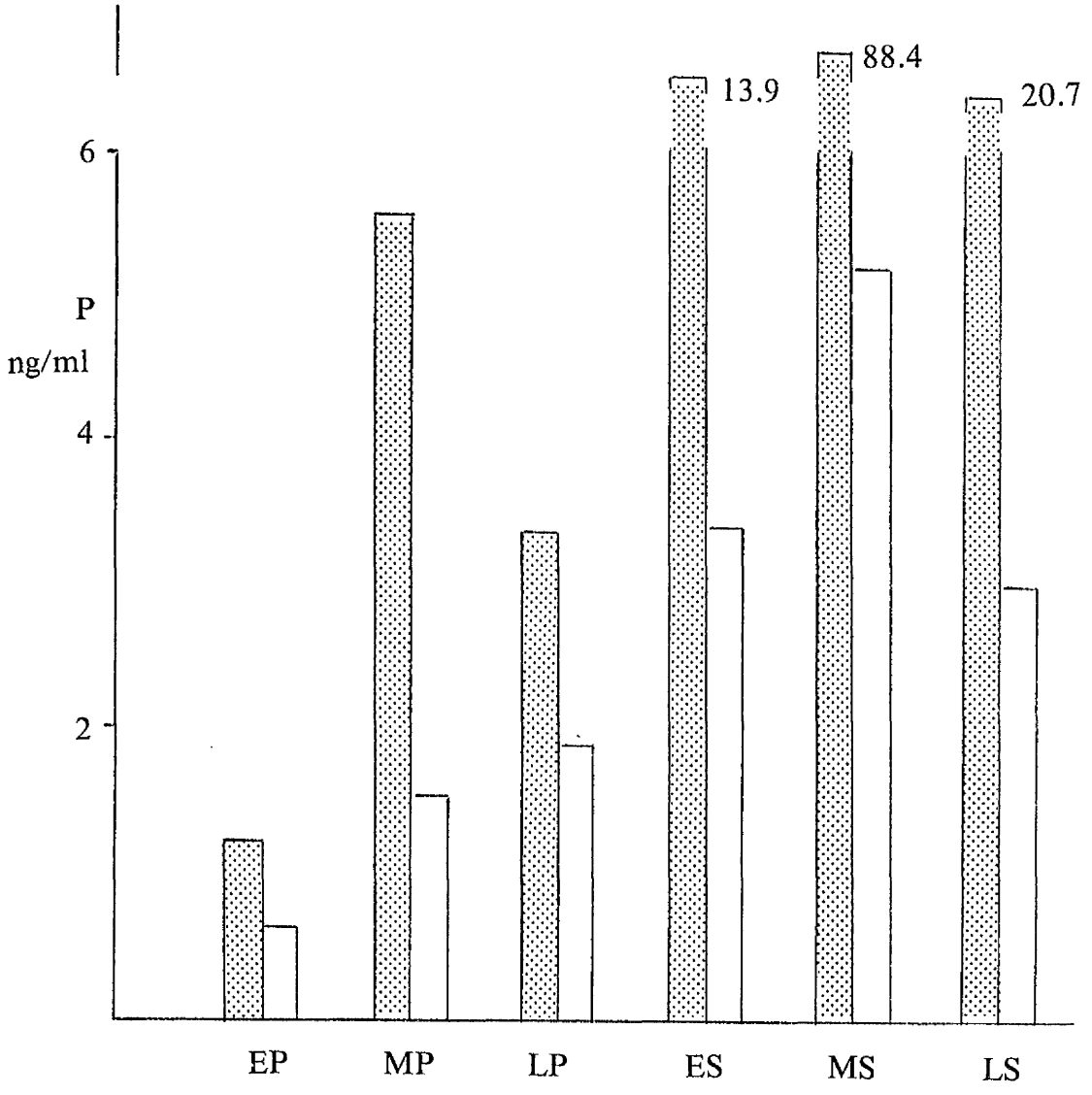


Figure 3.23
Comparision of 17OHP levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

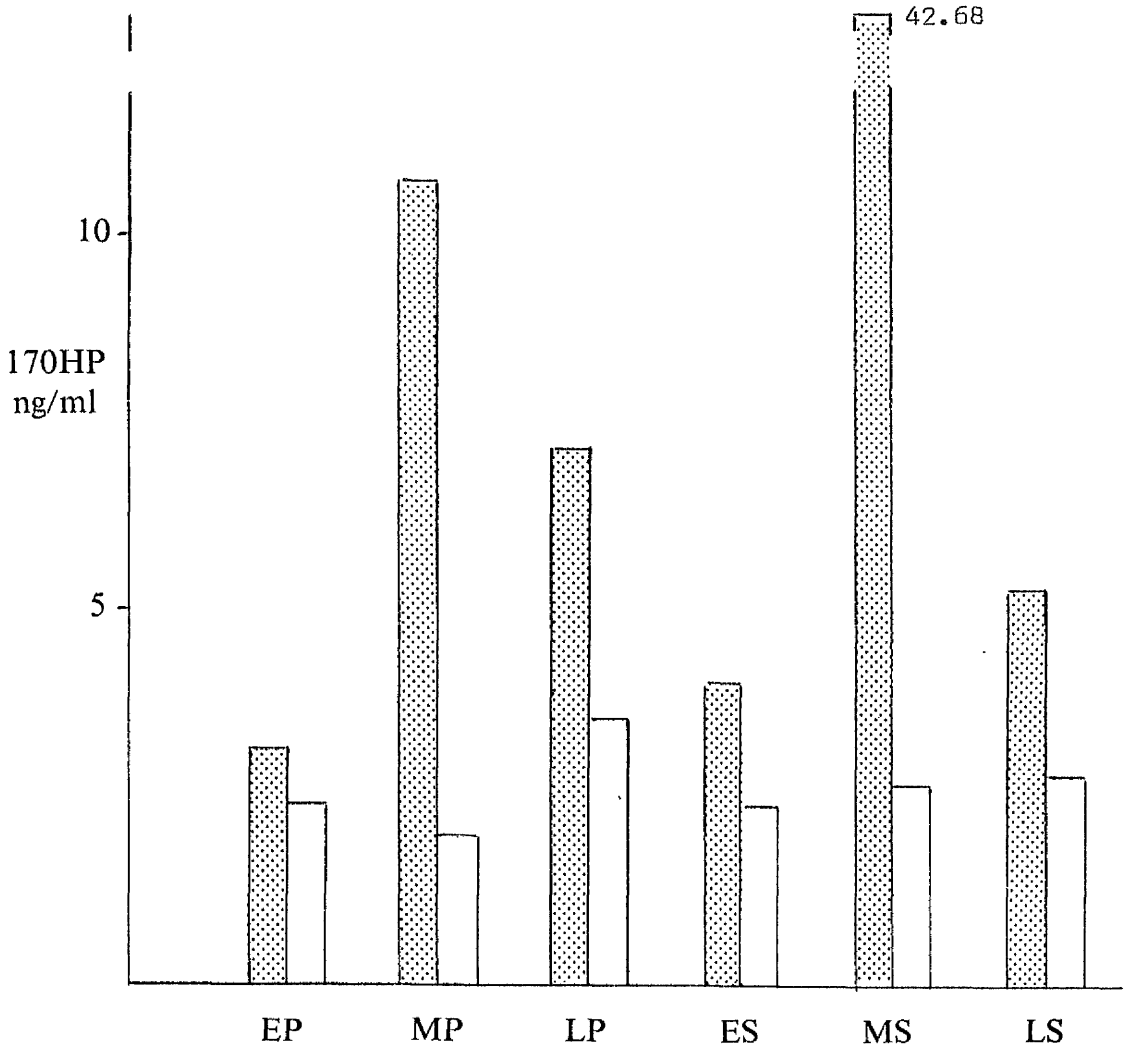


Figure 3.24
Comparision of OE_2 levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

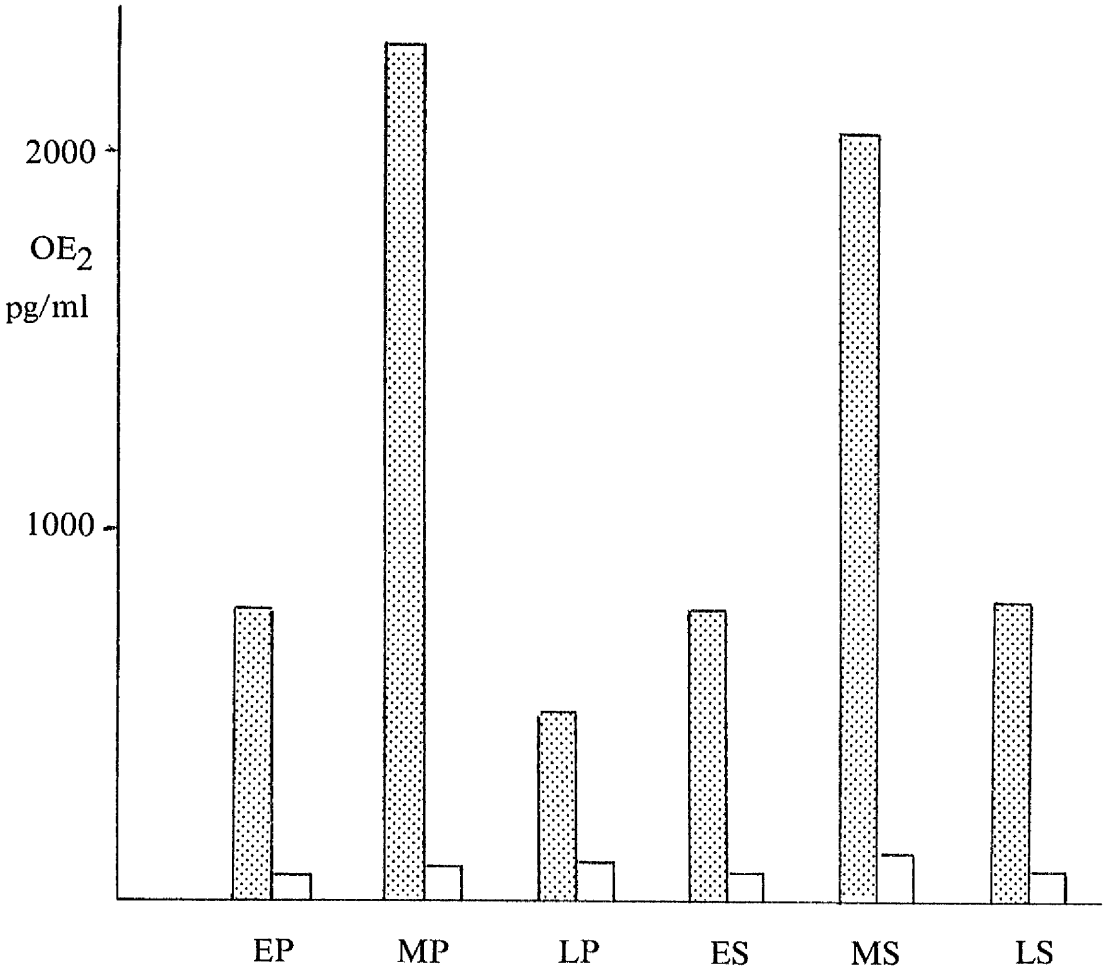


Figure 3.25
Comparision of A levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

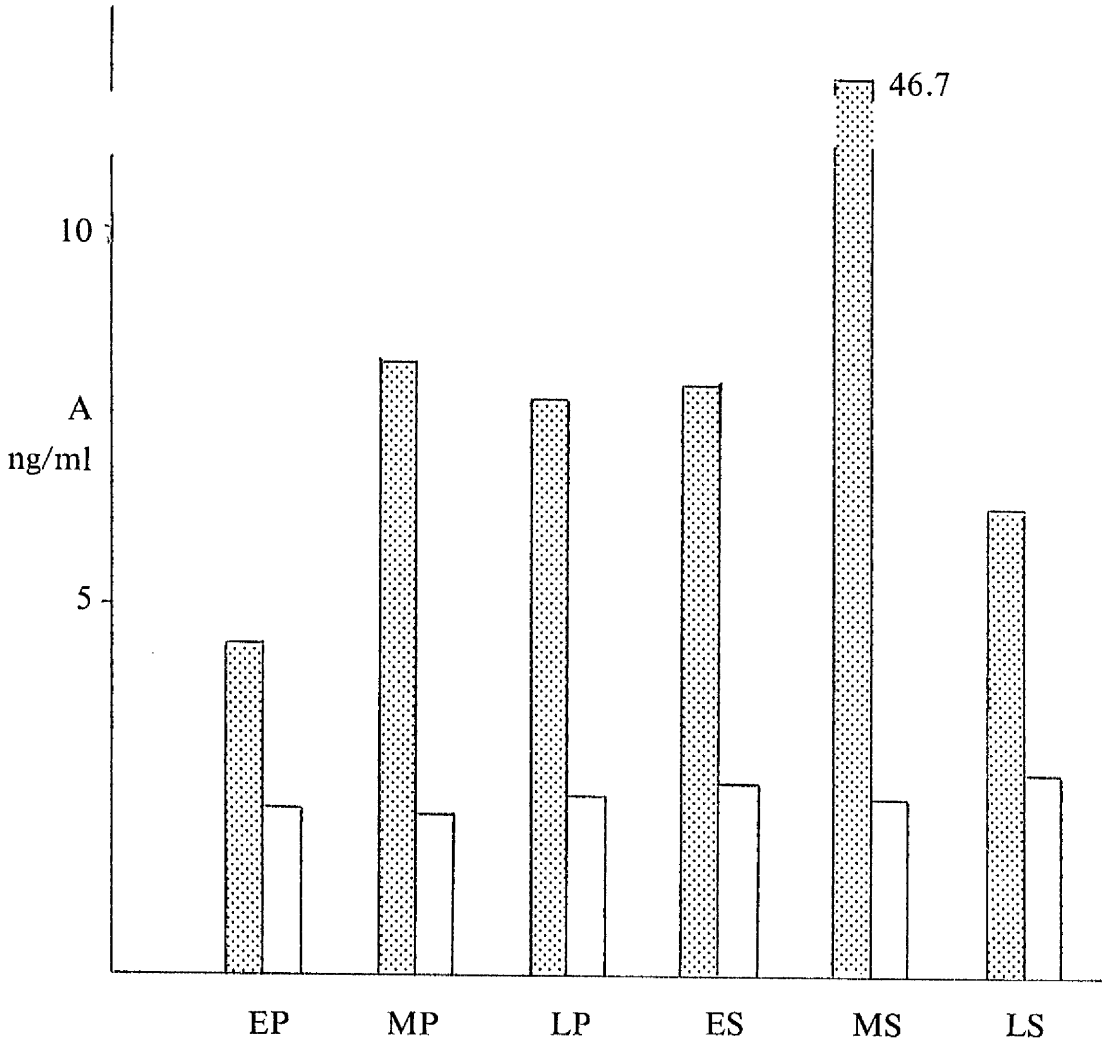


Figure 3.26
Comparision of DHT levels in *P* and *O* venous plasmas at the six stages of the menstrual cycle

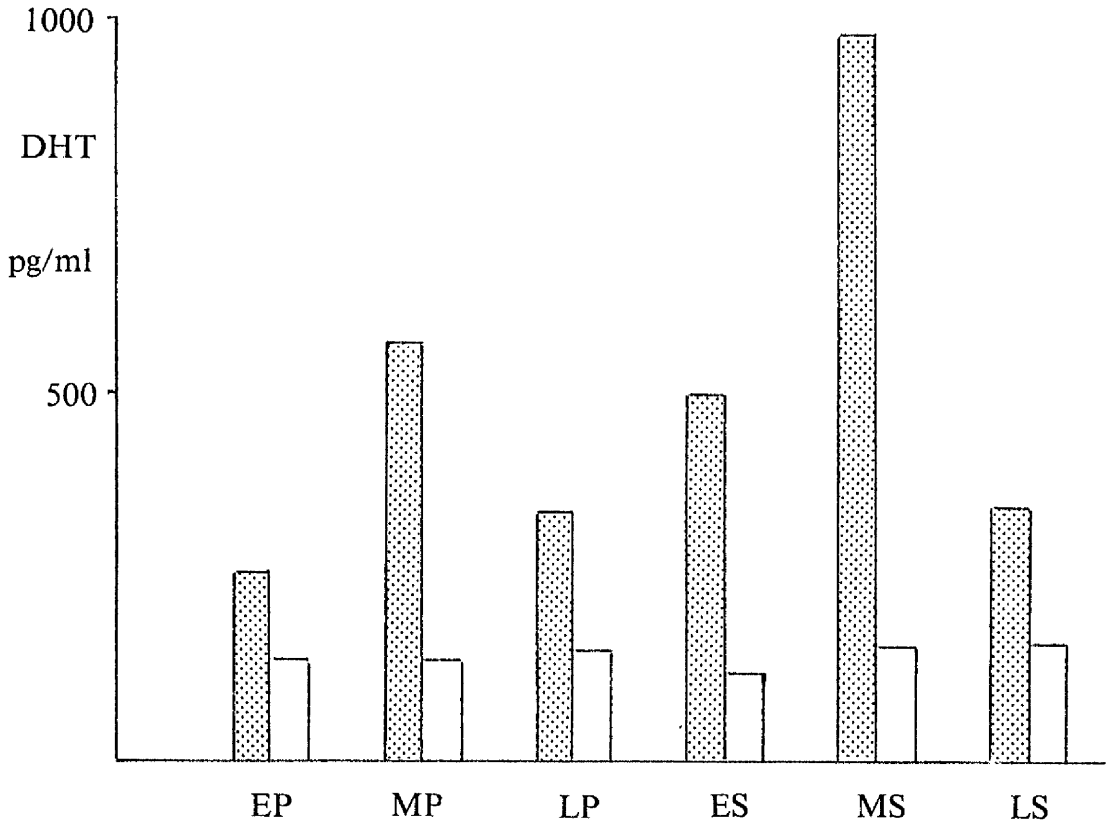


Figure 3.27
Comparision of T levels in *P* and *O* venous plasmas
at the six stages of the menstrual cycle

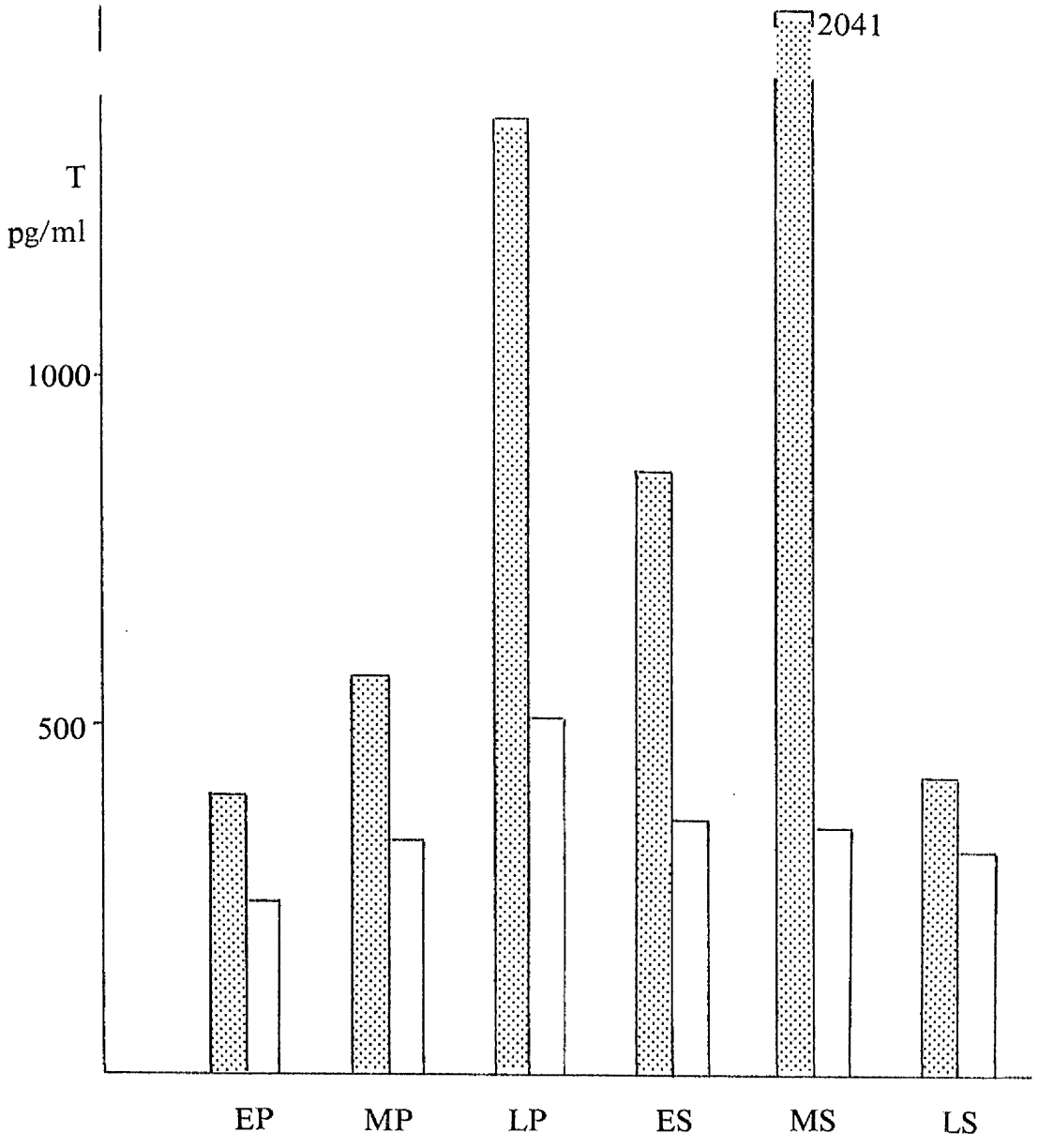


Figure 3.28

Levels of hormones in peripheral plasma and fluid aspirated from a corpus luteum in a patient in the LS stage of the cycle. (L=Luteal fluid)

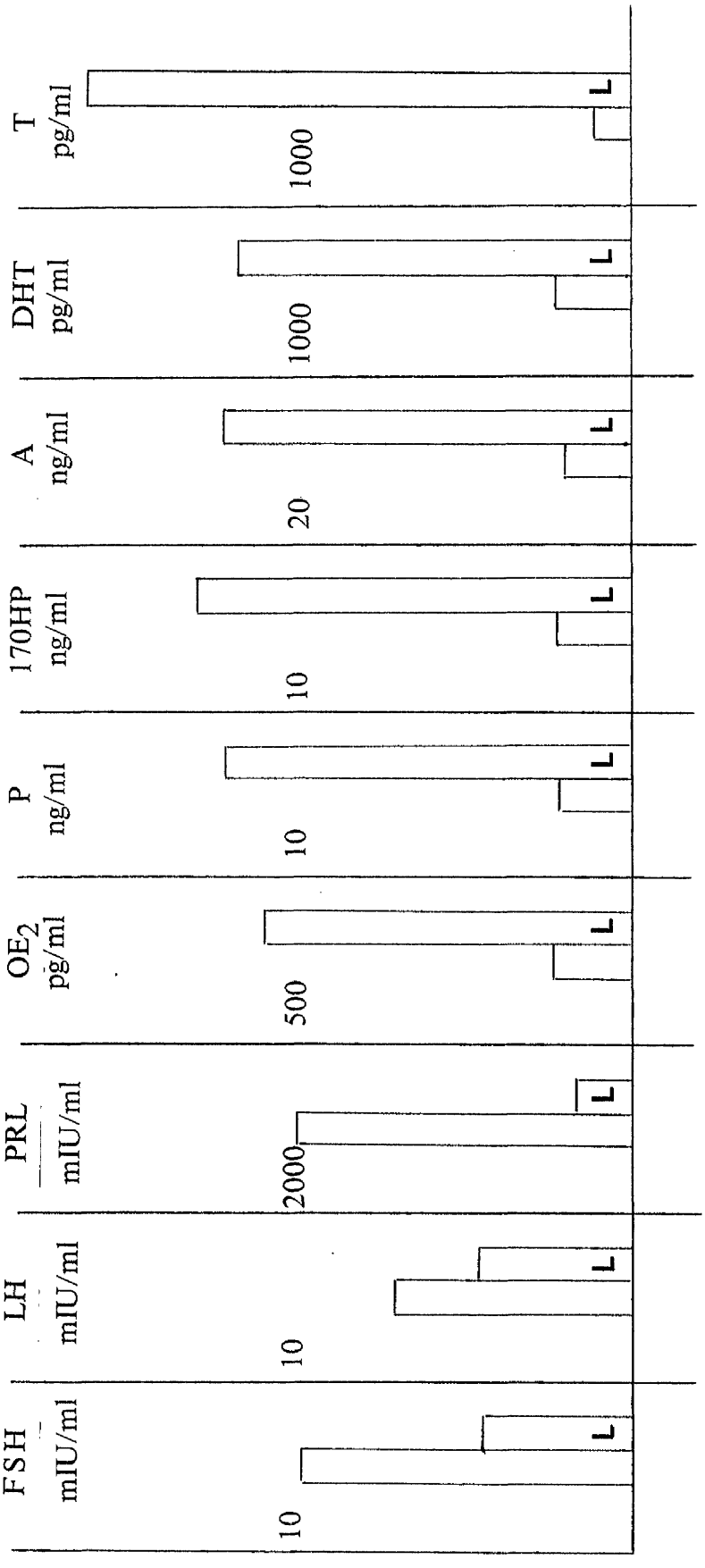


Figure 3.29

Levels of hormones in *P*, *O* and *C* venous plasmas in a patient in the MS phase of the cycle who had micropolycystic ovaries

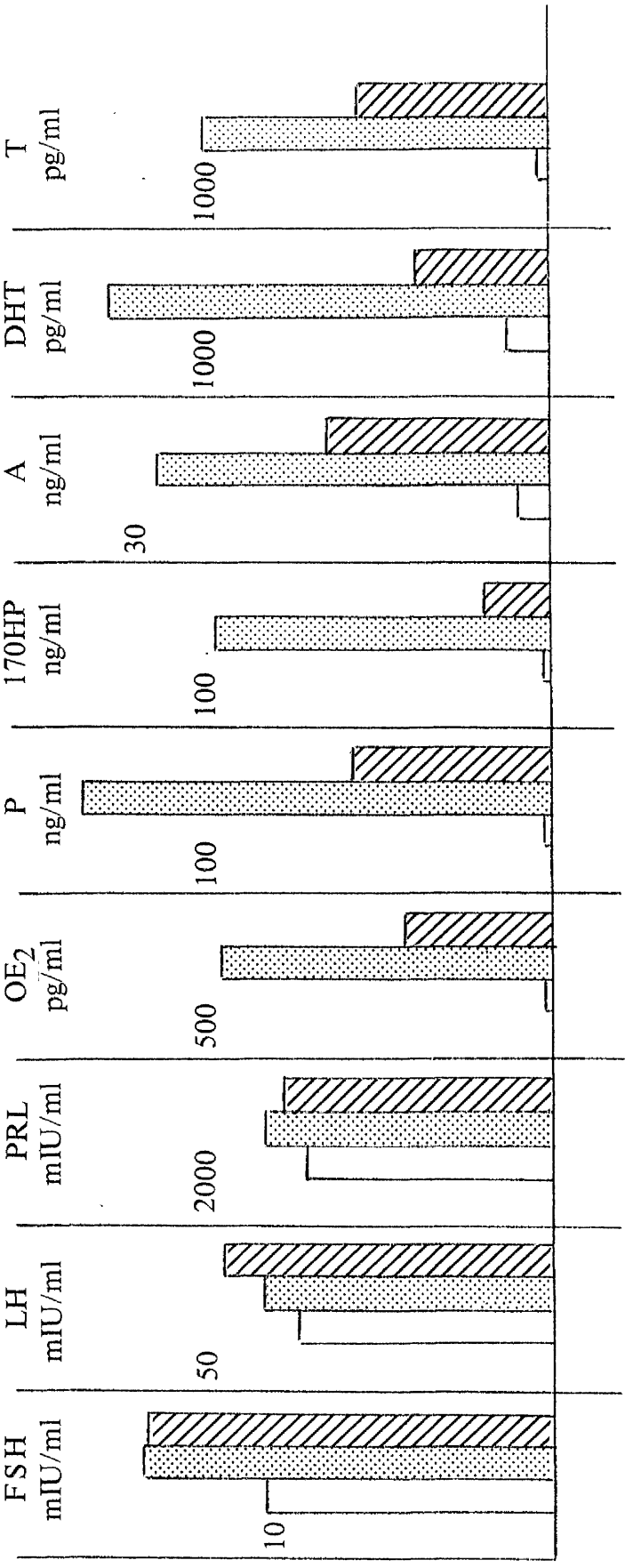


Figure 3.30

Levels of hormones in *P*, *O* and *C* venous plasmas in a patient in the LS phase of the cycle who had micropolycystic ovaries

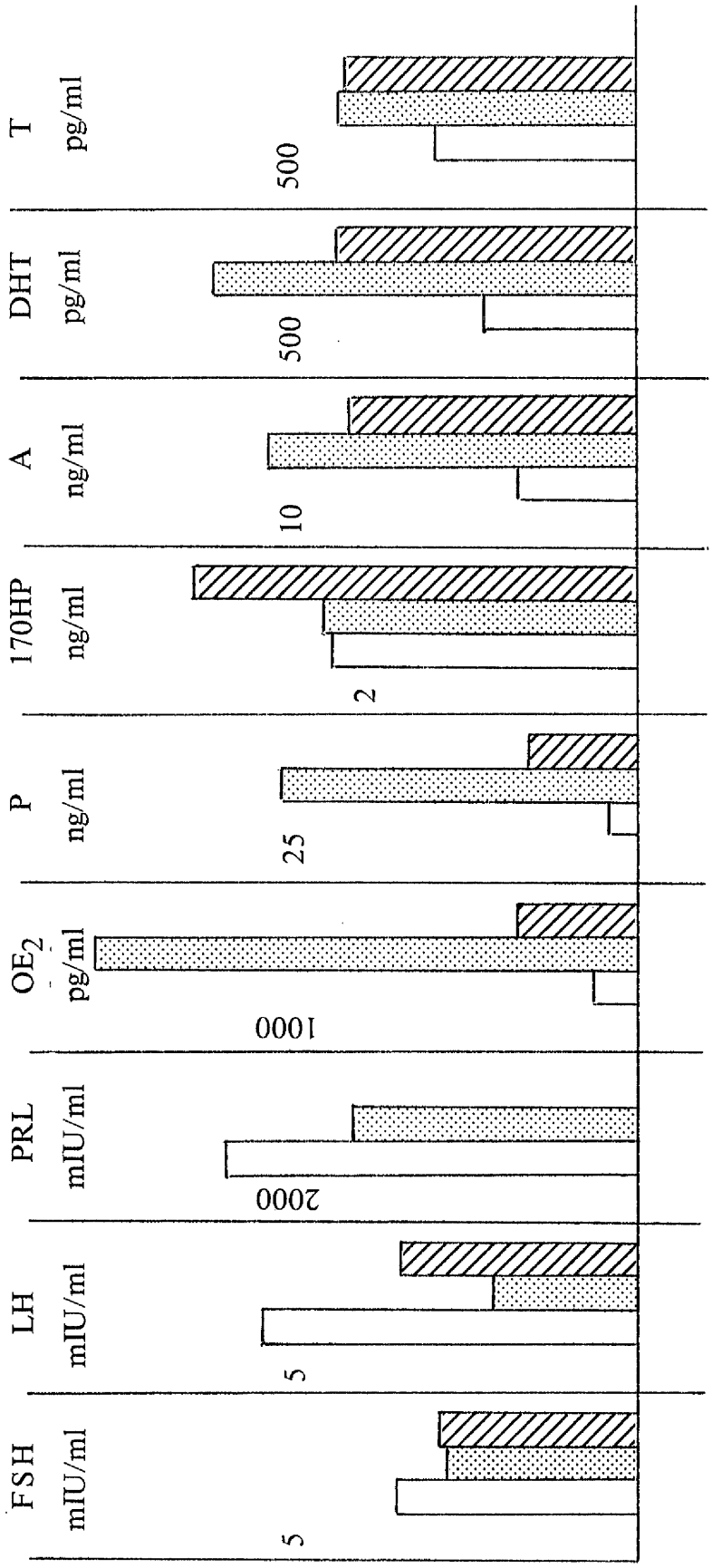


Figure 3.31

Levels of hormones in *P* and *O* venous plasmas and in tumour fluid (T) obtained from an infertile, hirsute woman

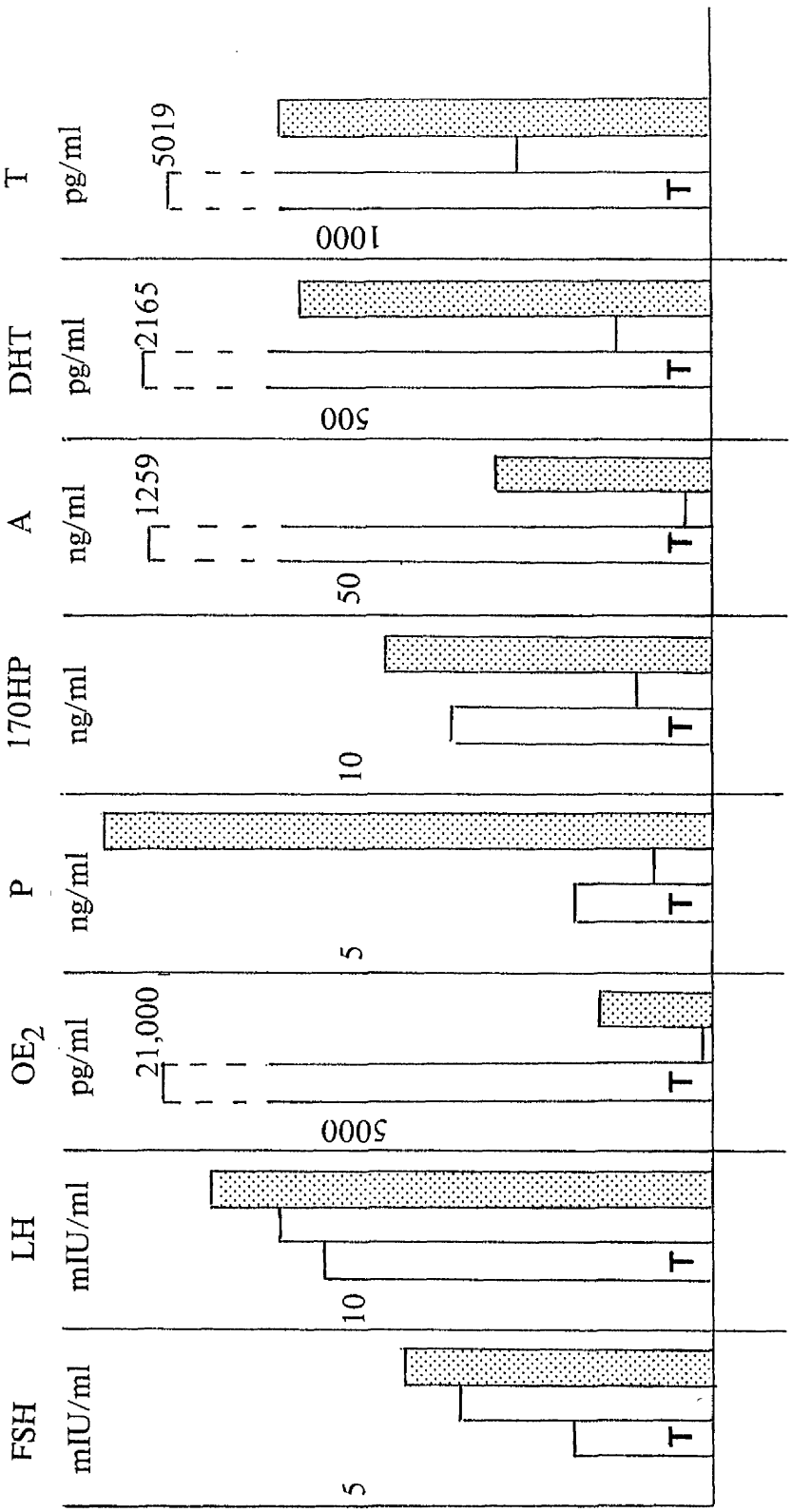


Figure 3.32

Levels of hormones in *P* and *O* venous plasmas in patient A.C. who was on oral contraceptives (samples collected on day 5 of the cycle, last 'pill' in previous cycle)

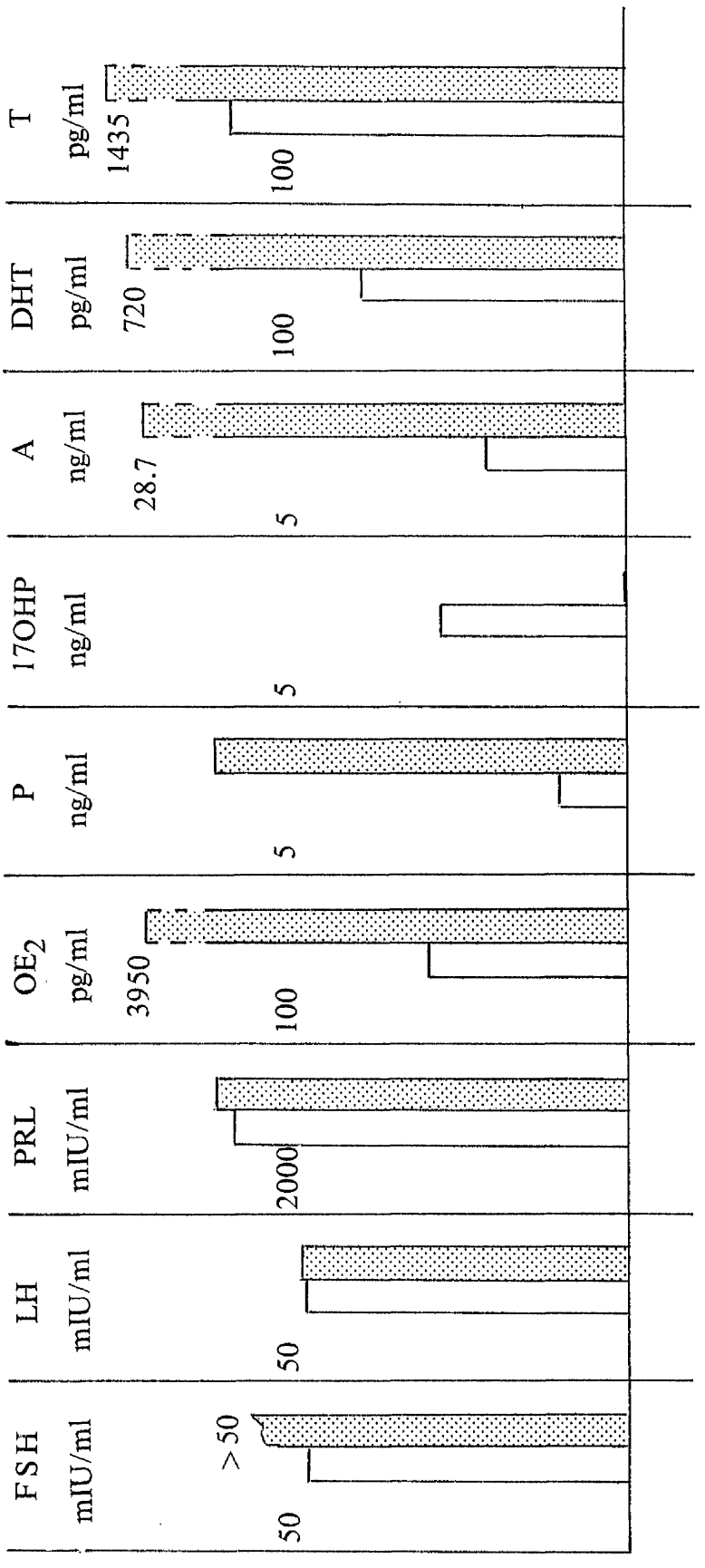


Figure 3.33

Levels of hormones in *P*, *O* and *C* venous plasmas in patient A.W. who was oral contraceptives. (samples collected on day 14, last 'pill' taken on day 10)

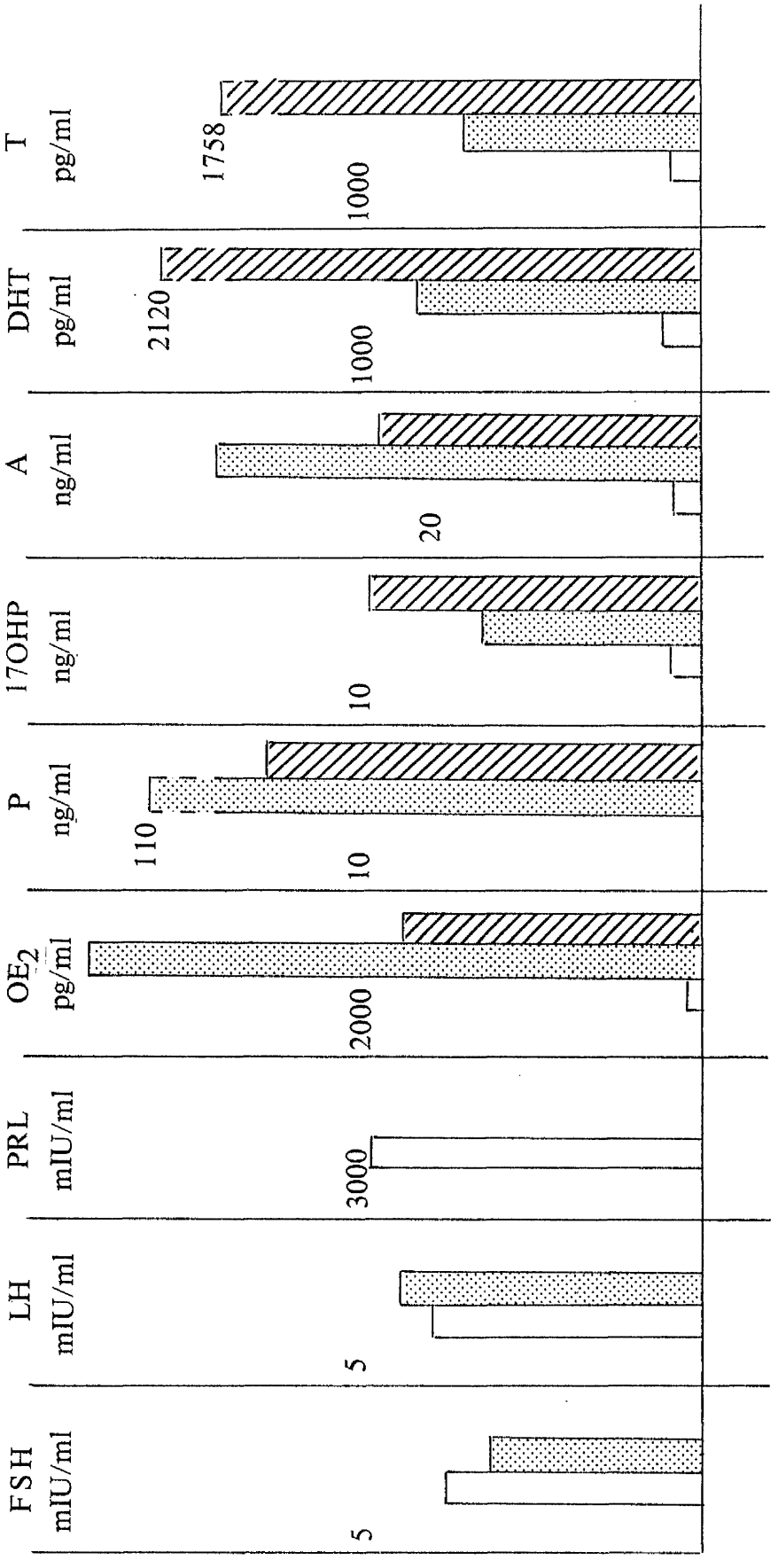


Figure 3.34

Levels of hormones in *P*, *O* and *C* venous plasmas in patient C.E. who was on oral contraceptives (samples collected on day 24, last 'pill' taken on day 20; left ovary which contained atretic follicles is plotted as *O*

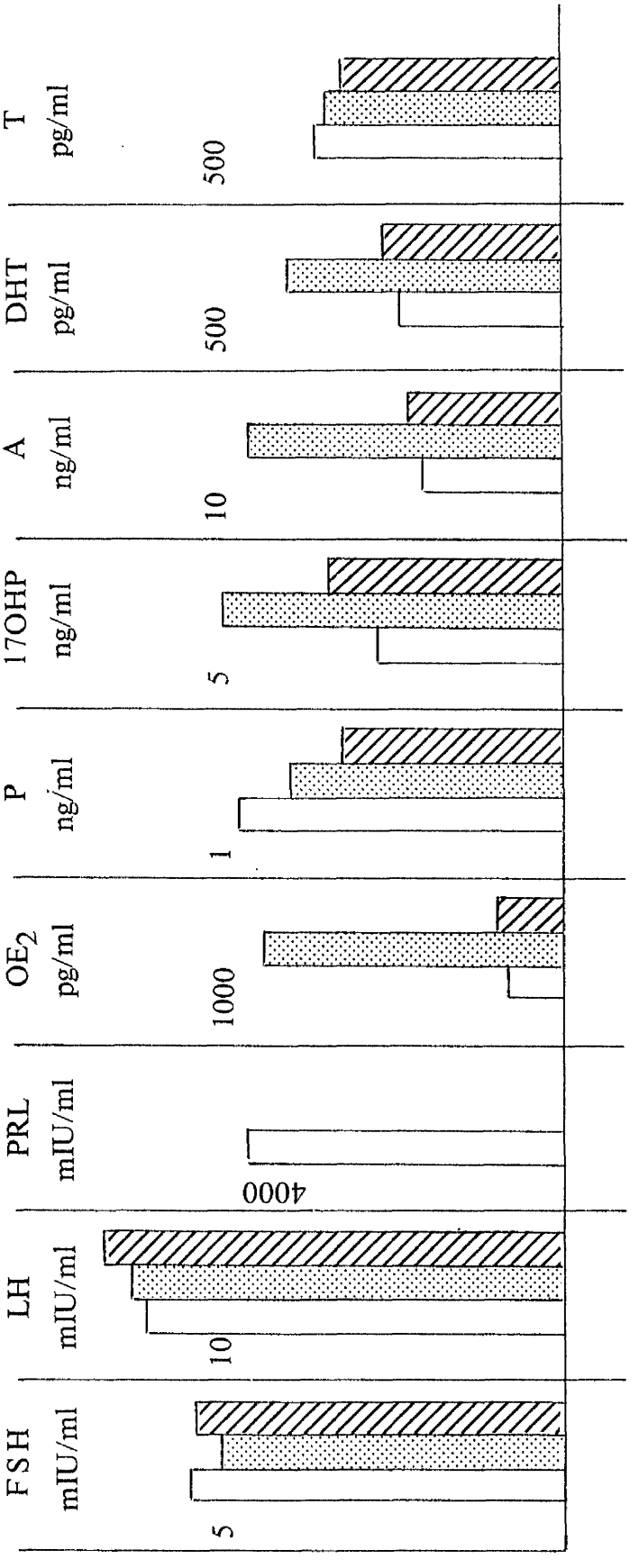


Figure 3.35

Levels of steroid hormones in fluid aspirated from retention cysts in patients who had been taking oral contraceptives (1=A.W.; 2=C.E.; 3=J.H.)

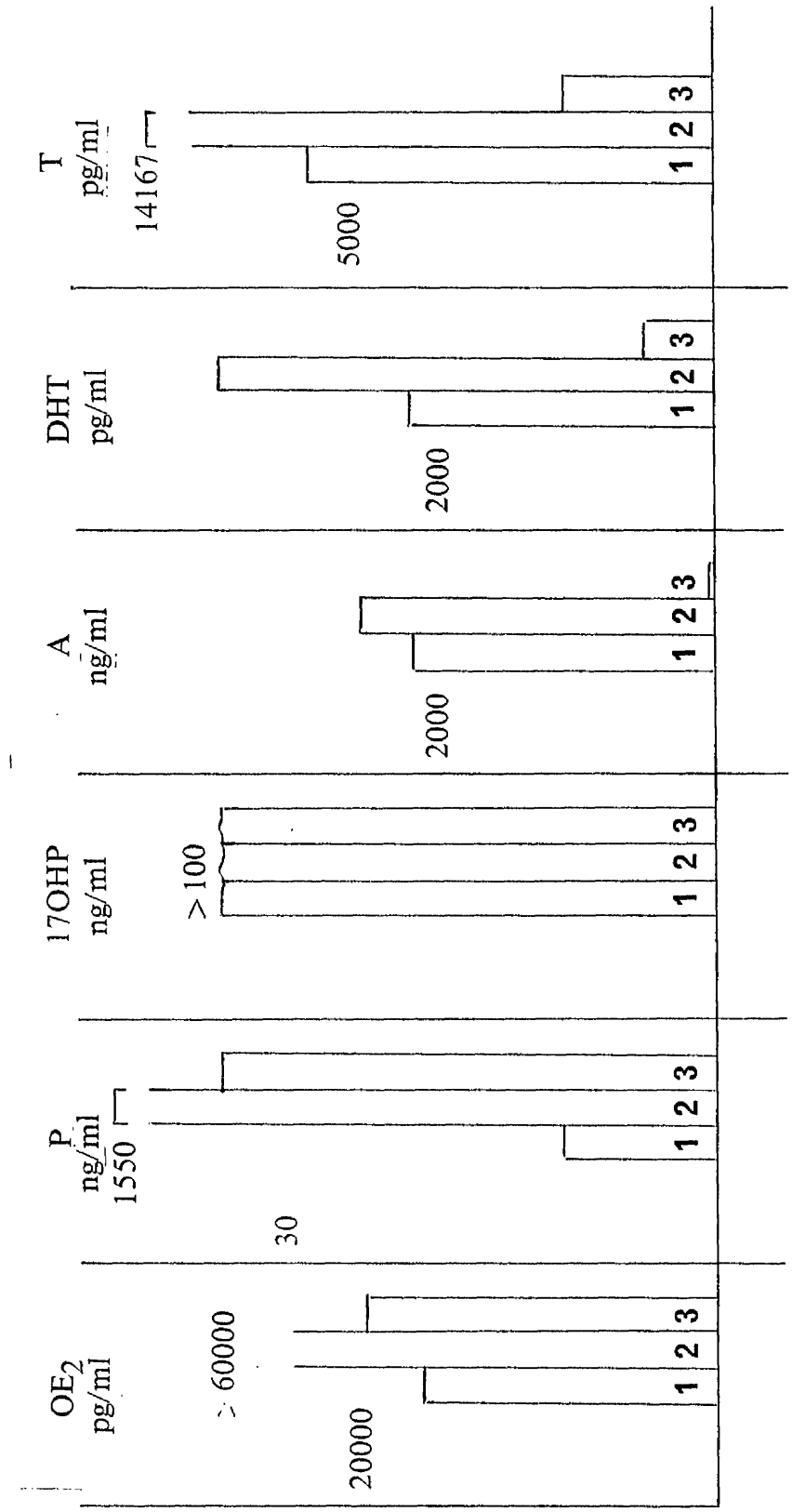
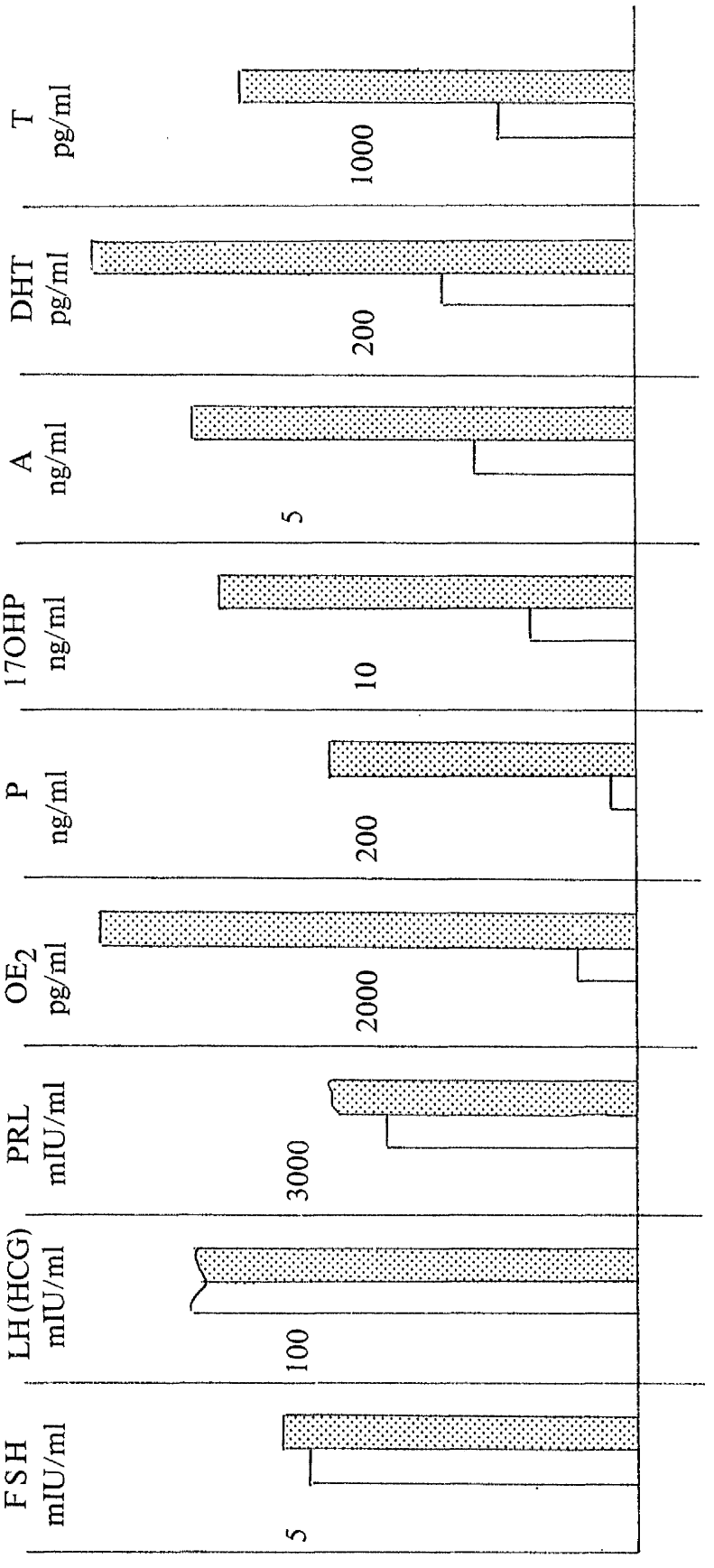


Figure 3.36

Levels of hormones in *P* and *O* venous plasmas
from a woman who was pregnant
(gestation = 6 weeks; O = ovary containing CL of pregnancy)



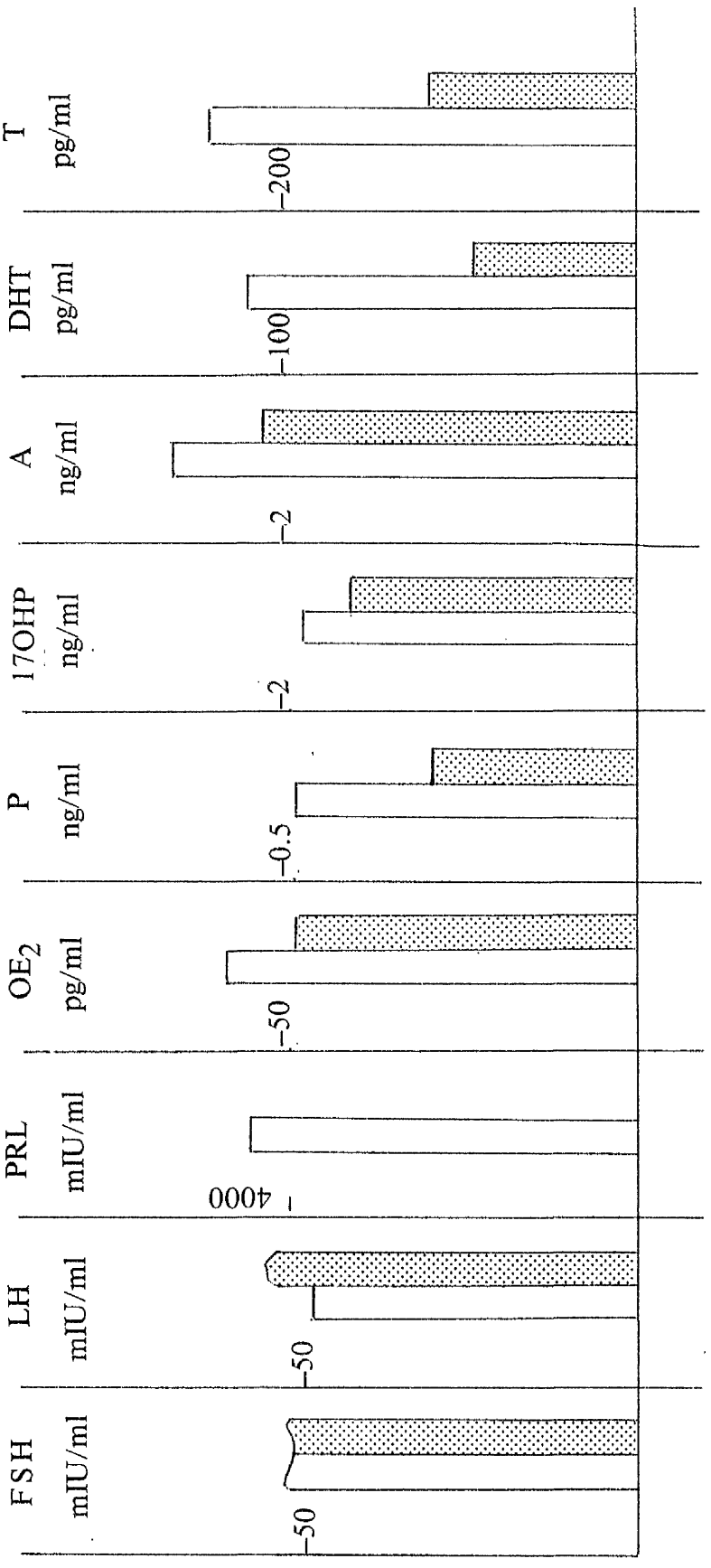
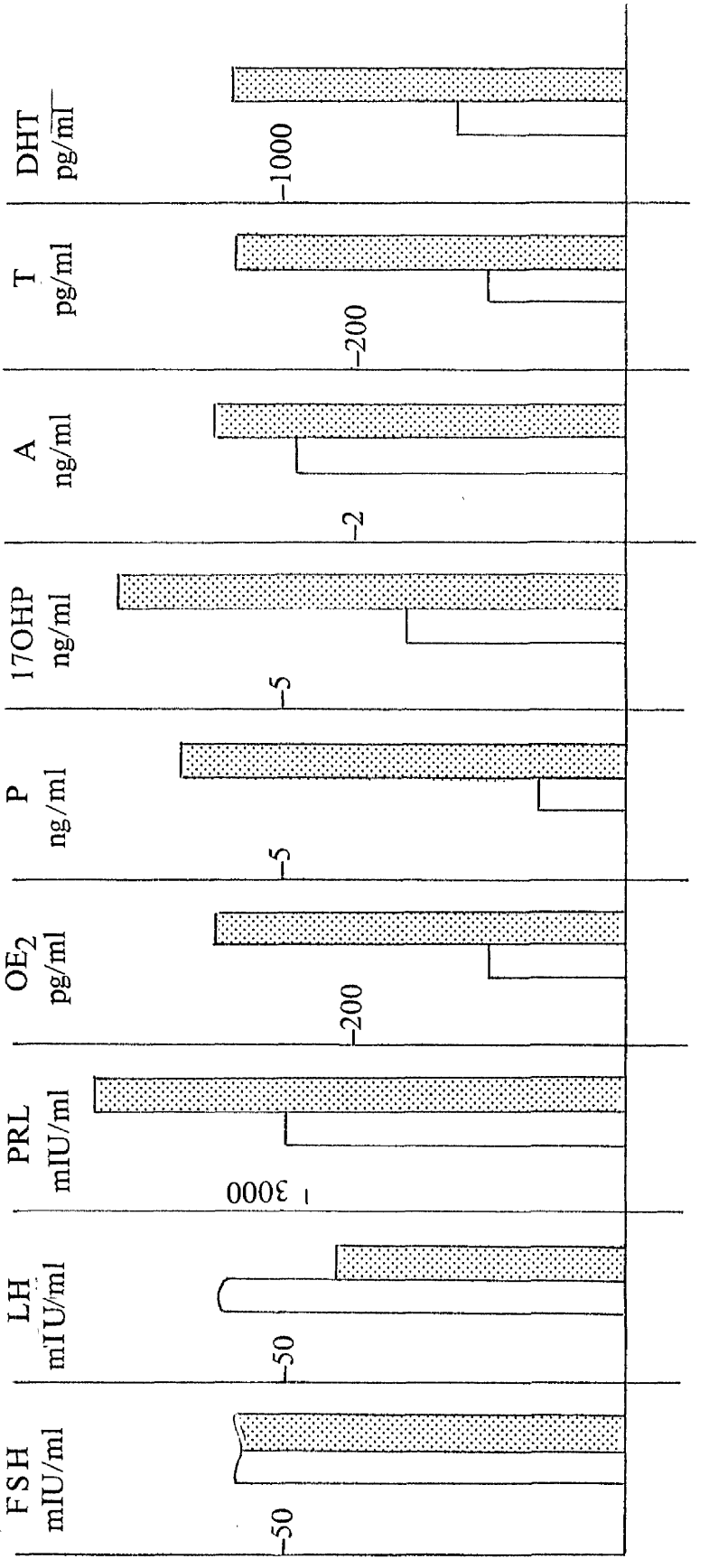


Figure 3.37
Levels of hormones in plasmas from a peripheral and one ovarian vein in a post-menopausal patient I.McV. (ovarian vein sample plotted as *O*)

Figure 3.38

Levels of hormones in plasmas from a peripheral and one ovarian vein in a post-monopausal patient M.McL. (The ovarian vein sampled drained the ovary which contained a 60 cm cyst.)



DISCUSSION

I) Radioimmunoassay – General

Radioimmunoassays have proliferated and proved to be of immense value as methods for measuring many different biological compounds and have made it possible for the investigator to achieve another dimension in physiological and clinical studies.

At the present time new assays based on these techniques appear in almost every issue of the endocrine journals. In the work described in this thesis radioimmunoassay was employed for the determination of protein and steroid hormones in plasma samples and cyst fluids.

One of the most important considerations in the development of a radioimmunoassay is the production of specific antisera for the compound to be assayed. This is a particular problem in the field of steroids since these hormones are themselves not antigenic. To render the steroid antigenic a protein molecule must be linked to the steroid to produce a complex which on innoculation into animals elicits the formation of antibodies.

An assay method must be validated by determination of precision, accuracy, and sensitivity, and once established assay conditions should be standardised. It is important that these parameters are continually assessed while the method is in routine use.

Once a viable assay has been established, the biggest problem encountered in saturation analyses has traditionally been the presence of a methodological blank. Steps must be taken to ensure that the blank due to methodological interfering factors is insignificant.

Specificity of a method is determined by (1) the specificity of the antiserum employed and (2) any purification procedures used prior to assay. Experience has shown that most specific antibodies were produced by conjugating the steroid through a position in the steroid nucleus remote from the distinguishing functional groups of the compound of interest; e.g. OE_2 has been conjugated to bovine serum albumin through the "3" position (Thorneycroft et al 1970) the "17" position (Caldwell 1971) and the "6" position of the steroid nucleus (Exley, Johnson and Dean 1971). Antisera raised to the 6 –linked conjugate are most specific and have been employed in the present study. However, Walker, Clark and Wotiz (1973) have shown that specificity depends not only on the site of conjugation but also on the immunisation procedure and the carrier protein. The most specific antiserum raised to the antigen would be the one of choice, although a relatively non–specific antiserum such as the one conjugated at position "17" in the case of OE_2 may be useful if more than one oestrogen is to be measured quantitatively in a single sample of plasma. The oestrogens can then be separated chromatographically prior to assay of the different fractions.

Once specificity has been achieved the next major problem in producing a viable assay is the employment of a suitable method for the separation of "free" and "antiserum bound" fractions.

The antisera used for all of the assays described in The METHODS section were stable on storage for at least two years at –20° and were not altered by freezing and thawing.

Thimerosal was included in all solutions prepared for radioimmunoassay containing gelatin to act as an inhibitor of bacterial growth.

II) Extraction of Steroid Hormones from Biological Fluids

It is not possible to routinely perform steroid radioimmunoassays directly upon samples of biological fluid unless the fluid has a very low protein content (e.g. Liquor Amnii). In more complex fluids the presence of protein creates assay blank problems, and as a result the hormones must be extracted using organic solvents. The extracting solvents used in the steroid assays employed in this thesis are shown in the MATERIALS and METHODS section and the efficiencies of the extraction are reported in the RESULTS section – Table 3.I.

These results showed that OE_2 and 17OHP were extracted quantitatively with diethyl ether and P quantitatively with a mixture of petroleum ether/ethanol (15/1). In the assays for these hormones extractions were assumed to be 100% and no corrections were made. Testosterone and DHT were also extracted quantitatively with diethyl ether but, because the methods employed a chromatographic purification, internal radioactive standards were added to each plasma and individual samples were corrected for their own recovery. Androstenedione extraction was initially a problem. Quantitative recoveries were obtained using diethyl ether but some component(s) of the ether caused blank problems with the antiserum used for A radioimmunoassay. As a result a compromise was reached and hexane (67°–70°) was used for A extraction. This solvent gave no blank problem and reproducibly extracted A but only with an efficiency of 80%. All A results were corrected for this recovery of 80%.

Thus good and reproducible extractions of all steroids assayed in this study were obtained and the results presented take into account any losses where they occurred. More details of the extraction of the C.19 androgens, in the new assays developed in this study are discussed later.

III) Radioimmunoassay – Specific points applied to new assays in this study

(1) SEPARATION OF FREE AND ANTISERUM BOUND STEROIDS

A wide variety of techniques has been employed for the separation of free and bound fractions in steroid assays including charcoal (Miller 1957), ammonium sulphate (Mayes and Nugent 1968), florisil (Murphy 1967), fullers earth (Murphy 1967) and DCC (Herbert, et al 1965).

The addition of gelatin solution prior to charcoal precipitation has been used by several workers (e.g. Wu and Lundy 1971). It is conceivably useful, in assays employing protein precipitation as a method of separation of free and bound hormones, since addition of extra protein may increase the precipitation of very small amounts of protein present in the assay system.

When DCC is used as the method of choice careful attention must be paid to the contact time of the DCC with the contents of the antigen–antibody incubation mixture, to ensure that all non–specific binding has been removed and that significant stripping does not occur. Figure 3.1 shows the standard curve obtained for A with different DCC contact times of 0, 10, and 20 minutes. Significant stripping occurred prior to 10 minutes followed by a plateau where further significant reduction in the percentage bound did not occur. Presumably during the inital period non–specific weakly bound hormone was being stripped off protein by the DCC. When the plateau occurred this process was complete. A contact time of 10 minutes was therefore utilised for the A assay, this 10 minutes being timed from the addition of DCC to the last tube thus ensuring that the 10 minutes was a minimum figure.

In the case of the radioimmunoassays for DHT and T the ideal

DCC contact times were 20 minutes and 10 minutes respectively (Figures 3.2 and 3.3), the timing again being started from the moment of addition of DCC to the last tube to ensure that the time stated was minimal.

For steroids no method of separation of free and bound fractions is absolute but the important parameter for these assays is reproducibility. In all assays described in this thesis "no As points" were included with the standard curve to ensure that the separation of free and bound (i.e. the absorption of the free) was reproducible. Charcoal concentrations were used which reproducibly absorbed more than 90% of the free fractions.

(2) RADIOIMMUNOASSAY OF A

(i) Specificity

Androstenedione levels in plasma and cyst fluids studied in this thesis were determined by radioimmunoassays using an antiserum raised to A–7–oxo–bovine serum albumin (Weinstein et al 1972).

Cross–reaction data provided by Miles Yeda Ltd. showed significant cross–reaction with only 5α androstenedione and epiandrosterone. These compounds are reduction products of A and are either further metabolised and/or conjugated rapidly for their removal from the circulation. Significant levels of these in their unconjugated form have not been reported in human plasma. No significant cross–reaction was observed with other major C.19 androgens – T, DHT and dehydroisoandrosterone. This antiserum is therefore suitable for use in a specific radioimmunoassay for A without chromatography. To confirm this, samples from the same pool of mixed plasma were assayed employing

methods with and without chromatography. Table 3.II shows the results of this experiment; no significant difference being observed between the results obtained with the two techniques. Indeed, if anything, the results from the method employing chromatographic purification were more variable than those obtained using the direct method. This was presumably a function of the additional manipulations required in the chromatographic method. As a result of these findings, in this study, A was assayed, using an antiserum raised to A–7–oxo–bovine serum albumin without chromatography.

(ii) Extraction

Androstenedione was extracted from the samples as described in the RESULTS. Hexane was used as the extracting solvent for A because in preliminary experiments extraction with diethyl ether, although more efficient, resulted in significant methodological blank values. Using hexane as the extracting solvent removed this problem. Extraction recoveries of 80 ± 1.2% were achieved for A by this method (Table 3.I). Since the recovery was so consistent, each sample assayed for A was assumed to be extracted with 80% efficiency and the results were corrected accordingly.

(iii) Sensitivity

The radioimmunoassay of A had a blank value of 1.4 ± 2.1 pg/0.05ml of adrenalectomised, ovariectomised plasma. 10pg of unlabelled A caused a significant drop in the percentage of labelled hormone bound by the antiserum and was significantly different from the blank. Therefore, the limit of sensitivity for radioimmunoassay of A was 10pg per 0.05ml sample (i.e. 200pg per ml where 0.05ml samples were used).

(iv) Accuracy and Precision

Intra–assay precision and accuracy were determined by assaying plasma (adrenalectomised, ovariectomised) to which 20 replicates of each of 30pg and 70pg were added. This gave values for accuracy of 107.7% and 103.6% and intra–assay precision values of ± 16.4% and ± 9% at the 30pg and 70pg level respectively. Precision and accuracy were better at 70pg which fell on the more sensitive part of the standard curve. Inter–assay precision was ± 8.7% determined on internal quality–control plasmas assayed with each batch of samples. These results show that the radioimmunoassay method developed and used in this study for A was specific, precise, accurate, sensitive and reproducible.

(3) RADIOIMMUNOASSAY DHT AND T

(i) Specificity

Attempts have been made to produce specific antisera for T by coupling the steroid to proteins through a number of positions on the steroid molecule. Despite using positions which, for other steroids containing functional groups similar to T, have produced specific antisera only limited success has been achieved . The major problem has been the cross–reactivity of DHT with T. Thus, specific antisera distinguishing between the two compounds had not been achieved at the initiation of this study. Antisera have been raised after coupling T to proteins through C atoms 1, 3, 6, 7, 11 and 17 of the modecule (Rao and Moore, 1976). The resultant antisera for T showed large cross–reactions with DHT (mean ca. 48%). Methods employed for measuring either DHT or T accurately therefore require a chromatographic step to separate the two steroids.

In this study celite partition column chromatography with ethy-

lene glycol as the stationary phase was employed (cf METHODS section). Columns were eluted with benzene/isooctane mixtures – 5/95 (v/v) followed by 40/60(v/v).

The antiserum used in this study was raised to T – 3 –oxime bovine serum albumin conjugate and had a cross–reaction with DHT of 55%. No significant cross–reaction was obtained with any other major androgens (Table 2.II). The cross–reaction with DHT was not regarded as a drawback, in the context of this study, since it was desired to measure the levels of DHT and as well as T in the samples. Whilst it would have been convenient to have had specific antisera for T and DHT, which did not cross–react with each other, use was made of the cross–reaction of the T–3–oxime bovine serum albumin antiserum to assay both hormones. A method was therefore designed to isolate from plasmas extracts of both T and DHT with no cross contamination. After the initiation of the work reported in this study Rao and Moore (1976) described the production of specific antisera for both T and DHT using conjugates linked through position 15; such specific antisera were not available for use in this study.

Where specific antisera are not available radioimmunoassay may be rendered specific by purification, of the material to be assayed, by separation from any cross–reacting substances. The most common separation technique employed is chromatography. In the experience of our laboratory the most suitable type of chromatography for inclusion in a routine radioimmunoassay is one which (a) does not introduce methodological interfering factors; (b) gives reproducible and quantitative recoveries; (c) employs chromatographic material which may be reused and (d) can be performed rapidly without complicating the assay method unduly. For the purposes of separating T and DHT we were unable to

find a method which satisfied all of the above criteria. The method which was employed in this study was a compromise which fulfilled criteria (a) (b) and (c) above, but employed fairly large volumes of solvents and could not justifiably be described as rapid. Each chromatographic run lasted 40 minutes and 5 columns were the maximum number that one operator could handle at a time.

Chromatographic separations of DHT and T using celite have been described by other workers on much smaller columns (Abraham, 1974). In the experience of this author such chromatography on "Pasteur pipette" type columns led to considerable cross–contamination between the DHT and T fractions. Chromatographic separation was therefore performed in this study, as described in the METHODS, using bigger columns (19cm x 0.75cm) and larger eluting volumes of solvents (20ml DHT ; 15ml T).

This chromatography gave good and reproducible recoveries of DHT (79% ± 5.3%) and T (90% ± 7.5%) (Table 3.III). The recovery for DHT was slightly lower than that for T, because a conservative cut was taken of the DHT fraction to (a) limit the volume to 20 ml and (b) ensure, even if column variability occurred that there was no possibility of cross–contamination with T.

(ii) Extraction

Even allowing for those losses, associated with chromatography, the overall recoveries through the extraction and purification procedures were 69% for DHT and 75% for T (Table 3.IV). Although, these recoveries were, in hindsight, reproducible, internal standards (of both DHT and T) were added to each sample and the individual recoveries for the two hormones were used for correction purposes.

Prepared celite columns for chromatography were reused for up to 12 runs in one day without any detectable deterioration in the separation or increase in the column blanks. Although large volumes of solvents were used this did not introduce a significant blank provided all solvents were distilled immediately prior to use.

The isolated DHT and T fractions were assayed separately using the same antiserum but the radioligand being DHT or T depending upon which hormone was being assayed.

(iii) Sensitivity

The radioimmunoassays for DHT and T gave blank values for 0.05ml of ovariectomised, adrenalectomised plasma of 1.97pg ± .36pg (S.D.; n = 30) and 4.3pg ± 3.1pg (S.D.; n = 30) respectively. The limit of sensitivity was 10 pg for both hormones.

(iv) Accuracy and Precision

Intra–assay variations of DHT and T were computed from assays of 10 replicate standards of 50pg and 100pg of each hormone added to adrenalectomised, ovariectomised plasma. These gave accuracies at the 50pg and 100pg levels respectively for DHT of 102% ± 12.2% (S.D.) and 100.5% ± 11.6% (S.D.) and for T of 112.6% ± 7.4% (S.D.) and 101.3% ± 8.4% (S.D). Intra–assay precision values from the same experiment were ± 12% and ± 11.6% for DHT and ± 6.6% and ± 8.2% for T at the 50pg and 100pg levels respectively. Values for inter–assay precision determined by analysing the data from quality control samples assayed with each assay were ± 12.04% and ± 14.68% for DHT and T respectively.

These figures for validation of the assays demonstrate that specific, accurate, precise, sensitive and reproducible assays were developed for both DHT and T.

(4) RADIOIMMUNOASSAYS OF OTHER HORMONES ASSAYED IN THIS STUDY

Progesterone, 17OHP and OE_2 levels in samples were measured using radioimmunoassay employing the routine procedures existing in the Department of Obstetrics and Gynaecology. Validation of the specificity, sensitivity, accuracy and precision compare favourably with other methods currently available.(Table 3.VII). The assays for FSH, LH and PRL which were performed for us in other laboratories also employed well validated assay techniques.

IV) Levels of Hormones in Peripheral Plasma Samples from Normally Menstruating Volunteers

(1) LEVELS OF C.19 ANDROGENS

(i) Androstenedione

A specific, sensitive, accurate, precise and reproducible radioimmunoassay was developed for A. Using this assay, levels were determined in serial daily plasma samples from seven volunteers. These volunteers had apparently normal, regular menstrual cycles and fulfilled the criteria for normal cycles described in the MATERIALS section. Figure 3.4 shows the mean values of A $\pm$ SEM in these seven cycles. The mean levels of A were between 1ng and 2ng per ml and showed a rise at mid-cycle to an early secretory phase peak (2.3ng/ml; day +2). All previously published data for A levels in the normal menstrual cycle have employed chromatographic purification steps; alumina columns (Judd and Yen 1973), celite columns (Abraham 1974; Baird et al 1974;

Guerrero et al 1976) and paper chromatography (Frolich, Brand and van Hall 1976). Employing the more specific antiserum used in this study similar values to those reported by all of these authors were obtained without chromatography. A degree of controversy has existed with regard to the occurrence of peak levels of A in normal menstrual cycles. Some authors have reported significant luteal peaks of this hormone (Guererro et al 1976) whilst Abraham (1974) described a distinct peak on the day of the LH peak and others described elevated levels around the time of ovulation, i.e., in the late follicular and early luteal phases (Judd and Yen 1973; Baird et al 1974; Frolich et al 1976). In this study peak levels of A were observed in the early luteal phase, (day +2, 48 hours post LH peak) probably just after ovulation. However, in this study, these elevated values were not significantly higher than the levels immediately preceeding or succeeding them. Perhaps the situation was best described by Judd and Yen (1973) in the first publication of serial levels of A in normally menstruating women. These authors showed profiles for six individual cycles with rises in all patients, which did not always occur at the same stage in relationship to the LH peak. Such findings would explain the variability of the conclusions drawn by different authors and also the lack of statistical significance of the peaks which were found in this and other studies (Judd and Yen 1973). Where significant elevation was observed this might have been a result of a fortuitous choice of patients. In this study the levels of A found during menses (early follicular phase) were lower than those found later in the cycle when active growing follicles or corpora lutea would have been present in the ovaries.

(ii) Dihydrotestosterone

Dihydrotestosterone levels determined in daily peripheral venous samples from normally menstruating women in this study showed mean

values ranging from 100pg/ml to 173pg/ml. These levels were very similar to those previously reported for DHT in the normal menstrual cycle (Abraham 1974), but were slightly lower than those of Guerrero et al (1976). The scatter of the latter authors' results would however include the values obtained in this study. Guerrero et al (1976) found no fluctuation of DHT levels during the menstrual cycles and Abraham (1974) drew similar conclusions although in the latter study the levels rose (but not significantly) around the LH peak. In the present study, a more distinct elevation was observed around mid-cycle for DHT than has been described previously (Figure 3.5). This rise, although not significant, occurred in all patients. Again, the lack of significance might be related to patient variability with regard to the actual days of the peak values.

The conclusions of Ito and Horton (1971), that A is a major precursor of plasma DHT in adult females, would, together with the previous discussion of increased A levels at mid-cycle, lend evidence to support the findings in this study, that DHT levels rose at mid-cycle. The fact that the A peak day varied between individuals (Judd and Yen 1973) would, if this is the pre-hormone for DHT, mean a similar variability would be expected for DHT, thus explaining the lack of a significant rise, in this study and the apparent constancy of the levels described by Abraham (1974) and Guerrero et al (1976). Results reported by Coyotupa, Parlow and Abraham (1972) which indicated the possibility of a cyclical pattern for plasma DHT, in women, is in agreement with the present observations.

(iii) Testosterone

Testosterone levels have been determined previously throughout normal menstrual cycles by a number of authors including Judd and

Yen (1973), Dupon, Hosseinian and Kim (1973), Abraham (1974), Tyler, Newton and Collins (1975), Guerrero et al (1976) and Frolich et al (1976). These previous workers found mean levels of T of between 150pg/ml and 500pg/ml, and some controversy existed with regard to the pattern of levels throughout the cycle. In the present study mean levels of T (Figure 3.6) throughout the cycle ranged from 172pg/ml to 579pg/ml,; similar to the previously published data. A significant rise in the levels of T occurred at mid–cycle with maximal values being obtained on the day of the LH peak and the day following (day +1). The mid–cycle rise was followed by a fall and thereafter a secondary peak was observed in the late luteal phase (day +14). The presence of a mid–cycle rise in T levels has been observed by most workers – Judd and Yen (1973)., Abraham (1974)., Frolich et al (1976) and Guerrero et al (1976). Dupon et al (1973) and Tyler et al (1975) did not find a mid–cycle rise and in both of those studies mean levels were constant throughout the cycle. The latter group, however, observed a mid–cycle peak in three patients out of nine when they were examined individually. The finding of patient variability, by Tyler and his colleagues, confirmed similar earlier findings by Judd and Yen (1973) and again suggests a possible explanation for the inconsistencies reported by the different authors. In the present study a mid–cycle rise occurred and a secondary peak was observed in the late luteal phase (Figure 3.6) in all seven cycles studied. Abraham (1974) also reported a late luteal rise in mean values whilst Tyler et al (1975) observed luteal phase rises in two of their nine patients but did not indicate the exact timing of those peaks. The latter authors further complicated the picture with regard to T patterns in "mean cycles" by showing that the levels varied during the day in non–pregnant women. As a result, time of sampling might also affect the patterns obtained.

(2) PROLACTIN

Levels of PRL in peripheral samples from normally menstruating volunteers throughout the menstrual cycle are shown in Figure 3.7 The levels rose, from day –7 to reach peak values on day 0.

During the luteal phase a secondary rise was observed on days +11 and +12. Neither of these rises were statistically significant peaks but all seven cycles showed this trend. These rises in PRL levels at mid–cycle and in the late luteal phase were in agreement with previously published data from Franchimont et al (1976) but were contrary to the observations of McNeilly and Chard (1974) who found no difference in PRL levels throughout the cycle.

The occurrence of rises in PRL levels at mid–cycle and in the late luteal phase is not surprising since these are the times of the cycle when OE_2 levels are maximal and it is well established that the output of PRL by the lactotrophs is stimulated by oestrogen (Robyn and Vekemans, 1976).

(3) LH, FSH, OE_2, P AND 17OHP

The levels of the above hormones estimated in daily plasma samples throughout the menstrual cycle from normally menstruating volunteers confirmed our previously published results ((cf figures 1.4 to 1.8). These findings confirmed that these normally menstruating volunteers did indeed have "normal" cycles.

V) Levels of Hormones in Peripheral and Ovarian venous Plasmas obtained from Patients at Hysterectomy

(1) DATING OF THE STAGE OF THE MENSTRUAL CYCLE IN PATIENTS SAMPLED AT HYSTERECTOMY

One of the major criticisms of previous studies of ovarian venous levels of hormones has been, apart from the small numbers of patients studied, the lack of sufficient information regarding the stage of the menstrual cycle. No one parameter is sufficient to accurately date the stage of the cycle (e.g. Calabresi et al 1976) and this is particularly true in women who are attending for surgery because of some Gynaecological complaint.

In this study, therefore, a great effort was made to attempt to determine accurately the stage of the menstrual cycle.

As a result reference was made to as many as possible of the parameters listed below and the stage of the cycle was assessed from a consensus of all of the data available (Tables 3VIII and 3VIIIa).

Even on the basis of all of this information several patients had to be omitted from the study because the stage of the menstrual cycle could not be adequately assessed.

The following parameters were used to date the stage of the menstrual cycle.

(i) Menstrual History:

The menses or menstruation occurs at about monthly intervals. The first day of the menstrual loss denotes the beginning of the cycle. In the classical case of the normal twentyeight day menstrual cycle the

proliferative phase lasts for fourteen days and ovulation takes place at the end of this. Following ovulation the subsequent fourteen days are referred to as the secretory phase which lasts for at least thirteen days unless the luteal phase is deficient. Where the menstrual cycle is unduly prolonged, it therefore represents an extended proliferative phase. Reference to the regularity of the patients cycle and the date of the last menstrual period therefore gave an indication of the stage of the cycle in individual patients.

(ii) Macroscopic Examination of the Ovaries.

At laparotomy of cases involved in this study the ovaries were examined in detail for evidence of growing follicles and/or corpora lutea, thereby identifying the active ovary. Follicles smaller than 5mm in diameter were often seen in both ovaries in the EP stage; medium sized follicles (5mm to 15mm in diameter) were observed in the MP stage and bigger follicles with thin walls indicating impending rupture were associated with the LP stage. The presence of a fresh corpus luteum was usually identifiable by haemorrhages on the surface of the active ovary (ES). When mature corpora lutea were present the stage of the luteal phase was determined by reference to other parameters.

(iii) Microscopic Examination of the Ovary

Ovaries if removed were serially sectioned for evidence of follicles, corpora lutea and corpora albicantes in an attempt to further date the stage of the menstrual cycle.

(iv) Histological Study of the Endometrium

Samples for endometrial biopsies were transported to the Pathology Department for immediate analysis since prolonged fixation and/or

delay in immersing specimens in the fixative make precise dating of the endometrium difficult. The classical cellular changes in the endometrium as described by Noyes, Hertig and Rock (1950) were used as a reference for dating with regard to each patient's stage of the menstrual cycle.

(v) Levels of Hormones in Peripheral Plasma Samples

Mid-cycle peaks of the gonadotrophins LH and FSH (to a lesser degree) are classical reference points in the normal cycle. The LH surge (see INTRODUCTION section) occurs when the mature follicle is ready to rupture and ovulate. Thus high levels of LH immediately precede ovulation. Elevated levels of FSH are associated with the period immediately prior to and during menses when a group of follicles are being stimulated to grow. Thus high levels of FSH in the presence of a less distinct rise in LH than occurs at mid-cycle are indicative of the EP stage.

During the remainder of the proliferative phase OE_2 is secreted in increasing amounts culminating in the well established peak which causes the positive feedback release of LH. Throughout the proliferative phase P levels remain basal.

Following luteinisation and ovulation the corpus luteum secretes large quantities of P together with similar levels of OE_2 to those found a few days prior to the LH peak.

High levels of OE_2 alone are found in the MP and LP phases and elevated levels of both P and OE_2 are associated with the secretory phase. From the actual levels of P it is possible to approximately

allocate patients to the stages of the secretory phase – ES (> 1ng/ml to < 5ng/ml); MS (> 5ng/ml) and LS (< 5ng/ml) together with levels of OE_2 which are sub–maximal. The distinction between ES and LS on hormonal grounds alone is difficult and requires information from other parameters such as those described above – V) (1) (i) to V) (1) (iv).

The levels of LH, FSH, OE_2 and P in peripheral plasma samples from patients in this study were used mainly to confirm the stage of cycle assessed by the other parameters. However, in a few cases where the information from V) (1) (i) to V) (1) (iv) was incomplete or inconclusive hormone levels were used to date the stage of the cycle.

(2) OVARIAN VENOUS SAMPLING

Ovarian venous effluents were collected in this study from patients undergoing hysterectomy for conditions which did not affect their reproductive function (see MATERIALS and METHODS section).

Informed consent was obtained to collect concurrently bilateral ovarian and peripheral venous plasma samples. The vein draining the ovary which appeared more active was sampled first concurrently with an antecubital vein; the contralateral ovarian vein was sampled immediately thereafter. Where success in obtaining a sample was not immediate, only ovarian venous effluents draining active ovaries were collected to avoid prolonging operating time unduly. The ovarian vein leaving the hilum of the ovary on each side was selected in all cases. Variability of the pattern and calibre of the venation in the mesovarium posed difficulties in some cases. Previous workers (Horton, Romanoff and Walker 1966; Mikhail, 1970) overcame such problems by severing the veins in the mesovarium and collecting the pooled blood released

thereon. Blood samples collected in this way may not be pure venous samples but may be contaminated with arterial blood.

With progress of the study difficulties in blood collection became minimal presumably due to the increasing expertise of the operator.

Best results were achieved when the following guidelines were adhered to:–

(a) The abdomen, after opening, was lightly packed, to facilitate filling of the pampiniform plexus whilst still allowing sufficient room for manoeuvrability.

(b) The operation table was in a slight Trendelenberg position to bring the operating area into a more open view.

(c) A pair of Babcock forceps were applied at each pole of the ovary to permit easy handling of the ovary without any squeezing. This ensured that the ovarian venous effluent was collected under as physiological conditions as possible.

(d) '25' guage needles were used for sampling. However, in a few cases, where the venation was tortuous and grossly distended, '23' guage needles were found to be more suitable to facilitate aspiration without leakage between the needle and the venous wall.

(e) Small syringes were used (5ml or less). This allowed easy handling and prevented excessive suction, which might have collapsed the venous wall.

(f) Suction was applied intermittently allowing the vessels to refill in between.

Many previous authors have avoided sampling too close to the ovary for fear of associated bleeding. In the author's experience digital pressure, or compression with a warm pad for two minutes on the

puncture sites was sufficient to prevent bleeding or haematoma formation. If either complication had occurred it would have been necessary to sacrifice the ovary. No such occasion occurred in this study.

Using the methods described in this thesis good samples of pure ovarian venous plasmas were obtained with minimal complications.

(3) SIGNIFICANCE OF HORMONE LEVELS IN OVARIAN VENOUS EFFLUENTS

The design of the studies reported in this thesis assumed that comparison of the levels of hormones in veins draining ovaries (active or contralateral) with levels in corresponding peripheral plasmas would give information regarding the ovarian production of hormones. Two separate experiments were conducted in an attempt to confirm that this was the case. Firstly the levels of hormones were compared in one patient in peripheral plasma and in plasmas from the vein draining and the artery supplying the active ovary and secondly the effects of bilateral oophorectomy on the peripheral plasma levels of hormones over a number of days were examined.

The patients in whom peripheral and ovarian venous and arterial samples were compared was in the LP stage of the cycle. The results (Table 3XXVIII) showed that whereas the levels of all six steroid hormones were identical in the peripheral and ovarian arterial plasmas they were much higher in the ovarian venous plasma. Since the ovarian arterial and venous samples were virtually collected simultaneously the differences can only be explained by secretion of the hormones by the ovary. Secretion of steroid hormones by ovaries was confirmed by examination of the effects of bilateral oophorectomy in a patient in the

EP stage (Table 3.XXIX). Peripheral plasma samples were collected before and for six successive days after ovariectomy.

Even though this patient was at the least active stage of the cycle an immediate (by 1 day) reduction was observed, in the levels of all six steroids measured, after ovariectomy. These effects of bilateral oophorectomy have now been confirmed in a much larger series of patients (Barlow et al, 1981).

The results of these two studies were interpreted as evidence that peripheral–ovarian venous plasma differences in the levels of steriod hormones were indicative of hormone production by the ovaries. Due to the inherent risks associated with arterial sampling ovarian arterial plasma samples were not collected from other patients.

(4) HORMONE LEVELS IN PERIPHERAL AND OVARIAN VENOUS PLASMAS OBTAINED FROM NORMALLY CYCLING WOMEN AT HYSTERECTOMY

Prior to the initiation of the studies presented in this thesis little information was available with regard to hormone levels in ovarian venous plasma. Isolated findings, usually from only a few patients, had been reported by several workers (Horton et al 1966; Rivarola et al 1967; Gandy and Peterson 1968; Mikhail 1970). Many of these samples were obtained from patients with polycystic ovarian disease who did not have normal cycles. It was difficult to correlate the findings of these studies with in vitro work on steroid biosynthesis, largely because, in most instances, no close attention was paid by the authors to the stage of the menstrual cycle of the patients at the time of sampling. Furthermore, there was considerable doubt in some instances as to the purity of the samples with regard to ovarian effluent. Often, where details of the

exact procedures used for sampling were given, it seemed likely that the samples collected might be contaminated with uterine effluent. In only a few cases did reports describe collection of blood from both ovarian veins thus allowing comparison of hormone levels in the 'active' ovary (containing the preovulatory follicle or corpus luteum) with the contralateral, 'inactive', ovary to ascribe the production of particular hormones to specific ovarian structures. Mikhail (1970) described steroid production from both ovaries in 7 normal patients, 6 of whom were in the luteal phase of the cycle. In Mikhail's study the stage of the menstrual cycle was well documented but his method of blood collection was not ideal since by his techniques he might possibly also have collected ovarian arterial blood thus obtaining lower results than the true hormone levels in the ovarian venous effluent.

The study described in this thesis was designed to collect peripheral and ovarian venous blood plasmas from patients who had normal reproductive function. Where possible venous blood draining both ovaries was collected. The number of patients in whom bilateral blood samples were collected was limited by a desire that these experimental techniques should not unduly prolong the surgery. In all patients the stage of the menstrual cycle was critically assessed by use of as many parameters as possible (Tables 3.VIII and 3.VIIIa) and the patients were assigned to each of the 6 groups – EP, MP, LP, ES, MS and LS – thus enabling a composite menstrual cycle to be constructed. Sufficient contralateral ovarian vein samples were obtained to ensure that conclusions could be drawn in both proliferative and secretory phases as to the secretory activity of the 'active' and 'inactive' ovaries. The composite menstrual cycle levels were compared with those in peripheral venous samples obtained from young healthy normally menstruating volunteers.

During the progress of the work described in this thesis a number of papers have been published which have produced results from normally menstruating women with better controlled sampling of ovarian venous plasmas and more rigorous assessment of the stage of the menstrual cycle than in the previous publications. In most cases, largely due to the problems of the human as an experimental animal, patient numbers have been small. Baird et al (1974) studied A levels in 10 normally menstruating patients (3, EP; 1, MP; 2, LP; 2, ES; 1, MS; 1, ES); de Jong, Baird and Van der Molen (1974) measured A, OE_2, OE_1, T, P and dehydroisoandrosterone and its sulphate in 4 normally menstruating patients (1 menstruating, 1 MP and 2MS); Baird and Fraser (1974) reported levels of OE_1 and OE_2 in 11 patients (2, EP; 3, LP; 2, ES; 3, MS; 1, LS); McNatty et al (1976) reported levels of OE_1, OE_2, A and T in 11 normally menstruating women (3 early follicular, 4 late follicular and 4 luteal) and Calabresi et al (1976) measured OE_2, P, A, T and DHT in 15 normally cycling patients who were all in the luteal phase. In a later publication an extension of the work of Calabresi and his colleagues was published (Serio et al 1976) in which in addition to the 15 luteal phase patients 7 proliferative phase patients (3, EP; 2,MP; 2, LP) were also studied and as well as the above levels those of OE_1 and dehydroisoandrosterone were also reported. In these two publications, however, (Calabresi et al 1976; Serio et al 1976) the stage of the menstrual cycle was assessed only from the menstrual history and macroscopic examination of the ovaries. Much more recently Aedo et al (1980 a; 1980 b) have published two papers regarding the contributions of the Graafian follicle (Aedo et al 1980 a) and the corpus luteum (Aedo et al 1980 b) to ovarian steroid secretion in normally menstruating women. These authors paid very strict attention to ovarian venous sampling and dating of the stages of the cycle but have only to date reported results from 9 patients in each of the LP and ES phases.

The results presented in this thesis are discussed under 3 separate headings:–

(a) a comparison of the mean hormone levels throughout the menstrual cycle in plasma from the peripheral circulation with those in plasmas draining both 'active' and 'inactive' ovaries;

(b) a comparison of hormone levels in peripheral plasmas, contralateral ovarian venous plasmas and active ovarian venous plasmas from patients in whom all 3 sites were sampled and

(c) with respect to their contribution to our understanding of hormone production by the human ovary.

(i) Hormone Levels in Ovarian Venous Effluents and in Peripheral Plasmas in Normally Cycling Women

The results from the whole group of women are discussed below under the nine individual hormones labelled A to I as in the RESULTS section.

(A) FSH

The levels of FSH found in the peripheral and ovarian venous samples – both active and contralateral – were not significantly different from each other. The levels, as expected from serial samples determined in normal cycles (Coutts 1976) were high in the early proliferative phase and were lower during the rest of the cycle (Figure 3.10). No evidence was obtained, from any patient, of a significant drop in the levels of FSH in the ovarian venous effluent compared to the levels in the peripheral samples (Tables 3.IX, 3.X). Such a drop might have been expected if FSH stimulates follicular growth after being bound to receptors in the growing follicles (McNatty et al 1975). Since the secretion of gonadotrophins is episodic (Boyer et al 1972) presumably the techniques of

blood sampling and/or radioimmunoassay of FSH were not sufficiently well designed and/or sensitive enough to observe the removal of FSH from the peripheral circulation during passage through the active ovary.

(B) LH

The levels of LH in the plasma samples were highest in the late proliferative stage of the cycle, presumably as a result of release of LH in response to the positive feedback effect of OE_2 on the hypothalamus (Yussman and Taymor 1970). The LH levels in the LP stage were variable, presumably because of the design of an LP group; some patients would be included just prior to the LH surge whilst others would be sampled during the time of peak release. No significant difference was observed between the levels of LH in peripheral and ovarian venous samples. Failure to detect a peripheral /ovarian gradient in LH values caused by binding of LH to mature follicles was again presumably a function of a combination of complicating factors such as, episodic secretion of LH and/or the inability of the assay to pick up minor fluctuations in LH levels.

(C) PROLACTIN

The levels of PRL observed in samples obtained at surgery were much higher (5 times) than those observed in peripheral venous samples from normally menstruating volunteers (Figure 3.7). This was presumably a response to anaesthesia and/or operative stress in the hysterectomy patients. Increases in PRL levels shortly after the induction of anaesthesia have been reported (Robyn et al 1973; Fournier, Desjardins and Friesen 1974). Whether this is an actual effect of the anaesthetic or is a result of collection of samples under the stress of surgery (Robyn et al 1976) is unknown and could not be determined since patients were not

subjected to major surgery without anaesthesia. McNatty et al (1975) also observed similar increases in peripheral plasma PRL concentrations in samples collected from patients at surgery compared with those of matched volunteers. Although the levels of PRL were increased the effect seemed to be consistant in individual patients in both ovarian and peripheral venous plasma samples. No significant differences were observed between the levels of PRL in ovarian and corresponding peripheral venous plasmas at any stage of the cycle. It was therefore impossible to demonstrate that follicles at different stages of the cycle bound different amounts of PRL (McNatty et al 1975) but such minor differences may have been obscured by the dramatic increases in response to stress and/or anaesthesia.

(D) PROGESTERONE

Levels of P found in peripheral plasmas from patients sampled at hysterectomy showed the same patterns as are well established in peripheral plasmas from normally menstruating volunteers (Dodson, Coutts and Macnaughton 1975); low levels were found in the proliferative phase and higher levels in the secretory phase (Figure 3.13). The levels of P present in plasma from veins draining active ovaries showed similar patterns to those described above but the levels were much higher than those in peripheral plasma during the secretory phase (17 times higher at MS) (Figure 3.13). These results confirm the conclusions of previous workers, Mikhail(1970); de Jong et al (1974), Calabresi et al 1976; Lucisano et al 1978, that the major source of secretion of P in the human female is the corpus luteum. The levels of P in the 'active' ovarian veins were significantly higher that those in the peripheral veins or the contralateral ovarian veins during the secretory phase ($p < .02$). Progesterone levels in the contralateral ovarian veins were not signifi-

cantly different from those in peripheral plasma at any stage of the cycle thus confirming the conclusions from other studies (Mikhail 1970; Calabresi et al 1976; Lucisano et al 1978) that with respect to P synthesis, the contralateral ovary was inactive. These findings are however at variance with those of Aedo et al (1980 a; 1980 b) who concluded that P was also produced by the contralateral ovary.

Evidence was obtained from both MP and LP samples that preovulatory luteinisation (Dodson et al 1975) occurred. The rise in P, prior to ovulation, was found in both peripheral venous samples and also plasmas from veins draining active ovaries. This proliferative stage progesterone was confirmed as a product of the active ovary since the levels in plasmas from veins draining active ovaries were much higher than those in the corresponding peripheral venous samples (Table 3.XV). The P levels in the 'active' ovarian venous plasmas were significantly higher than peripheral levels at the MP stage of the cycle ($p < 0.05$). Mikhail (1970), Lloyd et al (1971), de Jong et al (1974) and Serio et al (1976) have reported similar results with higher P levels being associated with the active ovarian venous plasma. Aedo et al (1980 a) on the other hand provided evidence that P was secreted by both ovaries and not only by the one containing the Graafian follicle. Perhaps by selecting patients very close to the LH peak when changes are occurring in P secretion these last authors introduced such patient variability that the contribution of the follicle to P secretion became difficult to assess.

(E) 17α HYDROXY PROGESTERONE

Peripheral plasma levels of 17OHP in serial daily samples from normally menstruating women showed a rise at about the time of the LH peak. In some patients this rise was followed by a nadir whilst in others

it formed a shoulder on the luteal peak which occurred in all patients (Dodson et al 1975). Strott et al (1969) suggested that the mid-cycle rise in 17OHP was a result of the release of this intermediate on the biosynthetic pathway to oestrogen, at the time when the follicle had matured and OE_2 synthesis was slowing down. Coutts (1976) questioned this hypothesis and suggested that the rise in 17OHP reflected pre-ovulatory luteinisation. The results from the peripheral samples obtained at hysterectomy showed similar patterns and levels to those from normally menstruating volunteers; maximum levels occurred in the LP stage with a secondary rise in MS and LS stages (Tables 3.XVII, 3.XVIII; Figures 3.14, 3.23). Levels of 17OHP in veins draining the active ovary were higher than corresponding peripheral levels at all stages of the cycle (Figure 3.23) being significantly so at MP. LP and MS stages (RESULTS section). The maximum levels of 17OHP in the active ovarian veins were found in the MS phase where levels of 16 times those found in peripheral samples were observed (Figure 3.23). Mikhail (1970) in his small series, also found very high levels of 17OHP in the active ovarian vein. The ratio of 17OHP in active ovarian venous plasma/17OHP in peripheral venous plasma was almost identical to that obtained for P levels in the same samples (Figures 3.22) suggesting that the latter hormone was a precursor of the former. In the MP and LP stages, levels of 17 OHP in active ovarian venous plasmas were of the order of twice those in peripheral plasmas (Figure 3.14). The highest levels of 17OHP found in the proliferative stage in venous samples draining active ovaries were at MP (Figure 3.23). At this same stage proliferative levels of OE_2 were also maximal (Figure 3.24). These findings were not in accord with the suggestion of Strott et al that peak levels of 17 OHP were associated with reduced secretion of OE_2. The increase observed in 17OHP levels at mid-cycle in peripheral venous samples (Figure 3.14) may well reflect further metabolism of P synthe-

sised as a result of the early stages of luteinsation (Figure 3.13). The findings that 17OHP is synthesised by both the Graafian follicle and the corpus luteum are in general agreement with the observations of Aedo et al (1980 a; 1980 b). Since only a few results were available for 17OHP levels in plasmas from veins draining contralateral ovaries it was not possible to draw definitive conclusions with regard to the synthesis of this steroid by the inactive ovary. However, from the few results presented the impression was that only in the MS stage might there be 17OHP secretion by the contralateral ovary.

(F) OESTRADIOL

The levels of OE_2 found in the peripheral plasma samples obtained from women at hysterectomy showed the same biphasic pattern which has been described by many workers from normally cycling volunteers showing peaks in LP and MS stages (Ross et al 1970; Abraham et al 1972; Shaaban and Klopper 1973; Dodson et al 1975). The actual levels of OE_2 observed from these patients were much lower than the equivalent peaks in normal volunteers at the same stages. No entirely satisfactory explanation can be given for this finding but the patients fitted a much older age group than most volunteers studied and also the grouping of the patients into stages of the menstrual cycle may well have resulted in slight overlaps causing distribution of the peak values obtained. Although these mean levels of OE_2 were low they were just within 2 S.D.s below the mean values of normal cycle levels (Coutts 1976) published from our laboratory.

The levels of OE_2 in the samples from veins draining active ovaries were much higher at all six stages of the cycle than the corresponding levels in peripheral plasma samples, being significantly higher

at all stages except EP where the significance was borderline ($p<0.1$). Peak active ovarian vein levels of OE_2 were found in the MP stage, i.e. before peak levels were found in peripheral samples. This was consistent with a nadir occurring prior to ovulation. The LP group would presumably have included patients in whom the positive feedback of OE_2 had been achieved, who were about to rupture their mature follicles and in whom levels of OE_2 would have decreased.

The secondary peak of OE_2 occurred in the MS stage samples where the ratio of OE_2 in active ovarian veins/OE_2 in peripheral veins was 16:1 – a very similar finding to those described for both P and 17OHP above. These results confirmed that the source of the vast majority of OE_2 in the human female of reproductive age was the active ovary (Mikhail 1970; de Jong et al 1974; Baird and Fraser 1974, 1975; Serio et al 1976 McNatty et al 1976; Aedo et al 1980 a). The failure of Aedo et al 1980 b to observe similar findings during the ES stage of the cycle was presumably related to their choice of subjects.

Levels of OE_2 in contralateral ovarian veins were also higher than their corresponding peripheral levels at all stages of the cycle where sufficient contralateral ovarian vein samples were available. Statistical assessment showed that the contralateral ovarian venous OE_2 levels were significantly higher than those in corresponding peripheral venous samples (RESULTS section). Increased levels of OE_2 in contralateral ovarian venous samples have been reported by other workers, usually without comment (Mikhail 1970; Baird and Fraser 1974). Baird and Fraser (1974) using elegant techniques determined the contribution of the active ovary to the blood production rate of OE_2 and concluded that this was $>$ 95% at some stages of the cycle. de Jong et al concurred

with these findings that the active ovary was the major source of OE_2 and also concluded from similar blood production studies that although the contralateral ovarian vein contained higher OE_2 levels than the equivalent peripheral venous samples the 'inactive' ovary produced an insignificant amount of OE_2. Calabresi et al (1976), who only examined patients in the luteal phase, did not find an increased level of OE_2 in the contralateral ovarian veins compared to their peripheral samples.

Although the contribution of the contralateral ovary was insignificant, by comparison with that of the active ovary, the finding that the contralateral ovarian vein contained significantly more OE_2 than the peripheral vein is important in consideration of whether the contralateral ovary is 'inactive' or not. The results produced by Aedo et al (1980 a; 1980 b) confirm those of this study showing that the contralateral ovary produces OE_2.

(G) ANDROSTENEDIONE

The levels of A in peripheral plasmas from women undergoing surgery were consistent with these in peripheral plasma samples from normally menstruating volunteers (Figure 3.4). Although there was a luteal phase rise this was not significant and the levels in the luteal phase were no different from those at mid-cycle or during the MP and LP stages.

Levels of A in plasmas from veins draining ovaries indicated that the major site of A production was the active ovary. The ratio of A levels in active ovarian venous plasmas to those in peripheral plasmas were 3.8:1 (MP) and 19.5:1 (MS). Thus the major sites of production of A by ovaries were the Graafian follicle and the corpus luteum.

The A levels in venous plasmas draining contralateral ovaries were much lower than those in venous plasmas draining active ovaries but nevertheless were higher than those in peripheral plasmas – significantly so in the MS phase ($p<0.01$). Other workers have observed production of A by the Graafian follicle (de Jong et al, 1974; Serio et al, 1976; McNatty et al, 1976) and the corpus luteum (Calabresi et al, 1976; McNatty et al, 1976), but some groups have concluded that A is produced by both ovaries equally throughout the cycle (Aedo et al, 1980 a; 1980 b) or during the luteal phase (de Jong et al, 1974). The latter author's conclusions must be related to their small numbers of patients whilst Aedo and his colleagues by the design of their patient selection did not study the situation during either maximal follicular or luteal activity.

The results presented here show quite clearly that during both proliferative and secretory stages of the cycle the active ovary is a major source of A production. In addition small amounts of A are also secreted by the inactive ovary particularly during the MS phase. Presumably the ovarian stroma is responsible for this A secretion by the contralateral ovary (Rice and Savard, 1966) and the biosynthetic capacity of that compartment for production of A is insignificant compared to that of the corpus luteum(Savard et al, 1965).

Despite the high levels of A observed in active ovarian venous plasmas and the cyclical changes therein the levels found in peripheral plasmas (both normal volunteers and patients) were relatively low and showed no distinct luteal phase peak. This suggests that rapid metabolism of a large percentage of the ovarian A must occur either in other organs or in peripheral tissues. A is a major prehormone for T (Ito and Horton, 1971) in women and has also been shown to be converted into

OE_1 in many peripheral tissues (Longcope 1971) especially adipose tissues (Nimrod and Ryan, 1975).

(H) DIHYDROTESTOSTERONE

The levels and patterns of DHT measured in the peripheral plasma of women undergoing hysterectomy (Figure 3.26) were similar to those seen in peripheral plasma samples from normally menstruating volunteers (Figure 3.5).

Dihydrotestosterone levels in plasmas from veins draining active ovaries were higher than those in corresponding peripheral plasma samples at all six stages of the menstrual cycle; maximal differential being observed in the MS phase when active ovarian vein levels were 6 times higher than peripheral plasma levels. The presence of 5α reductase activity in ovarian tissue ; previously established in vitro (Smith, Ofner and Verra, 1974), has been confirmed by these results. Since T, the substrate for the reductase enzyme, is largely synthesised from A in women (Ito and Horton, 1971) the DHT elevation in the MS phase is probably a reflection of the increased A production (Figure 3.25). In contrast to the DHT results reported in this thesis Calabresi et al (1976) reported no such DHT increase in the venous effluent of the active ovary during the luteal phase. Analysis of that author's data using a paired t test revealed, however, that Calabresi's findings were different from his conclusions (DHT $>$ in active ovary than in peripheral plasma; $p < 0.001$)! Serio et al (1976) also showed that during the follicular phase DHT levels were higher in active ovarian venous plasmas than in contralateral and/or peripheral plasmas but Aedo et al (1980 a; 1980 b) who sampled only in the LP and ES stages concluded that the levels of DHT were identical in peripheral and both ovarian venous plasmas.

In the present series DHT levels in contralateral ovarian venous plasmas where estimated were identical to or slightly higher than those in corresponding peripheral plasmas; the elevations were significant in the MS phase when A levels in contralateral plasmas were also significantly higher than those in peripheral plasmas.

These results confirm the ability of human ovarian tissue to produce DHT and suggest that significant DHT production only occurs when production of the precursor A is also increased.

(I) TESTOSTERONE

The profiles of T levels found in peripheral plasmas of women at hysterectomy (Figure 3.18) were similar to those determined in normally menstruating volunteers(Figure 3.6) showing a peak at mid-cycle and a further rise during the luteal phase. The elevation in peripheral plasma in the ES phase of the cycle was in agreement with the report of Aedo et al (1980 b).

Plasmas obtained from veins draining active ovaries contained higher levels of T than did corresponding peripheral plasmas at all 6 stages of the menstrual cycle, the elevations being significant in MP, MS and LS stages.

The highest levels of T in active ovarian venous plasmas were found during the LP and MS stages and at both of these stages there were great variations in the levels amongst the patients who composed the group. The LP peak of T values observed in the active ovarian veins (Figure 3.27) was in agreement with the observations during the normal menstrual cycle (Figure 3.6) but was different from the pattern

observed for A in the active ovarian vein suggesting that if A is the major prehormone for T in the human female (Ito and Horton, 1971) the extent of the conversion must vary considerably from patient to patient. This LP rise in T levels was coincident with a fall in active ovarian venous plasma OE_2 levels after the follicle had reached maturity (Figure 3.24). The levels of T observed might therefore reflect a compromise between conversion of the prehormone A and further metabolism.

The MS peak of T levels in active ovarian venous plasmas (6 times higher than peripheral plasma levels) was in agreement with the already described MS peaks of both A and DHT and adds credence to the suggestion that the rises occurring during the MS phase are due to increased synthesis by the corpus luteum of A which functions as a prehormone for T which in turn is converted to DHT.

Only very few samples were available for determination of T levels in venous plasmas draining contralateral ovaries. In the MS phase where a number of samples were available T levels were significantly higher in contralateral ovarian venous plasmas than in corresponding peripheral plasmas. Since similar increases in the contralateral ovarian venous plasma levels of A and DHT in the MS phase were reported earlier these increased levels of T might have been expected confirming that the three C.19 androgen levels in ovarian venous plasmas are closely inter–related.

Previous reports have indicated T production in approximately equal amounts by both active and inactive ovaries (Mikhail, 1970; de Jong et al, 1974; Aedo et al, 1980 a; 1980 b) whilst in agreement with

the present study Calabresi et al (1976); Serio et al (1976) and McNatty et al (1976) showed higher levels of T in the blood emanating from active ovaries than in that draining contralateral ovaries.

These results show that T is produced by both ovaries but in larger amounts by the active ovary. Presumably as described earlier for A the ovarian stroma in both ovaries produces T whilst during the times of maximal follicular and luteal steroid biosynthesis a further increment is added to the output of the active ovary.

(ii) Hormone Levels in Active Ovarian Venous Plasma, Contralateral Ovarian Venous Plasma and Peripheral Plasma from the same Patient

Where plasma samples were available from all three venous sites – active ovary, contralateral ovary and peripheral – Table 3 XXVII similar findings were observed to those described above for the mean data from all of the patients. The active ovarian venous plasmas were associated with significantly elevated levels of P and 17OHP at all stages except EP but particularly in MS, and of OE_2, A and DHT throughout the cycle. In the case of T the situation was more variable and less clear but the levels in the active ovarian venous plasma were usually higher than the corresponding peripheral plasmas. Contralateral ovarian venous plasmas were associated with increased levels compared with peripheral plasma of OE_2 but not of P and 17OHP. The levels of C.19 androgens coming from the contralateral ovarian veins although not significantly higher than peripheral plasma levels in this small series of patients were higher than the latter and suggestive that the contralateral ovary produces small amounts of these C.19 androgens.

The similarity of the conclusions from these patients who provided samples from all three venous sites and those from the mean data of all of the patients (V 4i) show that it is valid to draw conclusion with regard to ovarian production of steroids from the mean data.

(iii) Hormone Production by the Human Ovary

During the normal menstrual cycle the Graafian follicle is primarily responsible for production of OE_2 during the MP and LP stages. In addition the growing follicle also produces small amounts of P, 17OHP, A, T and DHT. After ovulation and formation of the corpus luteum that structure becomes the main producer of steroids and is responsible for secretion of large amounts of P, 17OHP and A; for maintaining OE_2 production at slightly lower levels than during the time of maximal follicular activity and for production of increased levels of DHT and T.

The contralateral ovary is not inactive but produces small amounts of OE_2 and the three C.19 androgens.

A study such as that presented in this thesis cannot give information with regard to which cellular type within the Graafian follicle or the corpus luteum is responsible for synthesis of steroid hormones. However, present work from a number of laboratories suggest that the follicular theca cells are responsible for the biosynthesis of 17OHP, C.19 androgens and some oestrogen during the follicular phase (Gaukroger 1979) whilst the follicular granulosa cells synthesize P per se and convert thecal C.19 androgens to oestrogen (Coutts et al 1981). The luteal granulosa cells synthesize P and 17OHP whilst theca lutein cells are probably responsible for production of C.19 androgens and oestrogens (Macnaughton et al 1981).

The site of production of OE_2 and androgens by the contralateral ovary requires further elucidation but is most probably the combined efforts of thecal cells of small follicles and ovarian stroma.

(5) HORMONE LEVELS IN PERIPHERAL PLASMAS AND OVARIAN FLUIDS OBTAINED AT HYSTERECTOMY FROM PATIENTS WHO HAD ABNORMAL CYCLES

(i) Polycystic Ovarian Disease

On macroscopic and microscopic examination of the ovaries after hysterectomy and blood sampling two patients were found to have had polycystic ovarian disease. Unlike many patients with polycystic ovarian disease (Van Look 1981) these patients showed ovulatory cycles and were assessed as being in the MS and LS phases respectively. In patients with polycystic ovarian disease elevated circulating levels of LH are found (McConway et al, 1981) which are apparently the cause of the thecal luteinisation responsible for the typical appearance of the ovaries. One of the patients, who was in the MS phase of the cycle, showed elevated levels of LH (Figure 3.29) at a time when such elevations do not occur in normal women (Figure 3.11). In these 2 patients the levels of all steroid hormones measured were higher in the plasmas from veins draining the contralateral ovaries than in the corresponding peripheral plasma samples. Although these patients were not clinically hirsute (a condition sometimes present in association with polycystic ovarian disease) they showed high levels of androgens in their ovarian venous effluents. This elevation of androgens was particular marked in the case of A levels in the contralateral ovarian veins especially of the MS phase patient (Figure 3.29). These results support the hypothesis

that in such patients the increased androgens present in the peripheral circulation are ovarian in origin (Van Look 1981).

(ii) Patient with Primary Infertility and Hirsutism

One patient with a history of primary infertility and hirsutism was sampled at laparotomy for removal of an ovarian tumour. Samples were obtained of peripheral and active (tumour containing) ovarian venous plasma and of tumour fluid. As far as could be assessed the patient was in the LP stage of the cycle and this was confirmed by the levels of hormones in the peripheral plasma (Figure 3.31). The levels of all steroid hormones were higher in the plasma from the vein draining the active ovary than in the peripheral plasma. In this patient the levels of OE_2 and A were considerably higher in both peripheral and active ovarian vein plasmas than those observed in patients at the same stage of normal cycles (Figures 3.15 and 3.16). Determination of the hormone levels in the tumour fluid showed very high concentrations of the steroid hormones – OE_2, A, DHT and T. Presumably the elevations of OE_2 and A observed in peripheral and active ovarian venous plasmas would at least in part have been due to secretion by the tumour.

The secretions of steroid hormones observed in this patient might explain her symptomatology. The elevated levels of androgens would be responsible for her hirsutism whilst the high circulating concentrations of OE_2 could exert a contraceptive type effect on the ovarian/pituitary/hypothalamic axis and as a result might prevent normal ovulation and produce infertily.

VI) Hormone Levels in Samples Obtained at Hysterectomy from Patients Who were not Cycling Normally

(i) Patients who were taking oral contraceptive therapy

In this study peripheral and ovarian venous samples were obtained from three patients who had been taking combined oestrogen/ progestogen oral contraceptives until a few days before the operation. These patients were operated on day 5 of the cycle (i.e. during the seven days when pills were not being taken); day 14 of the cycle but who had stopped taking the pill for four days prior to surgery and day 24 of the cycle. In addition to these three patients samples were also obtained from a fourth patients who had been on oral contraceptives for ten years until four months prior to surgery. The results of the hormone levels in the plasma samples from the patients who had currently been taking contraceptive therapy offer considerable insight into the processes which occur at the ovarian level during steroid contraception.

In patient A.C. (Figure 3.32), sampled on day 5 of the cycle, the hormone profiles obtained in the peripheral and active ovarian venous plasmas were consistent with the EP phase of the cycle – peripheral levels of steroids were basal, in the presence of elevated levels of gonadotrophins, especially FSH. The levels of the steroid hormones (OE_2, A, T and DHT) in the ovarian venous effluent were much higher than those observed in normally cycling patients.

In A.W. (Figure 3.33) who was on day 14 of the cycle the hormone patterns observed were consistent with her being in the MS phase of the cycle. This was confirmed by the presence of a corpus luteum and the steroid hormone levels in the plasma from the vein draining the active ovary being highest with respect to OE_2, P and A. The levels of steroid hormones found in the plasma from the vein draining the contralateral ovary were higher than those found from normally

menstruating patients at the same stage of the cycle (RESULTS section). Indeed the levels of 17OHP, DHT and T in the contralateral ovarian venous plasma were greater than those in the active ovarian venous plasma.

Hormone levels in patient C.E. (Figure 3.34) sampled on day 24 of the cycle showed that ovulation had not occurred. The ovaries were notable for the presence of many atretic follicles, particularly in the 'active' ovary. Although P levels were low, in the peripheral and in both the ovarian venous plasmas, samples from the vein draining the active ovary contained elevated levels of OE_2, 17OHP and A. Contra-lateral ovarian vein levels were basal for all 6 steroid hormones.

These result might be interpreted as showing that the following sequence of events occurred at the ovarian levels.

(a) During the period when the 'pill' was not taken a normal wave of follicular growth occured producing growing follicles.

(b) When the contraceptive steroids were then taken, the growth of these follicles was arrested but they appeared to remain dormant for a period of time.

(c) When the 'pill' was not taken for any reason at around the time of mid–cycle at least one of these follicles was capable of further growth and ovulation producing a corpus luteum.

(d) When the 'pill' was taken throughout the period, including mid–cycle, the follicles in which growth had been arrested, became atretic and the cycle was anovulatory.

This sequence of events explains why women on the 'pill' do not experience symptoms of oestrogen lack since they are producing OE_2 during the EP stage. This sequence of events also indicate that it is of paramount importance that the 'pill' be taken every night especially around the time of mid–cycle to ensure that ovulation does not occur.

These patients who were taking oral contraceptive therapy had elevated levels of some steriods in the contralateral ovarian veins. In fact, these patients resembled the two patients with polycystic ovarian disease described earlier with respect to contralateral ovarian vein steroid levels. This suggests that perhaps the small follicles showing excessive thecal luteinisation in polycystic ovarian disease patients and the atretic follicles found in 'pill' patients were similar functional units.

In addition, large (>2 cm) retention cysts were found in the ovaries of two of those patients A.W. and C.E. and also in patient J.H. who had stopped oral contraceptive therapy four months prior to operation after exposure for more than ten years.

Determination of the levels of steroid hormones in the fluids aspirated from these retention cysts showed very high levels of all six steroid hormones studied (Figure 3.35). The levels of these hormones in the cyst fluid from the patient who was four months post–oral contraceptive therapy were much lower than those in the 2 current 'pill' patients. Although these retention cysts are obviously not actively secreting their hormones into the general circulation (otherwise the peripheral levels would have been considerably higher) their existence and steroid content may account for the elevated levels of steroid hormones observed in the contralateral ovarian veins of these patients. It is of interest that these retention cyst fluids contained the highest

levels of androgens found in this study. Since excessive androgens have been linked in the female with lack of libido the presence of such cysts could explain why some women experience lack of libido as a side effect of oral contraceptive therapy.

Whist it must be remembered that these patients attended for surgery for gynaecological complaints and may not therefore, be entirely normal 'pill' patients these results give some insight into the mechanism of action of the 'pill'.

(ii) Early Pregnancy

In the one patient who presented for hysterectomy six weeks pregnant, plasma samples were obtained from the vein draining the ovary containing the corpus luteum and the peripheral vein. In this patient (Figure 3.36) high levels of HCG/LH, OE_2,P, and 17OHP were measured in peripheral and ovarian venous plasmas, confirming that at this stage of pregnancy the ovary is very active. The levels of hormones at the ovarian level were at least as high as those observed during the MS stage of the cycle. This finding adds further support to the well–established concept that early human pregnancy is maintained by the secretions of the corpus luteum (Csapo, Pulkinnen and Kaihola 1974).

(iii) Post–Menopausal Patients

Samples were obtained from two post–menopausal patients. One patient presented for hysterectomy for post–menopausal bleeding and had typical small atrophic ovaries. Peripheral and ovarian vein hormone levels in this patient (Figure 3.37) showed elevated gonadotrophin levels and very low levels of all six steroid hormones. The levels of the steroids in the ovarian venous plasma sample being less than the

corresponding peripheral plasma levels for all hormones.

The second post–menopausal subject who presented for surgery because of an abdominal swelling, was found to have one small white, streaky, typically atrophic, post–menopausal ovary while the other ovary contained a 60 cm benign mucous cyst. Hormone levels were determined in peripheral plasma and plasma from a vein draining the ovary containing the large cyst (Figure 3.38). As in the first post–menopausal patient gonadotrophin levels were very high. The peripheral plasma steroid levels were low and consistent with menopausal levels (Barlow et al 1981) but in some cases were higher than those observed in the first patient. The levels of all six steroid hormones in contrast to the situation in the first menopausal patient were higher in the ovarian venous plasma than in the peripheral sample, suggesting that, although benign, this cyst may have been responsible for a small amount of steroid biosynthesis.

Since there is great variability amongst post–menopausal patients with regard to the incidence of post–menopausal symptoms and this can be related to circulating OE_2 levels (Coutts 1976) it is of interest that the presence of such ovarian growth could alleviate an oestrogen lack.

CONCLUSIONS

The work presented in this thesis has led to the following conclusions.

(1) Specific, sensitive, accurate and precise radioimmunoassays have been developed for A, DHT and T. The assay for A was performed without chromatography, whilst the methods for DHT and T utilised chromatographic separation of the two steroids prior to separate determinations.

(2) Using these assays profiles of the three C19 androgens have been determined in serial daily peripheral plasma samples throughout the normal human menstrual cycle.

(3) Blood samples were obtained in patients at hysterectomy from a peripheral vein and where possible veins draining both ovaries. Samples were obtained (bilaterally where possible) from 54 patients of whom 42 were undergoing surgery for conditions which did not affect their reproductive functions. In 40 of these "normally cycling" patients, the stage of the menstrual cycle was accurately assessed by reference to, ovarian and endometrial histology, menstrual history, and peripheral plasma hormone levels. In all patients if possible the active ovary was diagnosed by visualisation and by ovarian histology. From the 40 normally cycling women, a composite menstrual cycle was constructed.

(4) The levels of LH, FSH, PRL, P, 170HP, OE_2, A, DHT and T were assayed in all of the ovarian venous samples – active and contralateral – and were compared and contrasted with those in the corresponding peripheral plasma samples.

(5)(a) From the results of the determinations in para (4) above on the samples in the composite menstrual cycle assessments have been made of the contribution of both ovaries to the circulating levels of these steroid hormones throughout the menstrual cycle.

(b) These data show that the active ovary at all stages of the cycle is responsible for steroid production. The Graafian follicle produces large amounts of OE_2 and smaller amounts of P, 170HP and the C19 androgens whereas the corpus luteum produces large quantities of P, 170HP, A and OE_2 and lesser amounts of T and DHT.

(c) The data also show that the contralateral ovary is not "inactive" but produces small significant amounts of OE_2 and the C19 androgens.

(6) The patients in whom peripheral and ovarian venous samples were obtained at surgery but who did not fit the criteria of "normally cycling" women included patients with abnormal cycles, patients using oral contraceptives a pregnant patient and post-menopausal patients. From the levels of hormones in their ovarian and peripheral venous plasmas and in fluids aspirated from ovarian cystic structures, if present, information has been obtained which aids understanding these conditions.

FUTURE WORK

(A) The results presented in this study have confirmed that the active ovary (containing either the Graafian follicle or the corpus luteum) synthesises a number of steroid hormones. It would be of interest to extend this study to determine;–

(i) the true ovarian secretion rate of the hormones, by attempting to measure ovarian blood flow.

(ii) the cellular origin of each of the hormones. This might possibly be performed by in vitro studies with pure, isolated cell preparations either by incubation or cell culture as is already being performed in a few centres. Another possible source of this information, in vitro, might arise if ovarian vein samples could be obtained from ovaries containing tumours of only one cellular type (e.g. granulosa cell tumours, thecoma.)

(B) The result presented in this thesis also showed that the contralateral ovary at certain stages of the cycle produced small amount of steroid hormones. It would be of interest to determine whether these were the products of the ovarian stroma or of the crop of small growing follicles which have been reported to be present in the contralateral ovary (Govan and Black, 1981)

(C) It would also be of interest to extend these preliminary studies presented in this thesis of the ovarian hormonal production in patients.

(i) on oral contraceptive therapy
(ii) with polycystic ovarian disease.
(iii) who are peri/postmenopausal.

REFERENCES

ABRAHAM,G.E. (1974). Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. Journal of Clinical Endocrinology and Metabolism, **39**, 340–346.

ABRAHAM,G.E., ODELL,W.D., SWERDLOFF,R.S. & HOPPER,K. (1972). Simultaneous radioimmunoassay of F.S.H., progesterone, 17α hydroxyprogesterone and estradiol–17ß during the menstrual cycle. Journal of Clinical Endocrinology and Metabolism, **34**, 312–318.

AEDO,A–R., PEDERSEN,P.H., PEDERSEN,S.C. and DICZFALUSY,E. (1980a). Ovarian steroid secretion in normally menstruating women I. The contribution of the developing follicle. Acta Endocrinologica, **95**, 212–221.

AEDO,A–R., PEDERSEN,P.H., PEDERSEN,S.C. and DICZFALUSY,E. (1980b). Ovarian steroid secretion in normally menstruating women II. The contributon of the corpus luteum. Acta Endocrinologica, **95**, 222–231.

BAIRD,D.T. & FRASER,I.S. (1974). Blood production and ovarian secretion rates of oestradiol and oestrone in women throughout the menstrual cycle. Journal of Clinical Endocrinology and Metabolism, **38**, 1009–1017.

BAIRD,D.T. and FRASER,I.S. (1975). Concentration of oestrone and oestradiol in follicular fluid and ovarian venous blood of women. Clinical Endocrinology, **4**, 259–266.

BAIRD,D.T., BURGER,P.E., HEAVON–JONES,G.D. & SCARAMUZZI,R.J. (1974). The site of secretion of androstenedione in non–pregnant women. Journal of Clinical Endocrinology, **63**, 201–212.

BARLOW,D.H., MACNAUGHTON,M.C., MOWAT,J. and COUTTS,J.R.T. (1981). Hormone profiles in the menopause. In "The functional morphology of the human ovary". Ed. Coutts, J.R.T. pp 223–234 MTP. Press Lancaster:

BARTELMEZ,G.W. (1933). Histological studies on the menstruating mucous membrane of the human uterus. Contributions to Embryology, **142**, 143–186. (Carnegie Institution of Washington Publication No. 443).

BAULIEU,E.E. (1973). Elements of a forward approach to the mechanism of androgen action. In "The Endocrine Function of the Human Testis", Eds. James, V.H.T., Serio, M. and Martini, L.I, pp 149–156. Academic Press, New York.

BOYAR,R.,PERLOW,M., HELLMAN,L. KAPEN,S. and WEITZMAN ,E. (1972). Twenty–four hour pattern of L.H. secretion in normal men with sleep stage recording. Journal of Clinical Endocrinology and Metabolism, **35**, 73–81.

BRAY,G.A. (1960) A simple efficient liquid scintillation method for counting aqueous solutions in a liquid scintillation counter. Analytical Biochemistry. **1**, 279–282.

CALABRESI,E., FIORELLI,G., FORTI,G., LOMBARDI, G., PAZZAGLI,M., DELL'ACQUA,E., and SERIO,M. (1976). Dihydrotestosterone in human ovarian venous plasmas. Acta Endocrinologica, **82**, 380–387.

CALDWELL,B.V. (1971) Personal Communication to J.R.T. Coutts.

COUTTS,J.R.T. (1976). Hormone production by the ovary. Clinics in Obstetrics and Gynaecology, **3**, 63–87.

COUTTS,J.R.T., GAUKROGER,J.M., SAMAD,A.K. and MACNAUGHTON,M.C. (1981). Steroidogenesis by the human Graafian follicle in "The functional morphology of

the human ovary" Ed. Coutts, J.R.T. pp53–72 MTP. Press Lancaster:

COYOTUPA,J., PARLOW,A.F. and ABRAHAM,G.E. (1972). Simultaneous radioimmunoassay of plasma testosterone and dihydrotestosterone. Analytical Letters, **5,** 329–340.

CSAPO,A.I., PULKINNEN, M.O. and KAIHOLA,H.L. (1974) The relationship between the timing of luteectomy and the incidence of complete abortions. American Journal of Obstetrics and Gynecology. **118,** 985–989.

DEANE,H.W., LOBEL,B.L. & ROMNEY,S.L. (1962). Enzymic histochemistry of normal human ovaries of the menstrual cycle, pregnancy and the early puerperium. American Journal of Obstetrics and Gynecology, **831,** 281–294.

DE JONG,F.H., BAIRD,D.T. and VAN DER MOLEN, H.J. (1974). Ovarian secretion rates of oestrogens, androgens and progesterone in normal women and in women with persistent ovarian follicles. Acta Endocrinologica (Copenhagen) **77,** 575–587.

DELL'ACQUA,S., SERRA,G.B., LUCISANO,A., MONTEMURRO,A. CINQUE,B., ARNO,E. and BOMPIANI,A. (1976) Release of C_{21}, C_{19} and C_{18} steroids by human ovaries stimulated in vivo by HCG. In "The Endocrine Function of the Human Ovary", Eds. James, V.H.T., Serio, M. and Giusti, G. pp 427–436. Academic Press, London.

DODSON,K.S., COUTTS,J.R.T. and MACNAUGHTON,M.C. (1975). Plasma sex steroids and gonadotrophin patterns in human menstrual cycles. British Journal of Obstetrics and Gynaecology, **82,** 602–614.

DORFMAN,R.I. & UNGER,F. (1965). Metabolism of Steroid Hormones. New York: Academic Press.

DUPON,C., HOSSEINIAN,A.H. and KIM,M.H. (1973). Simultaneous determination of plasma oestrogens, androgens, and progesterone during the human menstrual cycle. Steroids, **22**, 47–61.

EXLEY,D., JOHNSON,M.W. and DEAN,P.D.G. (1971). Antisera highly specific for 17ß oestradiol. Steroids, **18**, 605–620.

FALCK,B. (1959). Site of production of oestrogen in rat ovary as studied in microtransplants. Acta Physiologica Scandinavica, **47**, Supplement 163, 1–101.

FOURNIER,P.J.R., DESJARDINS,P.D., FRIESEN,H.G. (1974). Current understanding of human prolactin physiology and its diagnostic and therapeutic application. A review. American Journal of Obstetrics and Gynecology, **18**, 337–343.

FRANCHIMONT,P., DOURCY,C., LEGROS,J.J., REUTER,A., VRINDTS-GEVAERT,Y., VAN LAUWENBERGE,J.R. and GASPARD,U. (1975). Prolactin levels during the menstrual cycle. Clinical Endocrinology, **5**, 643–650.

FRANKEL,L. (1909) Die physiologischen und pathologischen bezie Lungen zwischen ovarien und uterus. Ztschr.f.artzl. Fortbild. (Jena) **6**, 65–69.

FRÖLICH,M., BRAND,E.C., VAN HALL,E.V. (1976). Serum levels of unconjugated aetiocholanolone, androstenedione, testosterone, dehydroepiandrosterone, aldosterone, progesterone and oestrogen during the normal menstrual cycle. Acta Endocrinologica, **81**, 548–562.

GANDY,H.M., & PETERSON,R.E. (1968). Measurement of testosterone and 17-ketosteroids in plasma by the double isotope

dilution derivative technique. Journal of Clinical Endocrinology, **28**, 949–977.

GAUKROGER,J.M. (1979). Steroidogenesis by isolated human ovarian cells. Ph.D. Thesis. University of Glasgow.

GOVAN,A.D.T. (1968). The human ovary in early pregnancy. Journal of Endocrinology, **40**, 421–428.

GOVAN,A.D.T. and BLACK,W.P. (1981). Follicular development in the first half of the human menstrual cycle in "The Functional Morphology of the Human Ovary" Ed. Coutts,J.R.T. pp37–52. MTP. Press Lancaster.

GUERRERO,R., ASO,T., BRENNER,P.F., CEKAN,Z. LANDGREN, B.M., HAGENFELDT,K. DICZFAULSY,E. (1976). Studies on the pattern of circulating steroids in the normal menstrual cycle. Acta Endocrinologica, **81**, 133–149.

GURAYA,S.S. (1971). Morphology, histochemistry and biochemistry of human ovarian compartments and steroid hormone synthesis. Physiological Reviews, **51**, 785–807.

HAMMERSTEIN,J., RICE,B.F. & SAVARD,K. (1964). Steroid hormone formation in the human ovary. I. Identification of steroids formed in vitro from acetate $1-^{14}C$ in the corpus luteum. Journal of Clinical Endocrinology and Metabolism, **24**, 597–605.

HERBERT,V., GOTTLIEB,C., LAU,K.S. and WASSERMAN,L.R. (1964). Intrinsic–factor assay. Lancet, **2**, 1017–1018.

HITSCHMANN,F. and ADLER,L. (1908) Der bau der uternsschleim haut des geschlechtsreifen weibes mit besonderer berucksichtigung der menstruation. Monatschr.f. Geburtsh,u. Gynak. **27**, 1–82.

HORTON,R., ROMANOFF,E. and WALKER,J. (1966). Androstenedione and testosterone in ovarian venous and peripheral plasma during ovariectomy for breast cancer. Journal of Clinical Endocrinology, **26**, 1267–1269.

ITO,T. and HORTON,R. (1971) The source of plasma dihydrotestosterone in man. Journal of Clinical Investigation. **50**, 1621–1627.

JACOBS,J.B. (1969). Selective gonadal venography, Radiology, **92**, 885–888.

JUDD,H.L. & YEN,S.S.C. (1979). Serum androstenedione and testosterone levels during the menstrual cycle. Journal of Clinical Endocrinology and Metabolism, **36**, 475–481.

KIRSCHNER,M.A. and JACOBS,J.B. (1971). combined ovarian and adrenal vein catheterization to determine the site(s) of androgen overproduction in hirsute women. Journal of Clinical Endocrinology, **33**, 199–209.

KIRSCHNER,M.A., ZUCKEV,I.R. and JESPERSEN,D. (1976). Ovarian and adrenal vein catheterisation studies in women with idiopathic hirsuitism. In "Endocrine function of the human ovary". Ed(s) James,V.H.T. Serio,M. and Giusti,G. pp 443–456. Academic Press London:

LLOYD,C.K.W., LOBOTSKY,J., BAIRD,D.T., McCRACKEN,J.A., WEISZ,J., PUPKIN,M., ZANARTU,J. and PUGA,J. (1971). Concentration of unconjugated estrogens, androgens and gestogens in ovarian and peripheral venous plasma of women, during the normal menstrual cycle. Journal of Clinical Endocrinology and Metabolism. **32**, 155–166.

LONGCOPE,C. (1971). The significance of steroid production by peripheral tissue. In"Endocrinology of the ovary". Ed.

Scholler, R. p.23 Proceedings of the International Symposium, Paris–Fresnes, France.

LUCISANO,A., DELL'ACQUA,S., MONTEMURRO,A., ROSETTI,A., CINQUE,B., LAFUENTI,G., ARMO,E. and BOMPIANI,A. (1978). Peripheral and ovarian vein plasma levels of 20 dihydroprogesterone in women with normal menstrual cycle. Journal of Steroid Biochemistry; **9**, 643–648.

MAYES,D. and NUGENT,C.A. (1968). Determination of plasma testosterone by the use of competitive protein binding. Journal of Clinical Endocrinology and Metabolism, **28**, 1169–1176.

McCONWAY,M.G., ENGLAND,P., BLACK,W.P., MACNAUGHTON M.C., GOVAN,A.D.T., and COUTTS,J.R.T. (1977). Hormone profiles and ovarian morphology in women with primary oligomenorrhoea. Journal of Endocrinology, **72**, 52p.

McCONWAY,M.G., ENGLAND,P., BLACK,W.P., MACNAUGHTON ,M.C., GOVAN,A.D.T. and COUTTS,J.R.T. (1981). Ovarian morphology and hormone proflies in women with primary oligomenorrhoea in "The functional morphology of the human ovary". Ed. Coutts,J.R.T. pp178–184 MTP Press Lancaster.

MACNAUGHTON,M.C., SAMAD,A.K., GAUKROGER,J.M. and COUTTS,J.R.T. (1981). Steroid hormone production by the human corpus luteum in "The functional morphology of the human ovary". Ed. Coutts,J.R.T. pp137–156 MTP. Press Lancaster.

McNATTY,K.P., HUNTER,W.M., McNEILLY,A.S. & SAWYERS, R.S. (1975). Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human graafian follicles throughout the menstrual cycle. Journal of

Endocrinology, **64**, 555–571.

McNATTY,K.P., BAIRD,D.T., BOLTON,A., CHAMBERS,P., CORKER,C.S. and McLEAN,H. (1971). Concentration of oestrogen and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. Journal of Endocrinology, **71**, 77–85.

McNEILLY,A.S. and CHARD,T. (1974). Circulating levels of prolactin during the menstrual cycle. Clinical Endocrinology, **3**, 105–112.

MEYER,R. (1920) Die anomalien der follikel und corpus luteum bildung im Zusammenhang mit den uterusanomalien. Ztschr. f. Geburtsh,u. Gynak. Stuttg., **33**, 842–843.

MIKHAIL,G. (1970). Hormone secretion by the human ovaries. Gynecological Investigation, **1**, 5–20.

MISHELL;D.R., NAKAMURA,R.M., CROSIGNANI,P.G., STONE,S., KHARMA,K., NAGATA,Y. & THORNEYCROFT,I.H. (1971). Serum gonadotrophin and steroid patterns during the normal menstrual cycle. American Journal of Obstetrics and Gynecology, **111**, 60–63.

MURPHY,B.E.P. (1967). Some studies of the protein binding of steroids and their application to the routine micro and ultra micro measurement of various steroids in body fluids by competitive protein binding radioassay. Journal of Clinical Endocrinology and Metabolism, **27**, 973–990.

NIMROD,A., and RYAN,K.J. (1975). Aromatisation of androgen by human abdominal and breast tissue. Journal of Clinical Endocrinology and Metabolism, **40**, 367–375.

NOVAK,E. (1915) A study of the relation between the degree of menstrual reaction in the endometrium and the clinical

character of menstruation. Surgical Gynecology and Obstetrics **21**, 336–354.

NOYES,R.W., HERTIG,A.T. and ROCK,J. (1950). Dating the Endometrial Biopsy. Fertility & Sterility, **1**, 3–25.

OLEARY,J.L. (1929). Changes in human uterine gland during menstrual cycle and in early pregnancy. American Journal of Anatomy, **43**, 289–346.

PATWARDHAN,V.V. & LANTHIER,A. (1969). A comparative study of the in vitro metabolism of C^{19} neutral steroids by the follicular and stromal compartments of normal human ovaries. Journal of Clinical Endocrinology and Metabolism, **29**, 1335–1340.

RAO,P.N. and MOORE,P.H.Jr.(1976). Synthesis of new steroids haptens for radioimmunoassay. Part I 15ß–carboxy–ethylmercapto testosterone–bovine serum albumin conjugate. Measurement of testosterone in male plasma without chromatography. Steroids **28**, 101–113.

RICE,B.P. & SAVARD,K. (1966). Steroid hormone formation in the human ovary. IV Ovarian Stromal compartment; formation of radioactive steroids from acetate – 1–^{14}C and action of gonadotrophins. Journal of Clinical Endocrinology and Metabolism, **26**, 593–609.

RIVAROLA,M.A., SAEZ J.M., JONES,H.W.jr., JONES,G.S., MIGEON,C.J. (1967). The secretion of androgens by the normal, polycystic and neoplastic ovaries. John Hopkins Medical Journal **121**, 82–90.

ROBYN,C., DELVOYE,P., NOKIN,J., VEKEMANS,M., BADAWI, M., PEREZLOPEZ,F.R., and L'HERMITE,M.L. (1973). Prolactin and human reproduction. In "Human prolactin"

Eds. J.C. Paskelas & C. Robyn: I.C.S. No. **308,** 167–188. Amsterdam, Excerpta Medica Foundation.

ROBYN,C., DELVOYE,P., VANEXTER,C., VEKEMANS,M., CAUFRIEZ,A., DENAYER,P., DELOGNE-DESNOECK,J., L'HERMITE,M. (1976). Physiological and pharmacological factors influencing prolactin secretion and their relation to human reproduction. In "Prolactin and human reproduction." Eds. Crosignani,P.G. and Robyn, C. p.71. Academic Press, London, 1976.

ROBYN,C. and VEKEMANS,M. (1976). Influence of low dose estrogen on circulating prolactin, LH and FSH levels in post-menopausal women. Acta Endocrinologica **83**, 9–14.

ROCK,J. (1949). Physiology of human conception New England Journal of Medicine **240**, 804–812.

ROLLAND,R., HAMMOND,J.M., SCHELLEKENS,L.A., LEQUIN, R.M. and DE JONG,F.H. (1976) Prolactin and ovarian function, In "The Endocrine Function of the Human Ovary", Eds. James,V.H.T., Serio,M. and Giusti,G. pp. 305–321, Academic Press, London.

ROSS,G.T., CARGILLE,C.M., LIPSETT,M.B., RAYFORD,P.L., MA MARSHALL,J.R., STROTT,C.A. & RODBARD,D. (1970). Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. Recent Progress in Hormone Research, **26**, 1–62.

RYAN,K.J. & PETRO,Z. (1966). Steroid biosynthesis by human ovarian granulosa and thecal cells. Journal of Clinical Endocrinology and Metabolism, **26**, 46–52.

RYAN,K.J., PETRO,Z. & KAISER,J. (1968). Steroid formation by isolated and recombined ovarian granulosa and thecal cells.

Journal of Clinical Endocrinology and Metabolism, **28**, 355–359.

SAVARD,K., MARSH,J.M. & RICE,B.F. (1965). Gonadotrophins and ovarian steroidogenesis. Recent Progress in Hormone Research, **21**, 285–365.

SCHRÖDER,R. (1915). Anatomische Studien zor normalen and pathologischen physiologic des menstruationszyklus. Archiv fier Gynakologie, **104**, 27–102.

SELYE,H. (1947). Textbook of Endocrinology–Universite de Montreal. Ed. Selye,H.

SERIO,M., DELL'ACQUA,S., CALABRESI,E., FIORELLI.G.,FOLTI, G., CATTANEO,S.,LUCISANO,A., LOMBARDI,G., PAZZAGLI,M. and BORRELLI,D. (1976). Androgen secretion by the human ovary: measurement of androgens in ovarian venous blood. In "The Endocrine Function of the Human Ovary" Eds. James,V.H.T., Serio,M., and Giusti,G. pp471–479. Academic Press. London.

SHAABAN,M.M. & KLOPPER,A. (1973). Plasma oestradiol and progesterone concentrations in the normal menstrual cycle. Journal of Obstetrics and Gynaecology of the British Commonwealth, **80**, 776–782.

SHORT,R.V. (1962). Steroids in the follicular fluid and the corpus luteum of the mare. A " two cell type " theory of ovarian steroid synthesis. Journal of Endocrinology, **24**, 59–63.

SMITH,O.E., OFNER,P. and VERRA,R.I. (1974). In vitro conversion of testosterone $-4^{14}C$ to androgens of the 5α androstane series by a normal human ovary. Steroids **24**, 311–315.

SOMMA,M., SANDOR,T. & LANTHIER,A. (1969). Site of origin of androgenic and estrogenic steroids in the normal human ovary. Journal of Clinical Endocrinology and Metabolism, **29**, 457–466.

STROTT,C.A., YOSHIMI,T., ROSS,G.T. and LIPSETT,M.B. (1969). Ovarian physiology; relationship between plasma LH and steroidogenesis by the follicle and corpus luteum: effect of HCG. Journal of Clinical Endocrinology and Metabolism, **29**, 1157–1167.

THORNEYCROFT,I.H., TILLSON,S.A., ABRAHAM,G.E., SCARAMUZZI,R.J. and CALDWELL,B.V. (1970). Preparation and purification of antibodies to steroids. In "Immunological methods in steroid determination." Eds. Peron,F.G. and Caldwell, B.V. pp. 63–86, Century–Croft Education Division, Meredith Corporation, New York.

TYLER,J.P.P., NEWTON,J.R. and COLLINS, W.P. (1975). Variations in the concentration of testosterone in peripheral venous plasma from healthy women. Acta Endocrinologica, **80**, 542–550.

VANDE WIELE,R.L. and TURKSOY,R.N. (1965). Treatment of amenorrhoea and of anovulation with human menopausal and chorionic gonadotrophins. Journal of Clinical Endocrinology and Metabolism., **25**, 369–384.

VAN LOOK P.F.A. (1981). Hypothalamic–pituitary–ovarian relationship in the polycystic ovarian syndrome in "The functional morphology of the human ovary." Ed. Coutts, J.R.T. pp.167–177, MTP Press Lancaster.

VEKEMANS,M., DELVOYE,P., L'HERMITE,M. and ROBYN,C. (1977). Serum prolactin levels during the menstrual cycle. Journal of Endocrinology and Metabolism, **44**, 989–993.

WALKER,C.S., CLARK,S.T. and WOTIZ,H.H. (1973). Factors involved in the production of specific antibodies to oestriol and oestradiol. Steroids, **21**, 259–283.