Chapter 1

INTRODUCTION

Progesterone or pregn-4-ene-3, 20-dione as it is called by a systematic name, is a steroid containing 21 carbon atoms (figure 1). It is an important female sex hormone produced mainly by the corpus luteum and the placenta; small amount is also derived from the adrenal cortex in both sex (Bengtsson, 1971).

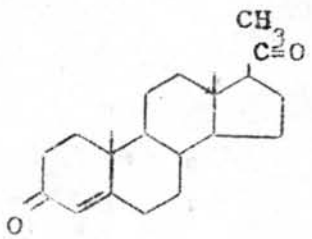

Figure 1. Structure of progesterone

Progesterone Biosynthesis

During menstrual cycle, the biosynthesis of progesterone is shown to proceed from acetate via mevalonic acid and cholesterol intermediates (figures 2, 3 pages 2 and 3). Although progesterone is produced mainly by the corpus luteum of the ovary, the biosynthesis of this steroid is not restricted to the tissue. Ryan and Smith (1961) was able to demonstrate

the conversion of acetate to progesterone in the human follicle even though the main products were estrogens and their intermediates.

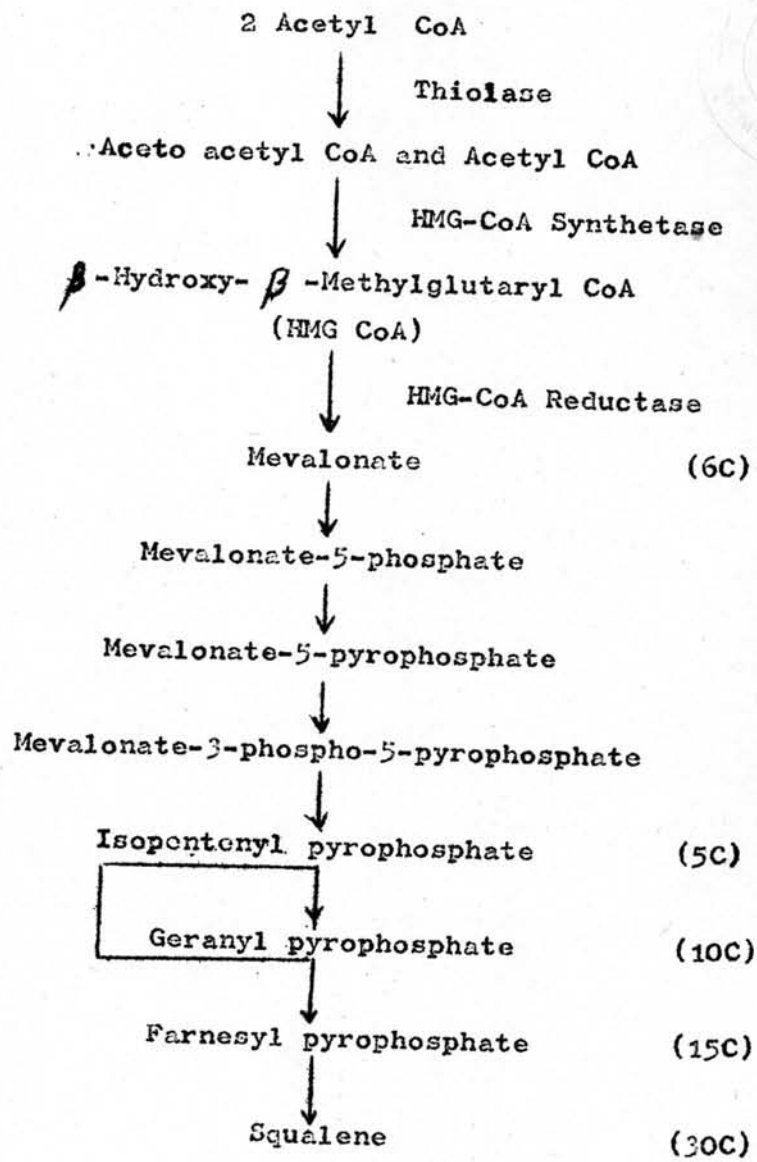

Figure 2 Biosynthesis pathway of progesterone (from Shearman, 1972).

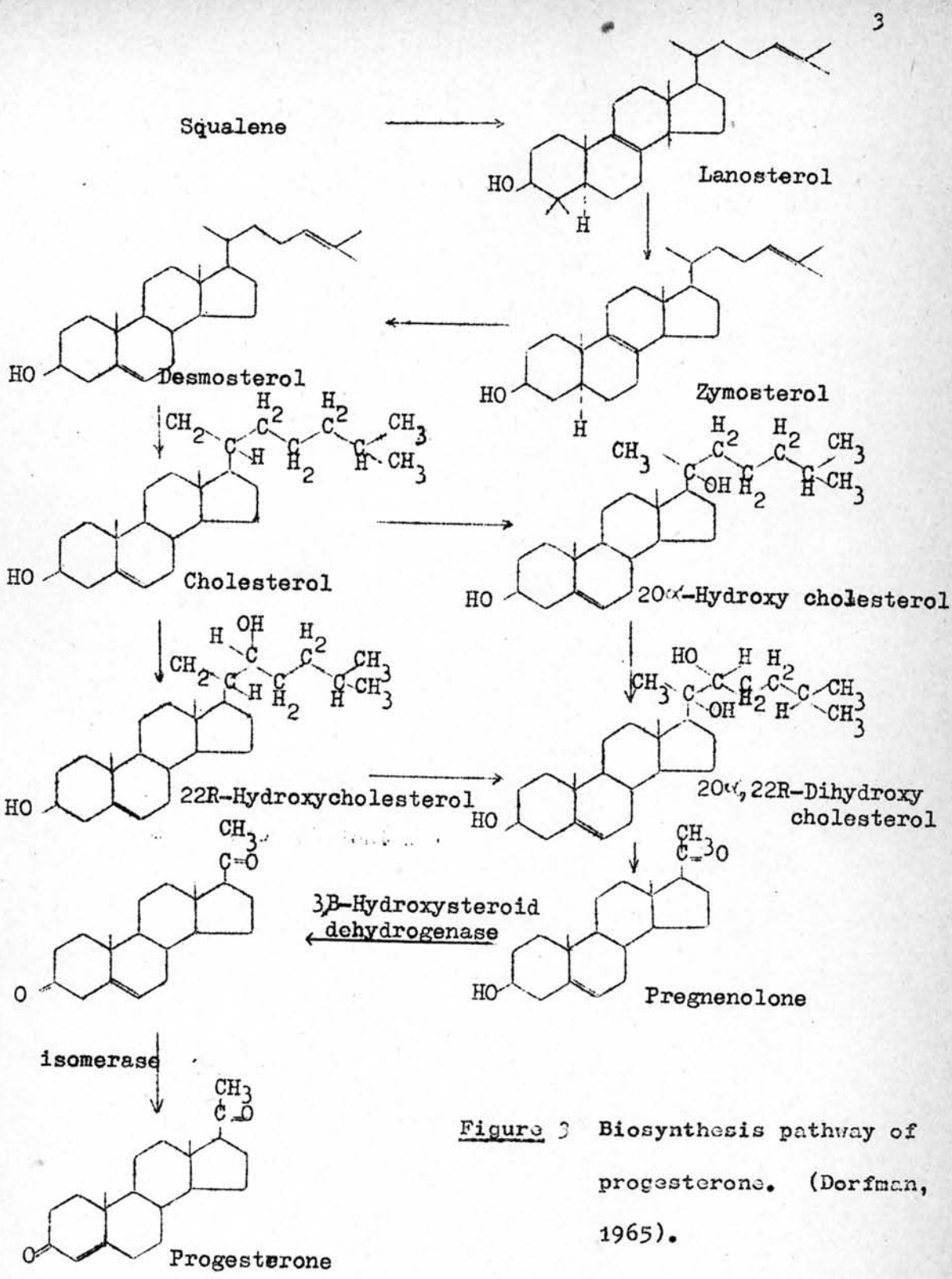

Figure 3 Biosynthesis pathway of progesterone. (Dorfman, 1965).

The placenta, instead of corpus luteum, is quantitatively recognized as the most important site of progesterone production in human pregnancy. One of the enzymatic deficiencies of the placental tissue in the pathways of progesterone synthesis is the lack of a 17-20 desmolase. This means that no small molecule precursor like acetate and mevalonate are indeed involved in placental cholesterol synthesis (Hellig, 1969). There is evidence suggested that the principal precursor of progesterone formed by the placenta is circulating maternal cholesterol (Diczfalusy, 1968). This is also supported by recent studies which demonstrated that the progesterone present in placental tissue and in maternal blood exhibits the same specific activity as labelled cholesterol present in the maternal circulation, as well as the pregnanediol excreted in pregnancy urine (Hellig et al., 1968). This data strongly suggested that the placental progesterone is derived totally from circulating maternal plasma cholesterol and indicating that a major quantity of progesterone is secreted by the placenta. Foetal cholesterol does not take part in the synthesis of placental progesterone and clinical situation, like foetal death, suggests a different mode of production for placental progesterone (Frandsen & Stakemann, 1961).

Physiological Role of Progesterone

During menstrual cycle, naturally, the prime duty of the hormone producing from the anterior pituitary gland control growth and development of the follicle, ovulation and menstruation. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) work in association to stimulate the growth of the follicle up to ovulation stage after which LH alone follows the change of ovulated follicle, by transforming it to corpus luteum and this finally produces estrogens and progesterone. It is generally assumed that corpus luteum exerts its endocrine function through the secretion of progesterone.

In the luteal phase of non-pregnant women, large amount of progesterone secreting from corpus luteum acts upon the uterus which has already been primed by entrogens during the follicular phase. In physiological conditions, progesterone invariably acts upon the genital tract which has previously been primed by estrogens. Estrogenic priming is of major importance in the response of target organs to progesterone.

Progesterone stimulates further growth of secretory glands in estrogen-primed endometrium. Thus the endometrium gets thickened and increases area for nidation of the fertilized ovum. Recently it has been suggested that human chorionic gonadotropin (HCG) of the fertilized ovum (embryo), stimulates

corpus luteum to produce progesterone (Saxena et al., 1974). If no fertilization accurs, no chorionic gonadotropin formation and there is no further progesterone formation from the corpus luteum. The levels of progesterone and estrogens are fairly low when they come to a point where there is no further growth of endometrium and the endometrium soon comes out as menses.

There is strong evidence to indicate that the progesterone plays an important role on the fate of a fertilized ovum during its transport in the oviduct, during implantation and later during pregnancy.

Progesterone has its special effect upon both tubule and uterine contraction while the eggs and sperms are travelling through the oviduct. Progesterone, in certain level, acts as an inhibitor of further ovulation. Progesterone alters the activity of the myometrium and inhibits the uterine contraction normally stimulated by oxytocin (Dorfman, 1962).

In pregnant women, after fertilization and nidation, corpus luteum of pregnancy under stimulation of chroionic gonadotropin secrets progesterone and estrogens in order to maintain the uterus in the condition appropriate to pregnancy. In the first 8-10 weeks of gestation, it has been shown that progesterone from corpus luteum plays a significant role of the pregnant uterus. After that period, the placenta is well develop and function and capable to synthesize in large amount of progesterone and estrogens.

Progesterone has been thought to be a steroid in delaying delivery at the termination of pregnancy and influences the activity of relaxin. This steroid also inhibits ovulation during pregnancy (Dorfman, 1962).

Metabolism of Progesterone

Progesterone is reduced at the double bond and keto groups to give its metabolites which can be divided into four main groups (Van der Molen and Aakvaag, 1967) namely; pregnanediones, pregnanolones, pregnanediol and compounds more polar than pregnanediol which are all derivatives of two parent hydrocarbons 5α-pregnane and 5β-pregnane, differing only in the spatial configuration of the hydrocarbon atom at C-5 of the steroid nucleus. The first three groups of compounds contain respectively 2, 4 and 6 more hydrogen atoms than progesterone itself, the final category contains additional oxo- and hydroxy- groups. Stereoisomers of pregnanediol which have been found include allopregnanediol and 5α-pregnane-3β, 20α-diol (figure 4, page 8).

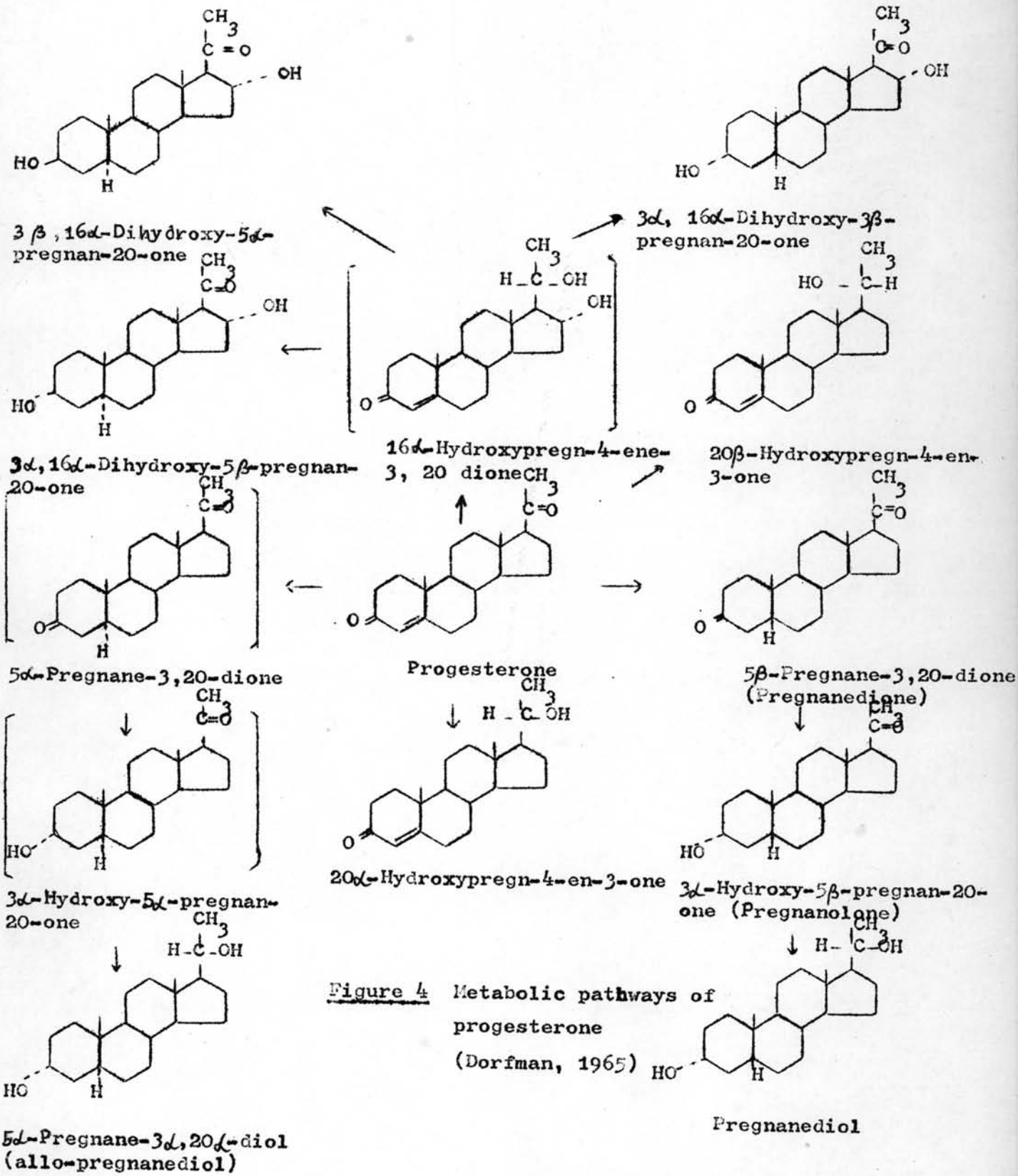

Figure 4 Metabolic pathways of progesterone (Dorfman, 1965)

Fotherby (1964) found progesterone metabolites excreted in 4 ways as uring, bile, expiration and skin. Pearlman (1957) discovered pregnanediol as the main metabolite excreted from the body in urine (about 6-27%). Later studies revealed that the level of pregnanediol in urine was in proportion to the level of circulating progesterone in blood (Woolever & Goldfein, 1963; Klopper et al., 1957). In view of these findings, one can presume that the levels of pregnanediol in urine reflect the levels of progesterone in blood.

Metabolic Effect of Progesterone

Progesterone is one of the precursor essential for the biosynthesis of a large number of steroid hormones including corticosteroids, androgens and estrogen (figure 5 page 10).

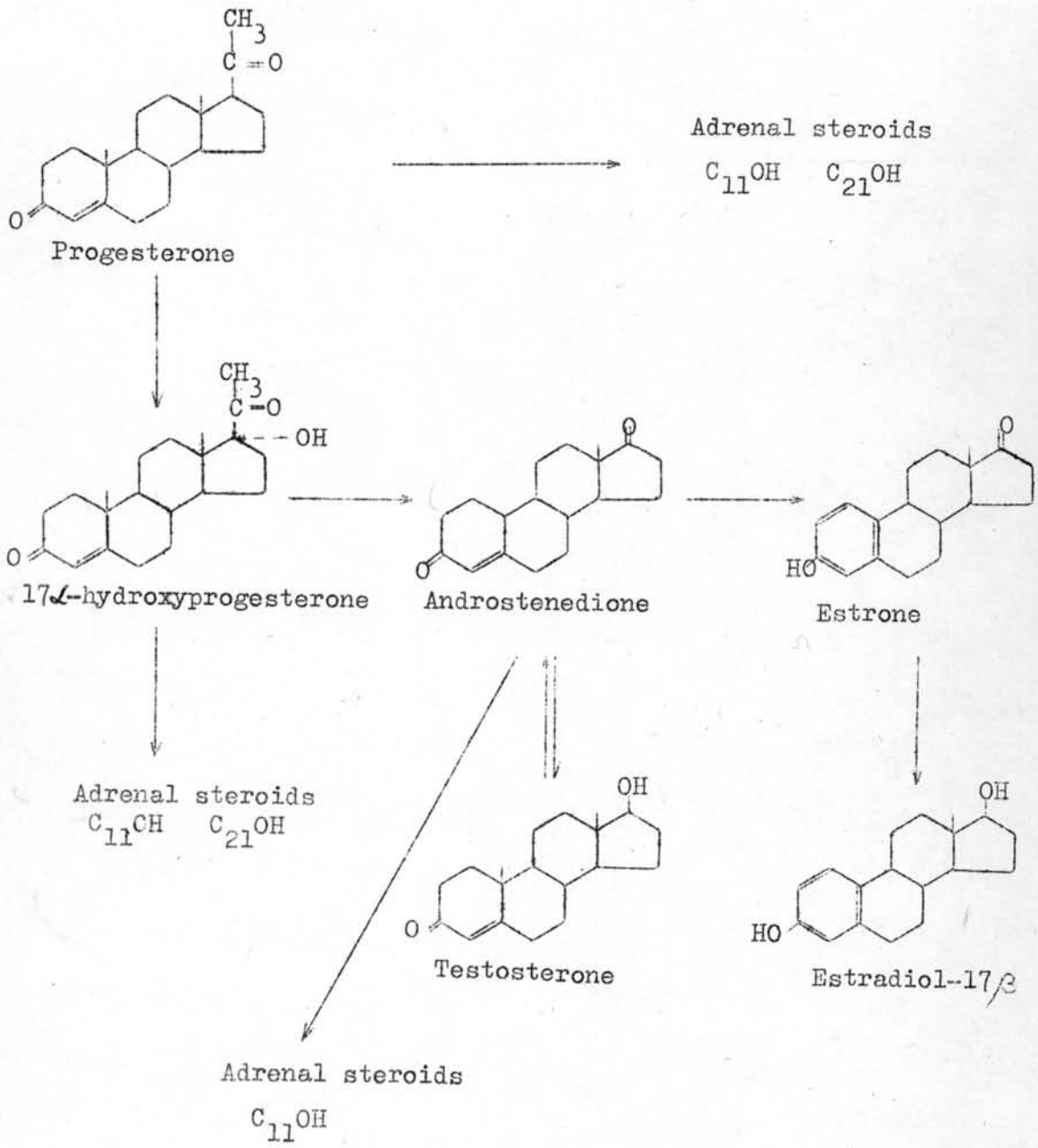

Figure 5 Pathways of steroid biosynthesis showing the key position of progesterone. (Short, 1961).

Progesterone either antagonizes or potentiates the activity of estrogen according to the balance between their dosages and progesterone in large amount acts as an anesthetic agent and inhibitor of the salt retaining corticoid. By antagonizing the effect of aldosterone on the renal tubules, progesterone tends to increase the excretion of sodium, but in large doses it causes sodium retention, presumably, because it is converted to deoxycorticosterone. Progesterone also increases urea total nitrogen excretion by stimulating protein catabolism (Shearman, 1972).

Assay Methods

There are different methods of determining progesterone and its metabolites both in blood and urine.

Various methods are used for direct determination of progesterone levels in blood.

1. Biological methods (Loraine and Bell, 1971)

1.1 Methods employing rabbits. This method depends on the production of progestational changes in the uterine endometrium but is too insensitive for clinical use (Pincus et al., 1957).

1.2 Methods employing rats (Velardo & Hisaw, 1951 and Zarrow et al., 1958). The sensitivity is also very low.

1.3 Methods employing mice (Salhanick et al., 1951; Zarrow & Neher, 1953). This method is claimed to be extremely sensitive but has a very poor precision and is not specific since other steroids like estrogenic hormones also interfere with the assay system.

2. Chemical methods

2.1 Colorimetric and fluorimetric methods

2.1.1 The Method of Zander and Simmer (1954). The accuracy of the methods when tested by recovery experiments gave an average yield of approximately 80%. The technique is capable of detecting as little as 0.05 µg progesterone.

2.1.2 The Method of Short (1958). The average recovery is 73%. The sensitivity and specificity of the method is within the acceptable limits.

2.1.3 The Method of Sommerville & Deshpande (1958). Alumina column was used instead of paper chromatography. The method is simple and suitable for clinical use but the sensitivity is low and the specificity is also questionable.

2.1.4 The Method of Oertel et al., (1959). This procedure yields high values but is of doubtful specificity.

2.1.5 The Method of Heap (1964). The sensitivity and specificity of the method are within the acceptable limits.

2.1.6 The Method of Touchstone & Muravec (1960). In this method, fluorescence reaction is used as an end point for the determination of progesterone in plasma.

2.2 Gas chromatographic methods

2.2.1 The Method of Collins & Sommerville (1964). The procedure is claimed to be capable of detecting as little as 0.01 µg progesterone, and recoveries of added hormone are stated to be greater than 80%.

2.2.2 The Method of Luisi et al., (1965). The accuracy of the method is 90%. Reproducibility and specificity are reasonable, while the method is capable of measuring 0.2 µg progesterone per 100 ml plasma.

As with most procedure involving gas-liquid chromatography, the technique is laborious, and is therefore not suitable for routine clinical use.

2.3 Isotopic methods

2.3.1 The Method of Woolever & Goldfein (1963). The method, although laborious, is reasonably sensitive being capable of detecting as little as 0.1 µg progesterone.

2.3.2 The Method of Wiest (1967). The method is capable of estimating progesterone level ranging from 0.05 to 1.0 µg with an error of 10% or less.

2.3.3 The Method of Riondel et al., (1965). This is a double isotopic procedure in which both the tritiated progesterone and the progesterone in the unknown plasma sample are labelled with S-35 thiosemicarbazide. Recoveries are low, the coefficient of variability being 7.5% and no interference by other steroids.

2.4 Competitive protein binding analysis

This procedure is an assay in which specificity is essentially dependent upon the specialized binding properties of a protein. The specificity of the method is assessed by analyzing the displacement effect of various steroids (de Souza et al., 1970). Among the related steroids, progesterone readily displaces ^{3}H-corticosterone, 9α , 10β -progesterone, $\Delta^{4,6}$-progesterone, 17α -hydroxyprogesterone and 20α -dihydroprogesterone which have the influence as strong binders with the cortisol binding globulin (CBG) binding activity of progesterone; while 9β , 10α -progesterone, $\Delta^{4,16}$-progesterone, 16α -hydroxyprogesterone and 20β -dihydroprogesterone are the weak binders or inactive.

2.4.1 The Method of Neill et al., (1967). This method indicated an average recovery of approximate 75% and is capable of measuring as little as 0.5 ng progesterone with a high degree of precision.

2.4.2 The Method of Yoshimi & Lipsett (1968). The accuracy as judged by recovery experiments is approximately 100%. Using a 5 ml sample of plasma, 3 ng progesterone per 100 ml could readily be detected, indicating that the method was highly sensitive.

However, the major problem which remains to be solved is the assessment of the specificity.

2.5 Radioimmunoassay methods (RIA)

Since the advent of RIA about a decade ago, this principle has been applied to almost all the known peptide hormone. More recently, this technique was used to measure steroid hormones with a detection method employing antisera raised against these steroids as specific binding reagents.

Principle of RIA

The hormone to be measured is represented as antigen "Ag". The first step consists of the addition of radioactive "Ag" to the biological medium. Following equilibration, Ag may be extracted and purified, recovery being obtained by the radioactivity present in the final extract. Subsequently the extracted hormone is mixed with a specific antibody "Ab" in such relative dilution, that part

of Ag reacts with Ab (bound Ag) and part remains in the unreacted form (free Ag). (See figure 6, page 15).

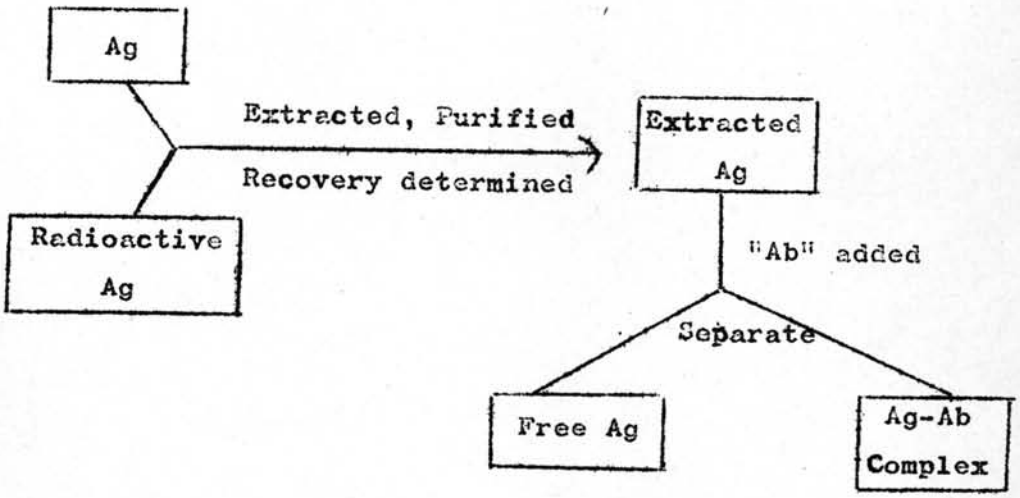

Figure 6 Fundamental principle of RIA

(Modified from Ekins and Newman, 1970)

Each of the given steroid and labelled steroid have the affinity to bind the same antibody. If the unlabelled steroid is increased, it will compete with the labelled steroid for the binding sites of antibody which is always constant.

According to Berson (1968), the two major advantages of RIA are its applicability to all material of antigenic nature whether they be hormones or not, and it provides a level of sensitivity which is generally much greater than those found with other types of procedure.

RIA also have other advantages. Specificity of RIA for a single compound makes the experiment more reliable also due to the high sensitivity of RIA, small amount of sample can be used for the assay. No special skill is required during RIA but cleanliness and carefullness must be taken into account. The other advantage is that one can assay a large number of samples in a considerably short period of time.

There are also some disadvantages as radioactivity handling and some of the reagents e.g. antibody, may not be available commercially. Lastly but not least, the cost of equipment is high.

To separate free steroids from bound steroids, 2 popular procedures, the double antibody and the activated charcoal, are used. The former technique requires a second antibody which is generally more expensive in comparison to the cost of the charcoal. In this study, the latter technique was applied in the separation of free and bound hormones.

The project was carried out in the following manner:

I Standardization of the method

II Physiological studies of progesterone levels in

(i) Menstrual cycle

a) Normal menstrual cycle

b) Contraceptive treated menstrual cycle

(ii) Pregnancy

a) Normal pregnancy

b) Postpartum period