REVIEW OF LITERATURE
INTRODUCTION

The progestational steroids used as contraceptives are of two categories:

(i) 19-nor steroids
(ii) 17α -acetoxy progesterone derivatives.

The 19-nor steroids are derivative of 19-nor testosterone. This category of compounds is characterized by the absence of the C-19 angular methyl group attached to the C-10 position. Another characteristic feature of the progestational steroids of this category is the presence of ethynyl group in the 17α -position of the steroid molecule which is responsible for the high oral activity of these synthetic steroids. Particular examples are norethynodrel, norethindrone, ethynodiol diacetate, lynestrenol, etc.

The 17α -acetoxy progesterone derivatives constitute another class of orally active steroids. They are characterised by the presence of an acetyl group in the 17α -position of the progesterone molecule.
They carry either a methyl group or a chlorine atom in the C-6 position. Some contain a double bond between C-6 and C-7. The substitution at C-6 enhances their oral potency. Examples of such compounds are megestrol acetate, medroxyprogesterone acetate, chlormadinone acetate etc.

Besides the above progestational steroids, compounds possessing estrogenic activity are also used in some oral contraceptive preparations. These are derived from estradiol-17β and possess an ethynyl group in 17α-position. The examples are ethynyl estradiol 3-methyl ether (mestranol) and 17α-ethynyl estradiol.

Over the years different modes of administration of these contraceptives have been developed. These are:

1. combination therapy, the progestational steroid is orally administered in combination with an estrogen.
2. sequential therapy, estrogen is given from day 5 - 15 or 20 and the progestagen from 16 or 21 to 25.
3. low dose continuous administration of a progestagen.
Besides the above there are other modes of administration which are being clinically tested. These include:

4. long acting injectable preparation
5. steroid releasing implants
6. post-coital contraceptives
7. long acting pills.

In combination and sequential therapy, the amount of the estrogen administered may vary from 50 μg to 150 μg, while the progestin may vary from 1 mg to 5 mg. The amount of the progestin in the combination preparation is being reduced. On the basis of accumulated experience, it has been decided that not more than 50 μg of mestranol or ethynyl estradiol be used in the combination therapy.

Low dose continuous administration of progestagens involves the administration of progestagens like chlormadinone acetate (0.5 mg) continuously. Norgestrel in as small an amount as 30 μg has been successfully tried as a continuous low dosage progestin (Kesserü, Larranga, Hertado and Benavides, 1972). Coutinho and De Souza (1968) have successfully used
norgestrel (0.5 mg) with (0.05 mg) ethynyl estradiol as an every-other-day pill for contraception. The drug is taken on alternate days from day 5 to day 25 of the menstrual cycle.

In once a month pill or long acting pill therapy, a cyclopentane ether of ethynyl estradiol (quinestrol) is administered as a 5.0 mg tablet, with a progestin of short duration of action like chlormadinone acetate.

Steroid releasing implants are being investigated for fertility control. Implants containing megestrol acetate, norgestrel and norethindrone are being clinically tested.

Newer delivery systems for the administration of contraceptive steroids in low doses is one of the current problems of investigation in many laboratories.

**MODE OF ACTION**

Pincus (1957) first reported that the probable mode of action of contraceptive drugs is by inhibition of ovulation. This was further confirmed.
by the studies on animals and women. Till recently inhibition of ovulation was considered as the principal mode of action of contraceptive drugs. However, due to a careful study of Martinez-Manautou, Cortez, Giner, Aznar, Cassasola and Rudel (1966), it is realized that inhibition of ovulation is not a "condition sine qua non", for antifertility effects of steroids. The antifertility effect could also be achieved by the action of contraceptive steroids at other sites like endometrium, fallopian tubes, cervical mucus, ovary etc., which are directly involved in the processes of reproduction.

In spite of extensive studies, very little is known about the exact mode of action of contraceptive steroids. Discrepancies arise probably due to the failure to realize that closely related compounds may have distinctly different modes of action at different dose levels and that compounds and their combination may have multiple mode of action. However, various sites of action of contraceptive steroids may be enumerated as follows:

1. Pituitary gland and hypothalamus
2. Ovarian responsiveness
3. Tubal factors
4. Cervical factors
5. Endometrial factors.

Pituitary gland and hypothalamus:

Normally the pituitary gland under the stimulation of the hypothalamus secretes two gonadotropic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH stimulates the ovarian follicular development and function and LH brings about ovulation and formation of corpus luteum. Since oral contraceptives inhibit synthesis or release of these gonadotropins, FSH and LH, ovulation is inhibited. This is also evidenced by the fact that the ovary at laparotomy shows an absence of fresh corpus luteum in women taking oral contraceptives (Garcia, Pincus and Rock, 1958, Østergaard, 1964). The ovaries are also reduced in size, acquire a smooth surface and white juvenile appearance. Their histological appearance reveals early arrest of follicular development.

Besides the examination of ovary at laparotomy, the ovulation inhibition is indicated by the estimation
of urinary or plasma FSH and LH levels in women taking oral contraceptives. The normal urinary LH excretion was estimated by bioassay method (Stevens and Vorys, 1967) and by radioimmunoassay method (Wide, 1966). Midgley and Jaffe (1966) estimated plasma levels of LH immunologically. LH secretion estimated by bioassay or by radioimmunoassay was characterized by a marked mid-cycle peak. This mid-cycle LH peak was much more marked in plasma than in urine probably due to the low renal clearance of LH as compared to FSH (Kellar, 1966).

Serum FSH levels have been found to rise during menstruation and are high early in the proliferative phase. They then decline to a preovulatory nadir just before mid-cycle LH surge (Taymor, Aono, Pheteplace, 1968, Ross, Cargille, Lipsett, Rayford, Maxwell, Strott, Rodbard, 1970) and rise again to a second peak roughly coincident with LH peak. This paraovulatory FSH peak is neither as dramatic nor as consistent as that seen for LH.

The effect of contraceptive drugs on LH and FSH levels has been studied by many workers (Taymor, 1964, Kaiser, Wide and Gemzell, 1966, Lauritzen, 1966,
Stevens and Vorys, 1967, Bell, Herbst, Krishnamurti, Loraine, Mears, Jackson and Garcia, 1967, Diczfalussy, Goebelsman, Johannisson, Tillinger and Wide, 1969, Larsson-Cohn, Johansson, Wide and Gemzell 1970). On the basis of their work it is concluded that both estrogens and progestagens interfere with pituitary gonadotropin function. The immediate effect of estrogens being directed towards the diminution of FSH secretion while that of progestagens towards the abolition or suppression of mid-cycle LH peak. Both together inhibit the FSH and LH secretion and thus prevent ovulation.

Progestagens given alone generally suppress the mid-cycle LH peak (Lauritzen, 1966, Daume and Kaiser, 1967) but when administered continuously in low doses such as 0.5 mg/day of chlormadinone acetate (Larsson-Cohn, Johansson, Wide and Gemzell, 1970, Diczfalussy, Goebelsman, Johannisson, Tillinger and Wide, 1969), 2.5 mg lynestrenol (Schmidt-Elmendorff, Kaiser and Kopera, 1967) and 0.1 mg norethisterone (Diczfalussy et al., 1969), these do not suppress the mid-cycle LH peak consistently. Instead, suppressed
or abortive LH peaks are formed which may or may not be accompanied by high pregnanediol excretion in the urine (Diczfalusy et al., 1969). Thus it is evident that the contraceptive effect of low dose progestagens may or may not be ovulation inhibition. They may have other sites of action.

**Ovarian responsiveness**

That contraceptive steroids may inhibit ovarian response to gonadotropic stimulation, is suggested by animal experiments (Starup and Ostergaard, 1966, Mears, 1965, Diczfalusy, 1965. Reviews) and also by reports that the administration of progesterone (Gemzell, Diczfalusy and Tillinger, 1960) or contraceptive steroid combination (Gual, 1967, Lauritzen, 1966), suppresses ovarian reaction of women to exogenous gonadotropins. Other investigators using different compounds and dosage schemes could not demonstrate such an effect of contraceptive steroids on the ovary. Indeed, in two cases, exogenous gonadotropins were shown to induce ovulation in 16 out of 18 treatment cycles on combination contraceptives (Starup and Ostergaard, 1966, Johannisson, Tillinger and Diczfalusy, 1965). However, in these two cases, the gonadotropin stimulation,
was undoubtedly excessive. Therefore, the possibility cannot be disregarded that certain contraceptive steroids diminish the ovarian sensitivity to gonadotropic stimulation, but that this effect can be overcome by the addition of increased amount of gonadotropins.

Contraceptive steroids may also interfere with the biogenesis (Ostergaard and Starup, 1968) and/or metabolism (Goldman, 1967) of progesterone in the ovary. This has been suggested on the basis of reports that in women on continuous low dosage progestagen, fresh corpora lutea and secretory endometrium have been found accompanied by low pregnanediol levels (Martinez-Manautou, Giner-Velasquez, Cortes-Gallegos, Aznar, Rojas, Guiterrez-Najar and Rudel, 1967b). The same was reported by Ostergaard and Starup (1966) in a study of 7 women on ethynyl estradiol and megestrol acetate, separately or in sequential therapy.

Tubal factors

Spermatozoa undergo certain physiological changes termed capacitation, in the female genital tract
before they are capable of fertilising the egg. In
most mammals, spermatozoa have to be capacitated in
the female genital tract. Uncapacitated spermatozoa
are unable to penetrate through the zona pellucida
of the eggs (Austin, 1967). This process which may
take place in the uterine or tubal cavities has the
role of enabling the spermatozoa to undergo an
acrosome reaction, which in turn causes release of
hyaluronidase and the enzyme makes it possible
for the spermatozoa to pass through the cumulus
(Austin, 1967). This mechanism seems to be operative
in human species too (Edwards, Donahue, Baramki
and Jones, 1966).

Contraceptive steroids may possibly interfere
with the process of capacitation is based upon the
observation that administration of progesterone to
rabbits inhibits the capacitation of spermatozoa in
the female genital tract (Chang, 1958). Further studies
by Chang (1967a) have shown that pretreatment of
rabbits with progestational steroids causes a distur­
bance in the sperm transport and sperm capacitation.
Tubal transport of ova may be one of the processes influenced by the contraceptive steroids. In rats (Pincus, 1965) and rabbits (Chang and Harper, 1966) it is established that administration of estrogens a day or two after insemination induces degeneration of blastocysts due to the rapid transport from the tube to the uterus and expulsion from the uterus. Chang (1967b) has reported that treatment of rabbits with progestational compounds produces a marked effect on the transportation of eggs, causing some disturbance in fertilization, degeneration of eggs in the uterus and their expulsion from the uterus. The motility of human fallopian tube is known to be influenced in vitro by pituitary, adrenal and ovarian hormones (Rorie and Newton, 1965, Sandberg, Ingelman-Sundberg, Lindgren and Rydon, 1960). The in vivo effect of hormones on the tubal motility in the human is being investigated (Coutinho, 1970).

Summarizing, one could say that tubal factors are certainly of importance in ensuring fertilization and proper transport of ovum. Estrogens and progestins are capable of interfering with these factors in several
species. Whether they do so in the human is not known.

Cervical factors

Cervical mucus undergoes cyclic changes, during the normal menstrual cycle induced by estrogens in the follicular phase and by progesterone in the luteal phase. Before or at the time of ovulation the cervical mucus is excessively thin and watery. It is characterized by low viscosity, a diminished concentration of albumin, a markedly increased concentration of non-migrating mucoid, as well as by its ability to show fern pattern and increased spinnbarkeit (Moghissi, 1966a and 1966b, Moghissi and Neuhaus, 1966). These changes are in favour of sperm penetration through cervical mucus (Botella-Llusia, 1964) and are estrogen induced. During the luteal phase cervical mucus is thick, tacky and cellular. It shows poor spinnbarkeit and lack of fern pattern. The ratio of albumin to mucoid is much higher than in the mid-cycle mucus. Such changes in the cervical mucus are hostile to sperm penetration (Botella-Llusia, 1964), and are induced by progesterone. These progesterone and estrogen induced changes in the cervical mucus can be duplicated by the administration of estrogen or progestagens (Moghissi, 1966a).
The administration of combined contraceptives results in progesterone-conditioned changes in the cervical mucus. These changes are considered significant for the contraceptive effects (Zanartu, 1964) as they definitely inhibit sperm-migration through cervical mucus (Moghissi, 1966a, Odeblad, 1966).

Sequential contraceptive therapy has no effect on the cervical mucus prior to the administration of progestagens (Cohen and Perez-Pelaez, 1965). Therefore, high contraceptive efficacy of this regimen must be involving other points of attack (Qual, Becerra, Rice-Wray, Goldzieher, 1967, Tyler, 1966).

The administration of low dosage progestagens results in non-suppression of endometrial changes accompanied by changes in the viscosity and spinnbarkeit of cervical mucus. Since low dosage progestagens are effective contraceptives, their antifertility mechanism has been suggested to involve an inhibitory effect on cervical mucus (Cohen and Perez-Pelaez, 1966). But recent studies of Martinez-Manautou and his associates (1967b) on the effect of 0.5 mg chlormadinone acetate in Sims-Hühner post-coital test of cervical mucus showed that in 80% of 115 cases, the sperm motility
was lacking, while in 20% the number of spermatozoa was fair to good and exhibited good motility. Therefore, the fertility protection offered by low dose progestagen could not be attributed to its effect on cervical mucus alone. It may have other loci of action too.

**Endometrial factors**

The human endometrium exhibits characteristic changes in each menstrual cycle under the sequential stimulation of ovarian hormones. In the first part of the cycle, the follicular phase, the endometrium is under the influence of estrogens. There is then a gradual transition at the time of ovulation and as the luteal phase progresses, the effect of progesterone becomes dominant. These morphological effects are associated with intricate biochemical changes. Animal experiments indicate that endometrial changes, regulated by delicate estrogen-progesterone balance, are critical for implantation in several species (Bishop, Borell, Diczfalasy and Tillinger, 1962, Pincus, 1965).

It is well known that all the changes characteristic of a proliferative endometrium can
be induced by the administration of estrogen and those seen in the secretory endometrium can be duplicated following administration of various progestins. *In vitro* effect of estrogen and progestins on endometrium in culture has also been shown to be similar to their *in vivo* effect (Csermely, Hughes and Demers, 1971). It can be expected, therefore, that the histochemical pattern of the endometrium exhibits significant difference according to the type of hormonal contraceptive administered. Indeed the patterns found in women taking combined pill were markedly different from those found in sequential treatment, whereas during continuous progestagen administration, two patterns emerge— one consistent with ovulation and another suggesting anovulation.

**Combined pill**— The combined estrogen and progestagen pill is generally administered from day 5 to day 26 of the menstrual cycle. In an ovulatory cycle of women, secretory changes begin in the uterus after ovulation and formation of functional corpus luteum around day 16 and reach a peak on day 19-20 to be followed by rapid regression. When a woman is administered
combined contraceptive on day 5 of the cycle, there is an early and transitory glandular response and secretory changes in the endometrium reach a peak within 4-5 days of drug administration. Glandular regression then begins to occur. At the conclusion of the 20 day regimen the endometrium may show complete absence of glandular secretion and tortuosity to one with some cellular and intraluminal secretion and minimal to moderate tortuosity of the gland. The stage of a fully developed secretory endometrium is rarely, if ever reached. (Maqueo-Topete, Perez-Vega, Goldzieher, Martinez-Manautou and Rudel, 1963, Maqueo-Topete and Rice-Wray, 1966). A thinning of the endometrium takes place after a few cycles, but cyclic changes are known to occur even after long term administration (Garcia, 1967). However, the menstrual flow, subsequent to the discontinuation of the treatment with the combined pill, is usually associated with nonsecretory atrophic appearing endometrium (Durkin, Lin and Kim, 1965). It shows no daily variation in volume and is significantly reduced as compared to that seen in women with normal cycles (Callard, Litoński and De Merre, 1966).
Sequential pill:— The sequential pill administration results in a marked proliferation of endometrium followed by the appearance of early secretory changes (Durkin et al., 1965). There is no appearance of exhausted or atrophic glands. The endometrium has the appearance of eighteenth to nineteenth day endometrium of an ovulatory cycle of a normally menstruating woman, and does not become thinner on continued administration. The menstrual flow following sequential regimen is associated with a secretory endometrium (Durkin et al., 1965) and its duration approximates the normal. Following a long term use of sequential contraceptive therapy, the post-treatment endometrial regeneration is significantly more rapid than in those taking combined pill. In sequential regimen where estrogen is given for 11 days and estrogen and progestagen for 10 days, the appearance of endometrium at various phases of treatment approaches that of normal (Mears, 1965).

Continuous progestagen administration:— When contraception is based on continuous progestagen administration, the histologic appearance of the
endometrium shows much greater variation than in other types of hormonal contraceptives. It ranges from inactive to secretory with a fair amount of irregular secretory type (Martinez-Manautou et al., 1966 and 1967b). It is of significance that secretory endometrium is found more frequently even after long term use of the progestins (Martinez-Manautou et al., 1967b).

The importance of endometrial changes introduced by contraceptive drugs may not be over-emphasized, but it is evident that any imbalance of estrogen and progesterone ratio, other than in normal cycle affects implantation in many species (Enders, 1963; Pincus, 1965). Blastocysts in many rodents will implant in almost any tissue (Kirby, 1967) but the endometrium certainly represents a most important exception in this respect (Enders, 1963; Pincus, 1965). The anti-implantation action of a drug like clomiphene in the rat is on the internal milieu of the endometrium rather than the blastocyst (Prasad and Kalra, 1967). Although much is not known about the human, yet it may be possible that estrogen and progesterone given
In the early proliferative phase may be able to create a "hormonal imbalance" interfering with normal endometrial development and implantation (Rudel and Martinez-Manautou, 1967).

**UPTAKE OF ESTRADIOL IN THE TARGET ORGANS**

Jensen and Jacobson (1962) have made the pioneering studies on the uptake of estradiol in the target organs of the rat uterus. After a single subcutaneous injection of 0.1 μg of 6,7-3H-estradiol of high specific activity (50 Ci/mM) the hormone is incorporated and retained in the rat uterus and vagina, in contrast to the other tissues like liver, kidney, adrenal, muscle, bone and blood. The peak of estradiol uptake in the uterus is reached between 1 to 2 hours and thereafter a gradual decline over the succeeding hours was obtained with a significant drop at 16 hours. The amount of estradiol present in the uterus is only a small fraction never exceeding
0.1 to 0.2% of the administered dose. Upto six hours after the administration of physiological amounts of tritiated estradiol the only significant radioactive substance present in the uterus is free estradiol. Furthermore when a mixture of 6,7-\(^3\)H estradiol and estradiol 17-\(^3\)H were employed, it was seen that neither the immature nor the actively growing uterus is capable of oxidizing estradiol to estrone. On the other hand two hours after the administration of tritiated estrone, the uterus contains tritiated estradiol which is 1/10 of the content resulting from the same dose of estradiol itself. This strongly suggests that estradiol stimulates uterine growth without undergoing metabolic transformation and that estrone exerts its hormonal action in the uterus, after being converted into estradiol.

Kato and Villee (1967) demonstrated preferential uptake and retention of estradiol by the anterior hypothalamus and the anterior hypophysis of the rat. The same selective uptake was also shown in vitro by Kato (1970). Other investigators (Eisenfeld and Axelrod, 1966, Laumas and Farooq, 1966a, Laumas, 1967, 1969) have reported similar observations on the
selective uptake of estradiol by the pituitary and the hypothalamus.

Stone and Martin (1964) studied the uptake of $^{3}$H-estradiol and estrone in the uterus of ovariecctomized mouse after local application. Both estradiol and estrone reached peak concentration in the uterus approximately one minute after application followed by a steady decline over next 12 hours. The rate of decrease in activity was more rapid with estrone than with estradiol, and may explain lower biological activity of estrone in local tests.

Studies of Sweat, Bryson and Young (1967) have shown that in human both endometrium and myometrium in the proliferative phase are able to transform estradiol-17β into estrone. The capacity of endometrium is 40 times that of myometrium. The reverse reaction i.e. estrone to estradiol is of less magnitude than that of the forward reaction. Krishnan, Hingorani and Laumas (1971) have reported that human endometrium and myometrium in both the proliferative and secretory phases of the cycle are able to convert estradiol to estrone. Such a transformation is found to be less
in the secretory phase than in the proliferative phase.

Jensen, Suzuki, Kawashima, Stumpf, Jungblut and De Sombre (1968) have shown that ethynyl, estradiol and mestranol administered in the same amounts as tritiated estradiol are taken up by uterine tissues in a manner similar to that found with estradiol. 3-Cyclopentane ether of this compound (Quinestrol) has been shown to be stored unaltered in the brain and fat (Meli, Steinetz, Beach, Wolff and Giannina, 1965).

**Sub-cellular distribution and binding of estradiol**

Centrifugal fractionation experiments demonstrated that 60% of the estrogen was present in the nuclear myofibrillar fraction and another 30% was present in the 105,000 x g (cytosol fraction). The remaining estrogen was found in the mitochondrial and microsomal fractions (Noteboom and Gorski, 1965). A number of other investigators have reported a similar localization of estradiol in the nuclear and cytoplasmic fractions of the rat uterus, vagina and pituitary (Talwar, Segal, Evans, Davidson, 1964,
King, Cowan and Inman, 1965a, King, Gordon and Inman, 1965b, Laumas, 1967, Laumas, 1969, Brecher and Wotiz, 1969, Shyamala and Gorski, 1969, Jensen et al., 1968). Stumpf (1968) using autoradiography technique has shown that after an injection of $^3$H-estradiol to immature rats, 68% of the radioactivity was associated with the nuclear compartment of all the uterine tissues. Further, Jensen et al. (1968) have suggested that the interaction of estradiol with target tissues involves a two-step mechanism of uptake and transport. The hormone associates spontaneously with an extra nuclear "uptake receptor" to form 9.5 S complex. This 9.5 S complex transfers estradiol to the nucleus by a temperature dependent process which consumes 9.5 S receptor. The formation of 5 S complex by the nucleus requires the presence of supernatant fraction containing estradiol in the form of 9.5 S complex. It is suggested 9.5 S complex may be transformed into the nuclear 5 S complex by cleavage of the 9.5 S receptor molecule.

Uptake of progesterone and other progesterational steroids

Earlier attempts to demonstrate the selective
localization of progesterone in the rat uterus were not successful because either very low specific activity progesterone was used in their studies or properly primed animals were not used (Riegel, Hartop, Kittinger, 1950). For the first time using estrogen primed rats it was demonstrated by Laumas and Farooq (1966b, 1967) that progesterone is rapidly taken up in the uterus with an initial rapid disappearance followed by a slow disappearance. The slow disappearance part of the curve indicated retention of the steroid. Higher uptake of the steroid was found in the uterus, ovary, pituitary and hypothalamus as compared with blood. Long term experiments on the uptake of \(^3\)H-progesterone showed that it is selectively taken up and retained by the hypothalamus and the pituitary, indicating the presence of progesterone binding receptors in these tissues. The uptake of progestational steroids norethindrone, norethynodrel and ethynodiol diacetate were studied by Watanabe, Saha and Layne (1968). They showed that there is selective uptake and retention of these steroids by the tissues of the rat. Various tissues
studied included ovary, uterus, vagina, pituitary, hypothalamus, adrenal, liver, kidney etc.

**Sub-cellular distribution and binding of progesterone.**

Maass, Trams and Wagner (1969) showed that following intracardial injection of $^3$H-progesterone to rats, 6-20% of the radioactivity was found in the uterine nuclear fraction and the bulk of the steroid was found in the supernatant and mitochondrial fractions. Wichmann (1967) found that after administration of $^{14}$C-progesterone to pregnant rats about 50% of the radioactivity as total or as progesterone was present in the nuclear myofibrillar fraction and about 25% in the 105,000 x g supernatant fraction. The rest of the steroid was present in the mitochondrial and micrornaial fraction. By using radioautography technique Stumpf (1968) showed that after the injection of $^3$H-norethynodrel to immature rats, radioactivity was not concentrated in the nuclei of uterine tissues but was randomly distributed especially over the extra cellular space and cytoplasm.

Milgrom and Baulieu (1968, 1970) showed a
specific binding of progesterone to a protein in the rat uterine cytosol. This protein was similar to corticosteroid binding globulin in physico-chemical criteria such as sedimentation coefficient, electrophoretic mobility and thermostability. The protein was found to increase in parallel with other proteins under the effect of estradiol (Milgrom and Baulieu, 1970). The sedimentation coefficient was found to be 4 S. The binding protein was not detectable in the kidney or diaphragm. Rao and Wiest (1970) demonstrated the presence of a progesterone binding protein in the pseudo-pregnant ovariectomized rabbit uterus. They found 60% of the radioactivity in the uterine cytosol fraction. The binding protein was shown to have sedimentation value of 5 S. Verma and Laumas (1973) have demonstrated that human endometrial and myometrial cytosols contain receptor proteins for progesterone. The sedimentation coefficient of the endometrial binding protein has been found to be 4.5 S and that of the myometrial binding protein 4.1 S. Evidence has been presented to show that the endometrial and myometrial progesterone binding proteins are different from cortico-steroid binding protein.
Milgrom, Atgar and Baulieu (1970) showed a progesterone receptor in the guinea pig uterine cytosol with a sedimentation value of 6.7S. The uterine receptor was shown to be different from that of the guinea-pig progesterone binding plasma protein. McGuire and De Della (1971) also showed a progestagen receptor in the rat and rabbit uteri. ^3H-norethindrone, chlormadinone and progesterone were found in the same region of the sucrose gradient and the binding of progesterone was completely antagonised by progestagens.

**METABOLISM OF PROGESTATIONAL STEROIDS**

The contraceptive steroids, like endogenously secreted sex hormones undergo inactivation in the body. Only a small part reaches the target site for eliciting its hormonal action. The major portion of the steroid is metabolized in the liver and other tissues and then excreted in the urine and faeces. Although considerable amount of information on the reactions leading to the metabolism and inactivation of naturally occurring
steroid sex hormones is available, yet similar information on the metabolism and excretion of synthetic contraceptive steroids is lacking.

Metabolism in the urine: Arai, Golab, Layne and Pincus (1962) studied the metabolism after the oral administration of $^3$H-norethynodrel in rabbits. It was found that the 50% of the radioactivity was excreted in the urine, although analysis of the bile of cannulated rabbits indicated that there was considerable enterohepatic circulation of the ingested material. Layne, Golab, Arai and Pincus (1963) studied the metabolic fate of orally administered $^3$H-norethynodrel and norethindrone in women. The urinary excretion accounted for nearly 33% of the radioactivity after the administration of norethynodrel and 50-70% after the administration of $^3$H-norethindrone. Considerable amounts of radioactivity was present in the bile and faeces also. One of the metabolites identified was 17α-ethynyl-19-nor-androst-4-ene-3β, 10β 17β-triol which was later found to possess estrogenic activity (Bialy, Layne and Pincus, 1965).
Fotherby and coworkers (Fotherby, 1971) have studied the metabolism of 17α-ethynyl steroids in women. The progestational steroids studied were lynestrenol (Kamyab, Fotherby and Klopper, 1968a), norethisterone (Kamyab, Fotherby and Klopper, 1968b), norgestrel (Littleton, Fotherby and Dennis, 1968). The total excretion of the steroid in the urine over 5 days was 53.9% for norethisterone, 43.7% for lynestrenol and 43.1% for norgestrel. However, in the case of each progestational steroid, the time taken for the administered radioactivity to decrease to 1% was approximately 5-6 days. Biological half life (the time required for the urinary excretion of radioactivity to decrease by one half) was 19 hours for norethisterone, 26.5 hours for lynestrenol and 24 hours for norgestrel. In contrast to these, the biological half life of 17α-ethynyl-estradiol was 31 hours (Kamyab, Fotherby and Steele, 1969).

Work of a number of investigators on the nature of the urinary metabolites after the administration of 17α-ethynyl-19-nor steroids in animals and man has shown that all the urinary metabolites retained the 17α-ethynyl group. (Layne et al., 1963,
Williams, Layne, Hobcrick, Nilsen, Hlahey, 1967, Cargill, Steinetz, Gosnell, Beach, Meli, Fujimoto and Reynolds, 1969, Fotherby, Kamyab, Littleton and Klopper, 1968). Fotherby et al. (1968) have presented evidence which suggests that urinary metabolites obtained after the administration of norethisterone or lynestrenol were similar in the free, glucuronide and sulphate fraction. This may be due to the conversion of lynestrenol into norethindrone in vivo. Conversion of lynestrenol into norethindrone has been shown in vitro by Mazaheri, Fotherby and Chapman (1970) using liver homogenate. An in vitro study of the metabolism of norethynodrel in the human endometrium and myometrium has shown that, amongst the metabolites, a considerable amount is present as norethindrone (Murugesan, Hingoranl and Laumas, 1973).

After the administration of MAP (medroxyprogesterone acetate) to women, Helmrich and Huseby (1962) reported the isolation of $6\alpha$-methyl-$6\beta$, 17,21 trihydroxy-pregn-3,20-dione-17-acetate as the major urinary metabolite of MAP. The major portion of
the metabolite was excreted as a glucuronide conjugate. Besch, Vorys, Ullery, Barry and Couri (1966) reported that the urinary excretion of $^{3}$H-
medroxyprogesterone acetate over 5 days, in pregnant and non-pregnant human patients, varies between 0.25% and 4.02% of the total dose.

Metabolism in blood: The disappearance of progesterone (Little, Tait, Tait and Erlenmeyer, 1966) and estradiol (Sandberg and Slaunwhite, 1958) has been investigated. The disappearance curve has been mathematically analysed and the metabolism discussed on the basis of a two compartment model. The initial half life and the subsequent slower half life have been calculated from the disappearance curve. Little et al. (1966) found an initial half life of 0.96 min and second half life of 10.7 min for progesterone while for estradiol Sandberg and Slaunwhite (1958) gave an initial half life of 20 min followed by a second slower half life of 70 min. The disappearance curve has also permitted the calculation of volumes of distribution and rate constants of metabolism (Tait and Burstein, 1964). The overall metabolism
of a hormone has been described by the calculation of its metabolic clearance rate (MCR), which has been defined as the amount of plasma which is cleared completely and irreversibly of its steroid content in a unit time. The MCR of 2,443 l/day and 1,350 l/day has been found for progesterone and estradiol respectively (Little et al., 1966; Longcope, Layne and Tait, 1968). In contrast to this high MCR value, contraceptive steroids show very low MCR value. Van der Molen et al. (1969) have reported an MCR value of 25 l/day for lynestrenol and its metabolites. The MCR values of 30 l/day and 42.6 l/day respectively have been reported for norethynodrel and its metabolites and chlormadinone acetate and its metabolites (Laumas, Murugesan and Hingorani, 1971; Dugwekar, Narula and Laumas, 1973a). These considerations thus suggest that the few contraceptive steroids which have been studied showed a much smaller MCR value compared with estradiol and progesterone, thereby showing that these progestational steroids are slowly cleared from the body. A lower MCR may be suggested as a criterion for the increased
potency of a drug since this would lead to persisting levels of the steroid in the blood and thus facilitate its access to the target organ.