GENERAL

INTRODUCTION
Phenomenal increase in the world population happens to be one of the major problems confronting mankind today, and is perhaps the most serious among all the biosocial and biomedical problems. Because of this population explosion all over the world more particularly in the developing countries, the search continues for a safe, effective and acceptable method of fertility control. During the last two decades a number of contraceptive techniques have been developed, such as -

(a) Pregnancy termination,
(b) Menstrual regulation,
(c) Female sterilisation,
(d) Male sterilisation,
(e) Intrauterine devices,
(f) Steroidal contraceptives.

(a) Pregnancy termination:

Abortion is the oldest method of pregnancy regulation. An increasing number of new methods which can improve the safety and reduce the cost are now available and in need of evaluation.

For terminating pregnancies in the first trimester, studies are in progress to compare dilatation and curettage (D & C) with vacuum aspiration for different gestational periods. A variety of cannulae have been designed (1-3) made of metal, hard plastic and soft plastic (4) with or without venting and with a number of aperture designs (5). Several vacuum sources are now available including electrically driven pumps, hand and foot pumps, vacuum syringes and heat evacuated bottles. Prostaglandins, which are involved in virtually all life processes, are also
potential abortificients. Csapo et al. (6) and Karim (7) have reported the successful use of intrauterine instillation of PGF2α in terminating first trimester pregnancy. However, prostaglandins are unable to compete with surgical methods of first trimester abortion (8). With new analogues (9) and improved delivery system (10) this may change. While artificial abortion by vacuum aspiration in the first trimester has rapidly become accepted, the induction of abortion in the second trimester still causes significant morbidity (11). Many methods have been suggested but few have carefully been tested on a comparative basis. The intra-amniotic administration of saline, urea and prostaglandins in different doses is under study. But these methods have serious complications.

Termination of pregnancy has its limitations. It requires trained medical personnel and a specific instrument to carry out the process. Severe pain and profuse bleeding are often associated with such processes. It may also cause adverse psychological effects on patients. Some of the more serious complications may occur with the use of saline, prostaglandin and urea such as hypofibrinogenemia, uterine rupture, and asthmatic attacks or uterine perforation. It may therefore, be concluded that though widely used, it cannot be considered to be a particularly suitable method of contraception.

(b) Menstrual Regulation (MR):

Menstrual regulation (MR) is the treatment of the missed menstrual period within 14 days of the expected onset (12). This
is a time when available pregnancy tests are not accurate. This is done by a vacuum aspiration using a convenient vacuum source such as a 50 ml syringe and 4, 5 or 6 mm soft plastic Karman cannula (13).

Though MR appears to be safer than performing first trimester abortions by vacuum aspiration, it has many complications also, such as, uterine perforation, apnea, continued heavy bleeding at the time of follow up, prolonged bleeding requiring curettage, pelvic infection etc. This shows that this method also cannot fulfill the demand of a suitable contraceptive.

(c) Female sterilisation:

Female sterilisation methods may be categorized in terms of approach to the fallopian tubes, as either laparatomy, laparoscopy, culdoscopy, colpotomy, hysteroscopy or blind instillation through the cervical os. Sterilisation may be performed for various categories of patients, such as patients in the interval, i.e., not recently pregnant, post-term delivery or post first or second trimester abortion periods. The various methods of occluding the fallopian tubes may be classified into ligature with and without excision, clips, silastic bands, cautery, intraluminal plugs and chemical sterilants.

None of the above methods of female sterilisation is inexpensive or simple. Moreover, careful and skilled hands are needed to carry out these procedures. It is thought that vaginal approaches will carry higher infection rates. Reports (14) of a high incidence of hysterectomy, usually due to heavy menstrual bleeding
among sterilised women necessitate long term study.

(d) Male sterilisation:

Vas occlusion by excision and ligation, either through single or double scrotal incisions, has long been a simple and effective method of male sterilisation. Many workers have obtained excellent results using fulguration of the lumina of the vas ends. Tantalum clips (15, 16) have also been successfully employed, two to each cut vas ends. This method is complicated and requires well trained and well equipped medical unit. Risk of infection is also there. Hence the sterilisation methods are also not the most acceptable method of contraception.

(e) Intrauterine devices:

In case definite contraception is wanted, male or female sterilisation is the best solution. If this is not wanted, intrauterine contraceptive devices (IUDs) are an acceptable method of contraception with a number of advantages. IUDs are reliable, do not influence any other part of the body, and finally IUDs are relatively cheap.

The introduction of copper-bearing IUDs (17, 18) constitutes the most important recent development in the field of contraceptive technology. This introduction was due to the fact that Zipper et al. (19) observed a local antifertility effect of copper in rabbits. Thereafter, much interest has been centred on copper releasing devices (20). The G.D. Searle Company is marketing their Cu-7 device in several countries, while the Population
Council are using 'Cu-T' devices of different surface areas (mainly 120 mm² and 200 mm²): the greater the copper area the lower the pregnancy rate. There are several published trials of Cu-releasing IUD in women (21-24).

IUDs are foreign bodies in the intra-uterine cavity and as such produce a characteristic morphological and biochemical response. In every species thus far studied, including humans, migration of increased quantities of polymorphonuclear leukocytes and other inflammatory cells have been noted in endometrial sections taken from uteri containing the IUD (25). The IUD may produce a U-shaped depression in the superficial endometrium particularly when the endometrium tissues are soft, in which ulceration may not be seen. In other patients microscopic ulcerations of the superficial epithelium can be seen underlying the areas of contact of the IUDs, with the resultant movement of the neutrophils into this area. Migration of the neutrophils into the uterine cavity and subsequent cytolysis release the intracellular components of the increased number of leukocytes. It is likely that these substances, which are not normally present, are responsible for the anti-fertility effect. The inflammatory response to the foreign body is a sterile response, cultures usually reveal no bacterial growth except during a transient bacterial infection upon insertion of the IUD through the cervical canal.

Mode of action of the intrauterine devices is rather controversial. Early workers felt that the device acted as an abortifacent and suggested that it might alter the endometrium to disturb
implantation. Other investigators have shown that tubal motility is increased while the device is in place, suggesting that the unfertilised ova may be rapidly expelled prematurely into the uterine or vaginal cavities before fertilisation can take place. It is known that sperm may be found at the usual site of fertilisation in the ampulla of the fallopian tube, indicating that sperm migration is not impaired. A more acceptable hypothesis for the mode of action of intrauterine device is that it produces both the foreign body reaction described and an increased amount of uterine fluid.

Although the IUDs have gained wide spread use, the method has its limitation/disadvantages by way of its application, usages and effectivity. For instance, (a) the foreign body reaction is nonspecific in action, and effective fertility control is only one of many end results, these may also include abnormal uterine bleeding, and myometrial and tubal contractions that result in expulsion of the device and in asynchronous maturation of the endometrial tissues. IUD requires a net work of trained personnel for their actual application. Further it has been observed that they have higher failure rate as compared to oral pills. Undesirable effects like pelvic infection and uterine perforation are also associated with the device.

(f) Steroidal contraceptive: 

Gregory Pincus was one of the pioneer workers on physiology of reproduction. His research on steroidal contraceptives is based on the fact already well known since 1937, that progesterone
suppresses ovulation in rabbits. However, until 1951, these steroids were not of practical value as antifertility agents in human being, either because of undesirable side effects or because of lack of therapeutic effectiveness on continued therapy. The estrogens were employed during 1940s to inhibit ovulation for the treatment of dysmenorrhea. However, it was soon found that the estrogens did not completely control ovulation in successive cycles of treatment and lack of continued control on ovulation was described by several authors as an 'escape' phenomenon.

Since Pincus, Rock and Garcia (26, 27) introduced, in 1956, the use of oral contraceptives, many modifications and improvements have been proposed with different results. Millions of women around the world use oral contraceptives. However, the ideal agent has not been described. Numerous reports have pointed out the untoward effects of the oral contraceptives, such as increased risk of thromboembolic disease, hypertension, breast and endometrial carcinoma (28, 29).

Most of the secondary effects of oral contraceptives are related to the metabolic effects of these steroids. The type of steroid, dosage, duration of use, and the individual characteristic of the users are all important factors in determining the magnitude and nature of the changes produced.

Hormonal oral contraceptives:

(i) **The combination oral contraceptive regimen** - The most widely used types of oral preparations consist of a progestin with an estrogen in combination ('the classical pill'), such as,
Norethisterone acetate 4.0 mg + Ethinylestradiol 50 µg; Norethisterone 10.0 mg + Mestranol 60 µg; Ethynodiol diacetate 1.0 mg + Mestranol 100 µg etc. Preparation of this type is given at the same uniform daily dose from fifth to the twenty fourth days of the cycle. This is followed by an interval during which the user normally has a bleeding resembling menstruation.

(ii) Sequential type :- A new type of oral contraceptive was introduced a few years after the 'combined type', which was variously known as a 'sequential' or 'serial' product. It was developed as an attempt to provide a series of hormone changes in women that more closely matched the endogenous ebb and flow of estrogen and progestogen during a normal menstrual cycle. In these products, the first course of tablets, beginning on day 5, contained only estrogen and were taken for 14, 15 or 16 days depending on the particular product. At the end of these tablets the women switched immediately to tablets containing both estrogen (at the same dose as before) and progestogen. These second tablets were taken for five, six or seventeen days according to the product, then followed by a 7 or 8-day tablet free period, during which time a withdrawal bleeding occurred. By reducing exposure to progestogen, it was hoped that certain side effects, such as, depression, thought to be caused by progestogens, would be reduced. Some sequential type oral formulations are:

Mestranol 100 µg (14 days) + Chloromadinone acetate 1.5 mg and Mestranol 100 µg (7 days).

Mestranol 100 µg (14 days) + Norethisterone 2.0 mg and Mestranol 100 µg (7 days).
Ethinylestradiol 100 µg (16 days) + Dimethisterone 25 mg
and Ethinylestradiol 100 µg (5 days) etc.

Products of this type have not been so widely used as combined oral contraceptives. Moreover, they are significantly less effective. A major setback to the use of sequential oral contraceptives came with reports that combined type products containing more than 50 µg daily estrogen are associated with a significantly higher incidence of thrombembolic diseases (30).

A hybrid of combined and sequential oral contraceptives has been marked, but also has failed to gain much acceptance. It provides a course of 16 daily 100 µg doses of mestranol with a very low dose of progestogen (ethynodiol diacetate 0.1 mg), then a final 7 days of estrogen (at the same dose as before), but with a much larger amount of progestogen (0.5 mg) (Fig. 1).

(iii) 'Mini pills' :- A more recent development in oral contraception has been the use of progestogen-only products, which have to be taken continuously, without the medication-free interval of combined or sequential products. It has been found that, given alone, progestogens have contraceptive efficacy at much lower doses than when used in combination with estrogen. The progestogens used are,

- Chlormadinone acetate 0.5 mg (31)
- Chlormadinone acetate 0.3 mg (32)
- Norethisterone 0.35 mg (33)
- Norethisterone acetate 0.3 mg (34)
- Megestrol acetate 0.5 mg (35) etc.
Fig. 1: Chemical structures of steroid hormones used in some of the combined or sequential oral contraceptives.
**Estrogens**

- Ethinylestradiol
- Mestranol

**Progestogens**

- Norethisterone
- Norethisterone acetate
- Ethynodiol diacetate
- Lynestrenol

![Chemical Structures](FIG. 1)
The minipill is capable of controlling fertility without inhibition of ovulation. Probably the minipill produces some combination of inhibited steroidogenesis, ovulation blockade, inhibition of transport of spermatozoa, accelerated ovum transport, and disrupted blastocyst implantation. Changes in the endometrium and/or cervical mucus may be responsible for the contraceptive effect (Fig. 2). All types of oral contraceptives mentioned above are eminently suitable for use by women in developed countries, but their use by women of underdeveloped countries causes considerable difficulties. Such products demand a certain minimum understanding of human reproductive physiology, together with a location in the house-hold where a packet of oral contraceptive tablets can be stored away from children and domestic animals. Most of these criteria are not met by many women from parts of Africa, Asia and South America. Moreover, many cannot afford oral contraceptives.

Largely to supply the needs for contraception in women of underdeveloped countries, studies have been undertaken to find depot products that can be injected at infrequent intervals, yet are effective and safe as oral contraceptives. Some depot contraceptives contain only progestogens, but others are used in combination with estrogens. The latter may be either incorporated into the oily vehicle with the progestogen, or given separately as intramuscular injections, or as orally active compounds (Fig. 3).

Besides these, post coital contraceptives, steroid releasing implants, steroid releasing vaginal ring and steroid releasing intrauterine devices are now under trials.
Fig. 2: Continuous dose, progesterogen-only, "mini-pills".

Fig. 3: Some depot contraceptives.
**FIG. 2**

- Quingestanol acetate
- Cingestol

**FIG. 3**

- Norethisterone enanthate (n-heptanoate)
- Hydroxyprogesterone caproate (n-hexanoate)
Regulation of human reproductive processes

The sequence of events following each menstrual cycle, during which structural, functional and chemical changes occur in the female reproductive system are controlled by the hormone of hypothalamic-pituitary-gonadal axis. In each menstrual cycle the endometrium is built up gradually in order to be prepared for the implantation of the fertilised ovum. This endometrial development is regulated by ovarian steroid hormones: estrogen during the first or proliferative phase, and progesterone estrogen during the second or secretory phase. The synthesis and release of these two ovarian hormones is regulated by pituitary-gonadotrophic hormones, namely, the follicular stimulating hormone (FSH) and luteinizing hormone (LH).

Under FSH stimulation an array of follicles are brought to considerable development in each cycle, until one (or in rare cases, two or more) matures or ripens and ruptures at midcycle, with release of its ovum, which is called ovulation. A mature unruptured follicle, containing an ovum, a graafian follicle, from which estrogen is released. The ruptured follicles become occupied by 'luteal cells' and 'corpus luteum' is formed. The other follicles become atretic, probably under the influence of ovarian androgen (56) and rapidly degenerate.

Whether or not gonadotrophins directly cause rupture of mature graafian follicles is more doubtful. It can be said that ovulation follows a rise in sex hormone release by the follicle, so that gonadotrophins induce ovulation indirectly through their
**Progesterone secretion begins immediately before ovulation, when small amounts are formed by the still intact Graafian follicles. After ovulation progesterone secretion increases due to its formation by the corpus luteum. Some 14 days after ovulation the steroid secretion of the corpus luteum decreases so markedly that the endometrium is left practically without hormonal stimulation. The endometrium is desquamated and its shedding occurs in the form of menstrual bleeding. Progesterone biosynthesis by the corpus luteum is heavily dependent on LH, probably by cAMP activation of cholesterol-20,22 hydroxylase and pregnenolone synthetase. The role of prostaglandins in this system is complex. Infusion of prostaglandin F₂α into an ovarian vein leads to regression of corpora lutea.**

**Cyclic variation of LH and FSH secretion from anterior-pituitary are dependent on a signal from hypothalamus (viz. Gonadotrophin releasing hormone, usually termed luteinizing hormone-releasing hormone, LH-RH) (37) which stimulates the secretion of both FSH and LH.**

**The secretion of the releasing hormone LH-RH in turn is controlled by feedback mechanism (long loop; short loop; negative positive etc.). A recent evaluation of evidence on the relative roles of releasing factors and sex hormones in regulation of gonadotrophin secretion by the anterior pituitary has been reported by Schally and Kastin (38). According to them the major controls for gonadotrophin release are a 'long' feedback of sex steroids onto the hypothalamus to regulate LH-RH secretion. Hence, although the hypothalamic pituitary gonad axis has the potential**
for control by short negative feed back inhibition, it is doubt-
ful whether these are of importance in normal reproductive phy-
siology.

Application of specific bioassays and particularly radio-
immunoassay, have shown that Serum LH concentration remains at
a relatively constant level during the early follicular phase,
begins to increase during the ovulatory phase culminating in a
sharp peak on about day 14 of the menstrual cycle. This peak
of LH production is so well defined that it is often used at
reference point in the menstrual cycle and is often referred to
as day 0. After the ovulatory peak production falls to their
lowest values during the luteal phase. FSH production also shows
an increase at midcycle coincident in most cases with the LH peak.

Serum estrogen rises significantly before any increase in
serum gonadotrophin activity can be detected. This phenomenon
which was originally described in rodents, has now been observed
in various primates (39, 40) and women (41, 42). Serum estradiol
begins to increase during follicular phase (day 1 to day 8)
reaching a peak on day 13 of the menstrual cycle, i.e. 1 day
prior to the midcycle, LH peak. There is then sudden decrease
in estrogen production till day 16; this is followed by a secondary
rise during the luteal phase, declining again prior to menstruation.

Progesterone production by the ovary is negligible during
the follicular phase, begins to rise after the midcycle, LH peak
reaches a maximum about 8 days later and declines abruptly
1-2 days prior to the onset of menses. Serum 17α-hydroxyproges-
terone follows a similar pattern, but increases prior to the onset of the LH peak (43, 44). The midcycle peak of LH is thought to precede ovulation by 24 hours (45) (Fig. 4).

The role of the third gonadotrophin prolactin in normal ovarian function is still uncertain. Administration of prolactin stimulates progesterone secretion and increases activity of cholesterol esterases in luteal cells. In rats there is a significant increase in serum prolactin concentration, though not in LH or FSH, during the afternoon of proestrus, so that the stimulus by which mating activates corpora lutea in rats is very likely to be prolactin. The existence of human prolactin is certain but its role in regulation of human gonads is not fully understood.

The evidence points to a +ve feed back of estrogen on gonadotrophin release, in contrast to the -ve feedback control occurring during basal secretion. That the basal secretion is a true -ve (or inhibitory) feedback is demonstrated by the observation that significant rises in serum LH and FSH occur in young women following ovariectomy (46). These rises can be reversed by estrogen administration.

Two problems are raised by these observations.

1. Why does ovarian estrogen secretion increase before there is any additional gonadotrophin stimulation?
2. Why does estrogen enhance gonadotrophin release at midcycle but inhibit secretion at other time?

There are a number of possibilities. During basal gonadotrophin stimulation of ovaries estrogen secretion will depend upon the
Fig. 4: The pattern of serum LH, FSH, estradiol, progesterone and 17-OH progesterone during the normal human menstrual cycle.

FIG. 4
number of gonadotrophin receptors activated, which will probably increase as follicles grow. If only a small amount of circulating gonadotrophin is stimulating estrogen production at early cycle, estrogen secretion should gradually increase with increasing follicle size, without the need for any additional gonadotrophin. Studies of urinary estrogen changes during menstrual cycle show a gradual increase up to the time of ovulation. Moreover, follicular fluid contains significant amounts of estrogen (47) which is released into the peritoneal cavity when a follicle ruptures at ovulation.

The more difficult question is why increase in serum estrogen should induce a dramatic surge of gonadotrophin from the pituitary. That the surge is due to estrogen, and not to some other substance liberated at the same time, is shown by the results of Millius et al. (48) who induced midcycle gonadotrophin peaks in young women with estradiol alone. Leigendecker et al. (49) have claimed that estradiol releases only LH, whereas progesterone releases both LH and FSH. In contrast to these findings the antiestrogens (clomiphene, epimestrol, cyclofenil, etc.) are also effective ovulation inducers under some circumstances. If ovulation is due to a +ve (i.e. stimulatory) feed back onto gonadotrophin release, then antiestrogen would be expected to partly block this action and inhibit ovulation. Boutselis et al. (50) compared conjugated-estrogen with clomiphene for ovulation induction in women and found very similar results with the two compounds. There is considerable evidence that ovulation is controlled mainly by hormone effects in the hypothalamus, or possibly higher brain centres, rather than by direct effects on
the pituitary. Estrogen receptor molecules have been found in hypothalamic tissues (51), but their uptake of estrogen cannot be reduced by noradrenaline, dopamine, MAO inhibitors, reserpine or acetylcholine. This is in contrast to in vivo effects of brain transmitter substances (and their inhibitors), which greatly alter gonadotrophin release when introduced into the hypothalamus, though not when infused directly into the pituitary (52).

At physiological doses, LH and FSH release is stimulated by dopamine, though prolactin is reduced. In contrast, small amounts of serotonin (or melatonin) have the reverse effect on all three hormones. It seems likely that control of gonadotrophin release, both at ovulation and for basal secretion is to be sought in feedback effects of steroid hormones onto the hypothalamus to alter the balance of neurotransmitter substances (inhibitory and stimulatory), controlling secretion of releasing factors and inhibiting factors for gonadotrophins and prolactin. A simple diagrammatic representation of control mechanism is given in Fig. 5.

Mode of action of oral contraceptives:

Considerable evidences indicate that the oral contraceptives of combined or sequential type primarily exert their contraceptive action by suppressing ovulation through their inhibitory action on hypothalamo-pituitary-gonadal axis. Secretion of both FSH and LH by the pituitary is reduced in women taking almost any oral contraceptive product (53). The characteristic midcycle surge of LH is eliminated, while even basal secretion of gonadotrophin at
Fig. 5: A simple diagrammatic representation of feedback controls for regulation of gonadal functions.

A. Buhl secretion

-ve feedback control

hormone receptors

neural — dopamine (+ve for LRF and PIF)

indoleamines (-ve for LRF and PIF)

hypothalamus

LRF (FRF ?) PIF

anterior pituitary

LH + FSH prolactin (?)

gonads (♂ and ♀)

sex steroids

growth stimulation

gametes (sperm formation; follicle growth)

B. Ovulation

+ve feedback control

hormone receptors

neural — dopamine (+ve for LRF and PIF)

indoleamines (-ve for LRF and PIF)

hypothalamus

LRF (FRF ?) PIF

anterior pituitary

estrogens

progestogens

ovaries

ovum

FIG. 5
other times of cycle is depressed. There is no conclusive evidence as to whether this gonadotrophin inhibition occurs at hypothalamic or pituitary levels or both. Administration of synthetic LH releasing factor (LRF) to women under treatment with combined type of oral contraceptives produces a rise in plasma LH, suggesting that secretion of LRF is blocked by contraceptive steroids.

While ovulation-inhibition may be the primary mode of action for combined and sequential oral contraceptives, effects at other sites undoubtedly occur. These presumably contribute to overall contraceptive efficacy of the products. The secondary 'sites' are:

**Ovary** :- Ovarian sensitivity to gonadotrophins is reduced by oral contraceptives, while steroidogenesis is abnormal. After even only one cycle on combined therapy, the size of ovary is decreased and it looks inactive. The density of follicles is increased due to condensation of cortex, follicles develop only to a few millimetres in diameter, and there is an increase in the number of atretic follicles. Prolonged use produces no lasting deleterious effect on either ovarian or pituitary function (55). Although it has been suggested that occasional breakthrough ovulation occurs, Shearman (56) suggested that ovulation is invariably suppressed while the patient is on combined tablet. On withdrawal of the drug the ovary reverts to normal. There is prompt and complete recovery of ovarian function including normal steroid excretion by the second cycle.
Cervix: - Secretion of mucus is reduced by steroid contraceptives and the condition is unsuited to sperm migration. The predominantly progestogenic combined tablets lower the sialic acid concentration of cervical mucus. Patients treated with combined tablets, particularly in higher dose, have been reported to show dose-related hyperplasia and hypersecretion increasing with duration of therapy. Numerous cases of atypical endocervical hyperplasia have been reported but premalignant changes have not been identified. The situation is confused by the finding of a higher prevalence rate of carcinoma in situ among women choosing oral contraceptives compared with women choosing a diaphragm (57). There is, however, no theoretical background for suspecting a carcinogenic effect on the cervix.

Endometrium: - Combined tablets regulate the menstrual cycle and reduce blood loss during the cycle by suppressing ovulation and substituting an artificial cycle. Although the different combinations of estrogen and progesterone produce quite different histological appearances in the endometrium, the usual pattern, when the tablets are given from the fifth day of the cycle, is to produce premature secretion in the glands followed by regression proceeding to atrophy together with a decidua-like transformation of the stroma and an alteration of the vascular pattern with prominent dilated venules.

During continuous combined therapy, striking pseudodecidual change develop but there is discrepancy between the glandular and stromal elements which clearly distinguishes from early pregnancy. Prolonged cyclic therapy produces small atrophic
endometrial glands in an inactive epithelium virtually devoid of mitosis, with dense postmenopausal type stroma.

Fallopian tubes: Passage of the fertilised ovum through the fallopian tube, which occurs while cleavage is in progress, takes at least 3 to 4 days (58) and a hostile environment during this passage could have a deleterious effect on the fertilised ovum. It is at least conceivable that certain contraceptive drugs may influence the mobility and secretion of the fallopian tube.

Uterine muscle: With estrogen progestogen mixture there may be a slight softening of the uterine body initially and perhaps a little enlargement. During prolonged cyclic therapy there is a tendency for the uterine body to become distinctly smaller.

Although no change has been seen in the size of fibroids in women taking combined tablets for contraception (59), marked increase of the size of fibroids has been noted in patients during steroid gynecological therapy. Although the estrogen fraction has gradually been regarded as responsible, it is possible that both the estrogen and progesterone contribute in an additive or synergistic way.

Sperm: It is possible though not definitely established that capacitation of sperm is inhibited in women treated by oral contraceptives.

Mode of action of "minipills":

It is less clear on which effect the primary contraceptive
action of "minipills" is based. Some products definitely induce inhibition of ovulation in some women (10 to 60% depending on progesterone and dose), but "minipills" do not inhibit ovulation in most women. It is likely that these products also act at the above mentioned sites, but little is known of these effects on these tissues. It has been reported that contraceptive efficacy of some compounds used in minipills closely parallels their effect on cervical mucus but others appear to be effective contraceptives at doses where cervical mucus is unchanged.

Side effects of oral contraceptives :

There are numerous reports on side effects of steroid contraceptives, which outline and quantify both subjective and objective hazards (60-64).

1. Subjective side effects :- As with all drugs, these are the most difficult to quantify for women vary widely in the ease with which they report symptoms of this type. Nausea, vomiting, dizziness, headaches, fatigue, nervousness, and general feelings of unease are the most frequent reports. Many are mentioned in only the first few cycles of treatment and thereafter disappear. There is some evidence that estrogen components are responsible for gastrointestinal symptoms. Most serious symptoms are changes in mental state, such as depression. These symptoms usually disappear if treatment is stopped, or even if the women are transferred to a different type of contraceptive.

2. Effects on menstrual cycle :- While most women experience regular cycle on combined oral contraceptive, a few
develop spotting or breakthrough bleedings. These symptoms often appear in only the first one or two cycles, but occasionally persist. They can sometimes be prevented by switching to another product of different estrogen : progestogen ratio.

**Body weight:** Some women gain weight on oral contraceptives, but majority show no change. The increase has a variety of different causes. In some cases it is a simple result of increased appetite and food intake, perhaps due to relief from fear of pregnancy. Other women may show mild fluid retention, due to the estrogen component; this can be prevented by changing to a more progestogenic product. Finally, there is evidence that some oral contraceptives have an anabolic effect, so that muscle mass increases (66).

**Skin:** Sebum formation and acne tend to be increased by androgenic drugs and reduced by estrogens. During the luteal phase progesterone appears to narrow the sebaceous duct orifice (67). The effects of contraceptive steroids depend on the type of progestogen and the overall balance in combined tablets. Generally increased skin pigmentation may occur particularly in olive skinned women, while patchy pigmentation (cholasma) develops occasionally in others.

**Liver function:** Sex hormones can be shown to influence significantly the synthesis of hepatic protein, including enzymes and plasma proteins. During treatment with contraceptive steroids liver microsomal drug metabolism appears to be diminished. This means that, although there may be compensating factors in tissue
binding and drug excretion, the dose required and the duration of effect of some drugs may be altered when patients are taking contraceptive steroids.

There is a close correlation between jaundice with contraceptive steroids and a previous history of jaundice or generalised pruritus during pregnancy. There are reports of hepatic dysfunction in 7 post menopausal women treated for 28 days with Lyndiol. This evidence consisted of pathological increase in serum transaminase levels in all cases, and of high bromosulphthalein retention in 3 cases; total bilirubin was elevated in one case, alkaline phosphatase in another case on the day following the treatment. It was shown that C17 alkylated steroids in general are capable of producing jaundice (68).

Combined preparations are therefore not advised for patients with hereditary or acquired defects of hepatic excretory function or in those with a previous history of pregnancy cholestasis or pregnancy pruritus.

**Blood pressure** :- Combined oral contraceptives stimulate increased synthesis and release of renin substrate by liver so that plasma angiotension II is formed in larger amounts (69). This results in decreased formation of renin by the kidney. In most women, these changes produce no significant change in blood pressure but a few individuals develop marked hypertension that is relieved only by withdrawing oral contraceptive.

**Cancer** :- Epidemiological surveys of breast cancer incidence (70) indicate a somewhat reduced risk among women who
have taken oral contraceptives than in those who have not. It has been reported that carcinoma of the cervix is more common in women taking oral contraceptives than in other groups (51) but definite evidence is still lacking and will take many years to collect.

**Fertility**: Return of fertility on stopping oral contraceptives occurs quickly in the majority of women, but a small number remain anovulatory, perhaps permanently. As the number involved are small, it is difficult to be certain that the effect is due to oral contraceptive, for secondary spontaneous amenorrhea of unknown etiology is known, though equally rare.

**Teratogenicity and Mutagenicity**: Studies of babies born to women after stopping oral contraceptives have shown no increase of genetic abnormalities (71) though it has been reported that spontaneous abortions from women who have at some time taken oral contraceptives show a higher incidence of chromosomal abnormalities than expected (72).

**Carbohydrate metabolism**: In patients on combined estrogen-progesterone tablets, elevation of plasma insulin and plasma glucose after an oral or intravenous glucose load has been generally reported. In a long-term survey before, during and after combined therapy (73) 13% of the patients developed 'chemical' diabetes during therapy and there was some impairment of oral glucose tolerance in 78%. Wynn and Doar (73) suggested that the impairment of glucose tolerance was, in fact, steroid diabetes due to elevated plasma hydro-cortisone levels secondary
to the metabolic effect of the estrogen component. Surprisingly the changes in glucose tolerance did not correlate with age, degree of obesity, parity or duration of therapy. Spellacy et al. (74), however, reported a rising incidence of abnormal tests over ten years. The changes in glucose tolerance on combined therapy resemble those found in the later stages of pregnancy.

The precise mechanism by which glucose tolerance becomes impaired has not yet been agreed. Pancreatic response, growth hormone levels, the rate of utilisation of glucose by the tissue and endogenous glucocorticoid activity are all involved. 50% of gestational diabetes show reduced capacity to metabolise a glucose load (75) and it is generally assumed that patients who put on weight rapidly or have any history suggesting prediabetes are at extra risk when taking oral steroids. Age may be a factor since Gershberg, Javiar and Hulse (76) found that estrogen could improve glucose tolerance in maturity onset diabetes.

It has been established that the effect on carbohydrate tolerance is dose dependent (77). Impairment of intravenous glucose tolerance has been reported with stilboestrol and Premarin but not with progesterone or Medroxy progesterone acetate. Garcia and Wallach (78) have not noted any increase in overt diabetes among patients who have been on combined oral contraceptives for 10 - 12 years.

In summary it is quite clear that estrogens in general cause significant impairment of glucose tolerance in approximately 15% of patients. The 19-norsteroids might aggravate the effect
but 17α-hydroxyprogesterone derivatives would, if anything, counteract it. The overall effect is dose related.

**Lipid Metabolism** :- In post menopausal women all estrogens decrease circulating cholesterol and low density β-lipoproteins which are rich in cholesterol and increase triglyceride rich and phospholipid rich α-lipoproteins and total triglycerides. Androgens have the opposite effect raising the β/α lipoprotein ratio whereas, progesterone and hydroxy progesterone derivatives have no effect on serum lipid levels. In premenopausal women both natural estrogen and mestranol raise the serum triglyceride level.

Wynn, Doar and Mills in 1966 (79) reported a rise in serum total cholesterol and low density lipoprotein in women taking ethynodiol diacetate plus mestranol or norethisterone acetate plus ethinylestradiol. 31% of the women had fasting serum triglyceride levels above the highest control value.

The effect of the different combinations varies, Svanborg (80) suggested that the overall effect reflected the competitive aspects of the component : Ethinylestradiol with hydroxy progesterone derivatives produced changes similar to those of the last trimester of pregnancy while Ethinylestradiol plus a 19-norsteroid showed relatively smaller changes in plasma lipids. The difference could be due partly to the antiestrogenic effect of the 19-norsteroid. With 30 μg Ethinylestradiol and 150 μg D-norgestrel cholesterol levels are actually reduced and serum triglyceride levels only slightly increased (81). Pribicevic in 1979 used an
oral contraceptive (0.5 mg Norgestrel + 0.05 mg Ethinylestradiol) on 100 women and studied their carbohydrate and lipid metabolism. The study showed no significant differences in lipid metabolism in women using oral or mechanical contraceptives.

**Blood clotting and thromboembolism:** Numerous changes in blood clotting factors due to combined hormone therapy have been reported. The changes are not dose dependant and in most cases have been directly related to the estrogen component.

Estrogens have long been used as haemostatic agents, and combined tablets reduce the amount of bleeding in patients with congenital clotting defects (83). Daniel et al. in 1968 (84) reported an increase in factor IX and an increase in thromboembolism associated with stilbesterol given to suppress lactation.

Both Premarin and Ethinylestradiol produced a rise in clotting factors VII and X and accelerated prothrombin time after three months' treatment (85) while Norgestrel has been shown to reverse the estrogenic rise in factor VII. Platelet aggregation is significantly accelerated with combined tablets. This effect is also estrogenic and not related to dose. Enhancement of platelet sensitivity to ADP with synthetic but not natural estrogen was reported (86). There have been mixed reports about the changes in fibrinolytic activity.

Thromboembolism probably depends on multiple factors including changes in blood vessels, blood viscosity, velocity of blood flow, the balance between the fibrinolytic and blood coagulation systems and hereditary tendencies. After considerable
debate it has been accepted that women taking combined contraceptive steroids are at greater risk from thromboembolism. The incidence of superficial thrombophlebitis is 50% higher and the risk of venous thromboembolism and of cerebral arterial thrombosis is five to six times greater. Thromboembolic mortality associated with various oral contraceptives is negligible when compared with maternal mortality (87). The risk does not apparently increase with duration of therapy but is greater in patients with a previous history. Severe varicose veins would generally be regarded as a contraindication to the use of combined contraceptives. Continued use does not increase the risk of thromboembolism and the risk does not continue after the pill is stopped (88). Contraceptive steroids would also appear to be contraindicated in patients with cyanotic heart disease, sickle cell anemia and polycythemia because of the existing risk of embolism. Although changes in clotting factors are apparently not dose dependent, the committee on safety of drugs came to the conclusion that the overall risk of thromboembolism was dose-related and they recommended that oral contraceptives containing not more than 50 µg of estrogen should normally be prescribed (89-90).
Injectable Contraceptives

From the preceding discussion it is evident that the oral contraceptive with the rigid dosage regimens require strict adherence to stipulated dosages. It has various adverse effects also. During the last ten years new development in contraception have been directed towards methods that do not disturb the hormonal regulation of the menstrual cycle. The use of low dose progestin was greeted as a great advance over the combined oral contraceptives as ovulation was not suppressed (90). It is very unlikely that the side effects of oral combined pills are simply a consequence of inhibition of ovulation. On the contrary, available studies indicate that the metabolic side effects (91-92) and the increased incidence of thromboembolic disease (93) are caused by the synthetic steroids used and are mainly due to the estrogen component.

Therefore attention has been focussed during the last decade to use progesterone-only contraceptive. Progesterone alone will block the estrogen induced LH and FSH release in primates (94-95), but progesterone has no effect on the high levels of LH and FSH observed in oophorectomised monkeys (96). However, high levels of progestins will decrease the plasma levels of both LH and FSH in normal men. Also synthetic progestin will block the midcycle surge of LH and prevent ovulation in most women, even when the dosage is as low as 0.5 mg of Norethindrone daily (97). At the dose level, the follicular development still occurs and occasional luteinization of these follicles
Junkmann in 1953 (98) reported that ester of a progestogen alcohol had long lasting effect when injected, and later synthesised esters of a progestogen, Norethindrone enanthate in 1958. In the same year 17-hydroxy progesterone and its derivative i.e. Medroxy progesterone acetate (MPA) were marketed. At that time it was used mainly for treatment of threatened and habitual abortion (99). A series of reports began to appear in 1959 regarding the treatment of endometrial carcinoma in situ and metastatic carcinoma with long acting progestogen compound (100-101). Since 1960, many investigators had reported on the use of progestogen compound in the treatment of endometriosis (102).

Depot formulation of medroxy progesterone acetate (DMPA) has been marketed by Upjohn, U.S.A. as Depoprovera. It has been extensively studied as a long acting contraceptive, when given in doses of 150 mg every three months or 300-400 mg every six months. By virtue of its poor solubility MPA is released very slowly after intramuscular injection of a sterile aqueous suspension resulting in prolonged activity (103).

Eichner in 1963 (104) indicated that this medication could be used for various gynecological and as obstetrical diseases like menorrhagia, amenorrhea, dysmenorrhea, premenstrual tension syndrome, recurrent or habitual abortion, threatened abortion, inadequate luteinisation etc. (105). Depoprovera was also used for treatment of idiopathic precocious puberty, though its effectiveness and safety for this purpose were not established (106-108).
A single massive dose of DMPA was given after the manage-
ment of premature labour, it was found that the women who received 
it became sterile for 12-21 months. Postpartum, follow up studies 
showed that this returning to normal menstrual cycles and normal 
pregnancy (109). Since then the contraceptive role of this drug 
was studied in different parts of the world. This drug was used 
alone or in various combinations with oral or injectable estro-
gens and given at intervals of 1 month to 1 year, the commonest 
being 150 mg every 3 months; the regimen of 300 mg every 6 months 
were also studied (110-115) (Fig. 6).

**Mechanism of action of DMPA as a contraceptive agent:**

DMPA, being a depot preparation, should act by constantly 
releasing small amounts of the drug which act in the body to 
produce contraceptive action. It is postulated that DMPA

i) inhibits ovulation,

ii) increases viscosity of cervical mucus forming a 
    barrier to spermatozoa,

iii) changes the rate of ovum transport through the 
    oviducts,

iv) makes the endometrium less suitable for implantation 
    of ovum.

DMPA probably affects ovulation by causing suppression of the 
surge of LH normally seen at midcycle just prior to ovulation 
(116-117). Zanartu *et al.* (118) postulated that DMPA does not 
block the follicle stimulating hormone required for follicular 
growth or luteinising hormone activity, but inhibits the preovu-
latory LH surge conditioning full follicle maturation and its 
rupture. To support the views they explored 27 women of proven 
fertility by surgical laparotomy after 6-37 months of contra-
Fig. 6: Depo-Provera (Depo-Medroxy Progesterone Acetate, DMPA) 17α hydroxy, 6α methyl, 4 pregnene, 3,20 dione acetate. Methyl group at C6 enhances its biological activity.
ceptive therapy with long acting injectable DMPA; in all the patients, the histology of the germinal epithelium, tunica albuginea ovarian cortex, stroma, hilam cells, egg cells and follicles from the primordial to graafian stage were normal, there was no formation of corpus luteum and an increase in ovarian connective tissue with congestion and haemorrhage of the thica interna was noted. A number of reports had described a variable decrease in total urinary gonadotrophin level (116) but no change had been noted in urinary levels of either 17-keto-steroids or 17-hydroxycorticosteroids (119). Studies by Goldzieher in 1972 (120) had shown that 7% of women, on conception control by means of progestogen administration had levels of pregnanediol in their urine comparable to values found in the urine of normally ovulating women. Therefore, he assumed that there are some factors other than inhibition of gonadotrophic hormone production by the pituitary gland by the progestogens; as such there might not be a viable ovum, the cervical mucus might have been made hostile to ovum or finally the endometrium might have been made unfavourable ovum implantation.

The injectable progestogens make cervical mucus scanty, viscous and sticky. The absence of both ferning and formation of spinbarkeit in the cervical mucus due to progestogen, is noted by many observers (121-122, 111). This mucus interferes with the movement of sperm into the uterus (115). But post coital tests (118) showed that the sperm actually had penetrated the viscous cervical mucus, however, no spermatozoa was recovered from the oviducts at laparotomy.
Under progestagen contraception, it has been observed that the endometrium becomes unable to support a fertilised ovum. DMPA causes thinning of endometrium, resulting in the production of an inactive tissue which is poorly suited for nidation of fertilised ovum (115). There is also decreased glandular activity (113).

According to Rosenfield (123), DMPA acts both centrally and peripherally but the primary contraceptive effect is on the hypothalamic pituitary axis. Recent data suggested the possibility that DMPA might produce a shock to the hypothalamus within 24 hours of administration with a resultant effect for 3-4 months. Supporting this view Johansson in 1974 (124) postulated that there might be less hazards of long term metabolic effects following the use of DMPA.

Drop out:

The drop out rate was only 25% according to Tyler et al. (114) and 39.1% in the series of Leiman (125) among the users of DMPA.

Excessive and irregular menstrual bleeding or spotting was blamed for the largest proportion of discontinuation between 1/4 and 1/2 of all the first year drop out as observed by Rinchart and Winter (126).

Drug effectiveness and failure:

No pregnancy was noted in the women taking DMPA for various purposes (112-114). Powel and Seymour (127) during
14.5 months of use of DMPA in 1,123 patients noted only 4 pregnancies of which 3 was attributed to patient failure (i.e. injection not taken in time). McDaniel and Pardihaisong (115) observed that 2.3% of the women taking DMPA experienced accidental pregnancies while under therapy (1.13 per 100 women year).

Richart and Winter (126) concluded in an abstract that only one in a hundred woman was likely to become pregnant during one year period while taking DMPA as a method of contraception.

Period of protection:

Mishell and his associates (112) had reported that a probable protection against conception persists for an additional month beyond the end of the third month. This observation was based on the fact that no pregnancy had occurred amongst 22 patients who received subsequent injection, 1-4 weeks later than scheduled (128).

Return of normal cycle and fertility:

Eichner in 1963 (104) first observed that normal pregnancy and delivery can occur following cessation of therapy with DMPA. Later in 1968 Zanartu and Navarro (111) reported that the interval of time between last injection of DMPA and occurrence of conception was apparently unrelated to dosage; and even after several years of use, fertility was resumed in the majority of the subjects. Return of fertility in two patients who failed to come after the first injection of DMPA, was observed, they
conceived in the 7th and 8th month respectively following discontinuation of therapy (113). Tyler et al. (114) in their 7 years study period observed that about one third of the patient resumed ovulation within half year after discontinuation of therapy and virtually all patients had ovulation within one year of the last injection of DMPA. In an experience with DMPA no correlation between the number of injections given and the time of return of regular menstrual cycles, or the interval between the last injection and conception was found. 68% of the patients conceived or established regular periods within 1 year and 84% of/conceived or established regular periods within 1.5 year after discontinuation of therapy (129). It was found that 75% of the women conceived with 1 year after discontinuation of DMPA (130).

Kora and Virkar (131) reported in their study that after stoppage of treatment with DMPA as a contraceptive agent 69% of the women had conceived by the end of 1 year and 87% had conceived by the end of 2 years.

Effect on fetus in subsequent pregnancy:

In the late 1950s and early 1960s researchers discovered that some progestagen when given in early pregnancy occasionally cause masculinization of the external genitalia of female fetuses (59, 132-133). Beurstein and Wasserman (134) showed that there was a minimal androgenic change in the human fetus where the mother used DMPA in early pregnancy. Kauman in 1973 (135), reported one case with congenital anomalies where the mother used oral stilbesterol and intramuscular DMPA for 2 months beginning
from 47 days after the last menstrual period.

It was suggested that in a few women steroid administration in early pregnancy might cause congenital anomalies (136).

Effect on lactation:

Using progesterone daily in small doses, Satterthwaite and Gamble in 1969 (137) noted cessation of milk secretion after first or second cycle. Orthonovum 10 mg daily caused depression or stoppage of lactation in 32.5% cases (138). Chinnatamby (139) also noted a depressive action of Enovid on lactation. In her later study she concluded that higher the dose of progesterone greater is the degree of suppression of lactation. Some observers (104) found no interference with lactation in the women who were undergoing conception control with DMPA.

Certain specific progestagens alone exhibit a dose-dependent suppression of lactation because they have been converted into estrogen in vivo (140); while another observer (90) showed that as chlormadinone or DMPA exhibit little or no estrogen conversion, they do not cause suppression of lactation. Mazhar and associates (141) comparing the ages at supplementation and weaning in 51 cases giving different gestagens with their previous lactational history found that though supplementation was early in onset in the present history, the total duration of lactation remained the same. There was no change in the composition of milk, namely production of fat, protein and lactose in women using DMPA as contraceptive (142).
During the study on the effect of oral contraceptive on lactation it was found that DMPA increased the milk production only initially and finally it inhibited the same, though oral contraceptive had no significant effect on specific gravity or the composition of milk (143). Gulloff et al. (144) in their study on the effect of contraception on lactation, noted that, when treatment with potential DMPA was began immediately post-partum, the median duration of lactation was significantly longer. The use of this compound soon after delivery even before the reflex is induced by suckling, does not inhibit lactation.

Metabolic milieu in DMPA therapy:

Carbohydrate metabolism: The effects of hormonal contraceptives on carbohydrate metabolism were studied both upon animal kingdom and human volunteers. According to some observers (145-146) progesterone has got no adverse effects on carbohydrate metabolism of pancreatectomized ferrets and diabetic rats. An increase in glucose level with high dosage of progesterone and a decrease with low doses was observed (147) while studying the pancreatectomised rats. The administration of different preparations of progestagens to rhesus monkeys caused dissimilation of plasma glucose during intravenous glucose tolerance test, though the insulin response was greater than during pretreatment test; implying that the progestagens studied, caused increased peripheral insulin resistance (148).

The interest began to grow regarding the effect of progesterone on the carbohydrate metabolism, when Lebhers and Pobes (149)
showed one case of diabetic whose disease was difficult to control while she was taking an oral progestin, Norlutin. The deterioration of the glucose tolerance test and the development of relative insulin resistance was reported (150) when adrenalectomised patients with breast cancer were given the Medroxyprogesterone preparation. There was no effect on the oral glucose tolerance tests in patients using 5 mg of progestin, (Norethisterone acetate) (151). When 34 women with breast cancers were treated with DMPA, one patient developed diabetes mellitus after she gained 20 lbs in weight while 4 diabetics were able to be adequately controlled while receiving the therapy (152). Goldman, Ovadia and Eckerling (153) performed intravenous glucose tolerance test on 22 women before, during and after treatment with DMPA, and noted no difference in the results of the tests. Significant elevation of blood glucose levels was observed (154) by oral glucose tolerance tests in 24 subjects after 12 months treatment with DMPA. Spellacy (155) in his reviews on carbohydrate metabolism and oral contraceptive found no adverse effect of progestins on the carbohydrate metabolism.

Larscon-Cohn et al. (156) in their study following progesterone administration in women as a contraceptive method found that glucose tolerance test and plasma insulin level both remained unchanged. Some observers (157) using intramuscular progesterone preparation 300-400 mg daily found no change in intravenous glucose tolerance test but there was increased insulin response. Spellacy, Birk et al. (158) concluded that there was definite rise of blood glucose level which probably was due to glucocorticoid like
activity. There was no change in plasma glucose or plasma insulin levels following daily administration of aprogestagenic contraceptive i.e. Megestrol acetate; whereas these levels fell considerably following combined estrogen-progesterone oral contraceptive (159).

Schwallie and Assenzo (160) noted no change in fasting blood sugar level after treatment with DMPA. Tuttle et al. (161) showed the effect of MPA on carbohydrate metabolism. The carbohydrate metabolism of 18 women receiving long-term treatment with medroxyprogesterone acetate (MPA) and 14 control women was investigated. Only 1 subject in the treated group had an impaired glucose tolerance and this was accompanied by excessive weight gain. The MPA caused an increase in serum insulin which was significant at the fasting and 3 hour periods. Serum growth hormone levels tended to increase, but the difference was significant at the 3 hour period only. The drug had no clear effect on plasma free fatty acids or serum triglycerides. In view of the hyperinsulinism, it is concluded that MPA is diabetogenic and the implications of this are discussed. In a recent study two injectable contraceptives DMPA and NET-0EN (Norethisterone enanthate) were given to 60 healthy women for 850 cycles, they were examined for all protein bound carbohydrates and the results did not show any significant statistical difference from the controls (162).

Other metabolic effects: In 1972 Simpson et al. (163) studied serum levels of calcium, phosphorus and magnesium in women employing two different hormonal contraceptive regimens.
In women using DMPA, serum phosphorus and magnesium levels were significantly elevated over the controls. There were no significant differences in serum calcium levels between DMPA and control groups. Again no significant changes in serum levels of phosphorus, magnesium or calcium could be demonstrated after prolonged contraceptive treatment. Dale et al. (164) measured serum magnesium levels in 348 women. The patients under study received a purely progestational agent, DMPA, had magnesium concentrations significantly higher than any other group (P<0.001). Briggs in 1973 (165) showed a depot preparation (DMPA) appeared to have little effect on platelet composition or functions.

Measurement of DMPA by Radio Immuno Assay (RIA):

In vivo metabolism of \(^3\)H-medroxyprogesterone acetate in pregnant and nonpregnant women and in fetus was studied by Besch et al. in 1966 (166). Medroxy progesterone acetate, 150 mg, was given at 3 months intervals, and plasma gonadotrophins assayed immediately before each injection. Plasma FSH level at this time were within the control range, and LH values, which depressed to 65% of control after the first injection, subsequently rose into the normal range (167).

A double antibody radioimmuno assay, which is capable of detecting 100 pg of Medroxyprogesterone acetate in 0.1 ml of unextracted serum was described by a group of workers (168). The antibody was produced to steroid conjugated at the 3 position with bovine serum albumin. The side groups attached at 17 and 21 positions are more important determinants of antibody
specificity than groups attached at 6. The concentration of DMPA in peripheral serum was determined for female monkeys and human that received the drug by various routes of administration. A rapid decrease to less than one half of the initial concentration was noted within 10 minutes after an intravenous injection. Blood levels were maximum at 2 or 4 hours after oral administration. Intramuscular injection resulted in fluctuating blood levels that were maximal at 2-15 days post injection.

Koetsawang (169) showed mean levels of MPA in blood taken 90 days after injection were not significantly different between women who had received a single injection of Depo-provera and those who had received eight injections at 90 days intervals. He also showed that in women who had received 31 to 45 injections of Cycloprovera, plasma levels of MPA 28 days after injection were significantly higher than those of women who had received a single injection. The levels were also higher than those found in women 90 days after injection of Depoprovera. The results suggest that the dose of MPA in Cycloprovera could be reduced.