CHAPTER I
GENERAL INTRODUCTION
We are living in a constantly changing chemical environment. Use of various drugs, such as, antibiotics, antiinflammatory steroids, anticonvulsants, laxatives, sedatives, whose list is evergrowing, contributes significantly to the changes in chemical environment. It is becoming increasingly evident that drugs interact with nutrients and increase their requirement, sometimes resulting in deficiency diseases. Drugs have been shown to cause nutrient depletion by different mechanisms such as impairment of absorption, displacement of nutrients from binding sites, impairment of normal metabolism or by antagonism.

The development of steroidal oral contraceptives as an efficient method of contraception has led to their widespread use. Oral contraceptive steroids are perhaps the most widely used among drugs. Although, their use was confined to women in affluent countries in the beginning, in recent years, they have gained popularity in developing countries like India as well. In view of the widespread prevalence of nutrient deficiencies in developing countries, exact information regarding effects of these hormones on nutrient metabolism and requirement is necessary.

The development of steroidal oral contraceptives started in the late 1950s, although, it was known even in 1930s that progesterone could inhibit ovulation in rodents. Selye et al in 1936 observed that injection of progesterone daily to rats inhibited estrous cycle. These results were
later confirmed in rabbits, guinea pigs and rats (Makepeace et al., 1937; Dempsey, 1937; Astwood and Fevold, 1939).

Many years later, Pincus (1956) used oral progesterone to induce anovulatory cycles in a group of normal women. Administration of 300 mg progesterone orally each day, from the 5th to the 25th days of the menstrual cycle, inhibited ovulation in 85 per cent of women in the first cycle and in 95 to 100 per cent of women in the second or third cycles. Using the same regimen of progesterone Ishikawa et al. (1957) obtained similar results.

However, use of oral progesterone had two disadvantages (Pincus, 1959). First the need for high doses of progesterone to obtain complete inhibition of ovulation and second the occurrence of premature menstruation or breakthrough bleeding (BTB) in a number of women. The problem of BTB was overcome initially by administering oral estrogen with oral progesterone, but, later on, again cycles of shorter duration ensued.

These problems associated with the use of oral progesterone, initiated the search for compounds which would be more potent progestogens when given orally and which would also result in a greater regulation of cycle length.

Among the various steroidal compounds tested, the derivatives of 19-nor-testosterone (Fig. 1) were found to be
Figure 1: Structures of 19-Nor-Testosterone Derivatives

19-Nortestosterone

Norethynodrel

Norgestimate

Norethisterone Acetate

Lyneostrol
effective inhibitors of ovulation in animals (Pincus et al., 1956) and in humans (Pincus and Garcia, 1956). Further, Rock et al. (1956) used these synthetic steroids to treat unexplained infertility in women and found that they inhibited ovulation. When these findings were presented at the Laurention Hormone Conference (1956), Greenblatt commented that "Dr. Rock has unwittingly given us an excellent oral contraceptive which may be employed with little untoward effect". These results opened the era of hormonal method of contraception.

Some of the synthetic progestogens tested were found to have inherent estrogenic activity and some were contaminated with estrogens. Progestogens which had no estrogenicity resulted in BTB (Pincus et al., 1956), suggesting that small amount of estrogen is also necessary for the maintenance of endometrium. These observations, thus, resulted in the use of estrogen-progestogen combination in oral contraceptives. The first field trials were conducted in Puerto Rico by Pincus et al. (1958) using a combination of norethynodrel and mestranol administered from 5th to 24th day of the cycle. These proved to be very effective in the control of fertility. It was also observed that women who had discontinued the use of these drugs after taking them for sometime became pregnant, suggesting that subsequent fertility is not interfered with the use of these hormones.
Kurzrock (1937) observed that the treatment of dysmenorrhea with estrogen inhibited ovulation and pointed out that this could be used as a method for fertility control. Lyon (1943) demonstrated inhibition of ovulation with 50/ug ethinylestradiol administered daily. Further, Fuller Albright (1945) proposed the administration of estrogen for some period followed by estrogen plus progesterone, as a method of contraception. The latter formed the basis for the "sequential type" of oral contraceptives. Studies carried out by Goldzieher et al. (1964) established the usefulness of sequential type of oral contraceptives. This method of oral contraception consists of the use of estrogen from 5th to 19th or 20th day followed by estrogen plus progesterone for the next 5 or 6 days.

The observation by Rudal et al. (1965) that the administration of small doses of the synthetic progestogen, chlormadinone acetate interfered with fertility, led to the development of "mini pill". Martinez-Manoutou et al. (1966, 1967) showed that the continuous administration of low doses (0.5 mg/day) of chlormadinone acetate could be effectively used for fertility control. Since then a number of other synthetic progestogens, such as, norgestrel, clomestone, norethindrone, lynestrenol, have been tested for their antifertility effects at various doses and have been found to be potent oral contraceptives (Foss et al., 1968; Eckstein et al., 1972; El Mahgoub et al., 1971; Moghissi et al. 1973; Board, 1971; Prisc et al., 1973).
We have, thus, mainly three types of oral contraceptives (OC) viz., the combined type, in which a progestogen is given in combination with an estrogen; the sequential type in which estrogen is given alone for 14-15 days followed by estrogen plus progestogen for the next 5 to 6 days; and the 'mini pill', in which a progestogen is administered in low doses continuously. The estrogen component of the pill is either ethinyl estradiol or mestranol (Fig. 2), while the progestogen is either a derivative of 19-nor-testosterone (Fig. 1) or 17-α(- hydroxy-progesterone (Fig. 3). Composition of some of the commercially available oral contraceptives are listed in Table 1.

**Biological activity of the synthetic steroids:**

Removal of the 19-methyl group from testosterone makes it less androgenic and to the resulting 19-nor-testosterone, addition of 17-α(- ethinyl group confers progestational activity (Saunders, 1970). Further modification of 17-α(- ethinyl-19-nor-testosterone results in compounds which differ slightly in their biological properties (Petrow, 1966).

Neither progesterone nor 17-α(-hydroxyprogesterone are active orally. However, esterification of 17-α(-hydroxyprogesterone makes the compound a potent progestogen (Saunders 1970). A major pathway of 17-α(-hydroxyprogesterone metabolism involves hydroxylation at C-6. Thus, substitutions at C-6 by chlorine (as in chlormadinone acetate) or methyl
Fig. 2: STRUCTURES OF SYNTHETIC ESTROGENS
Fig. 3: STRUCTURES OF 17α-HYDROXY PROGESTERONE DERIVATIVES
## Table 1
COMPOSITION AND DOSES OF SOME ORAL CONTRACEPTIVES

<table>
<thead>
<tr>
<th>Trade names</th>
<th>Progestogen (mg)</th>
<th>Estrogen (mg)</th>
</tr>
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<tbody>
<tr>
<td>Enovid, 5 mg</td>
<td>Norethynodrel 5</td>
<td>Mestranol 0.075</td>
</tr>
<tr>
<td>Norinyl, 10 mg</td>
<td>Norethindrone 10</td>
<td>Mestranol 0.06</td>
</tr>
<tr>
<td>Ortho-Novum, 1:80</td>
<td>Norethindrone 1</td>
<td>Mestranol 0.08</td>
</tr>
<tr>
<td>Norlestrin, 1 mg</td>
<td>Norethindrone</td>
<td>Ethinyl estradiol 0.05</td>
</tr>
<tr>
<td></td>
<td>acetate</td>
<td></td>
</tr>
<tr>
<td>Provest</td>
<td>Medroxyprogesterone acetate</td>
<td>Ethinyl estradiol 0.05</td>
</tr>
<tr>
<td>Ovulen, 1 mg</td>
<td>Ethynodiol acetate</td>
<td>Mestranol 0.1</td>
</tr>
<tr>
<td>Volidan</td>
<td>Megestrol acetate</td>
<td>Ethinyl estradiol 0.05</td>
</tr>
<tr>
<td>Ovral</td>
<td>Norgestrel 0.5</td>
<td>Ethinyl estradiol 0.05</td>
</tr>
<tr>
<td>Ovulen-50</td>
<td>Ethynodiol acetate</td>
<td>Ethinyl estradiol 0.05</td>
</tr>
<tr>
<td><strong>Sequential</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Quens</td>
<td>Chlomadinone acetate</td>
<td>Mestranol 0.08</td>
</tr>
<tr>
<td>Norquen</td>
<td>Norethindrone 2</td>
<td>Mestranol 0.08</td>
</tr>
<tr>
<td>Oracon</td>
<td>Dimethisterone 25</td>
<td>Ethinyl estradiol 0.1</td>
</tr>
</tbody>
</table>

* Estrogen alone 14 to 16 days followed by combination for 5 or 6 days.
group (as in medroxyprogesterone acetate) interferes
with the metabolism of this compound leading to an increase
in the biological activity (Bingel and Benoit, 1973). Some
of the biological properties of these compounds differ from
those of 19-nor-testosterone derivatives (Saunders, 1970).

When administered orally, estradiol is not active;
however, addition of 17-α(-ethinyl group to yield 17-α(-ethinyl
estradiol and the 3-methyl ether of this compound - mestranol,
are orally active (Allen, 1970). Both these synthetic steroids
are more potent estrogens than estradiol (Law et al, 1974).

Among the various synthetic progestogens,
d-norgestrel is the most potent, while medroxyprogesterone
acetate is the least potent (Law et al, 1974).

Menstrual cycle:

The ovaries contain a large number of premordial
follicles, each of which contains an immature ovum. Several
of these follicles enlarge at the start of each cycle, but
only one of them which is destined to ovulate grows rapidly
while the others become atretic. At the same time, the
ovum undergoes its meiotic division. Around the 14th day
of the cycle, the phase of follicular maturation - or the
follicular phase - is terminated by the rupture of the
follicle, accompanied by the discharge of the ovum into
abdominal cavity. The released ovum is taken up by the
fallopian tube and transported into uterus.
The ruptured follicle now changes into corpus luteum, which functions temporarily as a gland of internal secretion. The corpus luteum secretes estrogens and progesterone. This second phase of the cycle is referred to as the luteal phase. The corpus luteum persists if the discharged ovum gets fertilized. In the absence of pregnancy, however, it starts degenerating just four days before the next menstrual cycle.

The hypothalamic-pituitary-ovarian axis plays an important role in the regulation of ovulation and menstrual cycle. The anterior pituitary secretes gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), the synthesis and secretion of which are under the control of hypothalamic releasing hormone or hormones. This hormone(s) is referred to as gonadotrophin releasing hormone (GRH) (Shally et al., 1971), or luteinizing hormone releasing hormone or factor (LRH or LRF) and follicle stimulating hormone releasing hormone (FSH-RH) (Johansson et al., 1973; Currie et al., 1973; Bowers et al., 1973). Koering (1969) suggested that the growth of a follicle which is destined for ovulation begins during the luteal phase of the preceding menstrual cycle, under the influence of FSH, which begins to rise gradually during this period, and constant low levels of LH. The growth of follicle continues in the follicular phase of the next cycle, producing increased amounts of estrogens. Since estrogens have a negative feed-back action on FSH (Yen and Tsai, 1972; Monroe et al., 1972), an increase
in their production causes a slow decline in FSH levels. The positive feed-back action of estrogens on LH (Speroff and Van der Wiele, 1971) leads to a rapid increase in LH levels giving rise to a preovulatory LH surge. While the primary role of FSH is to stimulate follicular growth, LH plays a role both in follicular maturation and preovulatory steroidogenesis. Under the influence of FSH and LH, the follicle matures fully and ovulation occurs. Following ovulation, LH levels start declining. The newly formed corpus luteum from the ruptured follicle secretes increasing amounts of progesterone and estrogens under the influence of low post-ovulatory LH. The secretory capacity of corpus luteum decreases markedly after 9-11 days of ovulation (Speroff, 1971).

The ovarian steroid hormones, estrogens and progesterone, produce cyclic changes in the endometrium and the cervical mucus. Estrogens secreted during the 5th to 14th day of the cycle produce proliferative changes in the endometrium. Towards the end of the cycle, when the hormonal support from the regressing corpus luteum is withdrawn, menstruation occurs in the form of shedding of the endometrium.

During the follicular phase, particularly just before ovulation, the cervical mucus becomes thin and watery, and is favourable for sperm migration (Zussman et al., 1967). However, during the luteal phase, due to progesterone,
the cervical mucus becomes thick, scanty and cellular and is unfavourable for sperm migration.

**Mechanism of action of oral contraceptives:**

The anticonceptive action of both combined and sequential type of oral contraceptives is believed to be due to inhibition of ovulation. Rock *et al* (1957) observed inhibition of ovulation in women treated cyclically with norethynodrel or norethindrone plus mestranol. Zussman *et al* (1967) showed that in women treated with norethindrone plus mestranol, there were changes in ovarian morphology, such as, increased fibrosis, degeneration of follicles and decreased luteinization. Kopera *et al* (1964) and Ostergaard and Starup (1968) observed the absence of corpora lutea in women treated with Lyndiol and Enovid respectively, indicating inhibition of ovulation. It was, however, found that the inhibition occurred provided the medication was started earlier than 7th day of the cycle (Ostergaard and Starup, 1968).

Since ovulation is controlled by the hypothalamic-pituitary-ovarian axis, it appears very likely that the oral contraceptives exert their action on one or more of these centres. The most likely site of action seems to be hypothalamus, since it controls the endocrine function and since estrogens and progesterone have a feed-back control over it. Minaguchi and Mertes (1967) reported a
decrease in LH-RH in rats treated with Enovid. However, the capacity of LH-RH to stimulate the release of LH from the pituitary does not seem to be affected (Schally et al., 1968; Kastin et al., 1969). The hypothalamic suppression caused by an OC may result in a decrease in the pituitary gonadotrophin output. Both the FSH and LH peaks, which occur during menstrual cycle, have been shown to be suppressed by combined as well as sequential type of OC (Swerdloff and Odell, 1969; Abraham et al., 1970; Goldzieher et al., 1970; Dufau et al., 1970). Goldzieher et al. (1970) observed decrease in basal levels of LH on OC treatment.

It appears as though the estrogentic component of the pill plays a more important role in ovulation inhibition. Estrogens when used without progestogen in doses commonly employed in the OC preparations were found to diminish urinary FSH excretion (Vorys et al., 1965; Saunders, 1970). If the FSH levels are sufficiently suppressed by OC, follicular growth will not take place. In such an event, even higher LH levels, as found in sequential type OC (Goldzieher et al., 1970) cannot cause ovulation due to an absence of mature follicles. Combined type OC produces a greater suppression of pituitary, as evidenced by lower FSH and LH levels, which would result in a lesser degree of follicular maturation (Bingel and Benoit, 1973). They also provide secondary mechanisms to prevent conception. One such
mechanism being production of changes in the cervical mucus, typical of the luteal phase of the menstrual cycle, due to the progestogen present in the pill (Singer and Reid, 1970). The thick, scanty and cellular cervical mucus provides a physical barrier to the sperm migration. Endometrial changes produced by the OC may also provide one of the mechanism of action (Ronald, 1968 and Klopper, 1970).

Since ovulation is not always inhibited by low dose progestogen contraceptives, one of the main mechanisms by which they act seem to be the changes brought about in the cervical mucus (Eckstien et al, 1972; Moghissi et al, 1973; Boettcher, 1973). Other mechanisms of these OC involve impairment of corpus luteum function (Eckstien et al, 1972) or endometrial changes preventing implantation (Moghissi et al, 1973) or changes in hormonal profile (Larsson-Cohn et al, 1970). A direct action of these steroids on spermatozoa has also been suggested (Pedron et al, 1973).

Metabolism of oral contraceptive steroids

Metabolism of synthetic estrogens:

The metabolism of synthetic estrogens, ethinyl estradiol and mestranol, has been studied using labelled compounds. After the administration of \( ^{14} \)C-ethinyl estradiol to women (2 μCi in 50 μg, dose which is generally used in OC preparations), intravenously and orally, 28 and 48 per cent of the dose were respectively recovered from urine in 5 days (Kamyab et al, 1969). Reed et al (1970)
recovered half of the administered activity in 24 hours and complete recovery was obtained by 5 days. The biological half-life of ethinyl estradiol was shown to be 27 hours. Similar observations were made by Abdel-Aziz and Williams (1970) and Kulkarni and Goldzieher (1970) who showed that the biological half-life of ethinyl estradiol to be 28.5 hours.

Most of the radioactivity in urine was in the conjugated form, since hydrolysis with 3-glucuronidase released large amounts (74 per cent) of extractable radioactivity (Kamyab et al, 1970). Sulphation did not seem to be important in the metabolism of ethinyl-estradiol. Abdel-Aziz and Williams (1970) showed that 19.4 per cent of ethinyl estradiol was excreted unchanged, while conjugation with glucuronide was accounted for by 12.4 per cent.

Blood levels of ethinyl estradiol were higher on intravenous administration and within 1 hour of injection most of the activity was present in conjugated form (Kamyab et al, 1970). Reed et al (1970) found 9 per cent of the dose in plasma at 1 hour and this decreased to 4.7 per cent in 24 hours after oral administration.

After the oral administration of 6-7-3H-mestranol to 3 women, 2 of whom were taking Ortho-Novum (norethisterone plus mestranol), Williams (1969) recovered 29 per cent to 43 per cent of the dose in urine over an eight day period and the biological half-life was found to be 44 hours.
Wijmenga and Van der Mollen (1969) orally administered \( ^{14}\text{C} \)-mestranol to four women who were lactating and taking Lyndiol (mestranol plus lynestrenol) and recovered 30 to 52 per cent of radioactivity in urine at the end of 5 days. Mestranol was found to have two phases in plasma, a first rapid clearance corresponding to a biological half-life of 24 to 36 hours, followed by a second slower disappearance rate corresponding to a half-life of 40 to 50 hours. A small amount of the administered dose (0.0002 to 0.013 per cent) was excreted in milk. Kulkarni and Goldzieher (1970b) recovered 9.7 to 27.1 per cent (mean 18.8 per cent) of the activity in urine in 5 days, after the administration of \( ^{14}\text{C} \)-mestranol to women. The half-life was observed to be 71 hours. None of the administered compounds could be recovered in the unconjugated form (Williams, 1969); however, ethinyl estradiol could be isolated from the glucuronide fraction, showing that demethylation of mestranol did occur.

Metabolism of progestogens:

Metabolism of 19-nor-testosterone derivatives: The metabolism of norethisterone, lynestrenol and norgestrel were studied using \( ^{14}\text{C} \) labelled compounds (Kamyab et al, 1968a; Kamyab et al, 1968b; Littleton et al, 1968). These studies showed some similarities and dissimilarities in the metabolism of these compounds. The recovery of administered activity in urine was similar in case of norgestrel and lynestrenol (40 and 43.7 per cent respectively), whereas norethisterone
gave higher recoveries (53.9 per cent). The biological half-lives of norgestrel and lynestrenol were similar, being 24 and 26.5 hours respectively, while norethisterone had a shorter half-life of 19 hours. Further, differences in the pattern of conjugation among these steroids were observed. Norethisterone and lynestrenol had higher glucuronide conjugation (48.8 and 45.6 per cent recovery after glucuronidase treatment) compared to norgestrel (32.2 per cent). Sulphate conjugation for norgestrel seemed to be higher (25.8 per cent) compared to norethisterone (14 per cent) or lynestrenol (7.4 per cent). Both norethisterone and lynestrenol produced more of polar metabolites than norgestrel. The ethinyl group of these steroids does not seem to be metabolised.

The conversion of these progestogens to estrogens was very low, which was indicated by a low radioactivity (1.5 to 2.5 per cent) associated with the phenolic compounds. Brown and Blair (1960) have shown the conversion of norethisterone to ethinyl estradiol. Norgestrel has been shown to be metabolised to a weak estrogen (Edgren, quoted in Littleton et al., 1968). Similarly, ethinodioldiacetate has been shown to be converted to ethinyl estradiol after the removal of 3 /3, 17/3 - bis acetoxyl group and concomitant aromatization (Besch et al., 1963). Fotherby (1974), however, observed that most of the phenolic compounds found in urine after the administration of progestogens arise artefactually and that only small amounts of estrogenic
metabolites might be produced in vivo, as shown by the insignificant amount of in vitro conversion of norethisterone or norgestrel to estrogenic compounds.

**Metabolism of 17-α(-hydroxy progesterone derivatives):** The metabolism of these steroids have also been studied using labelled compounds. Slaunwhite and Sandberg (1961) administered medroxy-progesterone acetate to 6 women and recovered a mean of 33 per cent of the administered activity in urine. When labelled 17-α(-hydroxyprogesterone was administered, the recovery was more, being 58 per cent. This suggested that medroxyprogesterone acetate is retained in the body. Of the urinary radioactivity recovered after the administration of medroxyprogesterone acetate, only 8 per cent was in the free form, while the glucuronide fraction accounted for 50 to 60 per cent. The plasma clearance rate showed that this compound had two clearance rates, a first faster rate corresponding to a half-life of 52 minutes and a second slower rate corresponding to a half-life of 230 minutes, while 17-α(-hydroxyprogesterone, similarly had two half lives of 11 minutes and 108 minutes. Fotherby et al (1968), using tritiated medroxyprogesterone acetate, recovered 38 per cent of the activity in urine over a 5-hour period and the biological half-life was observed to be 14.5 hours. At 4 hours after the administration of the compound, the activity in plasma was 1.5 per cent and at 12 hours there was no detectable activity.
Cooper and Kellie (1968) studied the metabolism of megesterol acetate using $^{14}$C label at the 6-α(-CH$_3$) group, administered to 5 subjects; 4 subjects received 60 to 90 mg of the compound in 10 per cent alcohol, while the 5th subject received a dose of 4 mg, similar to the dose used in OC preparations. The four subjects excreted 56 to 78 per cent of dose in urine, whereas the 5th subject excreted 5 per cent of the dose. It was observed that 12 per cent of the dose was in the free form and 25.5 per cent in the conjugated form, while sulphate conjugation accounted for 5 to 7 per cent of this latter fraction. Dihydroxylation of medroxyprogesterone acetate at C-6 and C-21 was found to occur, yielding 6-α(-methyl-6/3, 17 α, 21-trihydroxy-4-en-3, 20-dione-17 acetate (Helmrich and Huseby, 1962). Most of this metabolite was in the conjugated form as glucuronide, probably conjugated at C-21. However, Castegnaro and Sala (1962) and Besch et al (1966) isolated 6-α(-methyl-6/3, 17 α, 21-trihydroxy-4-en-3, 20-dione-21-acetate, which seems to be an artefact, arising due to the transfer of the 17-α(-acetoxy group to C-21 during hydrolysis. Two important aspects of metabolism of the 6-methyl substituted compounds (such as medroxyprogesterone acetate and megesterol acetate) seem to be production of polar metabolites and retention of 4-en-3-one grouping in contrast to naturally occurring as well as other synthetic steroids (Fotherby and James, 1972).
Side effects

Use of OC is associated with side effects, some of which are of trivial nature, such as, nausea, cramps, indigestion, breast symptoms, nose bleed etc (Pincus et al, 1958), while others such as thromboembolism, hypertension, myocardial infraction, mental changes, liver tumours are more serious.

Thromboembolism associated with the use of norethynodrel was first reported by Jordan (1961). Inman and Vessey (1968) in a careful study observed a significantly higher incidence of pulmonary embolism and cerebral thrombosis in women taking OC. Vessey and Doll (1968) reported deep vein thrombosis on OC treatment. Sartwell et al (1969) found increased risk of thromboembolism on OC treatment, particularly with sequential type of OC. However, reports contradicting an association between OC and thromboembolism have also appeared (Drill and Calhoun, 1968; Drill, 1972; Drill and Calhoun, 1972).

OC treatment has also been shown to elevate blood pressure leading to hypertension (Spellacy and Birk, 1972; Wier et al, 1974; Weir et al, 1975). Rise in blood pressure is shown to occur during the first two years of OC treatment and in some women progressive rise of blood pressure occurs upto five years (Weir et al, 1962). Spellacy and Birk (1972) observed that progestogen alone type OC do not lead to hypertension, while the administration of ethinyl estradiol
in doses commonly used in OC preparations leads to hypertension, showing that the estrogen component of the pill is probably the responsible factor. OC treatment has been shown to stimulate renin-angiotensin system in women and this has been implicated in the development of hypertension (Saruta et al., 1970; Crane et al., 1971). Weir et al. (1975), however, feel that hypertension caused by OC might be due to the hemodynamic changes brought about by the estrogen component of OC.

Women using OC have been found to show altered liver function tests, such as, increase in the activities of serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT), and higher sulfobromophthalein (BSP) retention time (Larsson-Cohn, 1967). Liver tumours in OC treated women have also been reported recently (O’Sullivan and Wilding, 1974; Christopherson et al., 1975). Myocardial infarction has been shown to be associated with the use of OC, particularly in women who have predisposing factors (Oliver, 1970; Kubik and Bhowmick, 1974).

Number of reports have appeared on psychiatric disturbances in women taking OC (Nilsson et al., 1969; Herzberg et al., 1970). Women who have predisposing factors, such as previous history of psychiatric symptoms are more prone to develop these changes on OC treatment (Lewis and Hoghughi, 1969). The progestogen component of the OC has been implicated in their development (Lewis and Hoghughi, 1969). Further, it was observed that the endometrial monoamine oxidase (MAO) activity was higher in women using strongly
progestogenic type of OC, compared to other preparations, and in contrast women using strongly estrogenic sequential type of OC had weak MAO activity. These studies led to the suggestion that MAO may play a role in the development of depression and other psychiatric changes. Contradictory reports which show no association between OC and depression have also appeared (Goldzieher et al, 1969; Kutner et al, 1971).

**Endocrine effects:**

The effect of OC on the pituitary gonadotrophins has already been discussed in relation to the mechanism of anticonceptive action of these steroids. As a result of pituitary suppression, the endogenous production of sex hormones, progesterone and estrogen may be expected to be decreased. Lucis and Lucis (1972) have observed decreases in the urinary output of pregnanediol, pregnanetriol and estrogens in women taking OC.

Thyroid function in women on OC has been studied using a variety of tests. Estrogen (diethylstilbesterol) as well as OC have been shown to increase thyroid binding globulin (Engbring and Engstrom, 1959; Laurell et al, 1968) resulting in an increase in the protein bound iodine. The effects of ethinyl-estradiol, mestranol and progestogens on thyroid function were studied by Winikoff (1968). The results suggested that the changes observed were brought about by estrogens. The increase in plasma thyroxine level
was found to be confined to the protein-bound fraction with no change in free thyroxine (Lucis and Lucis, 1972).

Similar studies on the effects of OC on adrenocortical function have been carried out (Dodek et al, 1965; Lucis and Lucis, 1972). Enovid treatment to postmenopausal women significantly increased the plasma cortisol levels, while plasma clearance of cortisol was diminished (Dodek et al, 1965). The results suggested that OC treatment elevated the levels of plasma transcortin. Givens et al (1968) and Sandberg et al (1969) observed increases in transcortin as well as plasma cortisol levels in women taking OC. Besides, an increase in free cortisol was also observed. Lucis and Lucis (1972) found an increase in plasma cortisol levels, increase in urinary cortisol and a decrease in the urinary excretion of 17-ketosteroids. The plasma cortisol levels in OC treated women were found almost to resemble those found in patients with Cushing's Syndrome. The fact that these women do not show any of the symptoms of Cushing's Syndrome suggests that most of the increase in plasma cortisol must occur in the bound form, with a small increase in the free form, as reflected in its higher urinary output.

Metabolic effects of oral contraceptives

Carbohydrate metabolism:

Waine et al (1963) were the first to report an
impairment of carbohydrate metabolism in women taking OC. Since then many reports have appeared on this subject and these have been reviewed by Spellacy (1969). The abnormalities of carbohydrate metabolism observed in women using OC are (1) abnormal intravenous glucose tolerance; (2) abnormal oral glucose tolerance; (3) increased levels of circulating insulin; and (4) increased growth hormone levels. These abnormalities seem to be related to the type of OC used, family history of diabetes mellitus, age and body weight. It was speculated that increases in cortisol and growth hormone or altered liver function and alteration in peripheral tissue utilization of glucose may be responsible for this abnormality on OC treatment.

Wynn and Doar (1969) studied the effects of combined OC preparations on oral and intravenous glucose tolerance tests in 3 groups of women — one group tested before and during therapy, a second group during and after therapy, and a third group tested twice during therapy. The results of this study showed that intravenous and oral glucose tolerance deteriorated in 70 and 78 per cent of women respectively in the first group. In the second group, an improvement was observed in intravenous (85 per cent of women) and oral (90 per cent of women) glucose tolerance after stopping the OC therapy, and in the third group, there was no difference in glucose tolerance during two different time points of OC treatment. Mean fasting glucose levels were not altered in these women, whereas pyruvate levels were
significantly raised. The authors suggested that "steroid diabetes" is caused by elevated plasma cortisol levels as a result of OC treatment. Although various reports indicate that fasting glucose levels are not altered during OC treatment, in subclinical diabetes, OC elevate fasting glucose levels (Goldman and Eckerling, 1970). Vermeulen et al (1970) observed slight decreases in glucose levels during treatment with sequential type OC and ethinodiol diacetate. Posner et al (1975) showed that intravenous glucose tolerance was impaired in women treated with Ovulen within 4 to 13 weeks.

While there are some reports which show that fasting plasma insulin levels are not altered (Spellacy et al, 1968), contradictory reports have also appeared (Vermeulen et al, 1970). Plasma insulin levels have been shown to increase following oral or intravenous glucose load in women treated with OC, above the post load control values (Spellacy et al, 1968; Wynn and Doar, 1969). Discontinuing OC treatment, reverts this to normal.

It is not clearly understood whether the estrogen or the progestogen component of OC is responsible for the abnormal glucose tolerance seen in women taking OC. Available data, however, implicate estrogens rather than progestogens. Javier (1968) observed deterioration of glucose tolerance in women treated with mestranol. Goldman and Ovadia (1969) made similar observations in women treated with diethylstilbesterol or Premarin (which contains conjugated
natural estrogens). However, Spellacy et al. (1972a) did not find any significant alterations in glucose tolerance following treatment with Premarin or mestranol or ethinylestradiol. These authors suggested that the abnormal glucose tolerance associated with OC use may either be due to the progestogen component of the pill or due to a synergistic action of estrogen with progestogen.

Benjamin and Casper (1966) showed an improvement in glucose tolerance following treatment of women with 17-o-hydroxyprogesterone. Various studies on the effects of synthetic progestogens such as chlormadinone acetate, norethisterone, ethinodioldiacetate, medroxyprogesterone acetate and megestrol acetate have appeared (Goldman et al., 1968; Larsson-Cohn et al., 1969; Vermeulen et al., 1970; Goldman and Eckerling, 1970b; Adams and Wynn, 1972; Spellacy et al., 1972b). Most of these studies indicate that these progestogens do not affect glucose tolerance. In some cases plasma insulin responses were increased indicating increased resistance of peripheral tissue to the action of insulin.

Lipid metabolism:

Plasma triglyceride levels have been shown to increase in women taking OC (Barton et al., 1970; Kekki and Nikkila, 1971; Smith et al., 1975). Some of the studies showed that the increase in the levels of plasma triglycerides were associated with a concomitant increase in plasma lipo-proteins - very low density, low density and
high density (Wynn et al., 1966; Rossner et al., 1971; Osman et al., 1972). Seng et al. (1969), however, did not observe changes in lipoprotein levels along with triglycerides.

Cholesterol levels also have been shown to increase slightly in OC users (Wynn et al., 1966; Barton et al., 1970; Rossner et al., 1971). This increase was found to be associated with low density lipoproteins. Osman et al. (1972) have shown a decrease in cholesterol esterification in women using OC.

Estrogens have been implicated in the changes occurring in lipid metabolism in women using OC. Post-heparin lipoprotein lipase activity of serum has been shown to decrease in these women (Hazzard et al., 1969; Rossner et al., 1971). This decrease suggests an impaired clearance of plasma triglycerides. However, neither oral (Hazard et al., 1971, 1972) nor intravenous (Rossner et al., 1971) fat tolerance was found to alter during OC treatment, suggesting that increased triglyceride levels are not due to decreased clearance. Rise in serum triglyceride occurs in women using combination type OC but not progestogen alone type preparations (Barton et al., 1970; Donde and Virkar, 1975). While megesterol acetate did not change the serum triglycerides, norgestrel led to a fall in its levels (Donde and Virkar, 1975).

Studies on turnover of triglycerides using labelled compounds in women using OC have shown that both influx as well as efflux of triglycerides are increased, with a domination
of influx, thus, resulting in an increase in their level
(Kekki and Mikkila, 1971). OC increased both triglyceride
and insulin levels and it has been suggested that the
increase in triglyceride levels may be a result of
hyperinsulinaemia which might stimulate hepatic lipogenesis
(Hazzard et al, 1969).

The increases in serum triglyceride levels occur
after 2 weeks of OC treatment (Hazzard et al, 1969). Sachs
et al (1969) have shown that this rise continues upto
6 to 8 weeks and thereafter reaches a plateau.

Protein and mineral metabolism:

Changes in the levels of some plasma proteins have
been reported in women taking OC. Some of these proteins show
an increase while others show a decrease. As already pointed
out, thyroid binding globulin and transcortin levels increase
OC treatment has been associated with decline in haptoglobulin
and α₁-glycoprotein (Laurell et al, 1968; Song et al, 1970;
Briggs and Briggs, 1973) and increase in α₁-antitrypsin
and /3A globulin (Laurell et al, 1968; Song et al, 1970).
Albumin levels have been shown to decrease in these women
(Laurell et al, 1968; Briggs and Briggs, 1970; Prasad et al,
1975a). However, some reports indicate that OC do not alter
albumin levels (Song et al, 1970; Horne et al, 1971).
Laurell et al (1968) did not find changes in immunoglobulins
on OC treatment, while Horne et al (1970) reported increase
in their levels. However, in a later study, they could not
confirm these results (Horne et al., 1971). The changes occurring in some of the proteins on OC treatment have been observed in women treated with estrogens alone (ethinyl estradiol or mestranol) while a progestogen (norethisterone) reversed these effects (Briggs and Briggs, 1973). OC treatment also elevates $\alpha_2$-macroglobulin levels (Laurell et al., 1968; Horne et al., 1971) and this has been implicated in the clotting disorders associated with OC use.

Serum copper as well as the copper binding protein, ceruloplasmin, increase in women taking OC (Laurell et al., 1968; Song et al., 1970; Briggs et al., 1970; Schenker et al., 1971). Briggs et al. (1970) showed that treatment with norethindrone did not alter copper and ceruloplasmin levels, while Schenker et al. (1971) observed increases in serum copper on treatment with chlormadinone acetate. OC treatment has been shown to decrease serum zinc levels (Schenker et al., 1971; Hohn and Fuchs, 1974; Prasad et al., 1975). Norinyl treatment has been shown to increase erythrocyte zinc levels (Prasad et al., 1975). Combined and sequential OC did not differ in their effect on serum copper, but sequential type of OC led to a greater decrease in serum zinc compared to combined preparations (Schenker et al., 1971). Belavady et al. (1970) observed greater increases in ceruloplasmin levels in monkeys treated with OC fed a low protein diet compared to a group fed a high protein diet.

Transferrin and $\beta$-globulin which are involved in iron
transport, have been found to increase on OC treatment (Laurell et al., 1968, Song et al., 1970; Horne et al., 1971).

Briggs and Briggs (1970) did not find a change in total iron binding capacity (TIBC) with ethinyl estradiol, whereas norgestrel and norethisterone increased the TIBC significantly. Belavady et al. (1973), on the other hand, observed a decrease in TIBC, but an increase in serum iron, in monkeys treated with OC. Smith et al. (1975) and Prasad et al. (1975b) showed increases in both serum iron and TIBC in women using OC. OC do not seem to alter iron absorption (Norby et al., 1972).

Recently, Margen and King (1975) reviewed the literature on the effects of OC on trace element metabolism and also presented their own results. They observed that there was an increase in serum iron, TIBC, ceruloplasmin in women taking OC. Iron absorption was slightly decreased, whereas zinc absorption was not altered.

Serum magnesium has been shown to undergo cyclic changes during menstrual cycle, being lower at the ovulatory phase compared to the follicular phase, and further in women treated with OC, serum magnesium levels as well as urinary magnesium levels were found to decrease significantly (Goldsmith et al., 1970).

Since most of the proteins which show changes on OC treatment are synthesized in the liver, it has been suggested that these changes might be the result of action of OC steroids.
on liver (Song et al, 1970). Miale and Kent (1975) have recently described hundred laboratory tests which are altered in women taking OC.

Effects on vitamin metabolism and nutrition:

Besides affecting the metabolism of carbohydrates, proteins, lipids and minerals, OC alter the metabolism and nutritional status of a number of vitamins as well. Reports prior to 1973 (the present study was started towards the end of 1972) indicated that the use of OC led to biochemical changes suggestive of deficiencies of vitamins such as pyridoxine (Nutr. Rev. 1972), folic acid (Nutr. Rev. 1972), vitamin B₁₂ (Wertalik et al, 1972), vitamin C (Briggs and Briggs, 1972; Rivers and Devine, 1972) and vitamin A (Gal and Parkinson, 1971). Details of these reports will be discussed in the subsequent chapters.

Scope of the present investigation:

Most of the studies on the interaction of contraceptive steroids with nutrients such as vitamins, have been carried out in well-nourished women in Western countries. At the time the work was started no reports were available on the effects of contraceptive steroids on vitamin nutrition in Indian women of low income groups, whose nutritional status is known to be marginal with regard to most nutrients. No information whatsoever was available in literature on the effects of OC on riboflavin and thiamin status, except one report which showed a decrease in urinary output of these vitamins in female
monkeys treated with OC (Bamji quoted in Belavady et al., 1973). A physician from this Institute had observed development of oral lesions, characteristic of vitamin B deficiency in women treated with OC for prolonged periods of time. These women responded to treatment with riboflavin and pyridoxine (Lyengar, L. Personal Communication).

The present investigation was started against this background of information. The study was initiated by investigating the effects of combination type pills on the vitamin nutritional status of women belonging to low income groups. The details of this clinical study are described in Chapter III, Chapter II being devoted to a description of the methodology used throughout this study. Chapter IV deals with the effects of vitamin supplementation given along with OC to these women. In Chapter V, the results of experiments which were carried out in rats to elucidate the mechanism of "riboflavin defect" associated with OC use, are described. Chapter VI deals with general summary and conclusions.