PART A
CHAPTER I

REVIEW OF LITERATURE
1. INDUCTION OF CERVICAL CARCINOGENESIS

Cervical tumor induction has been tried since long using hormones, chemicals and virus. In the earliest studies, direct application of crude tar to the murine cervix and vagina had been found to produce a few carcinomas (Coli, 1929 and Fusco, 1932).

Hormones

Induction of vaginal and cervical carcinoma in mice has been demonstrated by the prolonged systemic treatment of estrogenic hormones (Loeb et al., 1936). Allen and Gardner (1941) reported that weekly injections of estrogens for a period of one year led to the induction of cervical tumors in 50 to 60% of mice. Mice of all strains responded in the same way and the highest tumor incidence occurred after 450 to 600 days.

Stilbestrol-cholesterol pellets attached to nylon thread and dipped in collodion when placed in the vagina of mouse could induce cervical and vaginal tumors. They were also seen in some mice after intravaginal instillation of stilbestrol, three times weekly (Gardner, et al., 1959).

Dunn's experiment confirmed the effectiveness of estrogens administered on the day of birth.
Diethylstilbestrol injections in three strains of mice led to the development of carcinoma of cervix and vagina after 13 months (Dunn and Green, 1963). Enovid, an antifertility drug, when administered in liquid diet to new born BALB/c mice for 2 years, led to the development of cervical lesions diagnosed as early cancer (Dunn, 1969).

Van Nie et al. (1961) reported the induction of cervical tumors in 26 out of 42 mice in about 15 months time by subcutaneous implants of testosterone pellets twice a week in C57B1 X DBA female mice.

Chemicals

Several chemical compounds have been used for the induction of cervical tumors in animals. Bi or triweekly placement of 5 mg fragments of benzo[a]pyrene (BP) in cholesterol into the vagina of 10 mice induces 100% cervical tumor incidence after 10-14 months (Fishman, 1942). The other methods practiced for exposing the cervix to carcinogens include; painting of BP solution in acetone on the cervix with cotton tipped wire loops (Van Haam and Scarpelli, 1955), later aided by the use of an infant-sized otic speculum (Koprowska et al., 1958) and insertion of carcinogen coated thread into the endocervical canal (Murphy, 1953; Vellios and Griffin, 1957).
Most experiments with BP have used the painting procedure and this model has been used to study enzyme histochemistry of changes in the upper vagina (Thiery and Villighagen, 1964), for studies of histogenesis of cervical carcinoma (Rubio and Lagelof, 1974a) and autoradiographic studies of early atypical changes (Rubio and Lagelof, 1974b).

Rats appear to be less susceptible than mice to cervical tumor induction by BP painting (Stein-Werblowsky, 1960).

Pan and Gardner (1948), reported the cervical tumor induction in the subcutaneously transplanted uteri, inserted with methylcholanthrene crystals, on the upper abdomen of hosts (brothers and sisters) of the same strain. Out of 104 mice of various genetic type 55 developed 61 tumors (32 carcinoma and 29 sarcomas), the majority appearing by the eighth week. Epidermoid carcinoma, adenocarcinoma, adenoacanthoma and sarcoma were the types of tumor that appeared in the present study.

Placement of MCA plus beeswax (in the ratio 1:3)-impregnated threads in the vagina of C3H mice led to the occurrence of malignant neoplasm of the cervix. In almost all the animals the cervix was involved and there were
extensions of tumors into the uterus and upper vagina. A few showed metastases to paraaortic lymph nodes, adrenal and lung (Reagen et al., 1955).

By comparing the string and painting methods with MCA and BP in C3H female mice it was observed that MCA-string method gave the highest incidence of cervical tumors (85%) then in order came BP string, MCA painting and BP painting methods (Scarpelli and VonHaam, 1957). The main advantage with the string method was the localization of tumors in the cervix only while painting gave many vaginal lesions in addition to cervical tumors. Many tumors were invasive and extended to pelvic nodes, with metastases seen in lung and liver. The lesions developed, showed a marked resemblance to human cervical cancer.

Painting of the mouse uterine cervix with MCA has been used to show the evolution of dysplasia of the cervix and vagina (Yang and Campbell, 1965).

Dimethylbenzanthracene (DMBA) has also been used to induce cervical tumors using knotted string method (Vellios and Griffin, 1955) or painting (Glucksman, 1971).
Virus

We come across very few studies concerning the viral induction of cervical cancer. Ultra-structural studies of BP-induced cervical carcinoma in C3HXA mice have shown virus-like particles which were not seen in the normal tissues of the mice. But it was speculated that these virus particles present in the tumors might be a result, rather than a cause, of the carcinomatous changes (Thiery et al., 1959).

Herpes Virus type 2 (HSV-2) has been used in an attempt to induce cervical carcinomas. In this effort animals were infected by applying the virus to the cervix with the help of cotton wool swabs. It is reported that rabbits treated in this way die with paralysis and encephalitis (Sever, 1973). In 19 BALB/c mice treated intracervically with HSV-2, only 2 cancerous and one precancerous lesion were seen and one of these cancers could be transplanted successfully (Munoz, 1973).

2. ORAL CONTRACEPTIVE PILLS AND CERVICAL CANCER

a. Epidemiological Studies

Equivocal findings have been obtained on the studies of cervical cancer and its precursors in users of oral
contraceptives. Some reported no link between the use of OC pills and the risk of developing malignant or premalignant cervical neoplasm while others have reported a positive relationship and few a negative one (see below).

Several factors have been attributed for the variability in epidemiological findings, the most important of which are:

1. The histologic interpretation of degrees of dysplasia and carcinoma in situ may vary from one pathologist to the other.

2. The important risk factors like, age at first intercourse, frequency of intercourse and number of sexual partners are difficult to determine accurately and these are not taken into consideration in most of the epidemiological studies resulting into the biased interpretation. Only one study before 1975 has specifically analyzed the issue of age at first intercourse and contraceptive choice (Merritt et al., 1975).

3. There is a tendency of oral contraceptive users to avail themselves of medical care more frequently than non users because of which certain diseases may remain underreported in populations not using contraceptives.
In the past few years numerous data have been published associating oral contraceptive pill use and increased risk of cervical neoplasia that leads to the growing consensus regarding an adverse effect on uterine cervix which is most disturbing.

Despite a large number of studies conducted so far we lack a conclusion suggesting a causal relationship between OC pill use and cervical cancer and still the question remains before us whether the association between oral contraceptives and cervical cancer is causal, or secondary to other factors?

Here only some of the important epidemiological findings are being discussed.

Melamed et al. (1969) surveyed the incidence of cervical carcinoma \textit{in situ} (CIS) among OC pill users and diaphragm users in New York City. Diaphragm users were predominantly whites of upper socioeconomic status and had first pregnancy after age twenty while the OC pill users were predominantly black or Puerto-Rican of low socioeconomic status and more likely to have had their first pregnancy before the age of 20. This study showed that prevalence rate for carcinoma \textit{in situ} for OC pill choosers and diaphragm users was 6.6/1000 women and 3.8/1000 women
respectively. The difference was small but significant. The possible explanations given for the difference are as follows:

a) a protective effect of the diaphragm acting as a barrier;

b) a causal effect of steroid acting on cervicovaginal epithelium;

c) some unknown factors in the makeup, behaviour or habit of a woman that would alter her probability of developing cervical carcinoma.

Ory et al. (1976) determined the prevalence rates for cervical dysplasia and carcinoma in situ for O.C pill users and IUD users at the Grandy Hospital family planning clinic in Atlanta. OC pill and IUD users showed similar characteristics that is all were black, between 15 to 44 years of age, having lower middle class income, similar marital status, age at first pregnancy and number of pregnancies. Result of this study showed that in IUD users (with two or more pap smears) the crude dysplastic rate was 1.3/100 women while in OC pill choosers 2.3/100 women. The calculated risk ratio of 1.5 in this series was considered statistically significant. O.C. pill acceptors with pap
smears before initial choice of contraceptive method had a 1.4 fold higher prevalence rate of cervical dysplasia than IUD acceptors. Quite interestingly for the same group of women with two smears the prevalence rate of CIS was 1.31/100 for IUD choosers and 0.34/100 for OC choosers. These differences were however not significant.

Subsequently Ory et al. (1977) again conducted a case control study of women with cervical dysplasia and with CIS comparing OC versus IUD use. Controls were matched for age, educational status, marital status, age at first birth and parity. The result showed a weak association between OC use and dysplasia. The relative risk of CIS for OC users as compared to non-users rose linearly from 1.3 (1 to 12 months use) to 4.7 (36 months use). This trend was statistically significant.

Stern et al. (1977) showed a significantly increased conversion of dysplasia to carcinoma in situ with extended pill use for more than 6 years. There was no suggestion of conversion from dysplasia to carcinoma in situ for less than 6 years of use.

Other case control studies before 1977 by Worth and Boyes (1972), Thomas (1972) and Boyce et al. (1977) indicated no increased risk of cervical neoplasia with OC
use. Vessey et al (1976) in their cohort study found that the incidence of dysplasia among IUD users and OC users was similar (0.28 and 0.31/100 women-year, respectively). In this study 12 cases were recorded but no valid statistical analysis was possible with so few cases.

Vessey et al. (1983) reported a relative increase in the incidence of both invasive and preinvasive cancer of the cervix in pill users. There was a positive relationship between the risk of neoplasia and duration of pill use. But because of the lack of data on sexual history the result remains inconclusive.

After 1979 a number of studies were conducted which attempted to determine age at first intercourse, age at first birth, number of sexual partners, incidence of sexually transmitted disease and other factors thought to be important in assessing the relationship between risk of cervical cancer and OC use.

One of the initial case control studies on the risks of cervical dysplasia and carcinoma in situ, in which there was ascertainment of age at first intercourse and number of sexual partners, was conducted by Harris et al. (1980). In this study significant risk factors were, age at first intercourse, multiple sexual partners and use of OC pills.
Statistically significant linear relationships were observed for the duration of pill use and risk of severe dysplasia and carcinoma in situ. This relationship remained significant after adjustment for number of sexual partners.

In another study by Swan (1981) which specifically controlled for sexual history, a 68% increase in the risk of cervical neoplasia was found among pill users. The risk of cervical cancer was highest in sexually active women who had used the pill for 4 to 6 years. After adjustment for sexual factors the increase in cervical cancer risk associated with pill failed to remain significant.

Result from the study of Royal college of General practitioners (Beral, Hannaford and Kay, 1988) described that among 47000 women followed since 1968, those who had used oral contraceptives (ever-users) had a significantly higher incidence rate of cervical cancer than never-users. After standardization of rates by age, parity, smoking, social class, number of previously normal cervical smears, and history of sexually transmitted disease, the excess was 41 per 100,000 woman-years for carcinoma in situ and 8 per 100,000 woman-years for invasive cervical cancer. Incidence increased with increasing duration of pill use and the standardized incidence rate for cervical cancer in women who
had taken the pill for more than 10 years was four times than never users.

Celentano et al. (1987) reported on the basis of his case control study that OC pill use is protective against invasive cervical diseases if used for a longer duration. But after adjustment for behavioural factors eg. age at first intercourse, smoking and gaps in pap test failed to remain significant.

Animal Studies

A large number of studies have been performed to assess the carcinogenic effect of oral contraceptive steroids and other estrogenic and progestogenic preparations, however only a little evidence exists related to their modulatory influence on the process of cervical carcinogenesis. Hormonal interaction with the process of cervical carcinogenesis seems to be very important aspect in the process of developing cancer. Regarding this aspect Clifton (1961) suggested that:

a) Specific hormones at normal levels may be necessary for the development of carcinomas secondary to an oncogenic virus, chemical carcinogen, or radiation, and once established some of these tumors may continue to
require normal hormone level for growth or successful transplantation;

b) Specific hormones in elevated levels may increase the susceptibility to an oncogenic virus, may reduce the threshold carcinogenic dose of chemicals or radiation, may increase the tumor frequency and may reduce tumor latency.

Considerable animal experimentation has been aimed at determining the carcinogenic action of estrogen, since mid 1930's. Injection of a total dose of 10,500 I.U. of estradiol benzoate over a period of 319 days led to the development of a large carcinoma of the cervix uteri in a mouse of C3H strain (Gardner, et al., 1938). This tumor could be grafted successfully in the male and female mice of the same strain and grew rapidly without any further estrogen stimulation. In the rest of the mice smaller invading epithelial growths were observed which are probably early carcinomas.

Topical application of stilbestrol for a prolonged period has been reported to produce cervical carcinoma in mice (Gardner and Frankenhuis, 1955). Dunn and Green (1963) reported that subcutaneous injection of 0.1 c.c of a 2% suspension of diethylstilbestrol in saline to BALB/c and
C3Hf mice on the day of birth produces cervical cancer after 13 to 26 months. Squamous metaplasia of uterine cervix in monkey was produced by the oestrin administration (Hisaw and Lendrum, 1936) but no attempt was made in this study to determine the minimum time or oestrin dosage required to bring about metaplasia.

Allen and Gardner (1941) gave estrogen injection to hybrid mice for more than 1 year. In one group, 20 of 53 survived, and 10(50%) had lesions and carcinoma of the uterine cervix. In a second group with 25 of 52 surviving, 15 (62%) had cervical lesions or carcinoma. Pan and Gardner (1948) found 13 epidermoid carcinoma or invasive epithelial lesions in 35 hybrid mice treated for more than 200 days with estrogen combined with androgens. Barbieri et al. (1958) gave weekly injections of estradiol to female mice for 155-250 days and found early lesions in the cervix. Gardner et al. (1959) produced epidermoid carcinoma in 3 and small invasive epithelial lesions in 5 in a group of 21 mice that received intravaginal instillation of an estrogen preparation 3 times weekly.

Some of the studies have reported the tumor inhibitory action of estrogen. 17β-estradiol, a naturally occurring estrogen has been shown to inhibit methylcholanthrene-induced cervical carcinogenesis in Swiss albino mice (Das et
al., 1988). When 17-β-estradiol was administered weekly at the dose levels of 0.01 ug, 0.1 ug, 5 ug and 50 ug for 16 weeks by intramuscular route, following carcinogen thread insertion, the cervical carcinoma incidence, as compared to the control mice (76%) were 66.6%, 61.5%, 55.5% and 42.1% (P < 0.05) respectively.

Reboud and Russfield (1969) have reported that simultaneous implantation of mestranol, at the dose level of 0.5 mg/mouse after every 3 weeks for 15 weeks, inhibits the increased percentage of MCA-induced cervical tumors following progesterone administration in B6AF mouse.

In a number of studies progesterone has acted as a co-carcinogen or promoter. Reboud and Pageaut (1973) have reported the co-carcinogenic effect of progesterone on 20-methylcholanthrene-induced cervical carcinoma in mice. In this study the progesterone administration for 9 weeks by subcutaneous implantation of pellets with a dose of 15 mg/mouse after every 3 weeks to C57BL6 mice led to the significant enhancement of MCA-induced cervical tumor incidence.

Progesterone and synthetic progestin 3-β-17-α-diacetoxy-6α-methyl-pregn-4-en-20-one (BL141) when administered as subcutaneous implant at the dose level of 5
mg/mouse after every 3 weeks to B6AF mice for 15 weeks, the MCA-induced cervical tumors got enhanced (Reboud and Russfield, 1969).

Besides this in some cases the progestins have been shown to act as inhibitor of carcinogenesis. Myre and Bjoro (1969) reported a 50% reduction in the incidence of cervical carcinoma induced by 1% DMBA solution in WLO mice. In this study 25 mg pellet of progesterone was subcutaneously implanted for 30 weeks.

In another study corporin treatment was found to inhibit the action of oestrin in evoking squamous metaplasia in the cervix of monkey (Hisaw and Lendrum, 1936).

Combinations of estrogen and progestins have been shown to display antagonistic or synergistic actions depending on the doses used. The progesterone treatment along with stilbestrol or estradiol has resulted in a marked inhibition of estrogen-induced tissue growth in the genital tract of New-Hampshire red chick (Hertz, 1947). A similar antagonism between estrogen and progesterone is demonstrated in the uterus of rabbit (Courrier, 1950). In a number of cases estrogen and progesterone in lower dosage combination have been reported to display synergistic action (Courrier, 1950).
Enovid, an antifertility drug, containing a synthetic estrogen norethynodrel and a progestin-like compound metranol have shown carcinogenic effect on uterine cervix in BALB/c mice (Dunn, 1969). In this experiment all the six mice receiving the Enovid through liquid diet for 518-721 days and 2 mice born to a mother on Enovid and continued on Enovid for 599 days, had cancerous lesions in the uterine cervix.

3. INJECTABLE CONTRACEPTIVE STEROID "MPA" AND CERVICAL CANCER

Since the introduction of steroidal injectable contraceptive, one of the major issues raised by the scientific community as well as social organizations, has been its possible association with neoplasia. Depot medroxyprogesterone acetate (DMPA) and Norethisterone enanthate (NE) are the two progestogen preparations that has been used popularly in the form of injection to provide an effective single dose contraception for 1 to 3 months.

Review of the available data has shown no increased risk of cancers of the breast, endometrium, ovary or liver in women using depot medroxyprogesterone acetate. The issue of causal association between DMPA use and cervical cancer
is as yet unresolved and will require the accumulation of additional data.

Epidemiological Studies

Epidemiological studies conducted so far lack sufficient numbers of women or followup periods to confirm or exclude an association of injectable contraceptive progestogens with risk of invasive cervical cancer.

In a case control study, conducted in costa Rica where DMPA has been a popular contraceptive method and approximately 11% of the currently married women between 15-49 years of age have used an injectable contraceptive, Oberele (1988) reported an association of DMPA use with slightly elevated risk of carcinoma in situ and invasive cancer. This slightly elevated risk has been considered as probably the result of chance or a detection bias.

In the WHO collaborative study (1985) of neoplasia and steroid contraceptive a strong association was observed between the use of injectable contraceptive and invasive cervical cancer in the preliminary analyses of data from Chile (1977-1983). But the analysis of additional data failed to confirm the initially observed association (Thomas et al., 1989). The original finding was considered as just a chance.
In another case control study Powell and Seymour (1971) followed up 1123 women in Texas (USA) who used DMPA. Only 51 of these women accumulated after more than 4 years of use. Both the abnormal cytology rate as well as rate of biopsy proven carcinoma in situ were higher in the DMPA users than was expected but no allowance was made for the effect of confounding factors such as age, social class, race or sexual behaviour.

Dabancens (1974) in his study compared IUD users with women who accepted injectable contraceptives in Chile. This study included 2409 IUD acceptors and 2684 comparable women using injectables. Of the women using injectables, 331 accumulated more than 4 years of exposure. In total 9 women in the injectable groups (1.26/1000 woman-years) and 6 in IUD groups (1.82/1000 woman-years) developed preinvasive or invasive (2 cases) cervical lesions.

Recently preliminary findings of a case control study conducted by WHO (1986) have been published which provide some evidence of increased risk of invasive cervical cancer associated with long term use of DMPA particularly among young women emphasizing the need for further evaluation of the issue.
In a latest case control study by Herrero et al., 1990 conducted in Latin America the association of injectable contraceptive use with the risk of invasive cervical cancer was analyzed while controlling for a variety of other risk factors, including female and spouse sexual behaviour and human papilloma virus infection. Results showed an association of high relative risks with long term injectable use among women with extended intervals since first or last use. The study suggested an adverse effect of injectable contraceptive use on cervical cancer risk particularly among women who cease participation in screening programs after terminating usage.

These studies suggest the need for close monitoring of long term injectable users which might give some conclusive results regarding their long term safety.

Animal Studies

The animals study conducted so far does not give any reason to associate Medroxyprogesterone use with increased risk of neoplasia (WHO, 1986).

The first toxicological studies in animals were carried out on several hundred mice and rats treated with DMPA doses 100 or 200 times than human dose. These animals were
compared with animals receiving no drug. The mortality rates and the incidence of neoplasia were found to be similar in all the treated and nontreated groups and no deaths could be attributed to the drug (WHO, 1986).

Another 7 year study which involved Beagledogs showed the occurrence of Mammary gland adenocarcinoma in 2 of the 16 high dose (25 times the human dose) treated bitches. At least some nodules in the other Beagledogs, including 2 of the control animals, were malignant. The breasts of healthy beagles have a reservoir of microscopic neoplasm which may grow and occasionally become malignant especially in response to progesterone for a longer duration (Cameron, et al. 1971). In contrast to this animal findings there is no such reservoir in healthy women. Besides this, DMPA treated Beagledogs show the condition of acromegaly, an abnormal growth process resulting from high level of growth hormone. The administration of DMPA to human or monkey does not result in elevated growth hormone levels (Dhall et al., 1977). Mammary gland nodules developed in all bitches that lived beyond first few years except for 2 from the control group.

Because of the susceptibility of beagledogs to mammary tumors and the development of acromegaly the Toxicology Review Panel in 1978 concluded that the beagledogs is an
unsuitable model to assess the adverse effect of MPA and the results can no longer be considered as indicative of significant hazard to women.

Another 10 years study, involving 52 rhesus monkeys, compared a control group of monkeys who received no drug with 3 groups that received, respectively, the equivalent of human dose, 10 times the human dose, and 50 times the human dose of DMPA. Approximately 50% of the animals died in each group during the study period. Monkeys which died spontaneously or sacrificed during the study showed nonmalignant mammary nodules. Animals from the control group and the group treated with low dose developed mammary nodules but were not found in animals given the medium or high doses.

Besides this, two of the monkeys from high dose treatment group showed endometrial carcinoma. This result cannot be extrapolated in human situation as endometrial carcinomas observed in women not receiving hormone treatment generally arise from a hyperplastic endometrium, whereas the carcinomas in two monkeys were found in an atrophic endometrium. It seems that the tumors in the monkeys arose from a cell type not found in women. In addition DMPA is used in high doses to treat endometrial carcinoma in women with considerable success. Additional evidence against the
hormonal action of DMPA causing the endometrial cancer is found in a variety of clinical and epidemiological studies.

4. CONTRACEPTIVE STEROIDS AND DRUG METABOLIZING ENZYMES SYSTEM

There are multiple pathways for drug metabolizing enzymes-catalyzed steroid and xenobiotic biotransformation in the liver. These drug metabolizing enzymes and the pathways responsible for biotransformation are modulated and regulated by a large number of environmental and hormonal factors.

As early as 1958 Quinn et al., reported the species, strain and sex differences in the metabolism of hexobarbitone, amidopyrene, antipyrene and aniline in different animal models and suggested the role of sex hormones in regulating the sex-differences in drug metabolism by modulation of microsomal enzyme system.

DMBA-induced skin carcinogenesis was found to be inhibited by endogenous estrogen in mouse (Bates, 1968). Besides other possible action of estrogen on skin tumor induction possibility of its effect on carcinogen metabolism was put forward.
Interaction of contraceptive steroids with liver drug metabolizing enzymes and in general with drug metabolism has been reported in a number of publications. However, various animal species and different experimental approaches and various contraceptive steroids combinations were employed with conflicting results.

Jori et al. (1969) reported that chronic treatment of steroid contraceptives increases the activity of liver microsomal enzymes in rats. Similar inducing effects were observed by Juchau and Fouts (1966) and Rumke and Noordhock (1969). Blackham and Spencer (1969) and Garg and Ahmed (1974) reported an increase in the metabolism of barbiturates after oestrogen and progestogen contraceptive treatments in mice.

In contrast to these results Freudenthal and Amerson (1974) failed to show any induction after treatment with synthetic progestins and on the contrary found a week inhibition of microsomal drug metabolism. An impairment of drug metabolism was also reported in women taking oral steroidal contraceptives (Crawford and Rudofsky, 1966; O'Malley, Stevenson and Crooks, 1972).

Briatico et al. (1976) assessed the effects of 3 different estrogen and progestogen combinations on drug
metabolism in various animal species. Lynestrenol plus mestranol, norethisterone plus mestranol and norethynodrel plus mestranol were given orally for 4 consecutive days (acute treatment) or 30 days (Chronic treatment) to rats, mice and guinea pigs. The results showed an enhancement in the liver microsomal enzyme activity in rats and mice. This effect was found to be produced because of the progestogenic compound alone which is in agreement with the results obtained by Jori et al (1969) and Blackham and Spencer (1969). The observed increase in microsomal enzyme activity was not found to be associated with an increase of the microsomal protein or of the cytochrome P450 content. These findings are at variance with the findings of Freudenthal and Amerson (1974) who showed that oestrogen progestogen combinations do not have any inducing action on microsomal enzymes. Guinea pigs in this study reacted very differently than in rats and mice and the doses of steroids used were completely ineffective in inducing microsomal enzyme activity.

In 1976, Marcucci et al., showed the modulatory effect of different estrogen-progestin combinations on the hepatic microsomal metabolism of mestranol. Three combinations of steroid contraceptive drugs i.e. mestranol plus lynestrenol, norethindrone or norethynodrel were given orally at
effective antifertility doses for 30 days to rats, mice and guinea pigs. Eighteen hours after the last treatment the animals were sacrificed for preparation of liver microsomal enzymes and the evaluation of mestranol metabolism in vitro. The results obtained clearly indicated that the animal species is an important variable. In rats and mice, but not in guinea pigs, there is on the whole, following steroids treatment an increased in vitro mestranol metabolism as evaluated by disappearance of mestranol and formation of ethinylestradiol with respect to control one. The type of contraceptive combinations were also found to be important in determining mestranol metabolic transformation, in vitro, into ethinylestradiol and its further conversion to more polar metabolites. In this study, in rats, the presence of ethinylestradiol after incubation with liver microsomal enzyme is decreased with respect to controls when the animals were pretreated with lynestrenol plus mestranol, is increased with norethynodrel plus mestranol and is unchanged with norethindrone plus mestranol. In guinea pigs although the changes are relatively very small, the pretreatment with lynestrenol plus mestranol increases the disappearance of mestranol and the presence of ethinylestradiol, while the combination norethynodrel plus mestranol gives the opposite effect and the treatment with norethindrone plus mestranol has no effect.
Ochs et al. (1986a) evaluated the effect of ethinylestradiol, estradiol, norethynodrel and norethisterone, its acetate and enanthate on microsomal enzyme induction in rat liver. Five different substrates - aminopyrene, ethylmorphine, benzphetamine, aniline and p-nitroanisole were used to check monoxygenase activities of isolated liver microsomes. Only a weak induction by ethinylestradiol was found. Treatment with estradiol, norethisterone or norethisterone esters did not cause distinct rise in the turn over of the substrates. In contrast aniline and p-nitroanisole metabolism were slightly depressed.

Ochs et al. (1986b) again reported the effect of progesterone on the induction of monoxygenase system in rats. Progesterone were administered orally via the diet or subcutaneous route. Progesterone treatment was found to enhance the activity of hepatic monooxygenases as measured with isolated microsomes in vitro. A dose dependent increase in the N-demethylation of aminopyrene, ethylmorphine and benzphetamine with a 3-fold increase in ethylmorphine demethylation was observed. The metabolism of aniline and p-nitroanisol showed no distinct increase.

These monooxygenase activities towards five different substrates in progesterone treated rats resemble that seen
after treatment with synthetic progestin - cyproterone acetate and pregnenolone-16\(^\alpha\)-carbonitrile.

In another study Schulte-Hermann et al. (1988) evaluated the effect of sixteen different steroids on growth and monooxygenase induction in rat liver to investigate the possible existence of any relation between these hepatic actions and structural or endocrine properties of steroids. According to their effects in the present study steroids were assigned into the following three groups:

a) Estrogens i.e. estradiol and ethinylestradiol as well as progestins i.e. norethynodrel and norethisterone which have estrogenic activities in rats, induced pronounced liver growth which was not associated with major monooxygenase induction;

b) Pregnenolone-16\(^\alpha\)-carbonitrile, progestins: progesterone, cyproterone acetate and medroxyprogesterone acetate, antimineralocorticoid: spirolactone and glucocorticoids: cortisol and dexamethasone displayed liver growth which was associated with pronounced monooxygenase induction

c) Levonorgestrel, testosterone and methyltestosterone did not show any effect.
Effects of group 'a' steroids have been related to their estrogenic actions as all of them show estrogenic actions in rats. This view is supported by inhibitory effects of clomiphene, an antiestrogen, on estrogen-induced liver growth and DNA synthesis (Schwarzole and Heim, 1970).

On the other hand effects of group 'b' steroids were apparently not related to any specific endocrine action but to certain structural features in particular to the presence of a saturated, at least two membered alkylsubstituent at C17 of the steroid ring system. Heuman et al. (1981) used an immunological assay to show that hepatic concentration of the pregnenolone-16α-carbonitrile-induced cytochrome P450 is increased following treatment of rats with spironolactone, cortisol and dexamethasone but not after estradiol, mestranol, testosterone and methyltestosterone treatment.

Yuen et al (1985) assessed the effect of some synthetic steroids on rat liver function. In this study norethynodrel at the dose level of 20 mg/kg body weight was found to have moderately potent effects on hepatic function in female rats. It causes increase in the cytochrome P450 level and protein content of the liver while ethinylestradiol in the same study failed to show any such effect.
Medroxyprogesterone acetate, a long acting injectable contraceptive steroid, has also been found to modulate the microsomal drug metabolizing enzyme systems. Collectively all the studies showed MPA to be a mixed type inducer of monooxygenases. Also NADPH cytochrome c reductase was found to be particularly responsive to MPA (Saarni, 1980). Saarni, (1980) showed that MPA has a dose dependent inducing effect on the hepatic drug metabolism in rats. In this study, the hepatic microsomal drug metabolism was examined in vitro in rats pre-treated with 10-600 mg/kg MPA intraperitoneally for 7 days. In both the sexes there was a significant increase in the liver weight, amount of cytochrome P-450, activities of NADPH-cytochrome C reductase, benzo[a]pyrene hydroxylase and 2,5-dephenyloxazole hydroxylase. The increase in 7-ethoxycoumarine-o-deethylase activity was also significant in female but not in male rats.

Long term treatment with MPA (10 mg/kg/day) for 30 consecutive days has been shown to induce metabolism of p-nitroanisole, aniline and aminopyrene in female rats (Jori et al., 1969).

Several rat hepatic P450s with specific microsomal hydroxylase activities have been identified that are sex dependent and developmentally modulated (Waxman 1988). Male specific P450s 2a and 2c have been found to be neonatally
imprinted by androgen in males while female specific P450 2d is expressed at a hormone independent basal level that can be stimulated by estrogen and suppressed by androgen.

In another study by Kretzschmar et al. (1989) levonorgestrel (LN), a synthetic sexual steroid, was administered to the rats at the dose level of 1 mg/kg and 10 mg/kg through oral route. The lower dose decreased the Cyt.P450 content significantly. The high dose did not influence this parameter but caused a significant stimulation of ethylmorphine N-demethylation and 7-ethoxycoumarin O-deethylation (EO). A statistically significant but small decrease of EO activity was found after administration of lower LN doses. Both the doses of LN decreased the glutathione (GSH) content in the liver significantly. The mitochondrial GSH pool was decreased more intensively than total GSH content. The low dose treatment showed a decrease in glutathione S-transferase (GST) activity.

Hepatic sulfation by aryl sulfotransferases is an important conjugation reaction in the metabolism of phenolic compound such as acetaminophen prior to urinary and biliary excretion (Mulder, 1984). Male rats have been found to eliminate acetaminophen more as sulfate conjugates and less as glucuronide conjugates than females (Raheja et al., 1983;
Green and Fisher, 1981). Galinsky et al. (1986) have reported that male rats have double the hepatic acetaminophen sulfotransferase activity compared to females and this high activity in males appeared to be due to increased activity of only one of the two acetaminophen sulfotransferases identified in hepatic cytosol (Galinsky 1986). Kane and Chen (1987) examined the influence of exogenous estrogen and progestogen upon hepatic acetaminophen sulfotransferases in normal as well as gonadectomized rats. The total hepatic arylsulfotransferase activity in mature male rats was 2 fold greater than that of mature females. In female oophorectomy alone had no significant effect upon total sulfotransferase activity but oophorectomy plus testosterone treatment resulted in a 61% increase in acetaminophen sulfotransferase activity versus oophorectomy alone. While in male rats, castration alone did not significantly affect the total sulfotransferase activity but ethinylestradiol treatment plus castration resulted in 27% decline in acetaminophen sulfotransferase activity versus castration alone. It was concluded that one of the factors in the sex differences in acetaminophen metabolism in rats was the influence of gonadal hormones on one of the two acetaminophen sulfotransferases identified in hepatic cytoplasm. The greater acetaminophen sulfate conjugation capacity observed in males compared with females appeared to
be due to enhancement of acetaminophen sulfotransferase 2 activity by testicular androgens and the lower activity due to the suppressive effect of ovarian estrogen on this same enzyme. Sulfotransferase 1 activity was similar in both the sexes and apparently not influenced by gonadal hormone.

In contrast to rat, the human male excretes more glucuronide conjugates than female with no apparent differences in the partial clearance of acetaminophen sulfates between the two sexes (Wojeicki et al., 1979; Mucklow et al., 1980) Gonadal hormones also appear to influence acetaminophen metabolism in man because oral contraceptives in female enhances the glucuronidation as well as oxidative metabolism of acetaminophen (Miners et al., 1983).