CHAPTER V

EFFECT OF COMBINED ORAL CONTRACEPTIVE PILLS "OVRAL" AND "NORACYCLINE" AND INJECTABLE CONTRACEPTIVE STEROID "MEDROXYPROGESTERONE ACETATE" ON PHASE I AND PHASE II DRUG METABOLIZING ENZYMES AND ACID SOLUBLE SULFHYDRYL LEVEL IN LIVER OF MOUSE
INTRODUCTION

The biotransformation of xenobiotic compounds is accomplished largely by Phase I and II hepatic drug metabolizing enzymes. Both exogenous and endogenous steroids are also metabolized by the same enzyme system (Conney, 1967). The multiple pathways involved in the biotransformation has been found to be regulated by a number of environmental and hormonal factors. Steroid hormones have been reported to influence the liver microsomal and cytosolic drug metabolizing enzyme activities (Carter et al., 1974; Briatico et al., 1976; Jori et al., 1976; Kretzschmar et al., 1989; Kane and Chen, 1987; Ochs et al., 1986; Vodicnik et al., 1981). It has also been shown that changes in the normal steroid balance could alter the metabolism and action of drugs (Marcucci, et al., 1976; Blackham and Spencer, 1969; Juchan and Fouts, 1966). Experimental studies have yielded conflicting results regarding the possible actions of different steroids and their combinations on drug metabolism and drug metabolizing enzyme activity. Wide spread use of oral contraceptive pills, by as many as 60 million women globally (Hatcher et al., 1967), and injectable contraceptive steroids, by more than 11 million women - years in 80 countries, raises the question whether these contraceptive steroids make the users
prone or resistant to the action of different xenobiotics, especially the environmental carcinogens, by altering the drug detoxifying system. The present study was designed to assess the modulatory influences of the two widely used combined oral contraceptive pills (OC), Ovral and Noracycline, and the injectable contraceptive steroid "medroxyprogesterone acetate", on phase I and II drug metabolising enzymes and acid soluble sulfhydryl level in liver of mouse.

MATERIALS AND METHODS

Randombred, 8-9 weeks old, female virgin Swiss albino mice (Source: Animal facility, JNU, New Delhi) were maintained in an air-conditioned animal room providing standard food pellets (Hindustan Lever Ltd., India) and tap water ad libitum.

Chemicals

Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB), reduced Nicotinamide adenine dinucleotide (NADH), reduced Nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH) and sodium dithionite have been obtained from Sigma Chemical Co., USA. Coomassie Brilliant Blue
(G-250) was obtained from Merck, Germany. Oral contraceptive pills Ovral (each containing 0.05 mg ethinylestradiol and 0.5 mg norgestrel) and Noracycline (each containing 0.05 mg ethinylestradiol and 1 mg lynestrenol), manufactured respectively by Wyeth Laboratory and Ciba-Giegy India, were purchased from local pharmacy. MPA (6\(\alpha\)-methyl 17\(\alpha\)-hydroxyprogesterone acetate) was a generous gift from Dr. Gokul Das, Cold Spring Harbor Lab, USA.

Preparation of Liver Subfractions

Animals were sacrificed, after an overnight starvation, by cervical dislocation. Excised livers were immediately rinsed in chilled Tris-KCl homogenizing buffer (pH 7.4), containing 0.15 M KCl and 50 mM Tris HCl. After perfusing with 0.9\% chilled NaCl, 1 gm of the liver from each animal was homogenized in the homogenizing buffer to yield a 10\% (w/v) homogenate. For the estimation of acid-soluble sulfhydryl groups (SH) a part of this homogenate was kept separately.

The homogenate was then centrifuged at 10,000xg for 20 minutes at 4\(^\circ\)C. The supernatant obtained (i.e. post-mitochondrial supernatant) was further subjected to centrifugation at 1,05,000xg for 1 hour at 4\(^\circ\)C. The pellet formed represents the microsomal fraction of the liver.
The microsomal pellet was resuspended in the homogenizing buffer and used for assaying cytochrome b$_5$ and P450 contents while cytosolic fraction was used for estimating glutathione S-transferase (GST) activity.

**Determination of Cytochrome b$_5$ and Cytochrome P450**

Omura and Sato method (1964) was used for cytochrome b$_5$ and P450 estimation. Microsomal pellet was resuspended in 50 mM Tris-KCl buffer (pH 7.4) at a suitable dilution.

**Cytochrome b$_5$**

Cytochrome b$_5$ is a hemeprotein which is readily estimated from its redox spectrum of NADH-reduced versus oxidised cytochrome. The reduction of cytochrome b$_5$ is catalysed by the presence of a microsomal flavoprotein enzyme namely NADH-cytochrome b$_5$ reductase.

1 ml of microsomal sample was mixed with 3.8 ml of 50 mM Tris-HCl (pH 7.4). The sample mixture was divided between two matched spectrophotometer cuvettes and scanned in dual beam spectrophotometer (UV-260, Schimadzu) between 400 and 500 nm for baseline correction. 100 $\mu$ of NADH (2% w/v) was added to the test cuvette and 100 $\mu$ of Tris-HCl buffer was added to the reference cuvette. After 2 minutes, the redox
spectrum between 400 and 500 nm was recorded. Cytochrome b₅ is determined as the absorption difference (ΔA) between 424 and 409 nm by UV 260 Schimadzu spectrophotometer.

Using the ΔA (424-409) nm value cytochrome b₅ content is calculated by following way and is expressed as nanomoles/mg microsomal protein

\[
\frac{\Delta A \times 5 \times 1000}{185 \times \text{protein in mg}}
\]

where 185 mM⁻¹ cm⁻¹ represents the extinction coefficient of cytochrome b₅.

Cytochrome P450

The reduced hemeprotein combines with carbon monoxide to give a characteristic absorption spectrum with a peak at 450 nm.

Sample used for b₅ was again used for estimating P450. Few mg of sodium dithionite was added to reduce the hemeprotein and the sample was divided equally between the two matched cuvettes which were then scanned between 400-500 nm in UV-260, Schimadzu spectrophotometer for baseline correction. The contents of the sample cuvette was gently
bubbled with carbon monoxide for about 1 minute, which results in the formation of cytochrome P450 and CO adduct with an absorbance maximum at 450 nm. The difference in the absorbance maximum at 450 nm and at 490 nm ($\Delta A$) was obtained directly by the spectrophotometer and then converted to the concentration of cytochrome P450 using an extinction coefficient of 91 mM$^{-1}$ cm$^{-1}$ in the following way

$$\Delta A\ (450-490) \times \frac{5 \times 1000}{91 \times \text{mg protein}}$$

Cyt.P450 content is expressed as nanomoles/mg microsomal protein.

Determination of GST Activity and Acid Soluble Sulphydryl (SH) Level

The activity of the cytosolic GST was determined spectrophotometrically, using Schimadzu, UV-260 spectrophotometer, at 30$^\circ$C with 1-chloro-2,4-dinitrobenzene as the substrate by the method described by Habig et al. (1974). The reaction mixture (3 ml) contained 100 umoles phosphate buffer (pH 6.5), 3 umol reduced glutathione and 3 umol 1-chloro-2,4-dinitrobenzene. The reaction was started by the addition of suitably diluted cytosol. The enzyme activity was observed at 340 nm for 5 minutes with reference
to a blank containing complete assay mixture minus the cytosol. The specific activity of GST is expressed as nanomoles of GSH-CDNB conjugate formed per minute per mg protein in the following way:

\[
\Delta A / \text{min} \times 1000 \times 3 = \frac{\Delta A}{9.6 \times \text{mg protein}}
\]

where 9.6 mM\(^{-1}\) cm\(^{-1}\) is the extinction coefficient.

Acid soluble sulfhydryl levels were determined by using Moron's method (1979). Homogenate (500 A) was precipitated with 100 \(\lambda\) of 25% TCA and precipitate was removed after centrifugation. 100 \(\lambda\) of the supernatant was added to test tubes containing 2 ml of 0.6 mM DTNB and 0.9 ml of 0.2 M phosphate buffer (pH 8.0). The yellow colour obtained was due to the complex formed between acid soluble SH groups with DTNB. Absorbance was measured at 412 nm against a blank which contained 100 \(\lambda\) of 5% TCA in place of the supernatant. SH content was calculated with the help of a standard graph made by using different concentrations of reduced glutathione and is expressed as umol/gm tissue.

Cytosolic and microsomal protein contents were determined by Bradford's method (1976) against a standard curve made by using BSA.
Student's t-test was used to calculate the statistical significance of the experimental results.

**Va. EFFECT OF COMBINED O.C. PILL OVRAL AND NORACYCLINE**

**EXPERIMENTAL DESIGN**

The animals were divided into control and experimental groups (see Table 7). Three different doses of the oral contraceptive pills Ovral and Noracycline used in this study are as follows:

**Ovral:**

(a) Dose $D_1$ (1/2000th of a pill) containing 0.025 ug of ethinylestradiol and 0.25 ug norgestrel;

(b) Dose $D_2$ (1/200th of a pill) containing 0.25 ug of ethinylestradiol and 2.5 ug norgestrel;

(c) Dose $D_3$ (1/20th of a pill) containing 2.5 ug of ethinylestradiol and 25 ug norgestrel;

**Noracycline:**

(a) Dose $D_1$ (1/2000th of a pill) containing 0.025 ug of ethinylestradiol and 0.5 ug lynestrenol;
(b) Dose $D_2$ (1/200th of a pill) containing 0.25 ug of ethinylestradiol and 5 ug lynestrenol;

(c) Dose $D_3$ (1/20th of a pill) containing 2.5 ug of ethinylestradiol and 50 ug lynestrenol;

OC pill treatment at all three different dose levels was given by oral route daily for a period of 15 days.

Animals of group 1 were treated with vehicle only (distilled water) for 15 days and these served as controls. Animals of groups 2, 3 and 4 were treated respectively with $D_1$, $D_2$ and $D_3$ doses of the OC pill ovrnal while animals of groups 5, 6 and 7 were treated respectively with $D_1$, $D_2$ and $D_3$ doses of noracycline for 15 days. Body weight of the animals were recorded initially and at the end of the experiment. Animals were sacrificed by cervical dislocation after 15 days treatment following overnight starvation and their livers were removed to proceed for different drug metabolizing enzyme assays. The statistical significance of differences between the control and experimental values were assessed by student's t-test.

RESULTS

Findings of the present study are depicted in the Table-7. Treatment with oral pills for 15 days did not show
any effect on the normal body weight gain in the mice (data not included in the Table).

Ovral treatments at $D_2$ and $D_3$ dose levels (Gr.3 and Gr.4) showed significant decrease ($P<0.01$) in Cyt.b$_5$ while dose $D_1$ almost did not show any change (Gr.2). However, treatment with noracycline at $D_1$ and $D_2$ dose levels showed significant decrease ($P<0.01$) in Cyt.b$_5$ (Gr.5 and 6) while dose $D_3$ caused significant enhancement ($P<0.01$).

A significant decrease was observed in Cyt.P450 with ovral treatment ($P<0.01$) at $D_2$ dose level (Gr.3) while treatment at $D_1$ and $D_3$ dose levels did not show any significant decrease (Gr.2 and 4). Noracycline treatment at $D_2$ dose level (Gr.6) showed significant decrease ($P<0.01$) in Cyt.P450 while $D_3$ dose (Gr.7) caused a significant increase ($P<0.01$). $D_1$ dose treatment of noracycline (Gr.5) did not appear to have any effect on Cyt.P450. A significant change (decrease) in the microsomal protein content was observed only in groups 2 and 7.

Only the $D_3$ dose of Ovral (Gr.4) was found to decrease the GST activity significantly ($P<0.01$) while $D_1$ and $D_2$ doses (Gr.2 and 3) did not show any change. Noracycline treatments with all the three doses (i.e. $D_1$, $D_2$ and $D_3$)
caused significant decrease (P<0.01) in GST activity (Gr.5, 6 and 7).

Both D1 and D2 doses of ovral (Gr.2 and 3) enhanced the acid soluble sulfhydryl (SH) level significantly (P<0.05, and P<0.01) while only D2 dose of noracycline (Gr.6) gave a significant enhancement (P<0.05). D3 and D1 doses respectively of ovral (Gr.4) and noracycline (Gr.5) showed a little enhancement in sulfhydryl level while D3 dose of noracycline (Gr.7) did not show any appreciable effect.

Animals of all the groups showed significant enhancement in the cytosolic protein content in the liver except those of group 5.

Vb. EFFECT OF MEDROXYPROGESTERONE ACETATE (MPA)

EXPERIMENTAL DESIGN

To evaluate the drug detoxifying enzymes and acid soluble sulfhydryl level the animals were divided into two groups (Table 8). Animals of group 1 were treated with vehicle only every 5th day for 30 days while animals of group 2 were treated with 50 ug MPA every 5th day for 30 days through intramuscular route. Body weights of animals were recorded initially at fortnightly interval and at the end of experiment. After 30 days animals were sacrificed
following overnight starvation and their livers were removed and processed for the estimation of different drug metabolizing enzymes and acid soluble SH level. The statistical significance of differences between the control and experimental values were assessed by student's t-test.

RESULTS

Estimation of drug metabolizing enzymes and acid soluble sulfhydryl level (see Table 8) showed that treatment of MPA at the dose level of 50 ug every 5th day for 30 days, decreases the Cyt.b$_5$ content significantly ($P < 0.01$) while Cyt.P450 remains unchanged. No significant alteration was observed either in GST activity or SH level. Cytosolic protein was enhanced significantly ($P < 0.01$).
### TABLE 7: EFFECT OF ORAL CONTRACEPTIVE PILLS ON DRUG METABOLIZING ENZYMES AND ACID SOLUBLE SULFHYDRYL LEVEL IN MOUSE LIVER*

<table>
<thead>
<tr>
<th>GROUPS TREATMENTS</th>
<th>@ EFFECTIVE SULFHYDRYL&lt;sup&gt;b&lt;/sup&gt; LEVELS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SULFHYDRYL&lt;sup&gt;b&lt;/sup&gt; NUMBER OF ANIMALS</th>
<th>CYTOCH&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;P450&lt;/sub&gt;</th>
<th>CYTOCH&lt;sup&gt;b&lt;/sup&gt; b5 PROTEIN</th>
<th>MICROSMAL&lt;sup&gt;b&lt;/sup&gt; PROTEIN</th>
<th>GST&lt;sup&gt;b&lt;/sup&gt; ACTIVITY</th>
<th>CYTOSOLIC&lt;sup&gt;b&lt;/sup&gt; PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>umol/gm</td>
<td>nanomol/mg Pr.</td>
<td>nanomol/mg Pr.</td>
<td>levels</td>
<td>umol of CDNB-GSH</td>
<td>levels</td>
<td>conjugate formed</td>
</tr>
<tr>
<td>1. VEHICLE</td>
<td>10</td>
<td>3.92±0.81</td>
<td>0.70±0.09</td>
<td>0.4±0.06</td>
<td>3.94±0.07</td>
<td>1.44±0.12</td>
<td>4.8±0.74</td>
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<tr>
<td>2. OVRAL (D&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>10</td>
<td>5.12±0.52&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.67±0.08</td>
<td>0.43±0.05</td>
<td>3.38±0.52&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.43±0.11</td>
<td>5.39±0.64&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>3. OVRAL (D&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>10</td>
<td>4.68±0.38&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.48±0.07&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.26±0.06&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.42±0.66</td>
<td>1.45±0.09</td>
<td>5.81±0.56&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>4. OVRAL (D&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>10</td>
<td>4.42±0.47&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.62±0.08</td>
<td>0.24±0.04&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.81±0.52&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.13±0.15&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.3±0.51&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>5. NORACYCLINE (D&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>10</td>
<td>4.15±0.47</td>
<td>0.70±0.05</td>
<td>0.32±0.06&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.59±0.35&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.20±0.10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.94±0.49</td>
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<tr>
<td>6. NORACYCLINE (D&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>10</td>
<td>5.52±0.67&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.55±0.08&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.22±0.05&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.26±0.45&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.18±0.14&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.39±0.20&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>7. NORACYCLINE (D&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>10</td>
<td>3.69±0.64</td>
<td>1.27±0.10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.50±0.08&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.28±0.29&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.02±0.11&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.47±0.48&lt;sup&gt;2&lt;/sup&gt;</td>
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* The experiment was repeated thrice with same design and the trends in the result were same as in the initial experiment.

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**Note:**
- **Pr.** - Protein
- **Mean ± S.D.**
- **D<sub>1</sub>** - 1/2000th of a Pill;
- **D<sub>2</sub>** - 1/200th of a Pill;
- **D<sub>3</sub>** - 1/20th of a Pill.

1. P < 0.01;
2. P < 0.05
TABLE 8: EFFECT OF MEIDOXYPROGESTERONE ACETATE (MPA) ON DRUG METABOLIZING ENZYMES AND ACID SOLUBLE SULPHHYDRYL LEVELS IN MOUSE LIVER*

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENTS @</th>
<th>EFFECTIVE NUMBER OF ANIMALS</th>
<th>SULPHHYDRYL LEVELS: umol/gm</th>
<th>CYTOCH$_b$ P450: nmol/ tissue</th>
<th>CYTOCH$_b$ GST: nmol/ mg Pr.</th>
<th>MICROSONAL b PROTEIN: mg/ml</th>
<th>G$S_b$ ACTIVITY: umol of CDNB-GSH LEVELS</th>
<th>CYTOSOLIC b PROTEIN: mg/ml</th>
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<tbody>
<tr>
<td>1.</td>
<td>VEHICLE</td>
<td>10</td>
<td>3.99+0.66</td>
<td>0.65±0.08</td>
<td>0.44±0.06</td>
<td>3.75±0.65</td>
<td>1.43±0.13</td>
<td>4.49±0.41</td>
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<tr>
<td>2.</td>
<td>MPA</td>
<td>10</td>
<td>4.28±0.74</td>
<td>0.61±0.06</td>
<td>0.27±0.02 **</td>
<td>4.16±0.64</td>
<td>1.34±0.21</td>
<td>5.35±0.23 **</td>
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</table>

* The experiment was repeated twice with same design and the trends in the results were same as in the initial experiment.

@ Intramuscular treatment every 5th day for 30 Days.

Pr. Protein

b Mean ± S.D.

** P < 0.01
DISCUSSION

The findings of the present study (Table 7 and 8) demonstrate that the administration of different doses of oral contraceptive pills Ovral and Noracycline and injectable contraceptive steroid "MPA" shows different modulatory influence on phase I and II drug metabolizing enzymes and acid soluble SH level.

The lowest dose of ovral (D1), which is equivalent to the human dose, did not show any effect on Cyt.b5 and Cyt.P450. However, the other two doses, i.e. D2 and D3, which are higher than the human dose, showed significant decrease in cytochrome b5 while a significant decrease in Cyt.P450 was observed only with dose D2. GST activity showed a significant fall with the treatment of highest dose (i.e. D3) of ovral while D1 and D2 doses did not show any change. SH level was found to be elevated with all the three doses but failed to show statistical significance at the highest dose used (D3).

The second OC pill Noracycline with a different progestogen component, used in the present study, showed a trend different from that observed with ovral in modulating the drug metabolizing enzymes and SH level except Cyt.b5, Cyt.P450 and SH group following D2 dose treatment, Cyt.P450 following D1 dose treatment and GST activity following D3 dose treatment.

The treatment of MPA at the dose level of 50 ug/animal every 5th day for 30 days brings about significant decrease in
cytochrome b$_5$ content and significant enhancement of cytosolic protein in liver of mouse. No change was observed in Cyt. P450 content and GST activity.

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, different experimental approaches and various contraceptive steroidal combinations have shown conflicting results.

Some workers (Carter et al., 1974; Briatico et al., 1976) reported an increase in the level of cytochrome P450 following prolonged administration of steroid combinations; others reported that the cytochrome P450 levels were not altered significantly (Kretzschmar et al., 1989; Marcucci et al., 1976; Omura and Sato, 1964) and yet another claimed a decrease in the microsomal enzyme (Sachan, 1976). With respect to microsomal protein reports showed an increase (Briatico et al., 1976), decrease (Sachan, 1976) or no significant change (Carter et al., 1974; Feuer et al., 1977; Sachan, 1976). Levonorgestrel at the dose level of 1 mg/kg body weight reduces the Cyt.P450 content significantly in rats (Kretzschmar et al., 1989). Estradiol has been reported to cause a marked lowering of cytochrome P450 levels in female rats (Vodicnik et al., 1981; Lee and Chen, 1971), however, one report suggests an increase (Carter et al., 1974), and other reports, with different doses of estrogen, failed to show any induction of
microsomal monoxygenase in rats (Al-Turk et al., 1980). Treatment of progesterone at high doses failed to show any inhibitory action on hepatic drug metabolizing enzymes in rat (Ochs et al., 1986). Acetaminophen sulfotransferase activity has been found to be regulated by gonadal hormones in rats showing suppression with estrogen administration (Kane and Chen, 1987). Levonogestrel at the dose levels of 1 mg/kg body weight and 10 mg/kg body weight decreases the GSH content significantly while lower dose decreases the GST activity (Kretzschmar et al., 1989).

Besides diverse modulatory actions on drug-metabolizing enzymes, different estrogen and progestogen as well as their combinations show a range of effects on the metabolism of different compounds. O-demethylation of mestranol by liver microsomal O-demethylase has been found to be enhanced by three combinations of contraceptive steroids, i.e. mestranol plus lynestrenol, norethindrone or norethynodrel in mice and rat when treated orally (Marcucci et al., 1976). Mestranol has been found to prolong while lynestrenol reduces the duration of pento-barbitone and hexobarbitone induced sleep in mice (Blackham and Spencer, 1969). Progesterone given to female rats causes an increased hepatic demethylation of p-chloro-N-methylaniline and microsomal protein (see Saarni et al. 1980). Norethynodrel and progesterone have been found to produce alterations in hepatic drug metabolizing system in vitro and the metabolic pathways most affected were those in
which hydroxylated or oxidized products are formed. Pathways involving reduction, N-demethylation, O-demethylation or sulfoxidation were not affected except at very high concentration (Juchan and Fouts, 1966). It is not clearly understood how estrogen or progestogen or their combinations achieve their different modulatory influences. Different animals species have displayed variations in drug-metabolism following exposure to various contraceptive steroids (Jori et al., 1976). A combination of mestranol and lynestrenol in the ratio of 1:5 has been shown to nullify the effects of each other (Marcucci et al., 1976). Ochs et al. (1986) have suggested the induction of monooxygenases to be dependent on certain structural features, particularly an alkyl sidechain at C17 of the steroid molecule rather than on any specific endocrine activity.

So far very few experiments have been conducted concerning the effect of MPA on liver drug-metabolizing enzymes. Saarni et al. (1980) and Stengard et al. (1984) observed a significant dose-dependent increase in liver size, cytochrome P450 and b$_5$ concentrations, and the activities of several hepatic microsomal monooxygenase in rats administered with 10 to 600 mg/kg MPA, i.p. daily for 7 consecutive days. In another study, significant induction of P-nitroanisole, aniline and aminopyrene metabolism was
observed in female rats following MPA treatment at the dose level of 10 mg/kg/day for 30 consecutive days. Dahm et al. (1978) demonstrated that MPA has an inducing effect on the hepatic proteins.

It is not clear how these steroids bring about the alteration in the hepatic enzymes. Divergence of the results can largely be explained in terms of (1) dosage; (2) poor availability or different kinetic behaviour of hormones; (3) differences in the induction characteristics of estrogen as compared with progestogens; (4) Differences in the sensitivities of the pituitary and the liver to the steroids employed; (5) route of administration; (6) widely recognized species differences in microsomal drug metabolism.

The possibility of the overlapping antagonistic and synergistic actions of different estrogen and progestogen components present in the OC pills, depending upon the doses, in modulating the drug metabolizing enzymes as well as SH level cannot be ruled out.