CHAPTER - IV

MICROSOMES

M I C R O S O M E S

Introduction

Microsomes being the main cell organelle that are involved in drug detoxication reactions (See Introduction - Chapter I); various parameters which are related to the microsomal metabolisms are studied to reveal the mechanism (s) of detoxication of liquid paraffin by microsomes of rat liver.

As it is already stated that alterations occurring in lipid peroxidation, cytochrome P-450, b_5, glucose-6-phosphatase activities, formaldehyde and RNA were studied in different experimental conditions.

Though the literature concerned with the metabolisms related to microsomes is already reviewed in Introduction (Chapter I); additions of some details in specific metabolisms connected with the concerned parameters will enlight the relationships within the parameters which will enable to evaluate the variations occurring in parameters during experimental conditions under stressed or altered metabolisms.

Lipid Peroxidation :

Microsomal lipid peroxidation is a complex process which involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in

unsaturated lipids and the eventual destruction of membrane lipids producing a variety of breakdown products, including alcohols, ketones, aldehydes and ethers (Gardner, 1975) e.g. peroxidation of linoeic acid alone results in the formation of at least 20 degradation products (Gardner et al., 1974). It begins with the abstraction of a Hydrogen atom from an unsaturated fatty acid, resulting in the formation of a lipid radical (Poyer and Stanley, 1975). The rearrangement of the double bonds results in the formation of conjugated dienes. Attack by molecular oxygen produces a lipid peroxy radical, which can either abstract a hydrogen atom from an adjascent lipid to form a lipid hydroperoxide, or form a lipid endoperoxide. The endoperoxides in unsaturated fatty acids containing at least 3 methylene interrupted double bonds can lead to the formation of malondialdehyde as a break down product. In isolated microsomal fraction lipid peroxidation is noted (Ernster and Nordenbrand, 1967; Poyer and McCay, 1971) as NADPH dependent and oxidized iron dependent and leads the destruction of microsomal membranes. Thus microsomal membrane lipids, particularly polyunsaturated fatty acids undergo degradation (May and McCay, 1968).

It has long been known that phenobarbitol and numerous other drugs and chemicals induce the expression of drug and steroid metabolizing enzymes in liver (Conney, 1967, Krishnamurthy 1985; Waxman and Azarott, 1992). These inductive responses can have a major impact on drug metabolism, pharmo-

kinetics, drug-drug interactions, toxicity and carcinogenicity of foreign chemicals and on the potency and disposition of circulating hormones (Jacoby, 1980; Conney, 1982; Barry and Feely, 1990; Dinonen and Lindros, 1995). All these enzymes need cytochrome P-450 for their metabolism (Ortiz de Montellano, 1986; Guengerich, 1987; Raton and Schuster, 1989; Porter and Coon, 1991). The expression of P-450 and related enzymes in liver is regulated by drugs and other foreign compounds many of which dramatically induce expressions of cytochromes P-450 (Nebert and Gonzalaz, 1987; Waxman and Azaroff, 1992). The enzymes (P-450 forms) are regulated both by endogenous factors including gonadal and Pituitary hormones (Waxmann, 1988) and by drugs and other foreign compounds. Although much is known about the molecular mechanisms by which 3-methyl cholanthrene and related polycyclic aromatic hydrocarbons induce in liver expression of selective P-450 genes (Adesnik and Atchison, 1985; Gonzalez, 1988; Nebert et al., 1990; Okey, 1990); still the mechanism (s) by which pentabarbitol inducer of P-450 expressions increase the expressions are still poorly understood. Genes of inducible P-450 belong to one super gene family CYP reviewed by Waxman and Azargoff, (1992) in case of phenobartial induction. Expression of CYP genes in liver is heterogenous (Gooding et al., 1978; Baron et al., 1981). CYP are expressed predominantly in perivenous region of liver (Gebhardt, 1992). The heterogenous expression of liver CYP genes has been suggested to be maintained by

gradients of hormones, substrates, metabolites or O_2/CO_2 tension of sinusoidal blood. The supergene family CYP is regulated by pituitary dependent hormones (Kato and Yamazoe, 1993) GH and other hormones regulated by pituitary could mediate the zonal expressions of the CYP genes. CYP 3A is expressed exclusively in the preveinous regions (Buhler et al. ,1993). CYP 3A gene catalytically active testosterone 6-β-hydroxylases and induced by pregnenolone, 16-α-Carbonitile, dexamethasone and phenobarbital (Oinone et al., 1993). These are differentiated into two types CYP 3A, and CYP $3A_2$ with their differential expression to GH, iodothyronine, hypophysectomy in males and females (Oinonen and Lindros, 1995) while GH suppresses the periportal expression of the rat CYP_2 B (Oinonen et al., 1993). Cytochrome P-450 enzymes dependent metabolisms represent the classical Phase I metabolism in which the substrate is oxygenated. Phase II enzymes often use the oxygen as a site for further metabolism (e.g. glucuronidation and sulfate glutathione or glycine conjugation). Detoxication usually requires both phase I & II enzymes (Nebert and Gonzalez, 1987).

Thus it can be revealed that cytochrome P-450, its super family of genes and related metabolisms has to be studied in detail. Cytochrome b_5 is also involved in the reactions (Chapter I - Introduction) but is poorly studied. But alterations in cytochrome P-450 are related with alterations in cytochrome b_5 and hence should be studied along with cytochrome P-450.

Cytochrome P-450 (Levin et al., 1973) and cytochrome b_5 (Tappel and Zalkin, 1960) are also inactivated during NADPH-dependent lipid peroxidation; although the mechanism of inactivation appears to involve the destruction of the heme group rather than the loss of membrane integrity. The loss of cytochrome P-450 during lipid peroxidation parallels the loss of drug metabolizing activity.

Glucose-6-Phosphatase :

Glucose-6-phosphatase is membrane integrated enzyme present in endoplasmic reticulum (Lehninger, 1982). The disrruption of membrane integrity resulting from the breakdown of the lipid constituents has been implicated as the cause for the decrease of glucose-6-phosphatase activity during NADPH dependent lipid peroxidation (Wills, 1971). Detergent disrruption of microsomal membranes causes a similar decrease in activity of glucose-6-phosphatase.

Formaldehyde

A variety of NADPH and oxygen dependent reactions catalysed by the microsomal fraction of many tissues has been recognized to yield formaldehyde as a product. The most common and frequently studied reaction resulting in the liberation of formaldehyde is the N-demethylation of secondary or tertiary amines. These reactions are mediated by cytochrome P-450 the

terminal oxidase of the microsomal mixed function oxygenase system (Cooper et al., 1965).

The reactions are depicted in following equations:

1. $$\begin{matrix} R \\ | \\ N\text{-}CH_3 \\ | \\ R \end{matrix} + O_2 + NADPH + H^+ \xrightarrow{\text{P-450}} \begin{matrix} R \\ | \\ N\text{-}H \\ | \\ R \end{matrix} + \begin{matrix} O \\ \| \\ CH_2 \end{matrix} + H_2O + NADP$$

2. $$\begin{matrix} OH \\ | \\ N - CH_3 \\ | \\ R \end{matrix} + O_2 + NADPH + H^+ \xrightarrow[\text{Oxidase}]{\text{Amine}} \begin{matrix} OH \\ | \\ N - H \\ | \\ R \end{matrix} + \begin{matrix} O \\ \| \\ CH_2 \end{matrix} + H_2O + NADP$$

3. $$\begin{matrix} OH \\ | \\ CH_3 \end{matrix} + \begin{matrix} O_2 + NADPH + H^+ \\ \downarrow \text{--- P-450} \\ H_2O_2 \end{matrix} \xrightarrow[\text{Catalase}]{} \begin{matrix} O \\ \| \\ CH_2 \end{matrix} + 2H_2O$$

The reaction is initiated by an amine oxidase catalyses N-oxidation of certain hydroxylamines. The highly unstable hydroxyl amine oxides formed undergo rapid dehydration and the resulting nitrone intermediates liberate formaldehyde upon non-enzymic hydrolysis (Poulsen et al., 1974). A third reaction sequence resulting in the formation of formaldehyde has been demonstrated to be associated with method oxidation (reaction3).

Table 12 : Variation in biochemical parameters assayed in liver microsomal fractions from liquid paraffin treated rats.

No.	Group of rats	Days of liquid paraffin treatment	Lipid Peroxidation nm/mg protein	Cytochrome P-450 M/mg protein	Cytochrome b_5 M/mg protein	Glucose-6-Phosphatase activity gPO_4 released/ mg protein	Formaldehyde M/mg protein	RNA ng/mg protein
1	Group I	-	87.09 ±4.23	15..05 ±0.98	8.92 ±0.36	1.22 ±0.06	2.05 ±0.13	65.22 ±3.60
2	Group II	3	150.89 ±9.75[c]	15.46 ±0.73[d]	11.35 ±0.61[b]	0.77 ±0.02[c]	5.76 ±0.29[c]	49.56 ±2.76[c]
3.	Group III	6	526.22 ±33.33[c]	6.81 ±0.30[c]	5.30 ±0.27[c]	0.50 ±0.03[c]	3.69 ±0.16[c]	83.72 ±3.97[c]
4.	Group IV	9	9.62 ±0.42[c]	1.47 ±0.05[c]	1.00 ±0.03[c]	0.41 ±0.02[c]	0.65 ±0.02[c]	162.50 ±8.00[c]
5	Group V	12	39.74 ±0.42[c]	1.13 ±0.04[c]	0.85 ±0.05[c]	0.05 ±0.04[c]	0.61 ±0.04[c]	1400.00 ±86.00[c]
Group VI		15	279.41 ±13.80[c]	2.72 ±0.083	2.19 ±0.67[c]	0.77 ±0.06[c]	0.97 ±0.07[c]	1898.97 ±86.00[c]

Values are mean of ± SE of 5 animals.

P - values are as [redacted] - a < 0.05, b < 0.01
c < 0.001 & d > 0.05

These reactions may involve the cytochrome P-450 containing electron transport syystem as a source of H_2O_2 (Hildebrandt et al., 1975), which in the presence of methanol is reduced peroxidatically by catalase (Oshino et al., 1973) contaminating many microsomal preparations.

RNA :

Microsomal RNA content includes ribosomal RNA and mRNA. Ratio of RNA to protein indicates the protein synthesizing activity of rough microsomes as one can reveal. On the basis of the literature concerned with these above parameters that are directly involved in the drug detoxication metabolism which reflect the alterations taking place in the cells and particularly in involved cell organelle one can evaluate the role of the cell organelle and can predict the status of metabolism.

Observations

Liver

Isolated microsomal fractions were used for the bioassays of various parameters studied.

Lipid peroxidation - Table 12

Group I - Normal rats.

The estimated values of lipid peroxidation in liver micro-

somal fractions were 87.09 nM/mg protein.

Group II - 1 ml/kg body wt. liquid paraffin daily administered for 3 days.

Lipid peroxidation showed 1.73 folds increase over the values reported in hepatic microsomal fractions of normal rat.

Group III - 1 ml/kg body wt. liquid paraffin daily administered for 6 days.

A significant increase of 3.48 folds was recorded over the values reported in liver microsomal fractions of Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin daily administered for 9 days.

A sudden fall in lipid peroxidation products by 54.7 folds was reported as compared to the lipid peroxidation noted in hepatic microsomal fractions of Group III rats.

Group V - 1 ml/kg body wt. liquid paraffin daily administered for 12 days.

The products of lipid peroxidation noted in hepatic microsomes of rats belonging to this group was 4.13 folds less as compared to the products of lipid peroxidation reported in hepatic microsomal fractions of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin daily administered for 15 days.

Malondialdehyde quantities estimated in the microsomal

fractions of liver in this group showed 7.03 fold increase over the values of malondialdehyde in microsomal fractions of Group V rats.

Cytochromes P-450 - Table 12

Group I - Normal rats.

15.05 mM/mg proteins cytochromes P-450 were noted in microsomal fractions of livers of normal rats.

Group II - 1 ml/kg body wt. liquid paraffin daily administered for 3 days.

No difference was noted in cytochromes P-450 as compared to the values estimated in microsomal fractions of livers of normal rats.

Group-III - 1 ml/kg body wt. liquid paraffin daily administered for 6 days.

Cytochromes P-450 showed 2.27 folds decrease as compared to the values estimated of cytochromes-P-450 in microsomal fractions of livers of Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin daily administered for 9 days.

4.63 folds decrease in the content of cytochromes P-450, was noted as compared to the values estimated in hepatic microsomal fractions of Group III rats.

Group V - 1 ml/kg body wt. liquid paraffin administered daily for 12 days.

2.40 folds decrease was noted in cytochromes P-450 content assayed in microsomal fractions of liver of Group IV as compared to the values reported in microsomal fractions of livers of Group V rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered daily for 15 days.

Marginal increase of 0.415 folds was noted in the cytochromes P-450 content from hepatic microsomal fraction of rats of these groups as compared to the values of cytochromes P-450 noted in hepatic microsomal fractions of rats belonging to Group V.

Cytochromes b_5 - Table 12

Group I - Normal rats.

Cytochromes b_5 content estimated was 8.92 µM/mg proteins in hepatic microsomal fractions of normal rats.

Group II - 1 ml/kg body wt. liquid paraffin administered daily for 3 days.

A marginal increase was noted in the microsomal fractions of livers of rats that belong to Group II as compared to the values estimated in hepatic microsomal fractions of normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered daily for 6 days.

5.141 fold decrease was observed in the content of cytochromes b_5 as compared to the hepatic microsomal cytochrome b_5 content in rats that belong to Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered daily for 9 days.

Cytochromes b_5 content from hepatic microsomal fraction showed 5.30 folds decrease as compared to the values represented in microsomal fractions of liver of Group III rats.

Group V - 1 ml/kg body wt. liquid paraffin administered daily for 12 days.

A marginal decrease was noted as compared to the cytochromes b_5 content reported in liver microsomal fractions of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered daily for 15 days.

2.57 folds increase was observed in the hepatic microsomal cytochrome b_5 as compared to the hepatic microsomal cytochrome b_5 content noted in rats of Group V.

Glucose-6-Phosphatase activities - Table 12

Group I - Normal rats.

1.22 µg phosphate released was estimated per mg proteins of hepatic microsomal fraction of normal rats due to glucose-6-phosphatase activity.

Group II - 1 ml/kg body wt. liquid paraffin administered daily for 3 days.

Glucose-6-phosphatase activities showed 1.58 fold decrease as compared to the enzyme activities noted in hepatic microsomal fractions of normal rat.

Group III - 1 ml/kg body wt. liquid paraffin administered daily for 6 days.

1.54 fold decrease was estimated in glucose-6-phosphatase activities of the microsomal fractions of liver as compared to the enzyme activities reported in microsomal fractions of liver in Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered daily for 9 days.

Enzyme activities decreased by 1.21 folds in hepatic microsomal fraction as compared to the enzyme activities reported in hepatic microsomal fraction of rats belonging to Group III.

Group V - 1 ml/kg body wt. liquid paraffin administered daily for 12 days.

Glucose-6-phosphatase activities assayed in hepatic microsomal fractions of these rats showed 8.2 folds decrease as

compared to the Glucose-6-phosphatase activities estimated in hepatic microsomal fractions of the Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered daily for 15 days.

8.2 folds decrease in Glucose-6-phosphatase activities were reported in the microsomal fractions of liver as compared to the enzyme activities that were noted in microsomal fractions of liver of rats that belong to Group V.

Formaldehyde Table - 12

Group I - Normal rats.

Formaldehyde estimated in hepatic microsomal fraction of normal rats showed value 2.05 μ M/mg proteins.

Group II -1 ml/kg body wt. liquid paraffin administered for 3 days.

2.8 folds increased was noted in the production of formaldehyde in hepatic microsomal fraction as compared to the formaldehyde content that was estimated in hepatic microsomal fraction in normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

The alterations in formaldehyde content from hepatic microsomal fractions of present rats showed 1.56 fold decrease

as compared to the formaldehyde that was assayed in hepatic microsomal fractions of rats of Group II.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

5.676 fold decrease was noted in the formaldehyde estimated from hepatic microsomes as compared to the formaldehyde content noted in hepatic microsomal fraction of Group III rats.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

There was no significant alteration noted in the formaldehyde content of hepatic microsomal fraction as compared to the hepatic microsomal fraction of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

1.59 folds increase was observed in the formaldehyde content of hepatic microsomal fraction as compared to the formaldehyde content of hepatic microsomal fraction of Group V rats.

RNA - Table 13 12

Group I - Normal rats.

RNA content of hepatic microsomal fraction was 65.22 ng/mg protein in normal rats.

Group II - 1 ml/kg body wt. of liquid paraffin administered for 3 days.

1.315 folds decrease was noted in RNA content of hepatic microsomes as compared to RNA content that was noted in microsomal fraction of liver of normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

1.689 folds increase was observed in RNA content in hepatic microsomal fraction as compared to the RNA content noted in hepatic microsomal fraction of Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

1.94 folds increase was noted in hepatic microsomal RNA as compared to hepatic microsomal RNA of rats of Group III.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

Hepatic microsomal RNA content showed 8.61 folds increase as compared to the hepatic microsomal RNA content noted in rats of Group IV.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

RNA content of microsomal fraction of liver showed 1.35

Table 13: Variations in biochemical parameters assayed in kidney - microsomal fractions from liquid paraffin treated rats.

No.	Group of rats	Days of liquid paraffin treatment	Lipid peroxidation nm/mg protein	Cytochrome P-450 M/mg protein	Cytochrome b_5 M/mg protein	Glucose-6-Phosphatase activity gPO released/ mg protein	Formaldehyde M/mg	RNA ng/mg protein
1	Group I	-	29.36 ±16.04	20.98 ±0.67	11.65 ±0.54	1.55 ±0.03	3.26 ±0.11	63.64 ±3.33
2	Group II	3	384.46[c] 18.00	7.25[c] ±0.32	7.58[c] ±0.33	1.36[b] ±0.05	5.60[c] ±0.22	67.71[d] ±6.00
3	Group III	6	573.02[c] ±47.86	9.98[c] ±0.47	6.48[c] ± ±0.29	1.15[c] ±0.03	9.43[c] ±0.46	110.61[c] ±8.44
4	Group IV	9	40.22[c] ±2.79	2.01[c] ±0.06	0.67[c] ±0.03	0.49[c] ±0.01	8.32[c] ±0.36	245.10[c] ±12.08
5	Group V	12	54.49[c] ±3.31	5.13[c] ±0.29	4.27[c] ±0.18	0.43[c] ±0.02	7.78[c] ±0.41	268.00[c] ±14.52
6	Group VI	15	83.61[c] ±8.00	3.82[c] ±0.13	3.52[c] ±0.15	0.17[c] ±0.01	1.31[c] 0.04	208.70[e] ±9.64

Values are mean ± SE of 5 animals. p values are as in Table 12

folds increase over the RNA content of microsomal fraction of liver of rats of Group V.

Kidney :

Microsomal fractions were used for the bioassays of various parameters studied.

Lipid peroxidation - Table 13

Correct the value in table.

Group I - Normal rats.

291.36 nM/mg proteins malondialdehyde was noted in the renal microsomal fraction of normal rats.

Group II - 1 ml/kg body wt. liquid paraffin administered for 3 days.

1.34 folds increase in the products of lipid peroxidation was noted in the renal microsomal fractions as compared to the malondialdehyde estimated in the renal microsomal fractions of normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

1.490 folds increase was noted in the renal microsomal fraction as compared to the renal microsomal fraction of rats of Group II.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

A decrease of 14.247 folds was noted in lipid peroxidation in microsomal fraction of kidney as compared to lipid peroxidation in microsomal fraction of kidney observed in rats of Group III.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

Renal microsomal fractions showed 1.354 folds decrease in lipid peroxidation as compared to the lipid peroxidation that was noted in microsomal fractions of kidneys of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

Renal microsomal fractions showed 1.534 folds decrease as compared to the lipid peroxidation that was noted in microsomal fractions of kidneys of Group V rats.

Cytochrome P-450 - Table 13

Group I- Normal rats.

Cytochromes P-450 estimated from renal microsomal fraction of normal rat were 20.98 µM/mg proteins.

Group II - 1 ml/kg body wt. liquid paraffin administered for 3 days.

2.89 folds decrease was noted in kidney microsomal fraction as compared to the cytochrome P-450 noted in renal microsomal fractions of normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

1.376 folds increase in cytochromes P-450 content of renal microsomal fraction was noted over the Cytochrome -P-450 content of renal microsomal fractions observed in rats of Group II.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

Cytochromes P-450 content of renal microsomal fraction showed 4.965 folds decrease as compared to the cytochrome P-450 content of renal microsomal fractions of rats that belong to Group III.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

Cytochrome P-450 content showed 2.552 folds increase in renal microsomal fractions over the Cytochrome P-450 values noted in renal microsomal fractions of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

1.342 folds decrease was observed in Cytochrome P-450

content of renal microsomal fractions as compared to the Cytochromes P-450 content of renal microsomal fractions of Group V rats.

Cytochrome b_5 : Table 13

Group I - Normal rats.

Cytochrome b_5 estimated were 11.65 µm/mg proteins in microsomal fractions of rat kidneys of normal rats.

Group II - 1 ml/kg body wt. liquid paraffin administered for 3 days.

Decrease of 1.53 folds was noted in the renal microsomal fractions cytochrome b_5 content as compared to the content of cytochromes b_5 from renal microsomal fractions of normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

1.169 folds decrease in Cytochrome b_5 were noted in kidney microsomal fractions as compared to the cytochrome b_5 content of renal microsomal fractions from Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

A decrease of 9.67 folds was noted in contents of Cytochrome b_5 of renal microsomal fractions which was evident over

the Cytochrome b_5 content of renal microsomal fractions of Group II rats.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

6.373 folds increase in content of Cytochrome b_5 of renal microsomal fractions was noted over the Cytochrome b_5 content reported in renal microsomal fractions of Group IV rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 15 days.

1.21 folds decrease in Cytochrome b_5 content was noted in renal microsomal fraction as compared to the Cytochrome b_5 content of renal microsomal fractions from Group V rats.

Glucose-6-Phosphatase activities - Table 13

Group I - Normal rats.

1.55 µg phosphate/mg proteins was liberated as the activities of the enzyme in kidney microsomal fractions of normal rat.

Group II - 1 ml/kg body wt liquid paraffin administered for 3 days.

1.13 folds decrease in Glucose-6-Phosphatase enzyme activities from renal microsomal fractions as compared to the Glucose-6-Phosphatase activities that were noted in the renal microsomal fractions of normal rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

A decrease of 1.18 folds was noted in Glucose-6-Phosphatase activities of renal microsomal fractions when compared with the enzyme activities of renal microsomal fractions from rats belonging to Group II.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

As compared to the Glucose-6-Phosphatase activities of renal microsomal fraction from Group III rats; 2.346 folds decrease was noted in the enzyme activities from microsomal fractions of kidneys of Group IV rats.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

1.139 fold decrease in Glucose-6-Phosphatase activities from microsomal fractions of kidneys was noted when compared to the enzyme activities from renal microsomal fractions of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

Glucose-6-Phosphatase activities from renal microsomal fractions showed 2.529 folds decrease as compared to the enzyme activities that were noted in the rats of Group V.

Formaldehyde - Table 13

Group I - Normal rats.

3.26 µM/mg proteins formaldehyde was noted in renal microsomal fractions of normal rat

Group II - 1 ml/kg body wt. liquid paraffin administered for 3 days.

1.171 folds increase was noted in formaldehyde content as compared to formaldehyde content noted in kidneys of Group I rats.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

1.683 folds increase was noted in formaldehyde content as compared to the content that was noted in kidneys of Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

A decrease of 1.13 folds was noted in formaldehyde content as compared to the content that was reported in Group III rats.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

The decrease of 1.06 folds was also observed in the formaldehyde content that was noted in kidneys of rats of Group IV.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

Formaldehyde estimated in kidneys of these rats was 5.938 folds lower in content than that was noted in kidneys of Group V rats.

RNA : Table 13

As per table Values it should be 63.64

Group I - Normal rats.

3.64 ng/mg proteins RNA was assayed in microsomal fractions of kidneys.

Group II - 1 ml/kg body wt. liquid paraffin administered for 3 days.

RNA content was increased by 1.063 folds in microsomal fractions of kidneys as compared to the RNA content that was noted in the renal microsomal fractions.

Group III - 1 ml/kg body wt. liquid paraffin administered for 6 days.

Renal microsomal fractions of these rats showed 1.633 folds increase in RNA content as compared to the RNA content that was noted in microsomal fractions of kidneys of Group II rats.

Group IV - 1 ml/kg body wt. liquid paraffin administered for 9 days.

Microsomal fractions of kidneys showed 2.228 folds increase in RNA content over the renal microsomal RNA that was noted in Group III rats.

Group V - 1 ml/kg body wt. liquid paraffin administered for 12 days.

1.093 folds increase was noted in renal microsomal RNA content as compared to the RNA content of the microsomal fractions of kidneys of Group IV rats.

Group VI - 1 ml/kg body wt. liquid paraffin administered for 15 days.

1.228 folds decrease was observed in microsomal fractions of kidneys as compared to the RNA content that was noted in renal microsomal fractions of kidneys of Group V rats.

Discussion :

The critical evaluation of alterations in different parameters studied in microsomal fractions will be helpful in understanding of the drug metabolism (Table 12 and 13) Graph 1.

The values of lipid peroxidation were low in normal rats, but of cytochromes P-450, and b_5 and activities of glucose-6-phosphatase were higher. Formaldehyde and RNA content was low. Three days treatment of liquid paraffin increased lipid peroxidation with very small decrease in cytochromes P-450 and

significant increase in cytochromes b_5 and formaldehyde, but showed significant drop in glucose-6-phosphatase activities and RNA content. Treatment of liquid paraffin for 6 days continued the increase in lipid peroxidation which was maximum in the hepatic microsomal lipid peroxidation reported in present study. This was coupled with the significant loss in cytochromes P-450 and b_5, glucose-6-phosphatase activities, formaldehyde but with significant increase in RNA content. Nine days treatment of liquid paraffin reduced, lipid peroxidation cytochromes P-450 and b_5, glucose-6-phosphatase activities and formaldehyde, but with significant increase in RNA content. Twelve days liquid paraffin treatment showed increase in lipid peroxidation but with further decrease in cytochromes P-450, and b_5, glucose-6-phosphatase activities and formaldehyde content coupled with tremendous increase in RNA content. Liquid paraffin treatment when continued upto 15 days the lipid peroxidation, cytochromes P-450 and b_5 showed significant increase with highly significant rise in enzyme activities of the glucose-6-phosphatase and RNA content (which was maximum in case of Liver microsomes) with little rise in formaldehyde content.

The above alterations indicate increase in lipid peroxidation with very little alterations in cytochromes P-450 but cytochromes b_5 showed increase indicating its involvement in drug processing at this stage. As expected a drop in glucose-

6-phosphatase activities indicate the damage to the enzyme coupled with increase in lipid peroxidation. Formaldehyde showed tremendous increase indicating its production during drug processing but coupled with a little drop in RNA synthesis may be attributed to increase in formaldehyde content. The increase in RNA content after 12 days and 15 days liquid paraffin treatments along with little rise in formaldehyde activity of glucose-6-phosphatase, Cytochromes P-450 and b_5 (after 15 days treatment particularly) simultaneously. This indicates the involvement of RNA in microsomal membrane building since all the parameter except formaldehyde are structural components of microsomal membranes. But these effects are also coupled with increased lipid peroxidation which indicated simultaneous membrane damage coupled with formaldehyde producing microsomal activities. Thus microsomal turnover is under stress as revealed by alterations in different parameters. Ultimately liver is under stress.

After 3-days treatment of liquid paraffin high lipid peroxidation low content of P-450 and b_5 cytochromes and glucose-6-phosphatase activities were noted. Formaldehyde content was increased coupled with marginally increase in RNA content. Six days treatment of liquid paraffin lead to further increase in lipid peroxidation, formaldehyde content, cytochrome P-450 and RNA content. But glucose-6-phosphatase activities and cytochrome b_5 contents showed marginal decrease.

Nine days treatment of liquid paraffin decreased lipid peroxidation significantly coupled with significant loss of P-450 and b_5 cytochromes and formaldehyde content(not significant) and glucose-6-phosphatase activities. This is indicating that lipid-peroxidation loss is apparent and microsomal membranes related parameters are lowered inspite of low lipid peroxidation; which may be the result of high formaldehyde content or excretion of peroxidation products showing apparent decrease in lipid peroxidation or synthesis of new membranes as RNA content is also increased. Twelve days of liquid paraffin treatment again increased lipid peroxidation with coupled increase in cytochromes P-450,and b_5 content but marginal loss in glucose-6-phosphatase activities and formaldehyde content along/with significant increase in RNA content. This explains the increase in Cytochromes content and also formaldehyde production revealing microsomal drug processing activity. Marginal loss in glucose-6-phosphatase activities may be due to presence of formaldehyde. Fifteen days liquid paraffin treatment increased lipid peroxidation significantly coupled with decrease in all the parameters studied except RNA which increased further. These results indicate heavy stress on microsomal membranes coupled with heavy RNA producing activity of cell.But all these indicate stress on microsomes and ultimately on kidneys.