C H A P T E R - VI

GENERAL DISCUSSION

The observations which are described in Chapter III - Histology, Chapter IV - Microsomes and Chapter V - Glutathione are discussed together to reveal the resultant toxicological effects. These effects have been studied by administration of 1 ml/ kg body wt. liquid paraffin male albino rat. The rats were sacrificed after the interval of 3 days, 6 days, 9 days, 12 days, 15 days to study histology, microsomes and cytosolic fractions from liver and kidney. The parameters studied include distributions of various types of cells and alterations in histology, cytochromes, P-450 and b_5 , glucose-6-phosphatase activity, lipid peroxidation, RNA and formaldehyde content from microsomes and Glutathione from cytosolic fraction.

All these alterations when considered together one can reveal the toxicological effects as reflected in histology where normal hepatocytes reduced progressively and foggy cytoplasm showing cells with either stained nuclei (in early intervals more), and unstained nuclei (more in late intervals); with few giant cells with stained nuclei, cells with vacuolated cytoplasm along with vacuolated giant cells and the degenerating cells made their appearance at specified intervals of treatment. The alterations show maximum number of foggy cells with unstained nuclei, giant cells with vacuolated cytoplasm, few vacuolated cells and few degenerating cells at the terminal dose. All these results indicate that liquid paraffin is not inert though we use it as vehicle

for drug treatment or base for ointments. It is surely hepatotoxic as well as nephrotoxic since cells having foggy cytoplasm were also observed in proximal, distal and collecting tubules coupled with swollen glomeruli followed by Bowman's capsule dialation and then appearance of vacuolated cells in glomeruli. All these alterations were coupled with the accumulation of blood in pools in Glomeruli and various other parts of kidney. These alterations were also coupled with the distribution of the trafficking cells of liver viz. Kupffer cells and sinusoidal cells. Both of them showed alterations in their distribution in centrolobular and periarterial region but at terminating interval Kupffer cells were more in lobular area than sinusoidal cells. Such type of positional heterogenecity related to function of Kupffer cells in the liver acinars is known and distribution of these cells in periarterial region is noted (Sleyster and Knook, 1982) as it is observed in present case but if phagocytotically triggered by latex then this zonal heterogenicity can be reduced (Sleyster and Knook, 1982). Therefore, the presently observed distribution is related to function and not triggered by any exogenous phagocytotic material. Kupffer cells are known to be involved in degradation of membrane components through their lysosomal system (Glaumann and Marzella, 1981). Both of them have not shown any significant morphologically evident toxic effects.

To study the mode of toxicity the drug metabolizing

system (microsomes) was studied using indicators of metabolism viz. lipid peroxidation, cytochromes P-450 and b_5, glucose-6-phosphatase activities, formaldehyde; RNA content to reveal the alterations in Rough microsomes. Cytosolic glutathione content which affects the consequences of lipid peroxidation in normal and drug metabolizing conditions is also studied. Formation of formaldehyde is a product of NADPH and O_2 dependent reactions catalyzed by microsomal fraction and hence reflect the integrity of microsomes. Special feature of formaldehyde alterations indicate that it was high in content in kidney and in liver it showed peak values in early intervals than late intervals.

The evaluation of alterations in the above parameters revealed that lipid peroxidation increased after 3 and 6 days treatment but decreased after 9 days treatment again showing many folds increase after 12 days and 15 days of treatments. This was coupled with progressive depletion in cytochromes P-450 and b_5, formaldehyde and glucose-6-phosphatase activities which continued from 3 days through 12 days of treatment but showed increase on 15 days of treatment but not on 9 days of treatment. Since lipid peroxidation is the indicator of the membrane damage (Shuster, 1966; Mehendale, 1986; Waxman and Azroff, 1992) producing free radicals and therefore, continuous drop in the contents of cytochromes P-450 and b_5, and activities

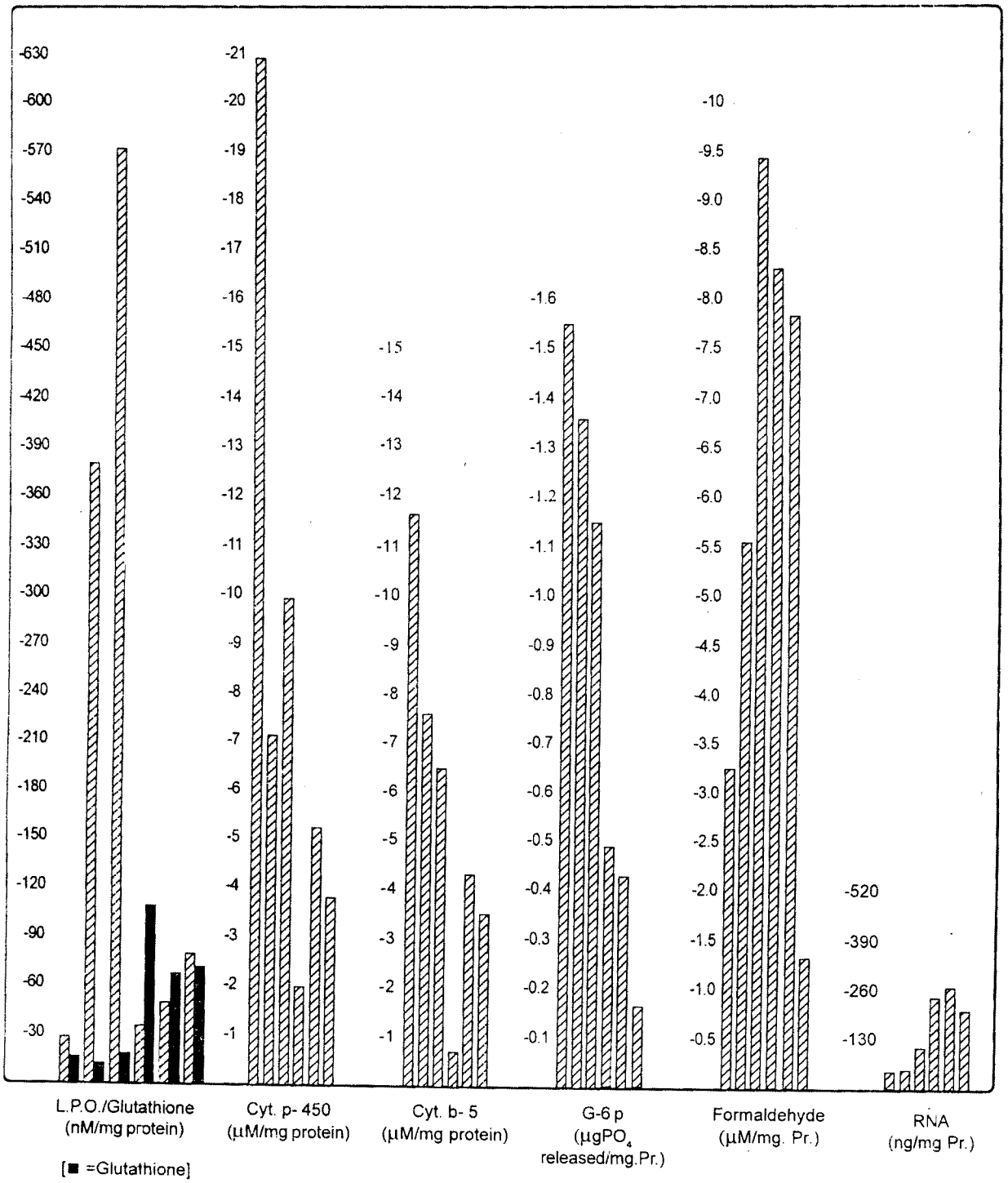

Fig. 1 -Variations in biochemical parameters assayed in kidney microsomes from liquid paraffin treated rats.

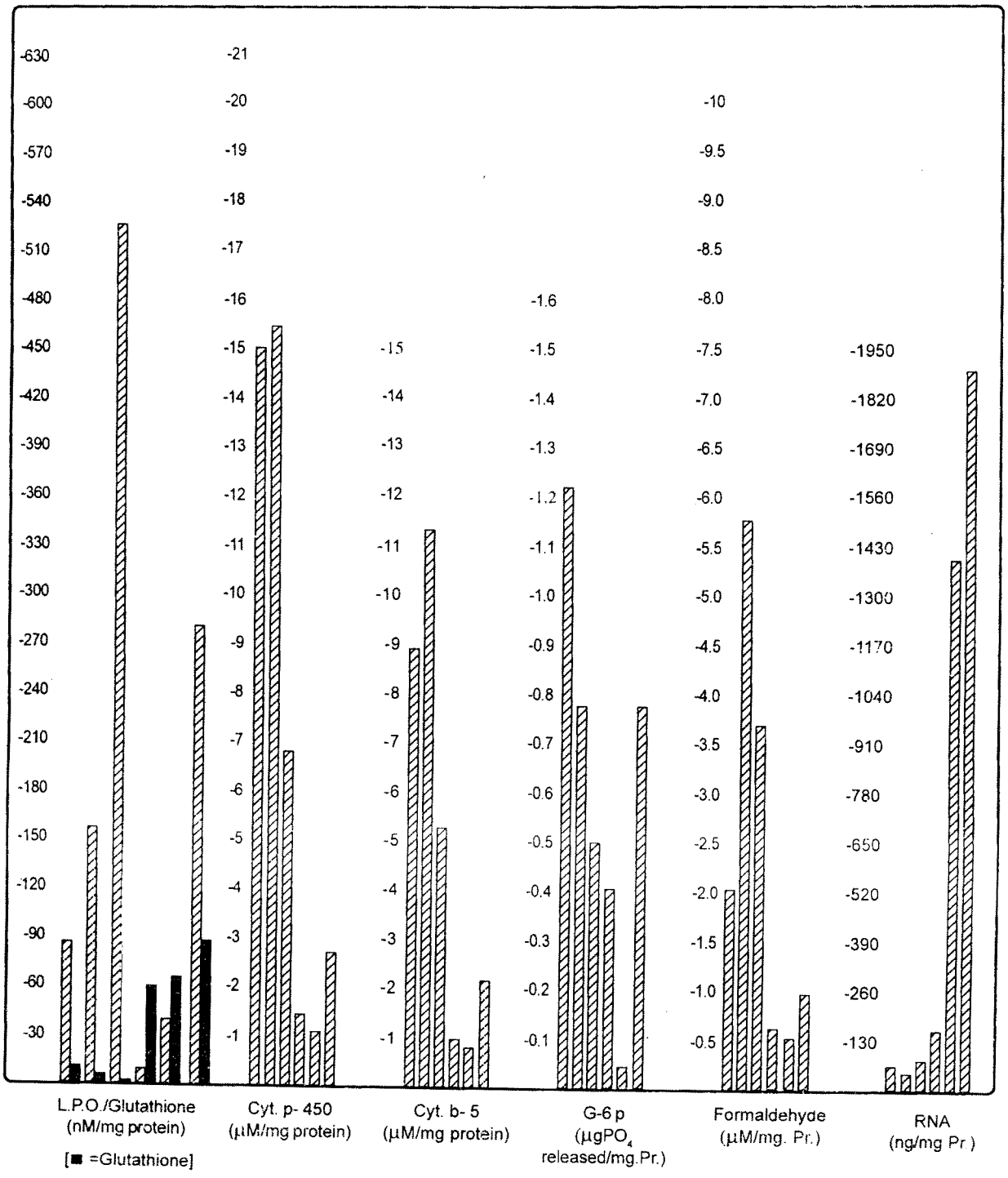

Fig. 2 -Variations in biochemical parameters assayed in liver microsomes from liquid paraffin treated rats.

of glucose-6-phosphatase is expected since these are the membrane components of microsomes and act when membranes are organized (Coon, 1978; Strittmatter et al., 1978) and therefore, integrity of the membrane is reflected in their metabolisms. Similar picture is also reflected in formaldehyde content which is product of microsomal metabolism and hence the coupled drop indicates the loss of microsomal activity. But all the parameters except lipid peroxidation were increased on 15 days of treatment which is actually expected after 9 days of treatment when lipid peroxidation was lowered. This drop in lipid peroxidation may be apparent since the free radicals formed as a result of lipid peroxidation might have been removed by endogenous ligands/ radical scavengers viz. glutathione etc. The glutathione content studied in cytosolic fraction have shown drop in rats given 3 days and 6 days treatment with tremendous increase after 9 days of treatment this may have dropped the values of lipid peroxidation apparently (Figs. 1 & 2). The increase in glutathione content was continued after 12 and 15 days of treatment indicate that in vivo glutathione transferases which are inducible and are required for glutathine actions may have been induced. Some of the reactions are non-enzymatic also (Mannerik & Danielson, 1988; Kefferer et al., 1988; Damon et al., 1996) and hence the disparity in content appears in observations. Glutathione transferases are involved in Drug resistance activities (Hayes et al., 1990). The increase after terminal treatment in other parameters possibly due to

proliferation of microsomes since it is indicator of the functional membrane system of microsomes (Coon, 1978; Strittmatter et al., 1978). Cytochromes, P-450 and b_5 , glucose-6-phosphatase activities indicate the structural and functional integrity of membranes which is confirmed further by increase in formaldehyde. Similarly total protein content is also increased with continuous increase in RNA content to show synthesis of proteins. This is supporting the increase observed in other parameters which suggest the proliferation of the smooth microsomes. Drug induced such proliferations in microsomes/smooth ER are well studied in rat viz. phenaborbital and Carcinogens (Comey and Burns, 1962) phenoborbital (Remmer et al., 1963),3 - methyl-4-dimethylamino-benzene (Porter and Burns, 1959) CCl_4(Kato et al.,1962) and many other toxic substances (Shuster, 1966). This induced drug metabolism is long lasting also (Burns et al., 1963). Thus liquid paraffin is hepatotoxic, microsomal system inducible, glutathione transferases inducible. The persistance of foggy nature of maximum cells may be due to increased protein synthesis and accumulation of proteins (due to membrane damage).

Liquid paraffin is nephrotoxic also since lipid peroxidation followed the same pattern of changes as in liver. But the decrease in cytochrome b_5 , glucose-6-phosphatase (with some brief increase in cytochrome P-450 and formaldehyde after 3 and 6 days of treatment, then followed the trend of other

parameter). All the results indicate the progressive damage of membranes as a result of lipid peroxidation but the other parameters were not increased as in case of liver. Increase in total proteins or RNA is not observed after 15 days of treatment (which showed increase after 9 days and 12 days of treatment). These parameters though showed mixed response they have not shown certainly the proliferation of microsomes after 15 days of treatment as in liver. Since number of types of biotransformed forms of products are taken to kidney for clearance. The response of various tubules may be heterogenous and hence some variations in results may be there. The histology is clearly indicating the retention of blood may be due to slow filtration/clearance and accumulation of proteins (due to foggy tubules) which is also noted in case kidneys of rats treated with isoparaffin (Short et al., 1986). Hyaline material (droplets) in proximal tubules was also observed in light hydrocarbon induced nephropathy (as the dense material is observed in present project) by Phillips and Cockrdl (1984) and the lesions were related to an alteration in normal cellular digestion of protein.

Isoparaffin treatment have shown to secrete B-microglobin excretion through kidney along with its accumulation in cytoplasm and very slow globular filtration.

Thus liquid paraffin treatment though appeared inert; even small doses are hepatotoxic and nephrotoxic and appearance

of giant cells makes them more risky in use since they appear in neoplasms also (Percy and Hulland, 1968) but they have reported to be vacuolated and degenerated even after 15 doses of liquid paraffin.

Prolonged treatment of liquid paraffin have to be studied to give the details of microsomal toxic stress and to evaluate the risk of giant cell appearance and renal toxicity; since these cells are observed as the carcinogenic effects. Proliferation of microsomal system throughout experimental schedule have shown the stress on drug processing constituents of cells. Prolonged treatment may help to resolve the fate of sustaineded stress on microsomes and the histological adaptations accordingly.

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