CHAPTER - I

INTRODUCTION

Introduction

For the safe use of chemicals, their toxicities have to be tested which make the toxicological studies important in medicine. Such type of studies are being carried out in recent years. These studies are now getting importance since mankind is constantly exposed to arrays of new chemicals through the pollution of all kinds. Growing industrial areas, heavy use of chemical pesticides and changing environment at global level compel these types of studies of varied sort of chemicals to evaluate the practical risk behind each of it and remedy for precautions to be employed.

The primary approach and development of these studies have been reviewed (Zbinden, 1963, Ariens, 1976; Gupta, 1985).

The toxicological responses depend upon the individual which is exposed to the chemical which are called as the Host factors. These include size, age, species, strain, sex, food and feeding habits. All these lead to changes in the internal environment; which is influenced by the exposure to drug, route of drug entry, repeatative exposure, rhythmic exposure. All these studies are classified under Idiosyncratic toxicity (Gupta, 1985) which deals with the toxicity peculiar to an individual or toxicity which appears in few persons/individuals but not in general population. There are four types of Idiosyncratic toxicity. 1. Dose-dependent reaction, 2. Toxicogenetic reactions, 3. Immunotoxicity,

4. Unexplained dose independent idio-syncreatic reaction.

The potential reaction expressed by the individual may be decreased (tolerance) or increased (dose-dependent response or in some cases hypersusceptibility). Among the other factors that are concerned with individual responses include the attitude of the animal (more conspicuous in human), aggression or fear expressed by the individual, presence of animals of the same species, male and female presence is also influential. In the individual animal effective dose may be different from the actual administered dose. It may vary organ to organ, cell to cell and their physiological status.

Some influences are also due to the properties of chemicals and route of the administration viz. solubility of chemical in water, vegetable oils, the suspending medium in which it is internalized, the chemical stability of the agent/drug, particle size, rates of disintegration, dissolution of formulations of chemicals, crystalline nature and the grittiness of inert substances given in bulk amounts. Routes and rate of administration influence the absorption rate which is reflected in lethal dose concentration/duration. If more than one chemicals are administered/internalized may show synergism or antagonism type of reactions.

As the properties of substance influence their effects on individual, so also the physical environment under which

the drug is administered/internalized viz. light, rhythmic cycles of day and night, temperature, relative humidity are known to influence the values of LD_{50} (Gupta, 1985). Even seasonal variations also influence the effects. The dose response curves with the graded doses or quantal response studied to specify the toxicity.

The extent of toxicity testing that is usually utilized is proposed by WHO (1978) and is briefed in Table 1.

Table 1 : The extent of toxicological Evaluation.

No.	Stages of technological development	Stages of toxicological evaluation	Toxicological Studies
1.	The critical concept and process flow diagram	Preliminary toxicological assessment	. Analysis of literature data on toxicity and hazards of raw materials, reagents, catalysers, semiproducts and additives. . Assessment of toxicological parameters on the basis of metabolic analogies, persistence the relationship between chemical structure and physical properties and biological activity. . Interpolation and Extrapolation in homologous series.

2.	Laboratory development of the technological process.	Acute toxicity	. Acute and subacute experiments in animals. . Toxicological evaluation of technological unit processes.
3.	Pilot plant stage	Subacute toxicity	. Subacute toxicity experiments on animals. - Studies of delayed effects. Medical examination of workers.
		Detailed toxicological evaluation.	. Chronic toxicity studies and when indicated, effects on reproduction, carcinogenicity, mutagenicity. . Formulation of medical and industrial hygiene requirements for full scale production.
4.	Design of industrial scale process	Additional studies	. Studies on the mechanism of action, early and differential diagnosis. Experimental therapy.
5.	Production and use of chemicals	Field studies	. Assessment of working and environmental conditions and health status of workers and general population. . Epidemiological studies. . Clinical evaluation of experimental, prophylactic, diagnostic and therapeutic methods.

. Adjustment and correction of requirements for health and environmental protection.

Being the student of Zoology we are always interested in animal toxicological studies which can be conducted by toxicological tests suggested by Gupta (1985). These are summerized in Table 2.

Table 2 :

General outline of Animal toxicological tests.

1. Acute toxicity (single)
   - A. LD_{50} determination (1-2 weeks observation)
     - a - Two species - one non-rodent
     - b - Two routes of administration
   - B. Irritation studies
     - a - Dermal (rabbit)
     - b - Eye irritation (rabbit)
2. Subacute toxicity
   - A - Duration 90 days
   - B - Two species (Usually rats and dogs).
   - C - Three dose levels
   - D - Route of administration according to intended route of use (exposure)
3. Chronic toxicity
   - A - Duration two years
   - B - Species (preferably two one non-rodent)

C - Three dose levels.

D - Route of administration according to intended route of use (exposure).

4. Special tests

A - Metabolism

B - Neurotoxicity (for organophosphorous or carbamates)

C - Reproduction toxicity and teratogenicity (at least one species).

D - Carcinogenicity

E - Mutagenicity

Usually the chemicals are primarily tested for toxicity rating. The basic rating doses that are recognized in human as worked out and basically used are suggested by Gleason et al. (1969) and given in Table 3.

Table 3 : Toxicity Rating Chart (Gleason et al., 1969)

Toxicity Rating or Class	Probable lethal dose (human)	
	mg/kg	for 70 Kg man
. Super toxic	Less than 5	A few drops
. Extremely toxic	5 - 50	A pinch to two table-spoonful.
. Moderately toxic	500 - 5000	1 ounce to a pint (1 pound)
. Slightly toxic	5000 - 15000	1 pint to 1 Quart (2 pounds)
. Practically non-toxic	Above 150,000	More than (2 pounds)

In statistical concept of toxicology LD_{50} is margin of safety Therapeutic margin is also studied using effective doses.

All these are primary approaches in toxicological studies to any chemical.

But approach to any one chemical to be studied by any individual worker or the group of the workers is decided by the aim of the study, need of the time, need of the work, proposed use of chemical, use of chemical in practice or any other specified need.

Considering all the above things, the limits of laboratories and availability of finance are the deciding factors when one decides to study the toxicological studies of any chemical.

To the students of Zoology and Cell-Biology the animal tests of the compounds are more meaningful for its toxicological evaluation and more analytical approach to its therapeutical use. Therefore to test the chemical/drug for its all types of effects in laboratory animal is a very natural approach.

In present project the approach is to study the effects of chemical/drug in laboratory animal. Basically the alterations occurring in animals are the results of the metabolisms those are altered by the presence of the foreign compound.

Such type of altered metabolisms related with the administrations of chemicals which are distantly related to the animal viz. different kind of pesticides, drugs, chemicals have been given a generalized approach in toxicological studies. In this approach the chemical in use is usually referred as the Xenobiotics.

On the entry of xenobiotic in any animal body, the body responses are metabolic as they are for any other substance which is normally involved in the routine physiological processes.

Absorption of Xenobiotics

The body metabolism basically includes the Absorption, Distribution and Excretion of Xenobiotics. The general aspect of these studies is reviewed by Parke (1968), Hathway et al. (1970, 1972) and Gupta (1985).

The over all fate of any Xenobiotic in body can be Schematically represented as in Table 4. This table indicates the importance of xenobiotics passage through membranes. Hathway et al. (1970, 1972) have briefed this transfer across biological membranes as given in Table 5.

Table 4 : Fate of xenobiotics in body

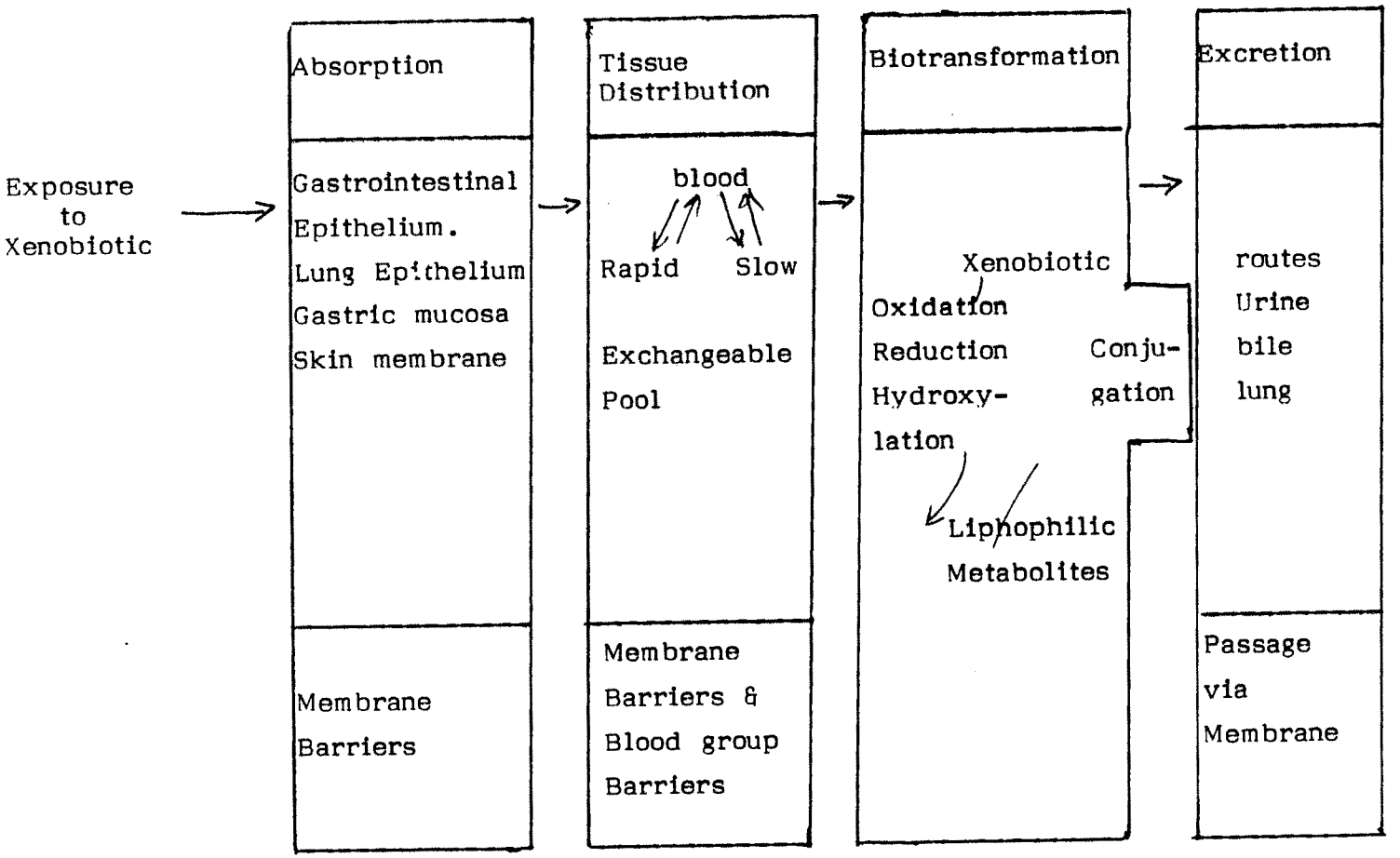

Table 5 : Transfer of xenobiotics across the membrane.

Transfer process	Mechanism	Sustrate Specificity
. Passive diffusion	Diffusion through lipoidal membrane down a concentration gradient	None Most foreign compounds
. Filtration	Diffusion through aqueous pores in the membrane down a concentration gradient	. Hydrophilic molecules . Ions of low molecular weight e.g. water, Urea.
. Facilitated diffusion	Carrier transport through membrane down a concentration gradient. Saturated by excess substrate	Narrow mainly for molecules concerned with process of intermediary metabolism e.g. sugars and amino acids.
. Active transport	Carrier transport through membrane down a concentration gradient requires metabolic energy saturated by excess substrate	
. Pinocytosis	Invaginations of the membrane absorbs extracellular material.	Uncertain

Absorption of Xenobiotics is influenced by the route of administration/entry of the chemical, characters of chemical and formulation components along with the chemical. Peritoneal entry or entry in direct blood increases the chances of absorption than entry through skin (dermal), lungs, muscles or gastro-intestinal tract.

Distribution of Xenobiotics :

After absorption into the blood stream the chemicals penetrate into the various fluid compartments viz. plasma, interstitial fluid, transcellular fluid and cellular fluids. It seems that the nonionized lipid/soluble fractions penetrate most readily. The rate of penetration of a chemical is not really a factor limiting chemical activity, because equilibrium occurs in most tissues quite rapidly. But longer time is required to achieve equilibrium in bone and adipose tissues.

In addition, some chemicals may accumulate in various areas as a result of binding or due to their affinity for fat.The accumulation may be at the site of action of chemical or may be in some other location.

In some cases the accumulation may be at the site of action of chemical or may be in some other location. In some cases, the accumulation may serve as a storage depot for the chemical. But usually it remains in equilibrium with the free chemical and maintains the effective concentration of chemical at the site of action.

Tissue permeability barriers are there to attain the concentration at the site of action viz. Blood-Brain barrier, placental barriers, Blood-testis barrier. The membranes also influence the transfer with their true transport characters.

Factors affecting distribution and tissue retention

The factors are blood flow, chemical interaction, age, sex differences and genetic factors etc.

Included among the above factors the most important one is binding to plasma proteins. This binding is important since the bound compound is temporarily localized, can seldom cross biological membranes or diffuse into tissues and its actions are reduced or abolished. Of the plasma proteins albumin has the capacity to bind many compounds but other plasma proteins such as globulins are also involved. This binding is affected by several factors such as species variation, changes in pH and hormonal influence.

Some of the compounds may be stored in brain e.g. psychotropic drugs. Some of them may be stored in erythrocytes e.g. heavy metals. Many tissues/organs/cells may store different type of chemicals depending upon their physical characters and influence of other factors discussed above may be included through which they may be redistributed.

Excretion of Xenobiotics

Some quantity of the Xenobiotics may be in the modified form or original form is excreted through renal cycles. Bilary excretion is another way of excretion. Through gastrointestinal tract before the drugs find their way in liver, significant amount reaches the general systematic circulation. The chemical may be excreted through bile which again undergoes through absorption-excretion cycles. The biliary excretion system and renal excretory system are interdependent and mostly either of it dominant in each type of chemical entering the body. In case of gastro-intestinal tract the compound may be excreted through bile or the gastrointestinal tract itself. Some gaseous compounds may be excreted through expired air. Some compounds may be excreted through sweat (Sulphonamides), Saliva (acidic drugs) milk (DDT) and genital secretions most particularly vaginal secretions.

The body also responds involving defence system of the body.

Under this system any new chemical - Xenobiotics is first scanned by the phagocytes in the region of entry. The macrophages may infiltrate to phagocytose the xenobiotic (may be in original, modified or any other altered form on entry). The chemical is presented to lymphocytes to set the immunological response involving circulating B and T lymphocytes.

If the entry of the xenobiotics is through blood the circulating immunoglobulin molecules bind to the compound to neutralize the compound as well as to attract other cellular events occurring through different types of immunological reactions (Gupta, 1985).

It is well established that the majority of drugs/toxics are cleared metabolically by liver at organ level. But almost all types of cells those are exposed to xenobiotics in one or the other form are influenced by xenobiotics and may take part in modifying the xenobiotics so as to remove it from the body or to make it harmless to the different parts of the body at cellular, tissue and organ level.

At the levels of the cell the entry may be by diffusion if the xenobiotic is lipophilic and if it is soluble aqueous form the entry may be through endocytosis (may be of any kind) to reach the lysosomes. As the result of the actions of lysosomal enzymes in lysosomal compartment the xenobiotics are degraded to convert them into the altered simple compounds. This is not only true in case of hepatic cells but also in the case of different cells that encounter with the xenobiotics.

As it is already mentioned that the compounds which are liphophilic have direct access in to the cell through the membrane. Thus the compounds belonging to this category; some of the compounds which are not susceptible to lysosomal degradation; or the compounds formed as the result of lysosomal degradation or in their original form are handled by the SER system

of the cell popularly known as the smooth microsomal compartment/fraction.

The handling of these xenobiotics by microsomes are the metabolic efforts to transfer or treat the compound in a way which lead the elimination/excretion of the compound as any other metabolite or compound that enters the body in normal, natural conditions.

These metabolic transformations are called as the biotransformations of xenobiotics. In all the different types of xenobiotics these transformations are not necessarily harmless but may lead to damage to the cells, organs or biotransformed compounds or may induce other pathological alterations or injuries in cells/organs/animals, viz., Carcinogenesis or altered metabolisms.

* Biotransformation of Xenobiotics

Xenobiotics undergo a variety of biochemical reactions depending upon their route of entry, physical properties particularly solubility or insolubility in lipids and their chemical activity towards ligands or binding molecules in the body.

Biotransformation word embraces both degradative or catabolic reactions mediating the detoxification of the xenobiotic and its elimination from the body as well as metabolic transfor-

mation of the parent compounds into highly toxic metabolites. This pattern of biotransformation differs from organism to organism. Parke, 1968, Hathway, 1970, 1972; Eto, 1974; Jacoby, 1980; Conney 1982; Krishnamurthy, 1985; Barry & Feely, 1990; Waxman and Azaroff(1992) reviewed these transformations in case of many types of foreign compounds.

During Biotransformation most lipid soluble compounds are changed into polar products to facilitate their secretion into bile or hydrophilic compounds excretion through urine. These reactions control the biological activity of a foreign compound; its duration of action and accompanying toxic symptoms.

* Cellular involvement in Biotransformation

Microsomes are studied in vivo and in vitro and is shown to involve in the transformation of xenobiotics. The list of the some of the transformed and original compounds is given in Table 6 reproduced from Krishnamurthy,(1985).

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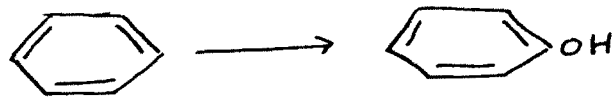
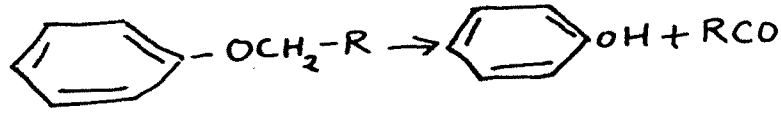
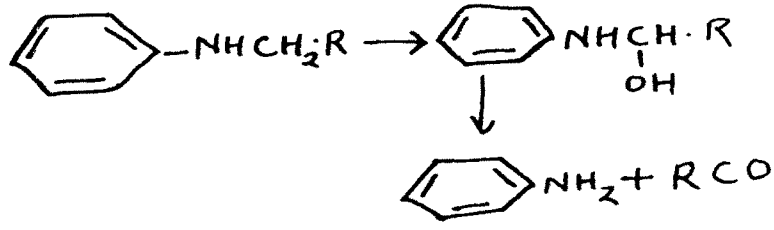
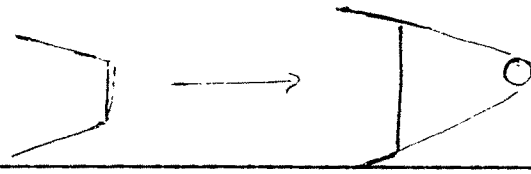
Table 6 :

No.	Original Compound	Active metabolite on transformation
1.	Prontosil rubrum	Sulfanilamide
2.	Acetanilid	P-hydroxy acetanilid
3.	Imipramine	Desipramine
4.	Antitriptyline	Nortriptyline
5.	Phenyl butazone	P-hydroxy phenyl butazone
6.	Bidrin	Azoclirin
7.	N-N-diallyl malenine	N-oxide of diallyl malenine
8.	3,5-Dimethyl pyrazole	3-Carboxylic Acid - methyl pyrazole
9.	Muracil D	4-hydroxy methyl muracil
10.	Parathion	Paraoxon
11.	Malathion	Malaoxon
12.	Cyclamate	Cyclohexylamine
13.	Diazepam	N-demethyl diazepam and Oxazepam.
14.	Dimethiote	P -- derivation
15.	Ethanol	Methanol
16.	Inorganic mercury	Methyl mercury

Table 6 gives the list of the transformations that occur in the Xenobiotics. They have been identified in Table 7.

Table 7 :

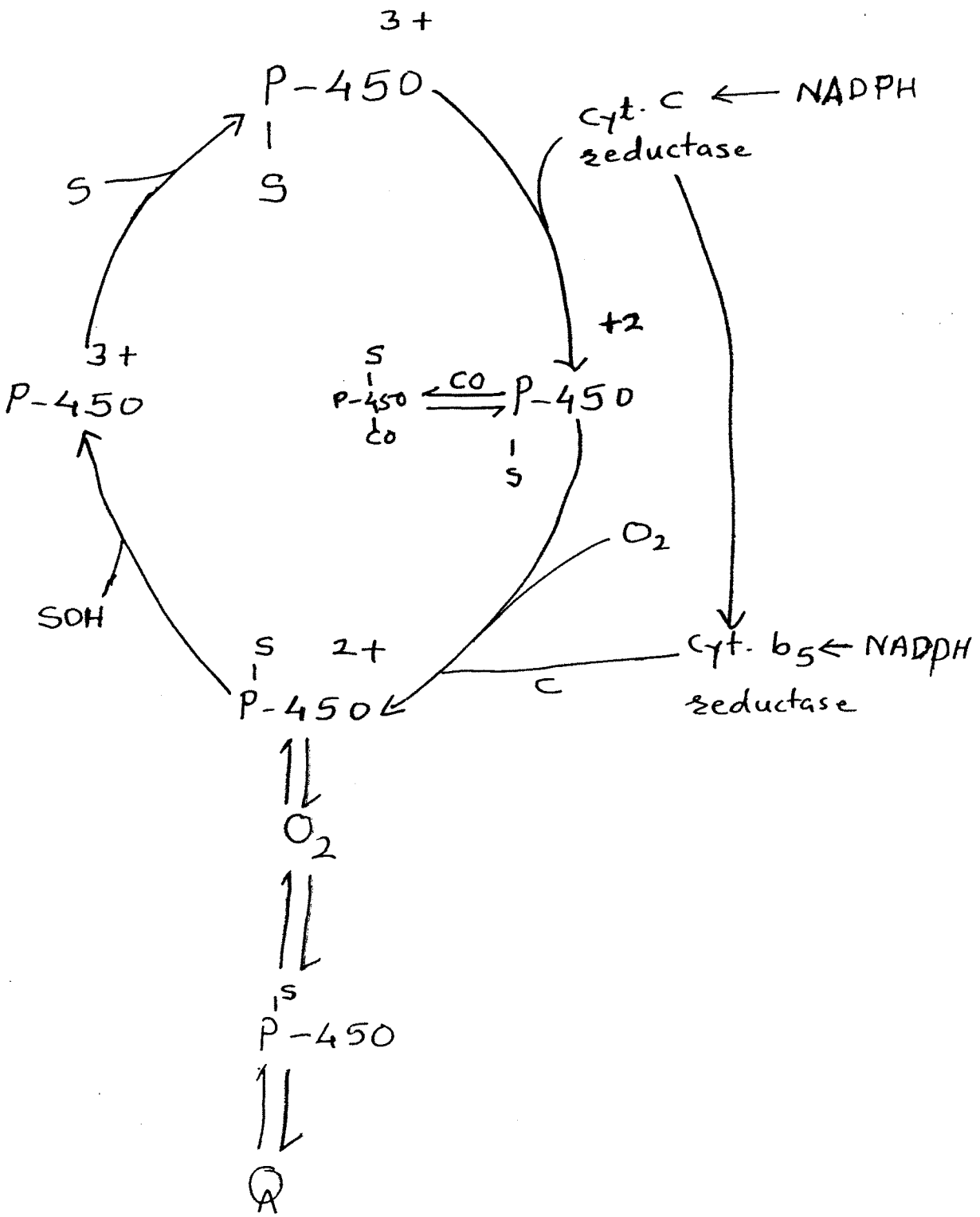
No.	Name	Structural Change
1.	Aromatic Hydroxylation	
2.	Aliphatic Hydroxylation	$R.CH_3 \longrightarrow R\text{-}CH_2OH$
3.	O-Dealkylation	
4.	N-Oxide formation	$R\text{-}N\text{-}(CH_3)_2 \longrightarrow RN\text{-}(CH_3)_2$
5.	N-Dealkylation	
6.	D-Sulfaration	$\longrightarrow P = S - \longrightarrow P = O \longrightarrow C = S -$  $C = O$
7.	Sulfide Oxidation	$R\text{-}S\text{-}R \longrightarrow R - SO - R \longrightarrow R - SO_2 - R$
8.	DeEsterification	$(RO)_2 \overset{S}{\underset{\|}{P}} - O\ Ar \longrightarrow (RO)_2\ PSon + HoAr$
9	Epoxidation	

The enzymes involved in these reactions are present in microsomes (Nebert and Negishi 1982; Nebert et al.,1983). These enzymes are generally referred to as mixed function oxygenases (MFO) and require a reducing agent (which is known to be NADPH), cytochrome P−450 and atmospheric oxygen. The reaction can be shown as

$$RH + NADPH + H + O_2 \longrightarrow ROH + NADP + H_2O.$$

This is well known microsomal electron transport system. The reviews indicate that mixed function oxygenases of microsomes are present in vertebrate liver, Malphighian tubules and the digestive tract of insects. Mixed function oxygenases activities are more in mammals as compared to birds and fishes. They have been studied in rat and are known to show high activities of enzymes in male than in female. The microsomal enzyme system exhibits poor substrate specificity and requires only high lipophilicity in the molecule to be biotransformed. Cytochrome P-450 which is obligatory for the enzyme activities is inactivated by carbon monoxide and also inactivates the enzymes. Oxidized cytochrome P-450 is reduced by NADPH providing the reducing potential and is catalyzed by the flavo-proteins cytochrome - C - reductase. The cytochrome b_5 electron transport depending upon NADPH is somehow inter connected with P-450 Pathway which is depicted in Diagram 1.

Diagram - 1


3+
P-450
S
S
Cyt. c ← NADPH
reductase
+2
S
P-450 ← CO ⇌ P-450
CO
S
3+
P-450
SOH
O2
S 2+
P-450
C
Cyt. b5 ← NADPH
reductase
O2
S
P-450
Q

Although mixed function oxygenases are non specific to xenobiotics to be qualified properties including polarity, electron nature and stereo or spatial configuration. Substrates do compete for sites on mixed function oxygenases (Krishnamurthy, 1985; Waxman and Azaroff, 1992). The multiplicity of enzymes is known which is evidenced by the distinction between cytochrome P-450 dependent toxygenetion and flavoprotein dependent N-oxidation. Isolation of cytochrome P-450 also decides the multiplicity. The electron transport is studied well; the components of which are NADPH-Cytochrome-C-reductase, cytochrome P-450, a heat soluble factor and lipids.

The binding of xenobiotics to the microsomal membrane is a complex process involving both binding sites and catalytic sites on P-450 and stimulatory sites on NADPH cytochrome-C-reductase. The rate limiting step in monoxygenation is the rate of reduction of the oxidized cytochrome P-450 substrate complex. Two electron equivalents are required for the coupled reduction of oxygen and the oxidation of the substrates. Cytochrome P-450 is one electron carriers. The flavoprotein NADPH-Cytochrome P-450 reductase mediates this reduction. The synergestic effect of NADPH on NADPH catalyzed xenobiotic oxidation and the alterations in the steady state level of cytochrome b_5 during NADPH initiated non-oxygenation have led to the assumption that reduced cytochrome b_5 interacts with oxygenated cytochrome P-450. Mixed function oxygenases activity is inducible. Many

drugs, food additives and chlorinated hydrocarbons can induce the activity. Three commonly used inducers are 3-methylchlolanthrene, phenobarbital and DDT. The inductions are coupled with significant morphological changes in liver and the inductions are considered as the adaptive responses of mammals to deal with increasing entry of chemicals in body; but long term effects are not studied.

* Extra hepatic monooxygenases

The increasing awareness of exposure to xenobiotics and better understanding of biochemistry of other organs viz. lungs, kidneys, placentae etc. have stimulated these studies in various organs. Kidney cortex microsomes catalyze hydroxylations that are normally carried out by liver.

The integrity of microsomes is essential component in enzyme activities of mixed function oxygenases. Therefore, membranes of microsomes is essential entity. The drugs that lead the phospholipid disintegrity of microsomal membranes, also show rapid turn over in lipids. In this condition the membranes are disintegrated by lipid peroxidation (LPO). The induction of LPO or increase in LPO by the metabolic activities induced by drugs concurrently destruct the enzyme activities of microsomes. NADPH-dependent LPO destructs cytochrome P-450 activity also. This increase in LPO and decrease in MFO are strongly exhibiting the microsomal functioning and membrane integrity.

* Lipid peroxidation :

Lipid peroxidation is well studied in many hepatotoxic drugs (Recknagel and Glende, 1978; Mehendale, 1985). Lipid peroxidation occurs as follows.

Some toxic event/s initiates LPO and organic free radicals are generated by the initiation process which serves to propogate the reaction.

The concept of LPO damage of membranes was advanced by Slater (1978) as the principle mechanism in CCl_4 induced liver injury which was further studied and accepted on the basis of ample amount of experimental evidence (Recknagel and Glende, 1978; Mehendale, 1979, 1985); which showed coupled destruction of cytochrome P-450 and glucose-6-phosphatase activity in vivo and in vitro preparation, representative microsomal monooxygenase activities, protein synthesis, formation and secretion of low density lipoproteins. This indicated the induced injury to endoplasmic reticulum (ER) altering its function.

In addition to above biotransformations, non-oxygenative oxidations in biotransformations are also studied. These studies include the reductions which are catalysed by liver alcohol dehydrogenase and NADH/NADPH as electron donor. The hydrolytic activities are also studied. Mammalian esterases are widely distributed and exhibit divergent properties of clearing and are

directly involved in hydrolytic reactions. Biotransformations may occur as combinations of xenobiotics with readily available endogenous substrates (e.g. glucuronic acid, sulphate, acetyl, methyl, glycine) to form conjugates. These make more polar and less lipid soluble conjugates; which can be eliminated through the kidney. The transfer of the endogenous substrates mediated through the coenzymes (UDP, COA, glutathione) to which they are bound by specific enzymes. There are some miscellaneous biotransformations which include ring scission, cyclization, dehalogenation etc.

The above review indicates the biotransformations of xenobiotics. In recent years there are other alterations are also worked out which play an important role in the protection of cells (liver, kidney and other organs). Some of the substances present in tissues/cells play the role as endogenous protective ligands.

Among these important is glutathione reduced. Decreased levels of glutathione in case of irreversibly binding compounds is reported by many workers [Mehendale, 1985). In this review role of glutathione is well discussed.

Glutathione is known to undergo conjugation reactions with electrophilic reactants either via enzymatically catalysed reactions by a family of glutathione transferases or via non-enzymatic reactions. Increased toxicities associated with depleted

glutathione levels was explained on the basis of diversion of biotransformation pathway via the increased accumulation of active intermediates which interacted with tissue micromolecules to cause toxicity.

In the condition of biotransformation of xenobiotics reduced glutathione levels may be increased by the administration of glutathione precursors such as cysteine or methionine to provide protection against bromobenzene and acetaminophen induced hepatic necrosis. Experimental manipulation of glutathione levels has become a standard experimental protocol in attempts to explain the toxicity of a variety of toxic chemicals.

It is important to realize that although depletion of protective agents such as glutathione can result in a diversion of the biotransformation pathway to a more toxic route, quantitatively such alteration only becomes important when depletion is more severe and when it falls below a certain critical level.

Similarly it is possible that the availability of sulfate and glucuronic acid two important ligands in the detoxication of a variety of foreign chemicals may be important in the expression of toxicity.

There are other types of ligands also viz. Vitamin E and ascorbic acid which can protect against bioactivation mediated cellular toxicities such as the free-radical mechanisms

(Gillette, 1978; Recknagel and Glende, 1978; Slater, 1978; Mehendale, 1985).

This foregoing review stresses the involvement of smooth microsomes in the handling of xenobiotics as well as the involvement of the organs (liver and kidney) in their clearance and therefore, it is but natural for research worker to concentrate on these two organs.

The above introduction is explaining the related background of the subject of the project which is dealt in the present M.Phil. thesis.

Selection of liquid paraffin to study its effects

1. Basically liquid paraffin (Laboratory grade LP) was used earlier as the vehicle for CCl_4 to induce the hepatic injury in albino rats (Devarshi et al., 1986). In this experimental work only LP treated rats (daily dose of 1 ml/kg body weight for 11 days) were studied as vehicle control. The histological observations of the liver of these rats showed that the staining properties of nuclei were altered so also the morphology of the liver coupled with the alterations in the activity of hepatic lipases showing 4 fold-increase in alkaline lipolytic activities (specific activities) coupled with 1.6 fold increase in Lipoprotein lipase activities (specific activities) but without any significant alterations in specific activities of Acid lipase. All these results

indicate that the significant alterations are taking place in liver metabolisms as the LP is given.

With the above observations it was felt at that time that there is a serious need of systematic approach towards the studies of effects of liquid paraffin on liver.

2. The above observations also compelled us to search for the use of liquid paraffin by human.

Following data reveals the common use of liquid paraffin in our daily life.

Use of liquid paraffin

Liquid paraffin is routinely used as the purgative and most commonly used by physicians using modern therapies for the treatment of constipation. This is taken orally. In addition Liquid paraffin is obligatory component of varied types of cosmetics which are in the market. The research on liquid paraffin in recent years is mainly dealing with the demanding properties by market and making them into patents as per the rules of states by market.(Szentmiklosi et al., 1984; Del Pozo, 1985; Leonidov et al. 1985). It is very commonly used in cosmetic creams and emulsions (in 18 V Kazmhisa and Yasunaga,1976), in Ointments for burns (1.15 V Gitlan et al., 1983), in foundations eye shadows and talc cakes (Hisashi, et al., 1984; Hideyuki and Yu, 1984) and in medicinal bath oils (Schmersahal 1985).

Overall role of synthetic and natural waxes as a new concept in cosmetics is reviewed by Carvalho and Lucia (1986). Liquid paraffin is also used in cosmetics containing immobilized enzymes (Calvo, 1985).

Thus it is commonly used for external application on skin as well as by oral uptake. This increases the need to study its toxic effects systematically so that the caution can be warned in use.

Effects of Liquid paraffin on organs and cells

The systematic effects of liquid paraffin on certain organs are scarcely available. There are few references which deal with the effects of liquid paraffin on some organs. The following paragraph includes the details of such references.

Devarshi et al. (1986) showed the alterations in liver and kidney cells on administration of 1 ml/kg body wt. daily dose of liquid paraffin for 11 days, SC. The hepatic cells showed foggy necrosis with 90 % cells showing no hematoxylene staining and remaining 10% with faint hematoxylene staining in Eosine-hematoxylin preparations. The renal cells also showed foggy necrosis. In addition to these altered staining properties in liver cells and morphological alterations in liver and kidney cells the lipoprotein lipase and alkaline lipase activities in liver cells showed 1.5 folds and 4 folds increase respectively

with insignificant increase in acid lipase activities. Concurrently the specific activities of acid lipase, alkaline lipase and lipoprotein lipase were decreased by 3.5 folds, 1.01 folds and 4 folds respectively in kidney and increased by 13 folds, 3.8 folds and 1.8 folds respectively in adipose tissue. Effect of chronic administration of Liquid paraffin (n-alkane) is also studied in the proliferative activity of lymphoid tissue and corneal epithelium of rats (Denisov, 1986). The proliferative activities increased in thymus which were especially pronounced by the third month. Thereafter the activity returned to the original level. The immunostimulating activity of the LP was also noted by an increased splenic weight (third month). No dose stimulation relationship was noted in the lymphoid tissues (thymus and spleen). Mitotic processes of the corneal epithelium was not substantially affected by the n-alkanes during the period of high functional activity of the lymphoid tissue. Thus n-alkanes showed specific stimulating effect on the lymphoid tissue. The inhibition of proliferation activity (6 month) is attributed to cytopathogenic effect of liquid paraffin. Eugene (1985) has tested many preparations with the skin as the barrier for the penetration of creams, oils and talcs.

Except the above described references there are few references available on the compounds which are from the category to which Liquid paraffin (N-alkane) belongs.

Viau et al. (1986) studied nepherotoxic effects of iso-

paraffinic solvents in rat. Prolonged inhalation of an isoparaffinic 6:5 mg/L consisting mainly of C_{10-12} aliphatic by hydrocarbons resulted in functional and morphological renal changes in male rats but not in female or castrated rats. Functionally, the increased excretion of lactate dehydrogenase in the absence of an increased β-N-acetyl-D-glucosaminidase excretion together with a decreased urinary concentrating ability during water deprivation and antinatriuretic response when no intake is reduced suggest a distal tubular alteration. β_2-microglobulin excretion is unchanged indicating good proximal tubular cell function. The increased excretion of albumin and slightly lower glomerular filtration rate suggest a moderate glomerular impairment. Light microscopy shows prominent hyaline droplet accumulation in proximal tubular cells and few scattered foci of regenerative epithelia in both proximal and distal cells of the deep cortex. The urinary clearance of the major male rat urinary protein α_2-globulin is similar in control and exposed rats; but the latter have a 10-fold greater renal accumulation of this protein whereas the hepatic levels are identical in both groups. Thus C_{10-12} aliphatic hydrocarbons inhalation causes moderate reversible tubular and glomerular changes in the male rat kidney.

Effects of some of the petroleum hydrocarbons in male albino rats have also been studied (Short et al., 1987) to indicate the intracytoplasmic protein droplet accumulation within renal tubular epithelial cells in the male albino rats exposed

to unleaded gasoline (0.2 to 50 mg/kg) 2,2,4 trimethyl pentane (2 to 2000 ppm/kg) in addition to the cell necrosis and regeneration of nephron (in 3 week exposure). Protein droplets are α-2μ -globulin and only in male. P_2 section of nephron showed proliferation which is related to the increased cell turn over. Short et al. (1987) have suggested that the protein accumulation may be essential factor in the development of renal cancer in male rats exposed to unleaden gasoline or other volatile hydrocarbons.

The above paragraphs deal with the toxic effects of Liquid Paraffin as well as related hydrocarbons on liver, kidney and immunological system also.

These observations indicate the need of the well oriented systematic work on Liquid Paraffin as well as related hydrocarbons. Since Liquid Paraffin is in frequent use we have studied effects of Liquid Paraffin in male albino rats in present project. The effects of Liquid Paraffin on liver and kidney studied by histological observations and metabolic stress are evaluated using some of the reactions that are related to bitransformation of Liquid Paraffin viz. glucose-6-phosphatase, Lipid peroxidation, formaldehyde, cytochrome P-450, and b_5, RNA, total proteins in microsomal fraction and glutathion in soluble fraction.

Selection of male albino rat for testing toxic effects of liquid paraffin.

Many laboratory animals are preferred by different research laboratories which include toxicological laboratories also that conduct the experimental work including albino rats.

Albino rats are small rodents with short oestrous cycle, pregnancy period and grow to maturity by three months. These short intervals are easy to utilize them for breeding. Our Department has well equipped animal house where albino rats are bred and maintained providing feed (Lipton India Ltd) and water ad libitum. Thus one can obtain needed animals for experimental work as per the convenience.

Male albino rats are preferred since this project is the beginning of the large systematic study of effects of liquid paraffin in albino rats. There are many metabolisms which are essentially influenced by male and female hormones. In female cyclic alterations in hormones (oestrous cycle) have to be considered when prolong treatment of particular xenobiotic decided to be used. In case of effects of liquid paraffin this type of project can be considered further once one knows the basic preliminary effects on the animals and therefore, such type of experimental work is not included here. The present project restricts to the effects of Liquid Paraffin in male albino rats.

Selection of route of administration for experimental studies in present project.

As per the uses of Liquid Paraffin that are already reviewed it is usually taken orally or applied on skin. To test the effects on skin again a different approach is needed in experimental work; therefore, SC administration of Liquid Paraffin is preferred for present studies.

Selection of dose and duration

The dose preferred is based on the use of liquid paraffin as a vehicle for dilutions of varied solvents or to increase the retention capacity. The details are discussed in Chapter II.

As the experimental work is initiated by the observations of alterations in staining properties of nuclei; it was taken as the criteria for prolonged treatment schedule. The duration of treatment is decided by the histological architecture of liver where total staining ability of nuclei in hematoxylin eosine preparation is totally abolished and almost all cells show totally foggy appearance. This condition deciding duration is considered as the terminal condition in experimental schedule.

To study the transitory alterations through which liver passes to abolish the hematoxylin staining.

The terminal condition showing period is divided into 5 intervals of equal time schedule; the details of which are given in Chapter II.

Selection of organs for experimental studies in present project.

The literature that is reviewed in foregoing part of Introduction critically indicates the metabolic route of biotransformation of any xenobiotic/drug/chemical is mainly liver. This makes the study of the liver a most essential part. As it is given already the excretion of xenobiotics/drugs/chemicals are mainly through two routes viz. bile excretion through faces and excretion through kidney.

Thus filtration and reabsorption of products of biotransformation of Xenobiotics/drugs/chemicals influence the kidney and metabolisms in kidney and therefore, it is essential to study the effects of liquid paraffin on kidney along with liver.

Selection of parameters for the studies.

The early part of the Introduction reveals the morphology of the organs may be changed during the metabolic transformation of xenobiotics/drugs/chemicals.

The brief review on effects of Liquid Paraffin and allied hydrocarbons indicate altered morphology of lymphatic organs, liver and kidney (Devarshi, et al., 1986; Denisov, 1986; Short et al. 1987). Therefore, it was decided to study the histological

alterations in liver and kidney. Histological picture is a mirror of metabolic status and can reflect the recovery or pathologic alterations.

The brief review on biotransformation of xenobiotics indicated that primarily the xenobiotic is circulated and/or absorbed by many cells. As it is already discussed the entry within the cell may be through endocytosis to enter in lysosomal system which handles the foreign material in cell or through diffusion as the lipophilic substances enter the cell which may be handled by the smooth microsomal system involving microsomal oxidations.

To deal with both smooth microsomal system and lysosomal system is a large extensive work and therefore, in the present project parameters concerning the microsomal system are studied.

Selection of the parameters from microsomal system.

1. Lipid peroxidation - Some of the drugs on their metabolism produce free radicals which generate sequence of free radicals acting on the membranes within the cell and damage foremost the microsomal membranes damaging the drug detoxication system and therefore, it is taken as the indicator of the microsomal integrity (Mehendale, 1985).
2. Cytochromes P-450 and b_5 - Microsomal oxidation and reduction reactions are mainly NADPH dependent and

cytochrome P-450 and b_5 dependent (discussed in Introduction). The alterations which are directly occurring in these cytochromes give directly the efficiency of the microsomal system and hence drug detoxing system (Krishnamurthy, 1985).

3. Glucose-6-Phosphatase - It is present only in microsomes and is used as the marking enzyme in cell fractionation studies (Appleman, 1955).

4. 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 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 MFO system (Cooper et al., 1965). This is also associated with the methanol oxidation (Hildebrandt et al., 1975).

5. RNA Content - This is used to study the retention system if any, developed by the cell. Additionally it provides the normal functioning of cell and particularly RER (Trump et al., 1965 .

Thus by studying 1 through 5 parameters most of the status of drug toxicating system can be realized.

6. Glutathione - It acts as endogenous ligand glutathione is

known to undergo conjugation reactions with electrophilic reactants either via enzymatically catalysed reactions by a family of glutathione transferases or via nonenzymatic reactions. Glutathione levels are used to explain the toxicity of a variety of toxic chemicals (Mehendale, 1985).

Thus the project of effects of Liquid Paraffin on liver and kidney of albino rat is carried out with the specification of doses, durations and using parameters those are involved in drug detoxication system to decide the status of toxicity induced by liquid paraffin.

The Dissertation is presented in six chapters.

Chapter I - Introduction

In introduction the subject is introduced in all rational respects which will help to understand the details of the subject. All these details are reasoned out viz. selection of problem animals, parameters.

Chapter II - Material and Methods

The methods used to maintain the animals, experimental schedule and details of methods of quantitation of parameters are included under this category.

Chapter III - Histology

Alterations in histology of liver and kidney is described in detail under this chapter and the results are discussed.

Chapter IV - Microsomal fraction

Using standard methods of cell fractionation microsomal fraction is isolated. It is used to study various parameters which are listed earlier viz. glucose-6-phosphatase, Lipid peroxidation, formaldehyde, Cytochrome P-450, Cytochrome b_5, RNA.

Chapter V - Glutathione

Since glutathione (reduced) is studied in cytosolic fraction; the alterations occurring in its content are dealt separately.

Chapter VI - Discussion

On the basis of the alterations that occurred in various parameters that are discussed to decide upon the status of the toxicity of liquid paraffin.

Chapter VII - Concluding Remarks

The results of present project are evaluated and future line of research is predicted.

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