4. BIOTRANSFORMATION OF XENOBIOTICS

In nature, the organisms are often exposed to a wide variety of chemicals, which are foreign to their body. Of these, the lipophilic substances easily penetrate the lipoprotein bilayers of their cell membranes and finally reach the target cells. If such chemicals are continuously or intermittently exposed to organisms and are gradually absorbed by the organisms but not eliminated from their body, they tend to accumulate. Thus, they may produce lethal effects. However, quite often during the course of their transport, they pass through certain tissues/organs (such as, liver) having some activity against those xenobiotics. Consequently, the lipophilic chemicals are biocatalytically converted into their hydrophilic forms that are easily excreted from the body of organisms. *The biologically catalyzed conversion of one form of xenobiotic compound into another form may be simply termed as* ***biotransformation***.

Almost all biochemical reactions in the biological system take place under the influence of certain enzymes and the biological conversion of one compound into another is termed as metabolism. But, the term biotransformation is used preferably to denote the biological conversion of xenobiotics and metabolism is referred to the biological conversion of useful and endogenous substances, such as nutrients or body constituents, *i.e.* carbohydrates, fats (lipids), proteins, nucleic-acids, *etc*.

The biotransformation of one xenobiotic compound into another often involves several changes in the parent compound. These changes may occur through a series of sequential reactions leading to one or more products. The new compound has distinct physical and chemical properties, hence different pharmacological and toxicological properties. Thus, biotransformation may lead to following alterations in the toxicological properties of the xenobiotics:

(*i*)Conversion of an active chemical into another inactive chemical;

(*ii*) Conversion of an active chemical into another active chemical;

(*iii*)Conversion of an inactive chemical into another active chemical; and

(*iv*)Conversion of an inactive chemical into another inactive chemical.

From the examples of above four types of reactions, it is obvious that although the basic purpose of biotransformation reactions is inactivation or detoxification of potent xenobiotic compounds to facilitate their elimination from the body of organisms. Sometimes, the biotransformation may also lead to the conversion of inactive or less active chemicals into more active products, which are not easily excreted and thus, highly toxic. Usually the hydrophilic substances are easily and quickly eliminated from the body of organisms. On the other hand, lipophilic substances are not easily eliminated from the body of organisms. Thus, quite often the biotransformation may cause biological conversion of lipophilic substances into their hydrophilic forms and facilitates their excretion. In other words, it may be stated that the biotransformations are often prerequisites to the excretion of xenobiotics.

(A) ***Biotransformation sites*** All organs and tissues are the sites of xenobiotics metabolism, including biotransformation. As xenobiotics are harmful to the organism, biotransformation of these xenobiotics can be considered as a defense mechanism, which may expedite the process of termination of the

xenobiotics from the body. Thus, the process of biotransformation if viewed as a defense mechanism can be best administered as soon as the xenobiotic exposure occurred through the portal, *i.e.* skin, lung and gastro-intestinal tract. This biotransformation process is catalyzed in the presence of enzymes present in the tissues of the above organs but this is best administered in the liver of vertebrates. The hepatocytes in the liver are the major site of xenobiotic biotransformation. These contain relatively high volume of

endoplasmic reticulum (ca 15%) and approximately 20 % of the hepatocyte protein, which are essential for most enzymes needed for biotransformation.

Kidney also receives a bulk of xenobiotics despite the fact it is not amongst the portals. It receives the toxicants during excretion process and is also rich in xenobiotic metabolizing enzymes. These enzymes also occur in adrenal cortex and medulla, placenta, testis, ovary, foetal and embryonic liver, corpus luteum, aorta and blood platelets. In man, these enzymes have been demonstrated in foetal and adult liver, placenta, kidney, testis, foetal and adult adrenal gland, skin, blood platelets and lymphocytes. In insects, such enzymes have been reported from the mid-gut, fat body and Malpighian tubules. Generally, the midgut is the site of greatest concentration of these enzymes in insects. It is also important to mention that these enzymes are not uniformly distributed within an organ. For instance, enzymes are most abundant in the centrilobular region of the liver and nonciliated branchiolar or Clara cells of lungs than the other cell types.

Thus, it is clear that almost every tissue has some activities against xenobiotics, but major enzyme systems are localized mainly in the liver of vertebrates. The primary function of liver is to process the chemicals received through portal circulation before they are distributed to other tissues. Because of this property, the liver has capacity to extract xenobiotics readily from portal circulation and modifies many of them before they are stored, secreted into the bile or released into the general circulation. Other tissues often also biotransform the xenobiotics, but they have limited capacity with respect to diversity of chemicals they can handle. Hence, their contribution to the overall biotransformation of xenobiotics is limited. The biotransformation of xenobiotics in extrahepatic tissue may have an important toxicological implication for that particular tissue.

(B) ***Nature of biotransformation enzymes*** One of the basic properties of enzymes is substrate specificity. But, the enzymes involved in biotransformation of xenobiotics have relatively low degree of substrate specificity in comparison to those involved in the metabolism of constitutive chemicals. Because these enzymes catalyze reactions of a wide variety of chemicals after the recognition of functional groups of the molecules rather than the recognition of entire molecules.

The biological significance for the presence of these enzymes may be either to evolve as a protective device against chemical insults encountered in the environment or they are relatively nonspecific biological catalysts, which normally biotransform certain body constituents, but have relatively low substrate specificity.

(I) Microsomal mixed function oxidases

The biotransformation of xenobiotics is accomplished by several remarkable enzyme systems. The biotransformation reactions are broadly of two types: (*i*) Phase I reactions, and (*ii*) Phase II reactions.

In phase I reactions, the functional groups (such as -OH, -SH, NH_2, -COOH) of xenobiotics are either added or exposed. These include simple reactions such as oxidation, reduction and hydrolysis. The phase II reactions consist of relatively complex conjugation and synthetic reactions. Phase I reactions are very important and predominant reactions in the biotransformation of toxicants and are catalyzed by enzyme systems, such as cytochrome P-450 and cytochrome P-450 reductase and these enzyme systems are termed as ***mixed function oxidase (MFO) system***. It is also termed as ***monooxygenases*** because they catalyze the incorporation of one atom of molecular oxygen into the substrate. But, the term mixed function oxidase is preferred. Some other hydrolytic enzymes, such as esterases and amidases, also expose the functional groups of xenobiotics.

The enzymes catalyzing phase I reactions are principally located in the endoplasmic reticulum, which is a continuous anastomosing network of lipoprotein membranes between plasma membrane, nucleus and mitochondria. The endoplasmic reticulum is of two types: (*i*) rough, and (*ii*) smooth. Rough endoplasmic reticulum (ER) is studded ribosomes on its outer surface whereas smooth endoplasmic reticulum is devoid of ribosomes. Although both these types contain all the components of mixed function oxidase system; the smooth form is rich in oxidative enzymes, hence the oxidative activity.

As a result of translocation, the lipophilic substances ultimately reach the lipoprotein bilayers of membranes. Hence, the presence of phase I enzymes within lipoprotein matrix of membranes, the actual site of biotransformation, is of great significance. The wide distribution of cytochrome P-450 system among various animal species and in various organs of a species along with its capability to catalyze incorporation of oxygen atoms into a wide variety of xenobiotics, make it the most important group of enzymes associated with the biotransformation of xenobiotics.

To isolate mixed function oxidase system, liver is taken out from the organisms and homogenized. On account of homogenization the endoplasmic reticulum breaks up and the fragments unite to form microvesicles often referred to as ***microsomes***. The microsomes are isolated by the differential centrifugation of liver homogenate. The liver homogenate is first centrifuged at 9000 X g to remove nuclei, mitochondria, ribosomes, unbroken cells, *etc*. and the resultant supernatant is again centrifuged at 1,05,000 X g, which yields pellets highly enriched in microsomes. The microsome contains mixed function oxidase system, *i.e.* catalysts of phase I reaction. The mixed function oxidases are also referred to as, ***microsomal mixed function oxidases***. The supernatant obtained after second step of centrifugation is referred to as ***cytosol,*** which contains soluble enzymes of Phase II reactions of biotransformation. Therefore, these enzymes may also be termed as ***cytosolic enzymes***.

(a) Constituent enzymes of microsomal mixed function oxidase

A number of research workers have suggested the mixed function oxidation of xenobiotics by the system consisted of cytochrome P-450, NADPH-cytochrome P-450 reductase and phosphatidylcholine. This system oxidizes xenobiotics in the presence of NADPH and oxygen, but this does not mean that:

(a) all xenobiotics are oxidized by this minimal enzyme system, and

(b) other microsomal constituents, such as cytochrome b_5 system, mixed function amine oxidase, *etc.* may not catalyze xenobiotic oxidations, although the role of cytochrome b_5 system in such oxidations is not well established.

(i) Cytochrome P-450 system The most important enzyme system of microsomal mixed function oxidase is cytochrome P-450. It is a coupled enzyme system consisted of:

(a) a haeme containing enzyme, cytochrome P-450, and

(b) NADPH-cytochrome P-450 reductase, which prefers NADPH as its cofactor.

Cytochrome P-450 is a haemoprotein of cytochrome b_5 type with unique redox potential and is named from the unique wave-length of absorption maximum (at 450 nm) of carbon monoxide derivative of the reduced form (reduced cytochrome P-450 (Fe^{2+}) forms a ligand with carbon monoxide). Only intact and biocatalytically functional cytochrome P-450 possesses this spectral property while denatured form loses its spectral peak and achieves maximum absorbance at 420 nm. At least 10 different forms of cytochrome P-450 have been isolated from rat liver microsomes. The different forms of enzyme differ in the structure of polypeptide chain as also the specificity of reactions catalyzed by them. Treatment of rats with certain xenobiotics shifts the absorption maximum of the enzyme. The types and amounts of cytochrome P-450 also vary with the species, age, sex and health of organisms. On the other hand, cytochrome P-450 reductase has been isolated from single source and its concentration varies between one tenth to one thirteenth of cytochrome P-450. Therefore, it is believed that this enzyme mediates reduction of different forms of cytochrome P-450.

(ii) Cytochrome b_5 system Associated with cytochrome P-450 system, there is another cytochrome system consisted of cytochrome b_5 and cytochrome b_5 reductase, but the function of this system in cytochrome P-450 mediated reactions is not properly understood.

(iii) Mixed function amine oxidase Another important oxidative enzyme is referred to as ***mixed function amine oxidase***. It is also present in endoplasmic reticulum and oxidizes nucleophilic nitrogen and sulphur atoms. This enzyme is not solely an amine oxidase, but it should be appropriately termed as *microsomal flavin containing monooxygenase*. Human beings and pigs possess remarkably high amounts of this enzyme while rats possess low level of the same.

(II) Microsomal hydrolytic enzymes

(i) Epoxide hydrolase Epoxide hydrolase is an important hydrolytic enzyme and is believed to be located in the microsomal fractions, *i.e.* in close proximity of microsomal cytochrome P-450 systems. It has been recorded in a wide variety of tissues, such as liver, testis, ovary, lung, kidney, skin, intestine, colon, spleen, thymus, brain and heart. The activity of this enzyme differs in various tissues. For example, clara cells of lung possess three to four times more activity of epoxide hydrolase than alveolar type I cells.

A distinct cytosolic epoxide hydrolase has also been reported in a number of animal tissues. The cytosolic enzyme has high levels in mice and rabbits in comparison to those of rats.

Epoxide hydrolase catalyses the hydration reactions of aliphatic and aromatic epoxides of xenobiotics; and thus inactivates a number of highly reactive epoxides. Hence, epoxide hydrolase is considered as a detoxication enzyme. The occurrence of enzyme in close proximity to the site of formation of its substrate (*i.e.* oxides of xenobiotics) may have great toxicological significance. In fact, it has been suggested that epoxide hydrolase has been evolved to detoxify various aliphatic and aromatic epoxides of xenobiotics.

(ii) Esterases and amidases A large number of nonspecific esterase and amidase enzymes occur in various mammalian tissues. Both of these hydrolyze ester and amide linkages of xenobiotics. These enzymes occur both in the microsomal and cytosolic fractions. The cytosolic esterases are

usually specific in reaction, such as acetylcholinesterase and pseudocholinesterases. On the other hand, the microsomal esterases are relatively nonspecific and can catalyze the hydrolytic reactions of ester of a diverse group of xenobiotics.

The amidases are comparatively more specific enzymes. Generally, the enzymatic hydrolysis of amides takes place more slowly than those of esters. The slow hydrolysis of amides by amidases has been ascribed to the comparatively high specificity of amidases.

(iii) Glutathione-S-transferases It is one of the most important enzymes of phase II biotransformation reaction. The initial step of glutathione conjugation to xenobiotics is catalyzed by a group of enzymes called as *glutathione-S-transferases*. These enzymes are present both in the soluble and microsomal fractions of tissues. But, their high concentration occurs principally in the soluble fractions. These enzymes are widely distributed in the animal kingdom, for instance, in protozoans (*e.g. Acanthamoeba*), insects, aquatic invertebrates, fishes and mammals. Besides, they are also known to be present in certain bacteria.

Various forms of glutathione-S-transferases have been reported from the liver of rats, mice and human-beings as also in insects. At least five different forms of enzyme have been reported from the soluble fraction of rat- and human liver.

Glutathione-S-transferases have been classified in accordance with their substrate specificity and pH optima. They may be grouped and named as follows:

(i) Glutathione-S-alkyltransferase,

(ii) Glutathione-S-aryltransferase,

(iii) Glutathione-S-aralkyltransferase,

(iv) Glutathione-S-epoxidetransferase,

As glutathione-S-transferases catalyze the conjugation of toxic electrophilic compounds with an endogenous molecule, glutathione, they protect endogenous critical nucleophiles, such as proteins and nucleic acids.

(IV) Factors Affecting Biotransformation of Xenobiotics

It is obvious that biotransformation of xenobiotics may take place by different pathways involving phase I and II reactions and they may give rise to different biotransformation products. Various factors related to the chemical, the environment and the physiological state of organisms affect the rate and relative importance of biotransformation reactions of xenobiotics. These factors can thus be divided into three categories: (1) Chemical factors, (2) Environmental factors, and (3) Factors related to organisms. The first two factors may also be referred to as *abiotic factors* and the third one as *biotic factors*.

(1) Chemical Factors

(i) Concentration The most important factor related to the chemical is that which affect rate and route of enzymatic modification of active concentration of the chemical at specific target site. Some of xenobiotics having immediate effects may cause pronounced damage to the tissue and thus the biotransformation enzymes may be inhibited on account of their binding with active sites. Therefore, such substances are believed to inhibit the xenobiotic biotransformation. On the other hand, there are certain substances, which may enhance the activity of these enzymes, hence may enhance their biotransformation. Such activator substances may increase the rate of biotransformation of xenobiotics in the body of organisms.

(ii) Chemical nature Another important factor related to the chemical is the nature of xenobiotics, *i.e.* whether lipophilic or hydrophilic or polar. The xenobiotics ionized at physiological pH have poor lipid solubility, hence they cannot easily cross lipoprotein layers of biological membranes. On the other hand, lipophilic chemicals are more easily absorbed through membranes. If such chemicals are inhibitors of biotransformation enzymes, they may either affect the process or at least slow down.

(iii) Interaction with endogenous molecules The xenobiotics interact with intracellular and extracellular proteins. This interaction reduces the concentration of xenobiotics at the active sites of enzymes involved in their biotransformation. This binding of xenobiotics with certain proteins has a definite effect on the intrinsic clearance of xenobiotics.

(2) Environmental factors

In vitro effects of light, temperature, *etc*. on xenobiotic biotransformation enzymes are similar to those of other enzymes. *In vivo* effects of environmental factors, such as temperature, light, moisture, ionizing radiation, *etc.* on the biotransformation of xenobiotics are being given under this section.

(i) Temperature It is often expected that variations in ambient temperature would not affect the biotransformation of xenobiotics in homeotherms, but actually it is not so. Temperature variations may work as a kind of stress and thereby produce changes mediated by hormonal variations. Seasonal changes in temperature also have a definite effect on hormonal levels. It is now known that changes in temperature clearly affect the biotransformation of xenobiotics. But, it is not clear whether these changes directly affect the biotransformation or through some other physiological mechanism.

(ii) Light Many xenobiotic biotransformation enzymes show a diurnal pattern in relation to light cycles rather than light intensity. Continuous darkness causes maintenance of high concentrations of *hydroxy indol-O-methyl transferase*. This enzyme has diurnal rhythm with greatest activity at night. Microsomal mixed function oxidase system also shows diurnal rhythm with greatest activity at the beginning of the dark phase. The activity of these enzymes may be correlated with the biotransformation of xenobiotics.

(iii) Moisture Effect of moisture on the biotransformation of xenobiotics in vertebrates does not seem to be investigated, but similar studies in insects have been made. It has been reported that housefly larvae reared at 40% moisture content have four times more activity of epoxidation of heptachlor than those of larvae reared on water saturated medium.

(iv) Ionizing radiation Exposure of rodents (*i.e.* rats and mice) to ionizing radiation reduces the rate of biotransformation of xenobiotics. This view has been supported by the fact that exposure to ionizing radiations reduce the hydroxylation of steroids, desulphuration activity and glucuronide formation. Inhibition in pseudocholinesterase activity following exposure to ionizing radiation has also been reported to be reduced.

(3) Factors related to organisms

(i) Species Different species differ in the toxicity of xenobiotics. These differences may be related to differences in the translocation and biotransformation of xenobiotics. The species differences in biotransformation of xenobiotics are quantitative rather than qualitative. These quantitative differences in biotransformation of xenobiotics may be ascribed to species differences in the enzyme concentration or its kinetics, availability of cofactors and the

concentration of substrate in tissues. For instance, rats have higher cytochrome P-450 concentrations than other mammalian species. It is found that rodents are well bestowed with the activity of enzyme sulphotransferase and relatively deficient in activity of enzyme glutathione transferase. The quantitative differences suggest that different biotransformation routes are favoured in different species, which may lead to different pharmacological and toxicological activity. In addition, the metabolic rates of a xenobiotic may be similar in some closely related species but the end product is different. For example, oxidative metabolism of amphetamines by cytochrome P-450 produces largely the deamination products in rabbit but it forms phenyl hydroxylation products in rats. Human beings, however, are capable to carry out almost all the metabolic transformation and do not show any particular difference in enzymatic pathways.

(ii) Strains Different strains of an organism differ in biotransformation of xenobiotics and these differences are under genetic control. The differences in biotransformation often may lead to variations in biological responses to xenobiotics. The strain-wise variation in phase I biotransformation reactions may be attributed to marked differences in cytochrome P-450 related enzymes activities. Variation in phase II biotransformation reactions in various strains are also known. For instance, Sprague- Dawley strain of rat and Fischer strain of rat are very similar but Gunn strain of rat shows a remarkable deficiency in one of the two major classes of *UDP-glucuronosyl transferase* activity. The cytochrome P-450 concentration in al three strains of rats is however very similar.

In humans, there is a tendency to respond differently towards the metabolism of various drugs. For instance, different populations of humans have different response to the antituberculosis drug, isoniazid. Variation in the acetylator phenotypes in humans is a reason of this differential response. High percent of Asian and Eskimo populations are fast acetylators and here the biotransformation of isoniazid drug leads to reactive metabolites which cause liver toxicity (hepatic necrosis). On the contrary, there is almost no effect on the metabolism of the isoniazid in the human population of slow acetylators. The unchanged drug accumulates causing the toxicity of peripheral neuropathy, which results from pyridoxal phosphate depletion by the unmetabolized drug. This accumulation of drug may cause bladder cancer when it gets exposed with arylamine compounds.

(iii) Sex The rate of biotransformation of xenobiotics varies according to sex of organisms. Gender-wise differences in biotransformation of xenobiotics by mammalian liver appear with the onset of puberty and are usually maintained throughout the adult life. Meager studies of sex dependent biotransformation in humans have shown that females have a better tendency for oxidative metabolism rates than the males. Adult male rats biotransform xenobiotics at higher rates than those of adult females. The differences in biotransformation of xenobiotics between males and females may be ascribed to differential activity of microsomal enzymes, which are normally under control of sex hormones.

(iv) Age Foetal and new born individuals have limited ability to biotransform xenobiotics, which provides the basis for increased sensitivity of young animals to the xenobiotics. But, all the pathways of biotransformation are not absent in new born individuals. The enzyme systems develop rapidly after the birth of individuals. For instance, new born rats possess almost negligible activity of cytochrome P-450 system that develops rapidly and reaches at peaks by 30 days of age. Thereafter, activity starts gradually declining with increase in the age of individuals. The age related changes in the activities of biotransformation enzymes may be ascribed to biochemical differentiation in hepatocytes.

In humans, the premature and neonatal infants have low activities of microsomal and nonmicrosomal metabolizing enzymes. The biotransformation o my exotic chemicals are fastest in adolescents.

(v) Diet The nutritional status of organisms also plays an important role in the biotransformation of xenobiotics. Human diets usually contain nutrients which are needed for the body growth and development. Apart from these they may contain pesticides residues and food additives, which can enter our physiological and biochemical systems and alter the activities of drug metabolizing enzymes. The flavonoids, naringin and quercetin present in the grape fruit juices have a tendency of inhibiting the Cytochrome P450 3A. There are other flavonoids, like catechin, myricetin and rutin, which can induce Phase II enzymes and provide protection against biotransformed products. Starvation of organims results in decrease in the levels of biotransformation enzymes. Therefore, starved animals are often more sensitive to toxicants than the normally fed individuals. Similarly the mineral and vitamin deficiencies in the diet decrease the levels of cytochrome P-450 system that in turn may decrease the rate of biotransformation of xenobiotics. Low protein and protein free diet also decrease the levels of microsomal enzymes. After the supply of nutrient rich diet to these animals, the enzyme activities return to normal levels. Burned carbon containing materials, like cigar, cigarette and charcoal broiled foods may largely modify and alter the biotransformation tendencies of enzymes.

(vi) Health The health status of organisms affects the biotransformation of xenobiotics. It is obvious from the previous accounts that the liver is most important organ and the site of biotransformation of xenobiotics. Any type of damage (either due to certain infections or due to the action of chemicals) may have pronounced effects on the levels of xenobiotic biotransforming enzyme systems, hence the biotransformation of xenobiotics. Hepatitis patients have been reported to show impaired ability to biotransform xenobiotics via microsomal mixed function oxidase system.

(vii) Circadian rhythms Daily and seasonal changes in the photocycle and other parameters are known to affect the endocrine functions of organisms. The biotransformation enzymes are under control of neuroendocrines. Therefore, changes in the season and the time of the day may have great influence on the levels of biotransformation enzymes, hence on the biotransformation of xenobiotics.

(V) Bioactivation

The biotransformation of xenobiotics may involve either the conversion of an active chemical to more active chemical or an inactive chemical to an active chemical. As a consequence to such biotransformation, *i.e.* conversion of an inactive chemical to an active chemical, the toxicity of parent compound is increased. *The process of bioconversion of inactive xenobiotics into more reactive products is termed as bioactivation*. Such biological conversions may have great pharmacological and toxicological consequences. One promising example of bioactivation is the conversion of organophosphorus pesticides belonging to the groups; phosphorothionates and phosphorodithioates having P=S group. These compounds are poor inhibitors of target enzyme, AChE. But, in the body of organisms both in the mammals and insects, they are biotransformed into more active form of organophosphates (having P=O group) by the microsomal mixed function oxidases, which are potent inhibitors of AChE, hence more toxic. For instance, less active malathion (having P=S group) is bioconverted into its oxygen analogue malaoxon (having P=O group) and parathion into paraoxon. Similarly, various other xenobiotics are also bioactivated by the organisms.