Chemical and Physiological Influences on Xenobiotic Metabolism

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9.1 INTRODUCTION

The metabolism of toxicants and their overall toxicity can be modified by many factors both extrinsic and intrinsic to the normal functioning of the organism. It is entirely possible that many changes in toxicity are due to changes in metabolism, because most sequences of events that lead to overt toxicity involve activation and/or detoxication of the parent compound. In many cases the chain of cause and effect is not clear, due to the difficulty of relating single events measured in vitro to the complex and interrelated effects that occur in vivo. This relationship between in vitro and in vivo studies is important and is discussed in connection with enzymatic inhibition and induction (see Section 9.5). It is important to note that the chemical, nutritional, physiological, and other effects noted herein have been described primarily from experiments carried out on experimental animals. These studies indicate that similar effects may occur in humans or other animals, but not that they must occur or that they occur at the same magnitude in all species if they occur at all.

9.2 NUTRITIONAL EFFECTS

Many nutritional effects on xenobiotic metabolism have been noted, but the information is scattered and often appears contradictory. This is one of the most important of several neglected areas of toxicology. This section is concerned only with the effects of nutritional constituents of the diet; the effects of other xenobiotics in the diet are discussed under chemical effects (see Section 9.5).

9.2.1 Protein

Low-protein diets generally decrease monooxygenase activity in rat liver microsomes, and gender and substrate differences may be seen in the effect. For example, aminopyrine *N*-demethylation, hexobarbital hydroxylation, and aniline hydroxylation are all

A Textbook of Modern Toxicology, Third Edition, edited by Ernest Hodgson
ISBN 0-471-26508-X Copyright © 2004 John Wiley & Sons, Inc.

decreased, but the effect on the first two is greater in males than in females. In the third case, aniline hydroxylation, the reduction in males is equal to that in females. Tissue differences may also be seen. These changes are presumably related to the reductions in the levels of cytochrome P450 and NADPH-cytochrome P450 reductase that are also noted. One might speculate that the gender and other variations are due to differential effects on P450 isozymes. Even though enzyme levels are reduced by low-protein diets, they can still be induced to some extent by compounds such as phenobarbital. Such changes may also be reflected in changes in toxicity. Changes in the level of azoreductase activity in rat liver brought about by a low-protein diet are reflected in an increased severity in the carcinogenic effect of dimethylaminoazobenzene. The liver carcinogen dimethylnitrosamine, which must be activated metabolically, is almost without effect in protein-deficient rats.

Strychnine, which is detoxified by microsomal monooxygenase action, is more toxic to animals on low-protein diets, whereas octamethylpyrophosphoramide, carbon tetrachloride, and heptachlor, which are activated by monooxygenases, are less toxic. Phase II reactions may also be affected by dietary protein levels. Chloramphenicol glucuronidation is reduced in protein-deficient guinea pigs, although no effect is seen on sulfotransferase activity in protein-deficient rats.

9.2.2 Carbohydrates

High dietary carbohydrate levels in the rat tend to have much the same effect as low dietary protein, decreasing such activities as aminopyrine *N*-demethylase, pentobarbital hydroxylation, and *p*-nitrobenzoic acid reduction along with a concomitant decrease in the enzymes of the cytochrome P450 monooxygenase system. Because rats tend to regulate total caloric intake, this may actually reflect low-protein intake.

In humans it has been demonstrated that increasing the ratio of protein to carbohydrate in the diet stimulates oxidation of antipyrine and theophylline, while changing the ratio of fat to carbohydrate had no effect. In related studies, humans fed charcoal-broiled beef (food high in polycyclic hydrocarbon content) for several days had significantly enhanced activities of CYPs 1A1 and 1A2, resulting in enhanced metabolism of phenacetin, theophylline, and antipyrine. Studies of this nature indicate that there is significant interindividual variability in these observed responses.

9.2.3 Lipids

Dietary deficiencies in linoleic or in other unsaturated fats generally bring about a reduction in P450 and related monooxygenase activities in the rat. The increase in effectiveness of breast and colon carcinogens brought about in animals on high fat diets, however, appears to be related to events during the promotion phase rather than the activation of the causative chemical.

Lipids also appear to be necessary for the effect of inducers, such as phenobarbital, to be fully expressed.

9.2.4 Micronutrients

Vitamin deficiencies, in general, bring about a reduction in monooxygenase activity, although exceptions can be noted. Riboflavin deficiency causes an increase in P450 and

aniline hydroxylation, although at the same time it causes a decrease in P450 reductase and benzo(a)pyrene hydroxylation. Ascorbic acid deficiency in the guinea pig not only causes a decrease in P450 and monooxygenase activity but also causes a reduction in microsomal hydrolysis of procaine. Deficiencies in vitamins A and E cause a decrease in monooxygenase activity, whereas thiamine deficiency causes an increase. The effect of these vitamins on different P450 isozymes has not been investigated. Changes in mineral nutrition have also been observed to affect monooxygenase activity. In the immature rat, calcium or magnesium deficiency causes a decrease, whereas, quite unexpectedly, iron deficiency causes an increase. This increase is not accompanied by a concomitant increase in P450, however. An excess of dietary cobalt, cadmium, manganese, and lead all cause an increase in hepatic glutathione levels and a decrease in P450 content.

9.2.5 Starvation and Dehydration

Although in some animals starvation appears to have effects similar to those of protein deficiency, this is not necessarily the case. For example, in the mouse, monooxygenation is decreased but reduction of *p*-nitrobenzoic acid is unaffected. In male rats, hexobarbital and pentobarbital hydroxylation as well as aminopyrine *N*-demethylation are decreased, but aniline hydroxylation is increased. All of these activities are stimulated in the female. Water deprivation in gerbils causes an increase in P450 and a concomitant increase in hexobarbital metabolism, which is reflected in a shorter sleeping time.

9.2.6 Nutritional Requirements in Xenobiotic Metabolism

Because xenobiotic metabolism involves many enzymes with different cofactor requirements, prosthetic groups, or endogenous cosubstrates, it is apparent that many different nutrients are involved in their function and maintenance. Determination of the effects of deficiencies, however, is more complex because reductions in activity of any particular enzyme will be effective only if it affects a change in a rate-limiting step in a process. In the case of multiple deficiencies, the nature of the rate-limiting step may change with time

Phase I Reactions. Nutrients involved in the maintenance of the cytochrome P450 monooxygenase system are shown in Figure 9.1. The B complex vitamins niacin and riboflavin are both involved, the former in the formation of NADPH and the latter in the formation of FAD and FMN. Essential amino acids are, of course, required for the synthesis of all of the proteins involved. The heme of the cytochrome requires iron, an essential inorganic nutrient. Other nutrients required in heme synthesis include pantothenic acid, needed for the synthesis of the coenzyme A used in the formation of acetyl Co-A, pyridoxine, a cofactor in heme synthesis and copper, required in the ferroxidase system that converts ferrous to ferric iron prior to its incorporation into heme. Although it is clear that dietary deficiencies could reduce the ability of the P450 system to metabolize xenobiotics, it is not clear how this effect will be manifested in vivo unless there is an understanding of the rate-limiting factors involved, which is a considerable task in such a complex of interrelated reactions. Similar considerations

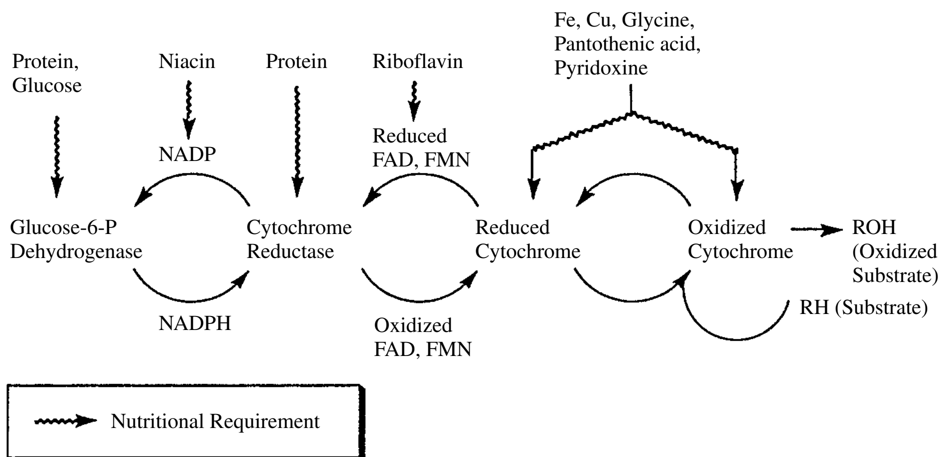


Figure 9.1 Nutritional requirements with potential effects on the cytochrome P450 monooxygenase system (From W. E. Donaldeson Nutritional factors, in *Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson and R. C. Smart, Wiley, 2001.)

could be made for other phase I reaction systems such as arachidonic acid cooxidations, the glutathione peroxidase system, and so on.

Phase II Reactions. As with phase I reactions, phase II reactions usually depend on several enzymes with different cofactors and different prosthetic groups and, frequently, different endogenous cosubstrates. All of these many components can depend on nutritional requirements, including vitamins, minerals, amino acids, and others. Mercapturic acid formation can be cited to illustrate the principles involved. The formation of mercapturic acids starts with the formation of glutathione conjugates, reactions catalyzed by the glutathione *S*-transferases.

This is followed by removal of the glutamic acid and the glycine residues, which is followed by acetylation of the remaining cysteine. Essential amino acids are required for the synthesis of the proteins involved, pantothenic acid for coenzyme A synthesis, and phosphorus for synthesis of the ATP needed for glutathione synthesis. Similar scenarios can be developed for glucuronide and sulfate formation, acetylation, and other phase II reaction systems.

9.3 PHYSIOLOGICAL EFFECTS

9.3.1 Development

Birth, in mammals, initiates an increase in the activity of many hepatic enzymes, including those involved in xenobiotic metabolism. The ability of the liver to carry out monooxygenation reactions appears to be very low during gestation and to increase after birth, with no obvious differences being seen between immature males and females. This general trend has been observed in many species, although the developmental pattern may vary according to gender and genetic strain. The component enzymes of the P450 monooxygenase system both follow the same general trend, although there

may be differences in the rate of increase. In the rabbit, the postnatal increase in P450 and its reductase is parallel; in the rat, the increase in the reductase is slower than that of the cytochrome.

Phase II reactions may also be age dependent. Glucuronidation of many substrates is low or undetectable in fetal tissues but increases with age. The inability of newborn mammals of many species to form glucuronides is associated with deficiencies in both glucuronosyltransferase and its cofactor, uridine diphosphate glucuronic acid (UDPGA). A combination of this deficiency, as well as slow excretion of the bilirubin conjugate formed, and the presence in the blood of pregnanediol, an inhibitor of glucuronidation, may lead to neonatal jaundice. Glycine conjugations are also low in the newborn, resulting from a lack of available glycine, an amino acid that reaches normal levels at about 30 days of age in the rat and 8 weeks in the human. Glutathione conjugation may also be impaired, as in fetal and neonatal guinea pigs, because of a deficiency of available glutathione. In the serum and liver of perinatal rats, glutathione transferase is barely detectable, increasing rapidly until all adult levels are reached at about 140 days (Figure 9.2). This pattern is not followed in all cases, because sulfate conjugation and acetylation appear to be fully functional and at adult levels in the guinea pig fetus. Thus some compounds that are glucuronidated in the adult can be acetylated or conjugated as sulfates in the young.

An understanding of how these effects may be related to the expression of individual isoforms is now beginning to emerge. It is known that in immature rats of either gender, P450s 2A1, 2D6, and 3A2 predominate, whereas in mature rats, the males show a predominance of P450s 2C11, 2C6, and 3A2 and the females P450s 2A1, 2C6, and 2C12.

The effect of senescence on the metabolism of xenobiotics has yielded variable results. In rats monooxygenase activity, which reaches a maximum at about 30 days

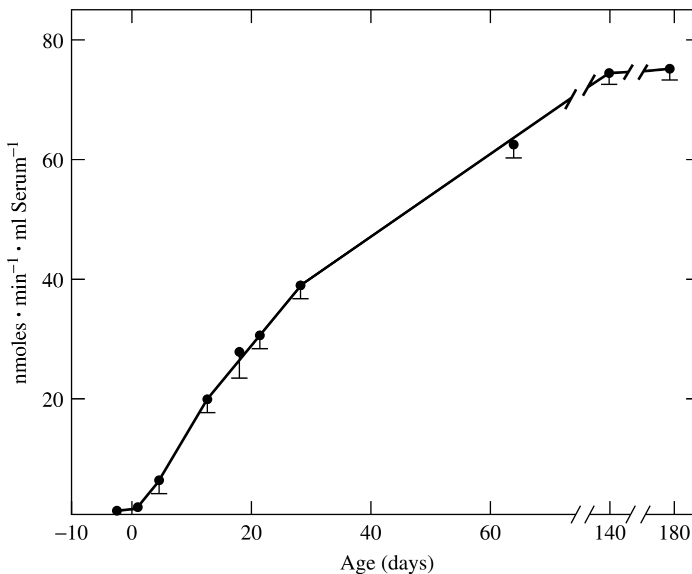


Figure 9.2 Developmental pattern of serum glutathione *S*-transferase activity in female rats. (Adapted from H. Mukhtar and J. R. Bend, *Life Sci.* **21**: 1277, 1977.)

of age, begins to decline some 250 days later, a decrease that may be associated with reduced levels of sex hormones. Glucuronidation also decreases in old animals, whereas monoamine oxidase activity increases. These changes in the monooxygenase activities are often reflected by changes in drug efficacy or overall toxicity.

In humans, age-related impairment of enzyme activity is highly controversial. Age-related declines in activity were not detected with respect to the activity of CYP2C and CYP3A isoforms among 54 liver samples from donors ranging in age from 9 to 89 years. Studies involving an erythromycin breath test in humans also suggested that there were no age-related declines associated with CYP3A4 activity. However, a study of CYP content and antipyrine clearance in liver biopsies obtained from 226 closely matched subjects indicated that subjects older than 70 had significantly less activity and clearance than younger subjects. Likewise, in older subjects, clearance of the drug omeprazole, a CYP2C19 substrate, was nearly half the rates observed in younger subjects.

9.3.2 Gender Differences

Metabolism of xenobiotics may vary with the gender of the organism. Gender differences become apparent at puberty and are usually maintained throughout adult life. Adult male rats metabolize many compounds at rates higher than females, for example, hexobarbital hydroxylation, aminopyrine *N*-demethylation, glucuronidation of *o*-aminophenol, and glutathione conjugation of aryl substrates; however, with other substrates, such as aniline and zoxazolamine, no gender differences are seen. In other species, including humans, the gender difference in xenobiotic metabolism is less pronounced. The differences in microsomal monooxygenase activity between males and females have been shown to be under the control of sex hormones, at least in some species. Some enzyme activities are decreased by castration in the male and administration of androgens to castrated males increases the activity of these sex-dependent enzyme activities without affecting the independent ones. Procaine hydrolysis is faster in male than female rats, and this compound is less toxic to the male. Gender differences in enzyme activity may also vary from tissue to tissue. Hepatic microsomes from adult male guinea pigs are less active in the conjugation of *p*-nitrophenol than are those from females, but no such gender difference is seen in the microsomes from lung, kidney, and small intestines.

Many differences in overall toxicity between males and females of various species are known (Table 9.1). Although it is not always known whether metabolism is the only or even the most important factor, such differences may be due to gender-related differences in metabolism. Hexobarbital is metabolized faster by male rats; thus female rats have longer sleeping times. Parathion is activated to the cholinesterase inhibitor paraoxon more rapidly in female than in male rats, and thus is more toxic to females. Presumably many of the gender-related differences, as with the developmental differences, are related to quantitative or qualitative differences in the isozymes of the xenobiotic-metabolizing enzymes that exist in multiple forms, but this aspect has not been investigated extensively.

In the rat, sexually dimorphic P450s appear to arise by programming, or imprinting, that occurs in neonatal development. This imprinting is brought about by a surge of testosterone that occurs in the male, but not the female, neonate and appears to imprint the developing hypothalamus so that in later development the growth hormone

Table 9.1 Gender-Related Differences in Toxicity

Species	Toxicant	Susceptibility
Rat	EPN, warfarin, strychnine, hexobarbital, parathion	F > M
	Aldrin, lead, epinephrine, ergot alkaloids	M > F
Cat	Dinitrophenol	F > M
Rabbit	Benzene	F > M
Mouse	Folic acid	F > M
	Nicotine	M > F
Dog	Digitoxin	M > F

is secreted in a gender-specific manner. Growth hormone production is pulsatile in adult males with peaks of production at approximately 3-hour intervals and more continuous in females, with smaller peaks. This pattern of growth hormone production and the higher level of circulating testosterone in the male maintain the expression of male-specific isoforms such as P450 2C11. The more continuous pattern of growth hormone secretion and the lack of circulating testosterone appears to be responsible for the expression of female specific isoforms such as P450 2C12. The high level of sulfotransferases in the female appears to be under similar control, raising the possibility that this is a general mechanism for the expression of gender-specific xenobiotic-metabolizing enzymes or their isoforms. A schematic version of this proposed mechanism is seen in Figure 9.3.

Gender-specific expression is also seen in the flavin-containing monooxygenases. In mouse liver FMO1 is higher in the female than in the male, and FMO3, present at high levels in female liver, is not expressed in male liver (Figure 9.4). No gender-specific differences are observed for FMO5. The important role of testosterone in the regulation of FMO1 and FMO3 was demonstrated in gonadectomized animals with and without testosterone implants. In males, castration increased FMO1 and FMO3 expression to levels similar to those observed in females, and testosterone replacement to castrated males resulted in ablation of FMO3 expression. Similarly, administration of testosterone to females caused ablation of FMO3 expression. Although these results clearly indicate a role for testosterone in the regulation of these isoforms, the physiological reasons for their gender-dependent expression remain unknown.

9.3.3 Hormones

Hormones other than sex hormones are also known to affect the levels of xenobiotic metabolizing enzymes, but these effects are much less studied or understood.

Thyroid Hormone. Treatment of rats with thyroxin increases hepatic microsomal NADPH oxidation in both male and female rats, with the increase being greater in females. Cytochrome P450 content decreases in the male but not in the female. Hyperthyroidism causes a decrease in gender-dependent monooxygenase reactions and appears to interfere with the ability of androgens to increase the activity of the enzymes responsible. Gender differences are not seen in the response of mice and rabbits to

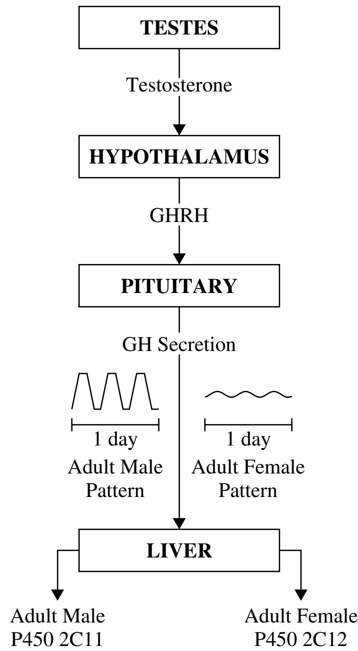


Figure 9.3 Hypothetical scheme for neonatal imprinting of the hypothalamus–pituitary–liver axis resulting in sexually dimorphic expression of hepatic enzymes in the adult rat. Neonatal surges of testosterone appear to play a role in imprinting. (From M. J. J. Ronis and H. C. Cunny, in *Introduction to Biochemical Toxicology*, 2nd ed. E. Hodgson and P. E. Levi, eds., Appleton and Lange, 1994, p. 136.)

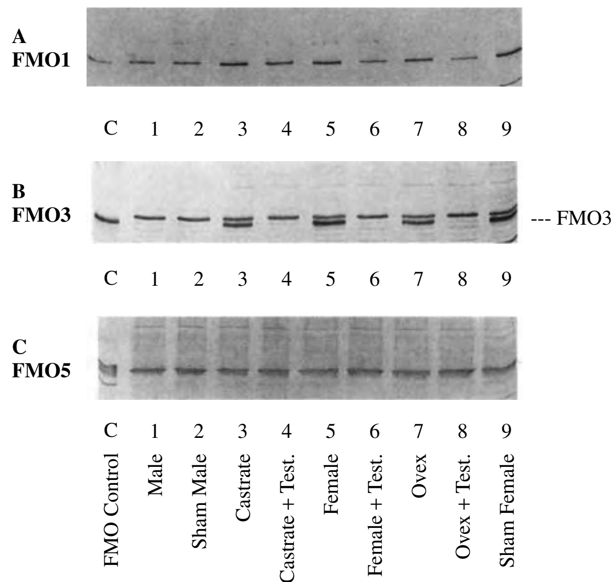


Figure 9.4 Immunoreactivity of liver microsomes from sexually intact control, sham control, gonadectomized mice, or mice undergoing gonadectomy and/or receiving testosterone implants (5 mg). (From J. G. Falls et al., *Arch. Biochem. Biophys.* **342**: 212–223, 1997.)

thyroxin. In mice, aminopyrine *N*-demethylase, aniline hydroxylase, and hexobarbital hydroxylase are decreased, whereas *p*-nitrobenzoic acid reduction is unchanged. In rabbits, hexobarbital hydroxylation is unchanged, whereas aniline hydroxylation and *p*-nitrobenzoic acid reduction increase. Thyroid hormone can also affect enzymes other than microsomal monooxygenases. For example, liver monoamine oxidase activity is decreased, whereas the activity of the same enzymes in the kidney is increased.

Adrenal Hormones. Removal of adrenal glands from male rats results in a decrease in the activity of hepatic microsomal enzymes, impairing the metabolism of aminopyrine and hexobarbital, but the same operation in females has no effect on their metabolism. Cortisone or prednisolone restores activity to normal levels.

Insulin. The effect of diabetes on xenobiotic metabolism is quite varied and, in this regard, alloxan-induced diabetes may not be a good model for the natural disease. The in vitro metabolism of hexobarbital and aminopyrine is decreased in alloxan-diabetic male rats but is increased in similarly treated females. Aniline hydroxylase is increased in both males and females with alloxan diabetes. The induction of P450 2D1 in diabetes (and in fasting) is believed to be due to the high circulating levels of endogenously generated ketones. Studies of activity of the enzymes mentioned show no gender differences in the mouse; both sexes show an increase. Some phase II reactions, such as glucuronidation, are decreased in diabetic animals. This appears to be due to a lack of UDPGA caused by a decrease in UDPG dehydrogenase, rather than a decrease in transferase activity, and the effect can be reversed by insulin.

Other Hormones. Pituitary hormones regulate the function of many other endocrine glands, and hypophysectomy in male rats' results in a decrease in the activity of xenobiotic metabolizing enzymes. Administration of adrenocorticotropic hormone (ACTH) also results in a decrease of those oxidative enzyme activities that are gender dependent. In contrast, ACTH treatment of female rats causes an increase in aminopyrine *N*-demethylase but no change in other activities.

9.3.4 Pregnancy

Many xenobiotic metabolizing enzyme activities decrease during pregnancy. Catechol *O*-methyltransferase and monoamine oxidase decrease, as does glucuronide conjugation. The latter may be related to the increasing levels of progesterone and pregnanediol, both known to be inhibitors of glucuronosyltransferase in vitro. A similar effect on sulfate conjugation has been seen in pregnant rats and guinea pigs. In some species, liver microsomal monooxygenase activity may also decrease during pregnancy, this decrease being accompanied by a concomitant decrease in P450 levels. An increased level of FMO2 is seen in the lung of pregnant rabbits.

9.3.5 Disease

Quantitatively, the most important site for xenobiotic metabolism is the liver; thus effects on the liver are likely to be pronounced in the organism's overall capacity in this regard. At the same time, effects on other organs can have consequences no less

serious for the organism. Patients with acute hepatitis frequently have an impaired ability to oxidize drugs, with a concomitant increase in plasma half-life. Impaired oxidative metabolism has also been shown in patients with chronic hepatitis or cirrhosis. The decrease in drug metabolism that occurs in obstructive jaundice may be a consequence of the accumulation of bile salts, which are known inhibitors of some of the enzymes involved. Phase II reactions may also be affected, decreases in acetylation, glucuronidation, and a variety of esterase activities having been seen in various liver diseases. Hepatic tumors, in general, have a lower ability to metabolize foreign compounds than does normal liver tissue, although in some cases the overall activity of tumor bearing livers may be no lower than that of controls. Kidney diseases may also affect the overall ability to handle xenobiotics, because this organ is one of the main routes for elimination of xenobiotics and their metabolites. The half-lives of tolbutamide, thiopental, hexobarbital, and chloramphenicol are all prolonged in patients with renal impairment.

9.3.6 Diurnal Rhythms

Diurnal rhythms, both in P450 levels and in the susceptibility to toxicants, have been described, especially in rodents. Although such changes appear to be related to the light cycle, they may in fact be activity dependent because feeding and other activities in rodents are themselves markedly diurnal.

9.4 COMPARATIVE AND GENETIC EFFECTS

Comparative toxicology is the study of the variation in toxicity of exogenous chemicals toward different organisms, either of different genetic strains or of different taxonomic groups. Thus the comparative approach can be used in the study of any aspect of toxicology, such as absorption, metabolism, mode of action, and acute or chronic effects. Most comparative data for toxic compounds exist in two areas—acute toxicity and metabolism. The value of the comparative approach can be summarized under four headings:

1. *Selective toxicity.* If toxic compounds are to be used for controlling diseases, pests, and parasites, it is important to develop selective biocides, toxic to the target organism but less toxic to other organisms, particularly humans.
2. *Experimental models.* Comparative studies of toxic phenomena are necessary to select the most appropriate model for extrapolation to humans and for testing and development of drugs and biocides. Taxonomic proximity does not necessarily indicate which will be the best experimental animal because in some cases primates are less valuable for study than are other mammals.
3. *Environmental xenobiotic cycles.* Much concern over toxic compounds springs from their occurrence in the environment. Different organisms in the complex ecological food webs metabolize compounds at different rates and to different products; the metabolic end products are released back to the environment, either to be further metabolized by other organisms or to exert toxic effects of their own. Clearly, it is desirable to know the range of metabolic processes possible.

Laboratory micro ecosystems have been developed, and with the aid of ^{14}C-labeled compounds, chemicals and their metabolites can be followed through the plants and terrestrial and aquatic animals involved.

4. *Comparative biochemistry.* Some researchers believe that the proper role of comparative biochemistry is to put evolution on a molecular basis, and that detoxication enzymes, like other enzymes, are suitable subjects for study. Xenobiotic-metabolizing enzymes were probably essential in the early stages of animal evolution because secondary plant products, even those of low toxicity, are frequently lipophilic and as a consequence would, in the absence of such enzymes, accumulate in lipid membranes and lipid depots. The evolution of cytochrome P450 isoforms, with more than 2000 isoform cDNA sequences known, is proving a useful tool for the study of biochemical evolution.

9.4.1 Variations Among Taxonomic Groups

There are few differences in xenobiotic metabolism that are specific for large taxonomic groups. The formation of glucosides by insects and plants rather than the glucuronides of other animal groups is one of the most distinct. Although differences among species are common and of toxicologic significance, they are usually quantitative rather than qualitative in nature and tend to occur within as well as between taxonomic groups. Although the ultimate explanation of such differences must be at the level of biochemical genetics, they are manifested at many other levels, the most important of which are summarized in the following sections.

In vivo Toxicity. Toxicity is a term used to describe the adverse effects of chemicals on living organisms. Depending on the degree of toxicity, an animal may die, suffer injury to certain organs, or have a specific functional derangement in a subcellular organelle. Sublethal effects of toxicants may be reversible. Available data on the toxicity of selected pesticides to rats suggest that herbicide use, in general, provides the greatest human safety factor by selectively killing plants. As the evolutionary position of the target species approaches that of humans, however, the human safety factor is narrowed considerably. Thus the direct toxicity to humans and other mammals of biocide toxicity seems to be in the following progression: herbicides = fungicides < molluscicides < acaricides < nematocides < insecticides < rodenticides. This formula is obviously oversimplified because marked differences in lethality are observed when different members of each group of biocides is tested against laboratory test animals and target species. One should also bear in mind that any chemical can be environmentally dangerous if misused because many possible targets are interrelated in complex ecological systems.

Interspecific differences are also known for some naturally occurring poisons. Nicotine, for instance, is used as an insecticide and kills many insect pests at low doses, yet tobacco leaves constitute a normal diet for several species. As indicated earlier, most strains of rabbit eat Belladonna leaves without ill effects, whereas other mammals are easily poisoned. Natural tolerance to cyanide poisoning in millipedes and the high resistance to the powerful axonal blocking tetrodotoxin in puffer fish are examples of the tolerance of animals to the toxins they produce.

The specific organ toxicity of chemicals also exhibits wide species differences. Carbon tetrachloride, a highly potent hepatotoxicant, induces liver damage in many species,

but chickens are almost unaffected by it. Dinitrophenol causes cataracts in humans, ducks, and chickens but not in other experimental animals. The eggshell thinning associated with DDT poisoning in birds is observed in falcons and mallard ducks, whereas this reproductive toxicity is not observed in gallinaceous species. Delayed neurotoxicity caused by organophosphates such as leptophos and tri-*o*-cresyl phosphate occurs in humans and can be easily demonstrated in chickens, but can be produced only with difficulty in most common laboratory mammals.

In vivo Metabolism. Many ecological and physiological factors affect the rates of penetration, distribution, biotransformation, and excretion of chemicals, and thus govern their biological fate in the body. In general, the absorption of xenobiotics, their tissue distribution, and penetration across the blood-brain barrier and other barriers are dictated by their physicochemical nature and, therefore, tend to be similar in various animal species. The biologic effect of a chemical depends on the concentration of its binding to tissue macromolecules. Thus substantial differences in these variables should confer species specificity in the biologic response to any metabolically active xenobiotic. The biologic half-life is governed by the rates of metabolism and excretion and thus reflects the most important variables explaining interspecies differences in toxic response. Striking differences among species can be seen in the biologic half-lives of various drugs. Humans, in general, metabolize xenobiotics more slowly than do various experimental animals. For example, phenylbutazone is metabolized slowly in humans, with a half-life averaging 3 days. In the monkey, rat, guinea pig, rabbit, dog, and horse, however, this drug is metabolized readily, with half-lives ranging between 3 and 6 hours. The interdependence of metabolic rate, half-life, and pharmacologic action is well illustrated in the case of hexobarbital. The duration of sleeping time is directly related to the biologic half-life and is inversely proportional to the in vitro degradation of liver enzymes from the respective species. Thus mice inactivate hexobarbital readily, as reflected in a brief biologic half-life in vivo and short sleeping time, whereas the reverse is true in dogs.

Xenobiotics, once inside the body, undergo a series of biotransformations. Those reactions that introduce a new functional group into the molecule by oxidation, reduction, or hydrolysis are designated phase I reactions, whereas the conjugation reactions by which phase I metabolites are combined with endogenous substrates in the body are referred to as phase II reactions. Chemicals may undergo any one of these reactions or any combination of them, either simultaneously or consecutively. Because biotransformations are catalyzed by a large number of enzymes, it is to be expected that they will vary among species. Qualitative differences imply the occurrence of different enzymes, whereas quantitative differences imply variations in the rate of biotransformation along a common metabolic pathway, the variations resulting from differences in enzyme levels, in the extent of competing reactions or in the efficiency of enzymes capable of reversing the reaction.

Even in the case of a xenobiotic undergoing oxidation primarily by a single reaction, there may be remarkable species differences in relative rates. Thus in humans, rats, and guinea pigs, the major route of papaverine metabolism is *O*-demethylation to yield phenolic products, but very little of these products is formed in dogs. Aromatic hydroxylation of aniline is another example. In this case, both *ortho* and *para* positions are susceptible to oxidative attack yielding the respective aminophenols. The biological fate of aniline has been studied in many species and striking selectivity in hydroxylation position has been noted (Table 9.2). These data show a trend,

Table 9.2 In vivo Hydroxylation of Aniline in Females of Various Species

	Percent Dose Excreted as Aminophenol		
Species	*Ortho*	*Para*	*P/O* Ratio
Dog	18.0	9.0	0.5
Cat	32.0	14.0	0.4
Ferret	26.0	28.0	1.0
Rat	19.0	48.0	2.5
Mouse	4.0	12.0	3.0
Hamster	5.5	53.0	10.0
Guinea pig	4.2	46.0	11.0
Rabbit	8.8	50.0	6.0
Hen	10.5	44.0	4.0

Source: Adapted from D. V. Parke, *Biochem. J.* **77**: 493, 1960.

in that carnivores generally display a high aniline *ortho*-hydroxylase ability with a *para/ortho* ratio of ≤ 1 whereas rodents exhibit a striking preference for the *para* position, with a *para/ortho* ratio of from 2.5 to 11. Along with extensive *p*-aminophenol, substantial quantities of *o*-aminophenol are also produced from aniline administered to rabbits and hens. The major pathway is not always the same in any two animal species. 2-Acetylaminofluorene may be metabolized in mammals by two alternative routes: *N*-hydroxylation, yielding the carcinogenic *N*-hydroxy derivative, and aromatic hydroxylation, yielding the noncarcinogenic 7-hydroxy metabolite. The former is the metabolic route in the rat, rabbit, hamster, dog, and human in which the parent compound is known to be carcinogenic. In contrast, the monkey carries out aromatic hydroxylation and the guinea pig appears to deacetylate the *N*-hydroxy derivative; thus both escape the carcinogenic effects of this compound.

The hydrolysis of esters by esterases and of amides by amidases constitutes one of the most common enzymatic reactions of xenobiotics in humans and other animal species. Because both the number of enzymes involved in hydrolytic attack and the number of substrates for them is large, it is not surprising to observe interspecific differences in the disposition of xenobiotics due to variations in these enzymes. In mammals the presence of carboxylesterase that hydrolyzes malathion but is generally absent in insects explains the remarkable selectivity of this insecticide. As with esters, wide differences exist between species in the rates of hydrolysis of various amides in vivo. Fluoracetamide is less toxic to mice than to the American cockroach. This is explained by the faster release of the toxic fluoroacetate in insects as compared with mice. The insecticide dimethoate is susceptible to the attack of both esterases and amidases, yielding nontoxic products. In the rat and mouse, both reactions occur, whereas sheep liver contains only the amidases and that of guinea pig only the esterase. The relative rates of these degradative enzymes in insects are very low as compared with those of mammals, however, and this correlates well with the high selectivity of dimethoate.

The various phase II reactions are concerned with the conjugation of primary metabolites of xenobiotics produced by phase I reactions. Factors that alter or govern the rates of phase II reactions may play a role in interspecific differences in xenobiotic metabolism. Xenobiotics, frequently in the form of conjugates, can be eliminated

through urine, feces, lungs, sweat, saliva, milk, hair, nails, or placenta, although comparative data are generally available only for the first two routes. Interspecific variation in the pattern of biliary excretion may determine species differences in the relative extent to which compounds are eliminated in the urine or feces. Fecal excretion of a chemical or its metabolites tends to be higher in species that are good biliary excretors, such as the rat and dog, than in species that are poor biliary excretors, such as the rabbit, guinea pig, and monkey. For example, the fecal excretion of stilbestrol in the rat accounts for 75% of the dose, whereas in the rabbit about 70% can be found in the urine. Dogs, like humans, metabolize indomethacin to a glucuronide but, unlike humans that excrete it in the urine, dogs excrete it primarily in the feces—apparently due to inefficient renal and hepatic blood clearance of the glucuronide. These differences may involve species variation in enterohepatic circulation, plasma level, and biologic half-life.

Interspecific differences in the magnitude of biliary excretion of a xenobiotic excretion product largely depend on molecular weight, the presence of polar groups in the molecule, and the extent of conjugation. Conjugates with molecular weights of less than 300 are poorly excreted in bile and tend to be excreted with urine, whereas the reverse is true for those with molecular weights higher than 300. The critical molecular weight appears to vary between species, and marked species differences are noted for biliary excretion of chemicals with molecular weights of about 300. Thus the biliary excretion of succinylsulfathioazole is 20- to 30-fold greater in the rat and the dog than in the rabbit and the guinea pig, and more than 100-fold greater than in the pig and the rhesus monkey. The cat and sheep are intermediate and excrete about 7% of the dose in the bile.

The evidence reported in a few studies suggests some relationship between the evolutionary position of a species and its conjugation mechanisms (Table 9.3). In humans and most mammals, the principal mechanisms involve conjugations with glucuronic acid, glycine, glutamine, glutathione and sulfate. Minor conjugation mechanisms in

Table 9.3 Occurrence of Common and Unusual Conjugation Reactions

Conjugating Group	Common	Unusual
Carbohydrate	Glucuronic acid (animals)	*N*-Acetylglucosamine (rabbits)
	Glucose (insects, plants)	Ribose (rats, mice)
Amino acids	Glycine	Glutamine (insects, humans)
	Glutathione	Ornithine (birds)
	Methionine	Arginine (ticks, spiders)
		Glycyltaurine (cats)
		Glycylglycine (cats)
		Serine (rabbits)
Acetyl	Acetyl group from acetyl-0CoA	
Formyl		Formylation (dogs, rats)
Sulfate	Sulfate group from PAPS	
Phosphate		Phosphate monoester formation (dogs, insects)

Source: Modified from A. P. Kulkarni and E. Hodgson, Comparative toxicology, in *Introduction to Biochemical Toxicology*. E. Hodgson and F. E. Guthrie, eds., New York: Elsevier, 1980, p. 115.

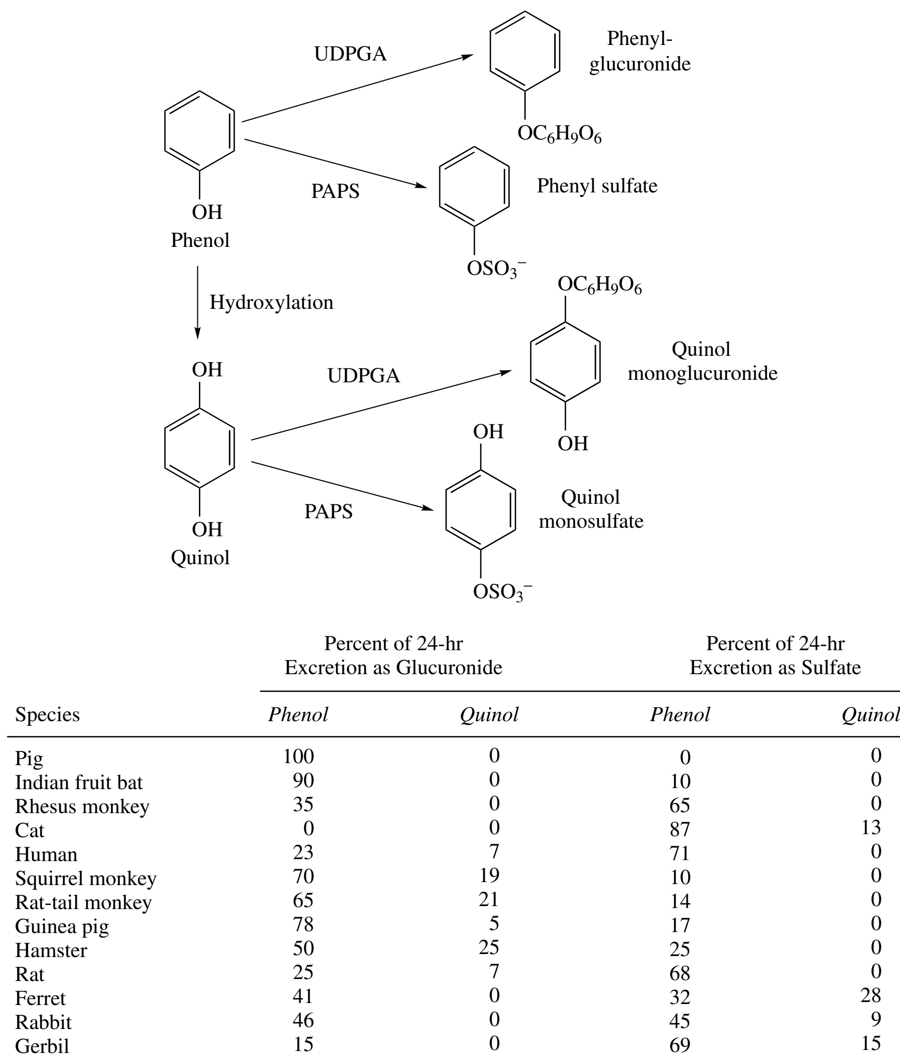
mammals include acetylation and methylation pathways. In some species of birds and reptiles, ornithine conjugation replaces glycine conjugation; in plants, bacteria, and insects, conjugation with glucose instead of glucuronic acid results in the formation of glucosides. In addition to these predominant reactions, certain other conjugative processes are found involving specific compounds in only a few species. These reactions include conjugation with phosphate, taurine, *N*-acetyl-glucosamine, ribose, glycyltaurine, serine, arginine, and formic acid.

From the standpoint of evolution, similarity might be expected between humans and other primate species as opposed to the nonprimates. This phylogenic relationship is obvious from the relative importance of glycine and glutamine in the conjugation of arylacetic acids. The conjugating agent in humans is exclusively glutamine, and the same is essentially true with Old World monkeys. New World monkeys, however, use both the glycine and glutamine pathways. Most nonprimates and lower primates carry out glycine conjugation selectively. A similar evolutionary trend is also observed in the *N*-glucuronidation of sulfadimethoxine and in the aromatization of quinic acid; both reactions occur extensively in human, and their importance decreases with increasing evolutionary divergence from humans. When the relative importance of metabolic pathways is considered, one of the simplest cases of an enzyme-related species difference in the disposition of a substrate undergoing only one conjugative reaction is the acetylation of 4-aminohippuric acid. In the rat, guinea pig, and rabbit, the major biliary metabolite is 4-aminohippuric acid; the cat excretes nearly equal amounts of free acid and its acetyl derivative; and the hen excretes mainly the unchanged compound. In the dog, 4-aminohippuric acid is also passed into the bile unchanged because this species is unable to acetylate aromatic amino groups.

Defective operation of phase II reactions usually causes a striking species difference in the disposition pattern of a xenobiotic. The origin of such species variations is usually either the absence or a low level of the enzyme(s) in question and/or its cofactors. Glucuronide synthesis is one of the most common detoxication mechanisms in most mammalian species. The cat and closely related species have a defective glucuronide-forming system, however. Although cats form little or no glucuronide from *o*-aminophenol, phenol, *p*-nitrophenol, 2-amino-4-nitrophenol, 1-or 2-naphthol, and morphine, they readily form glucuronides from phenolphthalein, bilirubin, thyroxine, and certain steroids. Recently polymorphisms of UDP glucuronyl-transferase have been demonstrated in rat and guinea pig liver preparations; thus defective glucuronidation in the cat is probably related to the absence of the appropriate transferase rather than that of the active intermediate, UDPGA or UDP glucose dehydrogenase, which converts UDP glucose into UDPGA.

Studies on the metabolic fate of phenol in several species have indicated that four urinary products are excreted (Figure 9.5). Although extensive phenol metabolism takes place in most species, the relative proportions of each metabolite produced varies from species to species. In contrast to the cat, which selectively forms sulfate conjugates, the pig excretes phenol exclusively as the glucuronide. This defect in sulfate conjugation in the pig is restricted to only a few substrates, however, and may be due to the lack of a specific phenyl sulfotransferase because the formation of substantial amounts of the sulfate conjugate of 1-naphthol clearly indicates the occurrence of other forms of sulfotransferases.

Certain unusual conjugation mechanisms have been uncovered during comparative investigations, but this may be a reflection of inadequate data on other species. Future



	Percent of 24-hr Excretion as Glucuronide		Percent of 24-hr Excretion as Sulfate	
Species	*Phenol*	*Quinol*	*Phenol*	*Quinol*
Pig	100	0	0	0
Indian fruit bat	90	0	10	0
Rhesus monkey	35	0	65	0
Cat	0	0	87	13
Human	23	7	71	0
Squirrel monkey	70	19	10	0
Rat-tail monkey	65	21	14	0
Guinea pig	78	5	17	0
Hamster	50	25	25	0
Rat	25	7	68	0
Ferret	41	0	32	28
Rabbit	46	0	45	9
Gerbil	15	0	69	15

Figure 9.5 Species variation in the metabolic conversion of phenol in vivo.

investigations may demonstrate a wider distribution. A few species of birds and reptiles use ornithine for the conjugation of aromatic acids rather than glycine, as do mammals. For example, the turkey, goose, duck, and hen excrete ornithuric acid as the major metabolite of benzoic acid, whereas pigeons and doves excrete it exclusively as hippuric acid.

Taurine conjugation with bile acids, phenylacetic acid, and indolylacetic acid seems to be a minor process in most species, but in the pigeon and ferret, it occurs extensively. Other infrequently reported conjugations include serine conjugation of xanthurenic acid in rats; excretion of quinaldic acid as quinaldylglycyltaurine and quinaldylglycylglycine in the urine of the cat, but not of the rat or rabbit; and conversion of furfural to furylacrylic acid in the dog and rabbit, but not in the rat, hen, or human. The dog and

human but not the guinea pig, hamster, rabbit, or rat excrete the carcinogen 2-naphthyl hydroxylamine as a metabolite of 2-naphthylamine, which, as a result, has carcinogenic activity in the bladder of humans and dogs.

In vitro Metabolism. Numerous variables simultaneously modulate the in vivo metabolism of xenobiotics; therefore their relative importance cannot be studied easily. This problem is alleviated to some extent by in vitro studies of the underlying enzymatic mechanisms responsible for qualitative and quantitative species differences. Quantitative differences may be related directly to the absolute amount of active enzyme present and the affinity and specificity of the enzyme toward the substrate in question. Because many other factors alter enzymatic rates in vitro, caution must be exercised in interpreting data in terms of species variation. In particular, enzymes are often sensitive to the experimental conditions used in their preparation. Because this sensitivity varies from one enzyme to another, their relative effectiveness for a particular reaction can be sometimes miscalculated.

Species variation in the oxidation of xenobiotics, in general, is quantitative (Table 9.4), whereas qualitative differences, such as the apparent total lack of parathion oxidation by lobster hepatopancreas microsomes, are seldom observed. Although the amount of P450 or the activity of NADPH-cytochrome P450 reductase seems to be related to the oxidation of certain substrates, this explanation is not always satisfactory

Table 9.4 Species Variation in Hepatic Microsomal Oxidation of Xenobiotics In vitro

Substrate Oxidation	Rabbit	Rat	Mouse	Guinea Pig	Hamster	Chicken	Trout	Frog
Coumarin 7-hydroxylase[a]	0.86	0.00	0.00	0.45	—	—	—	—
Biphenyl 4-hydroxylase[b]	3.00	1.50	5.70	1.40	3.80	1.70	0.22	1.15
Biphenyl-2-hydroxylase[b]	0.00	0.00	2.20	0.00	1.80	0.00	0.00	1.15
2-Methoxybiphenyl demethylase[a]	5.20	1.80	3.40	2.20	2.30	2.00	0.60	0.40
4-Methoxybiphenyl demethylase[a]	8.00	3.0	3.20	2.30	2.30	1.70	0.40	0.90
p-Nitroanisole *O*-demethylase[b]	2.13	0.32	1.35	—	—	0.76	—	—
2-Ethoxybiphenyl demethylase[a]	5.30	1.60	1.40	2.10	2.50	1.70	0.60	0.40
4-Ethoxybiphenyl demethylase[a]	7.80	2.80	1.80	2.30	1.80	1.50	0.40	0.90
Ethylmorphine *N*-Demethylase[b]	4.0	11.60	13.20	5.40	—	—	—	—
Aldrin epoxidase[b]	0.34	0.45	3.35	—	—	0.46	0.006	—
Parathion desulfurase[b]	2.11	4.19	5.23	8.92	7.75	—	—	—

Source: Modified from A. P. Kulkarni and E. Hodgson, Comparative toxicology, in *Introduction to Biochemical Toxicology*, E. Hodgson and F. E. Guthrie eds., New York: Elsevier, 1980, p. 120.
[a] nmol/mg/h.
[b] nmol/mg/min.

because the absolute amount of cytochrome P450 is not necessarily the rate-limiting characteristic. It is clear that there are multiple forms of P450 isozymes in each species, and that these forms differ from one species to another. Presumably both quantitative and qualitative variation in xenobiotic metabolism depends on the particular isoforms expressed and the extent of this expression.

Reductive reactions, like oxidation, are carried out at different rates by enzyme preparations from different species. Microsomes from mammalian liver are 18 times or more higher in azoreductase activity and more than 20 times higher in nitroreductase activity than those from fish liver. Although relatively inactive in nitroreductase, fish can reduce the nitro group of parathion, suggesting multiple forms of reductase enzymes.

Hydration of epoxides catalyzed by epoxide hydrolase is involved in both detoxication and intoxication reactions. With high concentrations of styrene oxide as a substrate, the relative activity of hepatic microsomal epoxide hydrolase in several animal species is rhesus monkey > human = guinea pig > rabbit > rat > mouse. With some substrates, such as epoxidized lipids, the cytosolic hydrolase may be much more important than the microsomal enzyme.

Blood and various organs of humans and other animals contain esterases capable of acetylsalicylic acid hydrolysis. A comparative study has shown that the liver is the most active tissue in all animal species studied except for the guinea pig, in which the kidney is more than twice as active as the liver. Human liver is least active; the enzyme in guinea pig liver is the most active. The relatively low toxicity of some of the new synthetic pyrethroid insecticides appears to be related to the ability of mammals to hydrolyze their carboxyester linkages. Thus mouse liver microsomes catalyzing (+)-*trans*-resmethrin hydrolysis are more than 30-fold more active than insect microsomal preparations. The relative rates of hydrolysis of this substrate in enzyme preparations from various species are mouse >> milkweed bug >> cockroach >> cabbage looper > housefly.

The toxicity of the organophosphorus insecticide dimethoate depends on the rate at which it is hydrolyzed in vivo. This toxicant undergoes two main metabolic detoxication reactions, one catalyzed by an esterase and the other by an amidases. Although rat and mouse liver carry out both reactions, only the amidase occurs in sheep liver and the esterase in guinea pig liver. The ability of liver preparations from different animal species to degrade dimethoate is as follows: rabbit > sheep > dog > rat > cattle > hen > guinea pig > mouse > pig, these rates being roughly inversely proportioned to the toxicity of dimethoate to the same species. Insects degrade this compound much more slowly than do mammals and hence are highly susceptible to dimethoate.

Hepatic microsomes of several animal species possess UDP glucuronyltransferase activity and with *p*-nitrophenol as a substrate, a 12-fold difference in activity due to species variation is evident. Phospholipase-A activates the enzyme and results of activation experiments indicate that the amount of constraint on the activity of this enzyme is variable in different animal species.

Glutathione *S*-transferase in liver cytosol from different animal species also shows a wide variation in activity. Activity is low in humans, whereas the mouse and guinea pig appear to be more efficient than other species. The ability of the guinea pig to form the initial glutathione conjugate contrasts with its inability to readily *N*-acetylate cysteine conjugates; consequently mercapturic acid excretion is low in guinea pigs.

9.4.2 Selectivity

Selective toxic agents have been developed to protect crops, animals of economic importance, and humans from the vagaries of pests, parasites, and pathogens. Such selectivity is conferred primarily through distribution and comparative biochemistry.

Selectivity through differences in uptake permits the use of an agent toxic to both target and nontarget cells provided that lethal concentrations accumulate only in target cells, leaving nontarget cells unharmed. An example is the accumulation of tetracycline by bacteria but not by mammalian cells, the result being drastic inhibition of protein synthesis in the bacteria, leading to death.

Certain schistosome worms are parasitic in humans and their selective destruction by antimony is accounted for by the differential sensitivity of phosphofructokinase in the two species, the enzyme from schistosomes being more susceptible to inhibition by antimony than is the mammalian enzyme.

Sometimes both target and nontarget species metabolize a xenobiotic by the same pathways but differences in rate determine selectivity. Malathion, a selective insecticide, is metabolically activated by P450 enzymes to the cholinesterase inhibitor malaoxon. In addition to this activation reaction, several detoxication reactions also occur. Carboxylesterase hydrolyzes malathion to form the monoacid, phosphatases hydrolyze the P–O–C linkages to yield nontoxic products, and glutathione *S*-alkyltransferase converts malathion to desmethylmalathion. Although all of these reactions occur in both insects and mammals, activation is rapid in both insects and mammals, whereas hydrolysis to the monoacid is rapid in mammals but slow in insects. As a result malaoxon accumulates in insects but not in mammals, resulting in selective toxicity.

A few examples are also available in which the lack of a specific enzyme in some cells in the human body has enabled the development of a therapeutic agent. For example, guanine deaminase is absent from the cells of certain cancers but is abundant in healthy tissue; as a result 8-azaguanine can be used therapeutically.

Distinct differences in cells with regard to the presence or absence of target structures or metabolic processes also offer opportunities for selectivity. Herbicides such as phenylureas, simazine, and so on, block the Hill reaction in chloroplasts, thereby killing plants without harm to animals. This is not always the case because paraquat, which blocks photosynthetic reactions in plants, is a pulmonary toxicant in mammals, due apparently to analogous free-radical reactions (see Figure 18.4) involving enzymes different from those involved in photosynthesis.

9.4.3 Genetic Differences

Just as the xenobiotic-metabolizing ability in different animal species seems to be related to evolutionary development and therefore to different genetic constitution, different strains within a species may differ from one another in their ability to metabolize xenobiotics. One reason for differences among strains is that many genes are polymorphic, or exist in multiple forms. A polymorphism is defined as an inherited monogenetic trait that exists in the population in at least two genotypes (two or more stable alleles) and is stably inherited. They arise as the result of a mutational event, and generally result in an altered gene product. The frequency of genetic polymorphisms is arbitrarily defined as having a population frequency of greater than 1%. Many polymorphisms are somewhat race specific, arising with greater frequency in one race than in another.

Observed differences between strains of rats and mice, as described below, may be the result of gene polymorphisms. In cases involving insecticide selection pressure, resistant populations may arise as a result of direct mutations of insecticide-metabolizing enzymes and/or insecticide target sites that are passed on to succeeding generations.

In vivo Toxicity. The toxicity of organic compounds has been found to vary between different strains of laboratory animals. For example, mouse strain C3H is resistant to histamine, the LD50 being 1523 mg/kg in C3H/Jax mice as compared with 230 in Swiss/ICR mice; that is, the animals of the former strain are 6.6 times less susceptible to the effects of histamine. Striking differences in the toxicity of thiourea, a compound used in the treatment of hyperthyroidism, are seen in different strains of the Norway rat. Harvard rats were 11 times more resistant, and wild Norway rats were 335 times more resistant than were rats of the Hopkins strain.

The development of strains resistant to insecticides is an extremely widespread phenomenon that is known to have occurred in more than 200 species of insects and mites, and resistance of up to several 100-fold has been noted. The different biochemical and genetic factors involved have been studied extensively and well characterized. Relatively few vertebrate species are known to have developed pesticide resistance and the level of resistance in vertebrates is low compared to that often found in insects. Susceptible and resistant strains of pine voles exhibit a 7.4-fold difference in endrin toxicity. Similarly pine mice of a strain resistant to endrin were reported to be 12-fold more tolerant than a susceptible strain. Other examples include the occurrence of organochlorine insecticide-resistant and susceptible strains of mosquito fish, and resistance to Belladonna in certain rabbit strains.

Several genetic polymorphisms have been recently described and characterized with respect to CYP enzymes. The first and best known example involves CYP2D6. In the course of a clinical trial for debrisoquine, a potential drug for use in lowering blood pressure, Dr. Robert Smith, one of the investigators who used himself as a volunteer, developed severe orthostatic hypotension with blood pressure dropping to 70/50. The effects of the drug persisted for two days, while in other volunteers no adverse effects were noted. Urine analysis demonstrated that in Dr. Smith, debrisoquine was excreted unchanged, while in the other volunteers the primary metabolite was 4-hydroxy debrisoquine. Subsequent studies demonstrated that CYP2D6 was responsible for the formation of 4-hydroxy debrisoquine and that the polymorphic form of 2D6 is prevalent in Caucasians and African-Americans, in which approximately 7% are poor metabolizers. In Asian populations the frequency of poor metabolizers is only 1%.

Another well-known genetic polymorphism has been described in the metabolism of drugs such as isoniazid. "Slow acetylators" are homozygous for a recessive gene; this is believed to lead to the lack of the hepatic enzyme acetyltransferase, which in normal homozygotes or heterozygotes (rapid acetylators) acetylates isoniazid as a step in the metabolism of this drug. This effect is seen also in humans, the gene for slow acetylation showing marked differences in distribution between different human populations. It is very low in Eskimos and Japanese, with 80% to 90% of these populations being rapid acetylators, whereas 40% to 60% of African and some European populations are rapid acetylators. Rapid acetylators often develop symptoms of hepatotoxicity and polyneuritis at the dosage necessary to maintain therapeutic blood levels of isoniazid.

Many other significant polymorphisms in xenobiotic metabolizing enzymes have been described, including those for several CYP genes, alcohol and aldehyde dehydrogenases, epoxide hydrolase, and paraoxonase. One interesting polymorphism affecting

metabolism of dietary trimethylamines involves FMO3. Individuals with FMO3 polymorphisms have a condition known as fish odor syndrome, or trimethylaminurea. Individuals with this syndrome exhibit an objectionable body odor resembling rotting fish due to their inability to *N*-oxidize trimethylamines, which are found in many foods including meat, eggs, and soybeans. This syndrome often leads to social isolation, clinical depression, and even suicide. Other toxicological implications of this polymorphism are still not known.

Metabolite Production. Strain variations to hexobarbital are often dependant on its degradation rate. For example, male mice of the AL/N strain are long sleepers, and this trait is correlated with slow inactivation of the drug. The reverse is true in CFW/N mice, which have short sleeping time due to rapid hexobarbital oxidation. This close relationship is further evidenced by the fact that the level of brain hexobarbital at awakening is essentially the same in all stains. Similar strain differences have been reported for zoxazolamine paralysis in mice.

Studies on the induction of arylhydrocarbon hydroxylase by 3-methylcholanthrene have revealed several responsive and nonresponsive mouse strains, and it is now well established that the induction of this enzyme is controlled by a single gene. In the accepted nomenclature, Ah^b represents the allele for responsiveness, whereas Ah^d denotes the allele for nonresponsiveness.

In rats, both age and gender seem to influence strain variation in xenobiotic metabolism. Male rats exhibit about twofold variation between strains in hexobarbital metabolism, whereas female rats may display up to sixfold variation. In either gender the extent of variations depend on age. The ability to metabolize hexobarbital is related to the metabolism of other substrates and the interstrain differences are maintained.

A well-known interstrain difference in phase II reactions is that of glucuronidation in Gunn rats. This is a mutant strain of Wistar rats that is characterized by a severe, genetically determined defect of bilirubin glucuronidation. Their ability to glucuronidate *o*-aminophenol, o-aminobenzoic acid, and a number of other substrates is also partially defective. This deficiency does not seem to be related to an inability to form UDPGA but rather to the lack of a specific UDP glucuronosyl-transferase. It has been demonstrated that Gunn rats can conjugate aniline by *N*-glucuronidation and can form the *O*-glucuronide of *p*-nitrophenol.

Rabbit strains may exhibit up to 20-fold variation, particularly in the case of hexobarbital, amphetamine, and aminopyrine metabolism. Relatively smaller differences between strains occur with chlorpromazine metabolism. Wild rabbits and California rabbits display the greatest differences from other rabbit strains in hepatic drug metabolism.

Enzyme Differences. Variation in the nature and amount of constitutively expressed microsomal P450s have not been studied extensively in different strains of the same vertebrate. The only thorough investigations, those of the Ah Locus, which controls aryl hydrocarbon hydroxylase induction, have shown that in addition to quantitative differences in the amount of P450 after induction in different strains of mice, there may also be a qualitative difference in the P450 isozymes induced (see Section 9.5.2).

9.5 CHEMICAL EFFECTS

With regard to both logistics and scientific philosophy, the study of the metabolism and toxicity of xenobiotics must be initiated by considering single compounds. Unfortunately, humans and other living organisms are not exposed in this way; rather, they are exposed to many xenobiotics simultaneously, involving different portals of entry, modes of action, and metabolic pathways. Some estimation of the number of chemicals in use in the United States are given in Table 9.5. Because it bears directly on the problem of toxicity-related interaction among different xenobiotics, the effect of chemicals on the metabolism of other exogenous compounds is one of the more important areas of biochemical toxicology.

Xenobiotics, in addition to serving as substrates for a number of enzymes, may also serve as inhibitors or inducers of these or other enzymes. Many examples are known of compounds that first inhibit and subsequently induce enzymes such as the microsomal monooxygenases. The situation is even further complicated by the fact that although some substances have an inherent toxicity and are detoxified in the body, others without inherent toxicity can be metabolically activated to potent toxicants. The following examples are illustrative of the situations that might occur involving two compounds:

- Compound A, without inherent toxicity, is metabolized to a potent toxicant. In the presence of an inhibitor of its metabolism, there would be a reduction in toxic effect.
- Compound A, given after exposure to an inducer of the activating enzymes, would appear more toxic.
- Compound B, a toxicant, is metabolically detoxified. In the presence of an inhibitor of the detoxifying enzymes, there would be an increase in the toxic effect.
- Compound B, given after exposure to an inducer of the detoxifying enzymes, would appear less toxic.

In addition to the previously mentioned cases, the toxicity of the inhibitor or inducer, as well as the time dependence of the effect, must also be considered because, as

Table 9.5 Some Estimates of the Number of Chemicals in Use in the United States

Number	Type	Source of Estimate[a]
1500	Active ingredients of pesticides	EPA
4000	Active ingredients of drugs	FDA
2000	Drug additives (preservatives, stabilizers, etc.)	FDA
2500	Food additives (nutritional value)	FDA
3000	Food additives (preservatives, stabilizers, etc.)	FDA
50,000	Additional chemicals in common use	EPA

[a]EPA, Environmental Protection Agency; FDA, Food and Drug Administration.

mentioned, many xenbiotics that are initially enzyme inhibitors ultimately become inducers.

9.5.1 Inhibition

As previously indicated, inhibition of xenobiotic-metabolizing enzymes can cause either an increase or a decrease in toxicity. Several well-known inhibitors of such enzymes are shown in Figure 9.6 and are discussed in this section. Inhibitory effects can be demonstrated in a number of ways at different organizational levels.

Types of Inhibition: Experimental Demonstration

In vivo Symptoms. The measurement of the effect of an inhibitor on the duration of action of a drug in vivo is the most common method of demonstrating its action. These methods are open to criticism, however, because effects on duration of action can be mediated by systems other than those involved in the metabolism of the drug. Furthermore they cannot be used for inhibitors that have pharmacological activity similar or opposite to the compound being used.

Previously the most used and most reliable of these tests involved the measurement of effects on the hexobarbital sleeping time and the zoxazolamine paralysis time. Both of these drugs are fairly rapidly deactivated by the hepatic microsomal monooxygenase

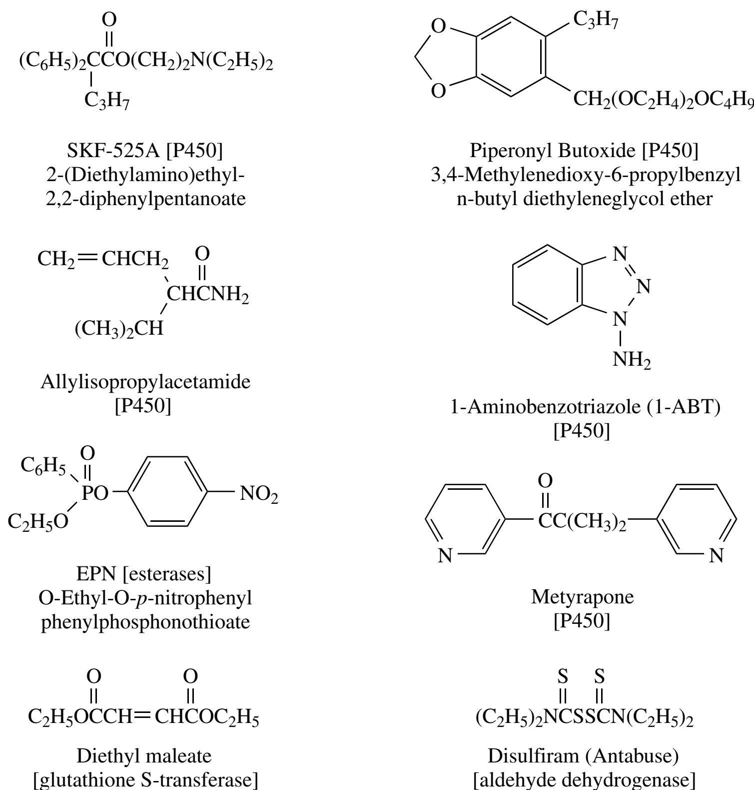


Figure 9.6 Some common inhibitors of xenobiotic-metabolizing enzymes.

system; thus, inhibitors of this system prolong their action. For example, treatment of mice with chloramphenicol 0.5 to 1.0 hour before pentobarbital treatment prolongs the duration of the pentobarbital sleeping time in a dose-related manner; it is effective at low doses (< 5 mg/kg) and has a greater than 10-fold effect at high doses (100–200 mg/kg). The well-known inhibitor of drug metabolism, SKF-525A (Figure 9.6), causes an increase in both hexobarbital sleeping time and zoxazolamine paralysis time in rats and mice, as do the insecticide synergists piperonyl butoxide and tropital, the optimum pretreatment time being about 0.5 hour before the narcotic is given. As a consequence of the availability of single expressed isoforms for direct studies of inhibitory mechanisms, these methods are now used much less often.

In the case of activation reactions, such as the activation of the insecticide azinphosmethyl to its potent anticholinesterase oxon derivative, a decrease in toxicity is apparent when rats are pretreated with the P450 inhibitor SKF-525A.

Cocarcinogenicity may also be an expression of inhibition of a detoxication reaction, as in the case of the cocarcinogenicity of piperonyl butoxide, a P450 inhibitor, and the carcinogens, freons 112 and 113.

Distribution and Blood Levels. Treatment of an animal with an inhibitor of foreign compound metabolism may cause changes in the blood levels of an unmetabolized toxicant and/or its metabolites. This procedure may be used in the investigation of the inhibition of detoxication pathways; it has the advantage over in vitro methods of yielding results of direct physiological or toxicological interest because it is carried out in the intact animal. For example, if animals are first treated with either SKF-525A, glutethimide, or chlorcyclizine, followed in 1 hour or less by pentobarbital, it can be shown that the serum level of pentobarbital is considerably higher in treated animals than in controls within 1 hour of its injection. Moreover the time sequence of the effects can be followed in individual animals, a factor of importance when inhibition is followed by induction—a not uncommon event.

Effects on Metabolism In vivo. A further refinement of the previous technique is to determine the effect of an inhibitor on the overall metabolism of a xenobiotic in vivo, usually by following the appearance of metabolites in the urine and/or feces. In some cases the appearance of metabolites in the blood or tissue may also be followed. Again, the use of the intact animal has practical advantages over in vitro methods, although little is revealed about the mechanisms involved.

Studies of antipyrine metabolism may be used to illustrate the effect of inhibition on metabolism in vivo; in addition, these studies have demonstrated variation among species in the inhibition of the metabolism of xenobiotics. In the rat, a dose of piperonyl butoxide of at least 100 mg/kg was necessary to inhibit antipyrine metabolism, whereas in the mouse a single intraperitoneal (IP) or oral dose of 1 mg/kg produced a significant inhibition. In humans an oral dose of 0.71 mg/kg had no discernible effect on the metabolism of antipyrine.

Disulfiram (Antabuse) inhibits aldehyde dehydrogenase irreversibly, causing an increase in the level of acetaldehyde, formed from ethanol by the enzyme alcohol dehydrogenase. This results in nausea, vomiting, and other symptoms in the human—hence its use as a deterrent in alcoholism. Inhibition by disulfiram appears to be irreversible, the level returning to normal only as a result of protein synthesis.

Use of specific metabolic enzyme inhibitors may often provide valuable information with respect to the metabolism of a particular drug. For example, quinidine is a potent

and selective inhibitor of CYP2D6. This drug has been used in clinical studies as a pharmacological tool to mimic the lack of CYP2D6 in humans. By demonstrating that quinidine substantially slows the metabolism of trimipramine (a tricyclic antidepressant), investigators have implicated CYP2D6 in its metabolism.

Effects on In vitro Metabolism Following In vivo Treatment. This method of demonstrating inhibition is of variable utility. The preparation of enzymes from animal tissues usually involves considerable dilution with the preparative medium during homogenization, centrifugation, and re-suspension. As a result inhibitors not tightly bound to the enzyme in question are lost, either in whole or in part, during the preparative processes. Therefore negative results can have little utility because failure to inhibit and loss of the inhibitor give identical results. Positive results, however, not only indicate that the compound administered is an inhibitor but also provide a clear indication of excellent binding to the enzyme, most probably due to the formation of a covalent or slowly reversible inhibitory complex. The inhibition of esterases following treatment of the animal with organophosphorus compounds, such as paraoxon, is a good example, because the phosphorylated enzyme is stable and is still inhibited after the preparative procedures. Inhibition by carbamates, however, is greatly reduced by the same procedures because the carbamylated enzyme is unstable and, in addition, the residual carbamate is highly diluted.

Microsomal monooxygenase inhibitors that form stable inhibitory complexes with P450, such as SKF-525A, piperonyl butoxide, and other methylenedioxphenyl compounds, and amphetamine and its derivatives, can be readily investigated in this way. This is because the microsomes isolated from pretreated animals have a reduced capacity to oxidize many xenobiotics.

Another form of chemical interaction, resulting from inhibition in vivo, that can then be demonstrated in vitro involves those xenobiotics that function by causing destruction of the enzyme in question, so-called suicide substrates. Exposure of rats to vinyl chloride results in a loss of cytochrome P450 and a corresponding reduction in the capacity of microsomes subsequently isolated to metabolize foreign compounds. Allyl isopropylacetamide and other allyl compounds have long been known to have a similar effect.

In vitro Effects. In vitro measurement of the effect of one xenobiotic on the metabolism of another is by far the most common type of investigation of interactions involving inhibition. Although it is the most useful method for the study of inhibitory mechanisms, particularly when purified enzymes are used, it is of limited utility in assessing the toxicological implications for the intact animal. The principal reason for this is that in vitro measurement does not assess the effects of factors that affect absorption, distribution, and prior metabolism, all of which occur before the inhibitory event under consideration.

Although the kinetics of inhibition of xenobiotic-metabolizing enzymes can be investigated in the same ways as any other enzyme mechanism, a number of problems arise that may decrease the value of this type of investigation. They include the following:

- The P450 system, a particulate enzyme system, has been investigated many times, but using methods developed for single soluble enzymes. As a result Lineweaver-Burke or other reciprocal plots are frequently curvilinear, and the same reaction may appear to have quite a different characteristics from laboratory to laboratory, species to species, and organ to organ.

- The nonspecific binding of substrate and/or inhibitor to membrane components is a further complicating factor affecting inhibition kinetics.
- Both substrates and inhibitors are frequently lipophilic, with low solubility in aqueous media.
- Xenobiotic-metabolizing enzymes commonly exist in multiple forms (e.g., glutathione *S*-transferases and P450s). These isozymes are all relatively nonspecific but differ from one another in the relative affinities of the different substrates.

The primary considerations in studies of inhibition mechanisms are reversibility and selectivity. The inhibition kinetics of reversible inhibition give considerable insight into the reaction mechanisms of enzymes and, for that reason, have been well studied. In general, reversible inhibition involves no covalent binding, occurs rapidly, and can be reversed by dialysis or, more rapidly, by dilution. Reversible inhibition is usually divided into competitive inhibition, uncompetitive inhibition, and noncompetitive inhibition. Because these types are not rigidly separated, many intermediate classes have been described.

Competitive inhibition is usually caused by two substrates competing for the same active site. Following classic enzyme kinetics, there should be a change in the apparent K_m but not in V_{max}. In microsomal monooxygenase reaction, type I ligands, which often appear to bind as substrates but do not bind to the heme iron, might be expected to be competitive inhibitors, and this frequently appears to be the case. Examples are the inhibition of the *O*-demethylation of *p*-nitronanisole by aminopyrine, aldrin epoxidation by dihydroaldrin, and *N*-demethylation of aminopyrine by nicotinamide. More recently some of the polychlorinated biphenyls (PCBs), notably dichlorbiphenyl, have been shown to have a high affinity as type I ligands for rabbit liver P450 and to be competitive inhibitors of the *O*-demethylation of *p*-nitronanisole.

Uncompetitive inhibition has seldom been reported in studies of xenobiotic metabolism. It occurs when an inhibitor interacts with an enzyme-substrate complex but cannot interact with free enzyme. Both K_m and V_{max} change by the same ratio, giving rise to a family of parallel lines in a Lineweaver-Burke plot.

Noncompetitive inhibitors can bind to both the enzyme and enzyme-substrate complex to form either an enzyme-inhibitor complex or an enzyme-inhibitor-substrate complex. The net result is a decrease in V_{max} but no change in K_m. Metyrapone (Figure 9.6), a well-known inhibitor of monooxygenase reactions, can also, under some circumstances, stimulate metabolism in vitro. In either case the effect is noncompetitive, in that the K_m does not change, whereas V_{max} does, decreasing in the case of inhibition and increasing in the case of stimulation.

Irreversible inhibition, which is much more important toxicologically, can arise from various causes. In most cases the formation of covalent or other stable bonds or the disruption of the enzyme structure is involved. In these cases the effect cannot be readily reversed in vitro by either dialysis or dilution. The formation of stable inhibitory complexes may involve the prior formation of a reactive intermediate that then interacts with the enzyme. An excellent example of this type of inhibition is the effect of the insecticide synergist piperonyl butoxide (Figure 9.6) on hepatic microsomal monooxygenase activity. This methylenedioxyphenyl compound can form a stable inhibitory complex that blocks CO binding to P450 and also prevents substrate oxidation. This complex results from the formation of a reactive intermediate, which is shown by the fact that the type of inhibition changes from competitive to irreversible as metabolism, in the

presence of NADPH and oxygen, proceeds. It appears probable that the metabolite in question is a carbine formed spontaneously by elimination of water following hydroxylation of the methylene carbon by the cytochrome (see Figure 7.8 for metabolism of methylenedioxyphenyl compounds). Piperonyl butoxide inhibits the in vitro metabolism of many substrates of the monooxygenase system, including aldrin, ethylmorphine, aniline, and aminopyrine, as well as carbaryl, biphenyl, hexobarbital, and *p*-nitroanisole among many others. Although most of the studies carried out on piperonyl butoxide have involved rat or mouse liver microsomes, they have also been carried out on pig, rabbit, and carp liver microsomes, and in various preparations from houseflies, cockroaches, and other insects. Certain classes of monooxygenase inhibitors, in addition to methylenedioxyphenyl compounds, are now known to form "metabolite inhibitory complexes," including amphetamine and its derivatives and SKF-525A and its derivatives.

The inhibition of the carboxylesterase that hydrolyzes malathion by organophosphorus compounds, such as EPN is a further example of xenobiotic interaction resulting from irreversible inhibition. In this case the enzyme is phosphorylated by the inhibitor.

Another class of irreversible inhibitors of toxicological significance consists of those compounds that bring about the destruction of the xenobiotic-metabolizing enzymes, hence the designation "suicide substrates." The drug allylisopropylacetamide (Figure 9.6), as well as other allyl compounds, has long been known to cause the breakdown of P450 and the resultant release of heme. More recently the hepatocarcinogen vinyl chloride has also been shown to have a similar effect, probably also mediated through the generation of a highly reactive intermediate (see Figure 8.2). Much information has accumulated since the mid-1970s on the mode of action of the hepatotoxicant carbon tetrachloride, which effects a number of irreversible changes in both liver proteins and lipids, such changes being generated by reactive intermediates formed during its metabolism (Figure 8.3).

The less specific disruptors of protein structure, such as urea, detergents, strong acids, and so on, are probably of significance only in vitro experiments.

Synergism and Potentiation. The terms synergism and potentiation have been used and defined in various ways, but in any case, they involve a toxicity that is greater when two compounds are given simultaneously or sequentially than would be expected from a consideration of the toxicities of the compounds given alone. Some toxicologists have used the term synergism for cases that fit this definition, but only when one compound is toxic alone whereas the other has little or no intrinsic toxicity. For example, the nontoxic synergist, piperonyl butoxide is often included in pesticide formulations because of its ability to significantly increase the toxicity of the active pesticide ingredient by inhibiting its detoxication in the target species.

The term potentiation is then reserved for those cases where both compounds have appreciable intrinsic toxicity, such as in the case of malathion and EPN. Malathion has a low mammalian toxicity due primarily to its rapid hydrolysis by a carboxylesterase. EPN (Figure 9.6) another organophosphate insecticide, causes a dramatic increase in malathion toxicity to mammals at dose levels, which, given alone, cause essentially no inhibition of acetylcholinesterase. The increase in toxicity as a result of coadministration of these two toxicants is the result of the ability of EPN, at low concentrations, to inhibit the carboxylesterase responsible for malathion degradation.

Unfortunately, the terms synergist and potentiation have often been used by some toxicologists in precisely the opposite manner. Historically, the term synergist has

been used by pharmacologists to refer to simple additive toxicity and potentiation either as a synonym or for examples of greater than additive toxicity or efficacy. In an attempt to make uniform the use of these terms, it is suggested that insofar as toxic effects are concerned, the terms be used according to the following: *Both synergism and potentiation involve toxicity greater than would be expected from the toxicities of the compounds administered separately, but in the case of synergism one compound has little or no intrinsic toxicity when administered alone, whereas in the case of potentiation both compounds have appreciable toxicity when administered alone. It is further suggested that no special term is needed for simple additive toxicity of two or more compounds.*

Antagonism. In toxicology, antagonism may be defined as that situation where the toxicity of two or more compounds administered together or sequentially is less than would be expected from a consideration of their toxicities when administered individually. Strictly speaking, this definition includes those cases in which the lowered toxicity results from induction of detoxifying enzymes (this situation is considered separately in Section 9.5.2). Apart from the convenience of treating such antagonistic phenomena together with the other aspects of induction, they are frequently considered separately because of the significant time that must elapse between treatment with the inducer and subsequent treatment with the toxicant. The reduction of hexobarbital sleeping time and the reduction of zoxazolamine paralysis time by prior treatment with phenobarbital to induce drug—metabolizing enzymes are obvious examples of such induction effects at the acute level of drug action, whereas protection from the carcinogenic action of benzo(a)pyrene, aflatoxin B1, and diethylnitrosamine by phenobarbital treatment are examples of inductive effects at the level of chronic toxicity. In the latter case the P450 isozymes induced by phenobarbital metabolize the chemical to less toxic metabolites.

Antagonism not involving induction is a phenomenon often seen at a marginal level of detection and is consequently both difficult to explain and of marginal significance. In addition several different types of antagonism of importance to toxicology that do not involve xenobiotic metabolism are known but are not appropriate for discussion in this chapter. They include competition for receptor sites, such as the competition between CO and O_2 in CO poisoning or situations where one toxicant combines nonenzymatically with another to reduce its toxic effects, such as in the chelation of metal ions. Physiological antagonism, in which two agonists act on the same physiological system but produce opposite effects, is also of importance.

9.5.2 Induction

In the early 1960s, during investigations on the N-demethylation of aminoazo dyes, it was observed that pretreatment of mammals with the substrate or, more remarkably, with other xenobiotics, caused an increase in the ability of the animal to metabolize these dyes. It was subsequently shown that this effect was due to an increase in the microsomal enzymes involved. A symposium in 1965 and a landmark review by Conney in 1967 established the importance of induction in xenobiotic interactions. Since then, it has become clear that this phenomenon is widespread and nonspecific. Several hundred compounds of diverse chemical structure have been shown to induce monooxygenases and other enzymes. These compounds include drugs, insecticides, polycyclic hydrocarbons, and many others; the only obvious common denominator

is that they are organic and lipophilic. It has also become apparent that although all inducers do not have the same effects, the effects tend to be nonspecific to the extent that any single inducer induces more than one enzymatic activity. Other enzymes often coinduced by P450 inducers include glutathione S-transferase, epoxide hydrolases and others; perhaps as a result of general induction of cellular processes including proliferative responses of endoplasmic reticulum, peroxisomes, and mitochondria.

Specificity of Monooxygenase Induction. The majority of studies involving monooxygenase induction have been conducted in mammals. Mammals have at least 17 distinct CYP families, coding for as many as 50 to 60 individual CYP genes in any given species. Many of these CYP families are fairly specific for endogenous metabolic pathways and are not typically involved in metabolism of foreign chemicals. As discussed in Chapter 7, CYP families 1–4 are the predominant families involved in xenobiotic metabolism. These CYP families are also known for their ability to respond to xenobiotic challenges by increasing their protein levels. Many of the genes within families 1–4 are transcriptionally activated through one of four receptor-dependent mechanisms. Others, such as CYP2E1, are regulated at the level of mRNA stabilization and/or protein stabilization. These mechanisms of regulation are discussed later in this section.

Inducers of monooxygenase activity fall into four principle classes, exemplified by TCDD (inducer of CYP1A1), phenobarbital (inducer of the CYP2B and 3A families), rifampicin (inducer of CYP3A and 2C families), and ethanol (inducer of 2E1). Inducers of the phenobarbital-type tend to share few structural features other than lipophilicity, while TCDD-like inducers are primarily polycyclic hydrocarbons. Other inducers, such as ethanol, dexamethasone, and clofibrate are more specific. Many inducers require either fairly high dose levels or repeated dosing to be effective, frequently >10 mg/kg and some as high as 100 to 200 mg/kg. Some insecticides, however, such as mirex, can induce at dose levels as low as 1 mg/kg, while the most potent inducer known, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is effective at 1μg/kg in some species.

In the liver, phenobarbital-type inducers cause a marked proliferation of the smooth endoplasmic reticulum as well as an increase in the amount of CYP content. Often these changes are sufficient to result in significant liver weight increases. Phenobarbital induction induces a wide range of oxidative activities including *O*-demethylation of *p*-nitroanisole, *N*-demethylation of benzphetamine, pentobarbital hydroxylation, and aldrin hydroxylation. CYP families that are primarily induced by phenobarbital and phenobarbital-like inducers include CYP2B, CYP2C, and CYP3A subfamilies.

In contrast with phenobarbital, induction by TCDD and polycyclic hydrocarbons does not cause proliferation of the endoplasmic reticulum, although the CYP content is increased. CYP1A1 is the primary isoform induced, although other non-CYP proteins such as uridine diphosphoglucuronyl transferase may also be induced. Induction of CYP1A1 by polycyclic hydrocarbons results in the induction of a relatively narrow range of oxidative activities, consisting primarily of reactions involving aryl hydrocarbon hydroxylase, the best-known reaction being the hydroxylation of benzo(a)pyrene.

Rifampicin and pregnenolone-16α-carbonitrile (PCN) induce members of the CYP3A family and represent a third type of inducer, in that the substrate specificity of the microsomes from treated animals differs from that of the microsomes from either phenobarbital-treated or TCDD-treated animals. Inducing substrates of this class include endogenous and synthetic glucocorticoids (e.g., dexamethasone), pregnane

compounds (e.g., pregenenolone 16α-carbonitrile, PCN), and macrolide antibiotics (e.g., rifampicin).

Ethanol and a number of other chemicals, including acetone and certain imidazoles, induce CYP2E1. Piperonyl butoxide, isosafrole, and other methylenedioxyphenyl compounds are known to induce CYP1A2 by a non-Ah receptor-dependent mechanism. Peroxisome proliferators, including the drug, clofibrate, and the herbicide synergist tridiphane induce a CYP4A isozyme that catalyzes the ω-oxidation of lauric acid.

All inducers do not fall readily into one or the other of these classes. Some oxidative processes can be induced by either type of inducer, such as the hydroxylation of aniline and the *N*-demethylation of chlorcyclizine. Some inducers, such as the mixture of PCBs designated Arochlor 1254, can induce a broad spectrum of CYP isoforms. Many variations also exist in the relative stimulation of different oxidative activities within the same class of inducer, particularly of the phenobarbital type.

It appears reasonable that because several types of CYP are associated with the endoplasmic reticulum, various inducers may induce one or more of them. Because each of these types has a relatively broad substrate specificity, differences may be caused by variations in the extent of induction of different cytochromes. Now that methods are available for gel electrophoresis of microsomes and identification of specific isoforms by immunoblotting and isoforms-specific antibodies, the complex array of inductive phenomena is being more logically explained in terms of specific isozymes.

Although the bulk of published investigations of the induction of monooxygenase enzymes have dealt with the mammalian liver, induction has been observed in other mammalian tissues and in nonmammalian species, both vertebrate and invertebrate. Many induced CYPs have now been cloned and/or purified from a variety of species. It is clear that many of these induced CYPs represent only a small percentage of the total CYP in the uninduced animal. For this reason the "constitutive" isozymes, those already expressed in the uninduced animal, must be fully characterized because they represent the available xenobiotic-metabolizing capacity of the normal animal.

Mechanism and Genetics of Induction in Mammals. Many different mechanisms may be involved in CYP induction. These include increased transcription of DNA, increased mRNA translation to protein, mRNA stabilization, and protein stabilization. Induction can only occur in intact cells and cannot be achieved by the addition of inducers directly to cell fractions such as microsomes. It has been known for some time that in most cases of increase in monooxygenase activity there is a true induction involving synthesis of new enzyme, and not the activation of enzyme already synthesized, since induction is generally prevented by inhibitors of protein synthesis. For example, the protein synthesis inhibitors such as puromycin, ethionine, and cycloheximide inhibit aryl hydrocarbon hydroxylase activity. A simplified scheme for gene expression and protein synthesis is shown in Figure 9.7.

Perhaps the best understood example of induction involves induction of the aromatic hydrocarbon receptor (AhR) by compounds such as TCDD and 3-methylcholanthrene. The use of suitable inhibitors of RNA and DNA polymerase activity has shown that inhibitors of RNA synthesis such as actinomycin D and mercapto(pyridethyl)benzimidazole block aryl hydrocarbon hydroxylase induction, whereas hydroxyurea, at levels that completely block the incorporation of thymidine into DNA, has no effect. Thus it appears that the inductive effect is at the level of transcription and that DNA synthesis is not required.

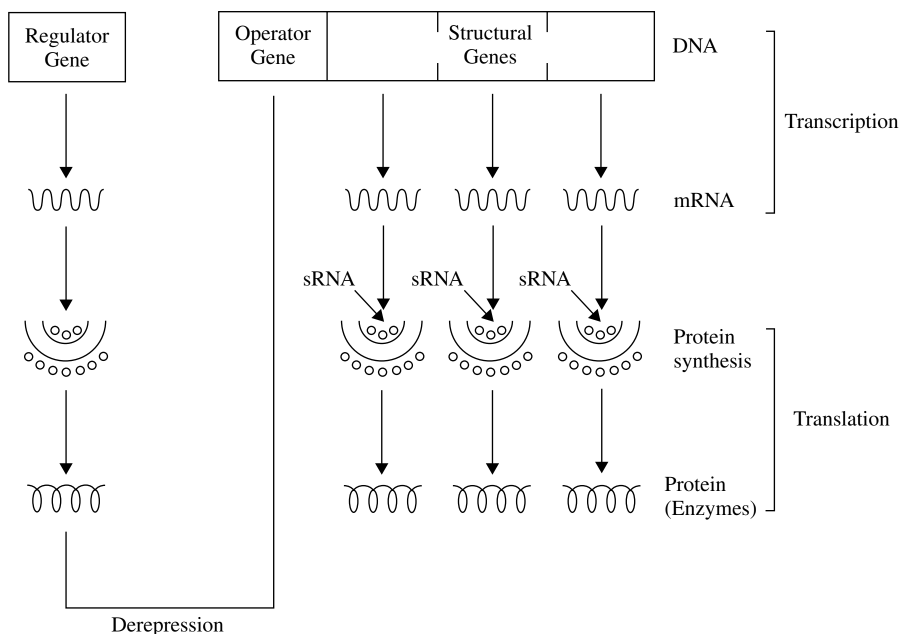


Figure 9.7 Simplified scheme for gene expression in animals.

These findings imply that compounds that induce xenobiotic-metabolizing enzymes play a role as derepressors of regulator or other genes in a manner analogous to steroid hormones—namely by combining with a cytosolic receptor followed by movement into the nucleus and then derepression of the appropriate gene. In the case of the AhR, TCDD, or some other appropriate ligand enters the cell through the plasma membrane and binds to the cytosolic Ah receptor protein (Figure 9.8). After ligand binding, the receptor translocates to the nucleus where it forms a dimer with another protein known as ARNT. In the nucleus the transformed receptor interacts with specific sequences of DNA known as xenobiotic responsive elements (XREs). Two XREs are located approximately 1000 or more base pairs upstream from the transcriptional start site in the 5′ flanking region of the CYP1A1 gene. A third site is likely to be an inhibitory or suppressor site (Figure 9.9). The protein-DNA interaction that occurs at the XREs is thought to result in a bending of the DNA, which allows for increased transcription followed by increased protein synthesis. Another promoter region is located just upstream from the transcriptional start site. Although several transcription factors may interact with this binding site, including the TATA-binding protein, it has no binding sites for the AhR/Arnt proteins. Transfection experiments indicate that the TATA-binding site is essential for promoter function, while the other sites are less important. This promoter region is silent unless the upstream XREs have been appropriately activated by the AhR and Arnt proteins. The fact that several genes may be responsive to CYP1A inducers is indicative of the fact that similar XREs are found on many Ah-receptor inducible genes.

Although phenobarbital induction has been studied for many years, the mechanism for induction has only recently been established. In bacteria a key feature of phenobarbital induction was demonstrated to involve barbiturate-mediated removal of a

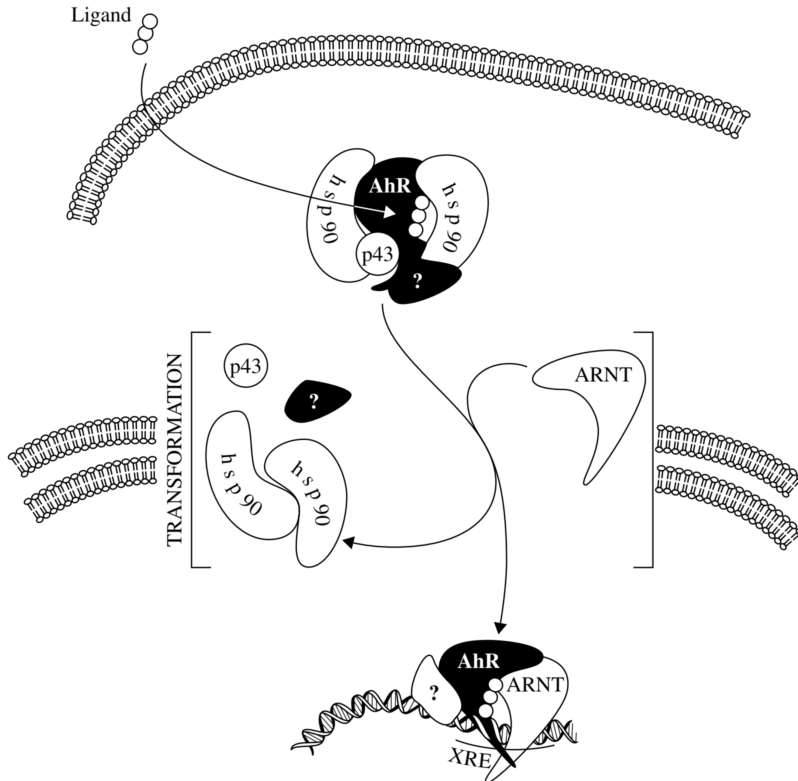


Figure 9.8 Proposed mechanism for ligand-activated AhR translocation and DNA binding (From J. C. Rowlands and J.-A. Gustafsson, *Crit. Rev. Toxicol.* **27**: 109, 1997.)

repressor protein from a 17-bp promoter regulatory sequence known as the "Barbie box." Although homologous promoter sequences have been observed in several phenobarbital responsive mammalian genes, ample evidence suggests that these sequences are not important in PB-induced transcription of mammalian CYP genes. Rather, in mammalian species the phenobarbital responsive sequences are found far upstream of the start codon.

The major advance in understanding phenobarbital induction came from a study using rat primary hepatocytes where phenobarbital responsiveness was demonstrated to be associated with a 163-bp DNA sequence at −2318 through −2155 bp of the CYP2B2 gene. Subsequent studies using in situ transfection of CYP2B2 promoter-luciferase constructs into rat livers confirmed this, as did similar studies involving mouse CYP2b10 and CYP2b9 genes. Additional deletion assays have narrowed phenobarbital responsiveness down to a minimum sequence of 51-bp from −2339 through −2289 of the Cyp2b10 gene; now known as the phenobarbital-responsive enhancer module (PBREM). The PBREM sequence has also been found in rat CYP2B1, CYP2B2, and human CYP2B6 genes. Multiple cis acting elements within this fragment cooperate to bring about increased DNA transcription. These include a nuclear factor 1 (NF1) binding site that is flanked by two nuclear receptor binding sites, designated NR1 and NR2. Inducibility requires at least one of the NR sites to be present to maintain phenobarbital inducibility. By

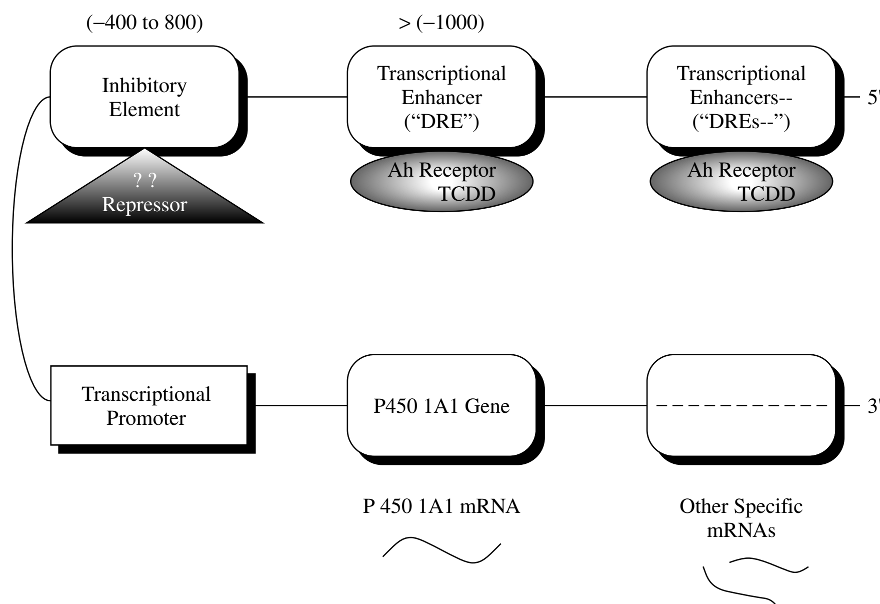


Figure 9.9 Interaction of the Ah-receptor-ligand complex with the 5′ flanking region of the P450 1A1 gene. Two dioxin responsive elements (DREs) appear to lie approximately 1000 or more base pairs upstream from the 1A1 transcriptional s tart site. These elements appear to be transcriptional enhancers, whereas less direct evidence indicates an inhibitory element ("negative control element") between 400 and 800 bases upstream. The negative control element may inhibit the 1A1 promoted although the conditions for this inhibition are, as yet, undefined. (Adapted from A. B. Okey, *Pharmacol. Ther.* **45**: 241–298, 1990.)

contrast, although the NF1 site is necessary for the maximum phenobarbital response, it is nonessential for the basic phenobarbital response. This conclusion is supported by the fact that in rodent and human CYP2B genes the NR sites are highly conserved while the NF1 site is not.

The key factor that interacts with the PBREM is the orphan nuclear receptor known as a "constitutive active receptor" (CAR). CAR binds to each of the PBRE NR sites as a heterodimer with the retinoid X receptor (RXR), a common heterodimerization partner for many orphan nuclear receptors. Although the CAR-RXR binding does not require treatment with phenobarbital for activity, hence the term "constitutive," inclusion of phenobarbital substantially increases the activity of CYP2B and other PBRE related genes. It is thought that this is due to the displacement of two endogenous inhibitory androstane steroids that bind to the CAR-RXR heterodimer and inhibit its activity in the absence of phenobarbital like ligands. Thus, in the presence of phenobarbital, the binding of the inhibitory androstanes to CAR is abolished and the intrinsic activity of CAR becomes manifest, leading to the activation of PB responsive genes. Recent studies using CAR knockout mice indicate that many drug metabolizing genes are under CAR regulation, including isoforms of CYP2B, CYP3A, NADPH cytochrome P450 reductase, and enzymes involved in sulfotransferase metabolism.

In the early 1980s a distinct group of CYPs was described by several groups, which was characterized principally by its inducibility by steroidal chemicals. This particular

group, belonging to the CYP3A subfamily, is well known for the diversity of substrates that it is capable of metabolizing. In humans the specific isoform CYP3A4 is responsible not only for the metabolism of endogenous compounds such as testosterone but also is credited for the metabolism of the largest number of currently used drugs. Many CYP3A substrates are further known for their ability to induce their own metabolism as well as the metabolism of other CYP3A substrates, resulting in the creation of potentially dangerous drug-drug type interactions. Regulation of the CYP3 family is likely to be primarily through enhanced transcription, although there are also some examples of post-translational regulation. For example, dexamethasone appears to increase CYP3A1 levels by stabilization of the mRNA while erythromycin acts by protein stabilization.

Several recent studies have begun to identify several elements on the 5′ upstream promoter region as well as receptors involved in CYP3 regulation (Figure 9.10). Deletion studies involving transfections of various chimeric reporter gene constructs into primary cultures of rat hepatocytes demonstrated the presence of a dexamethasone/PCN response element within the first 164 bp of the start of transcription. Subsequent studies demonstrated that for several CYP3A isoforms from different species contained nuclear receptor binding sites that are activated by DEX/PCN but exhibit low activation by rifampicin. Further work identified an additional 230-bp distal element called the xenobiotic-responsive enhancer module (XREM) located at −7836 through −7607 of the CYP3A4 that conferred responsiveness to both rifampicin and dexamethasone when combined with the proximal promoter region. XREM contains two nuclear receptor

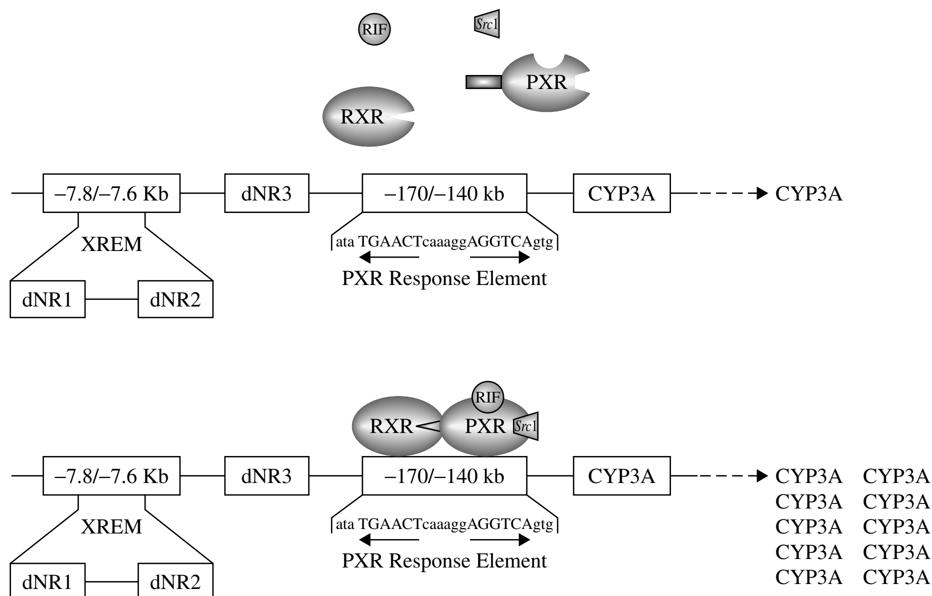


Figure 9.10 Illustration depicting DNA elements found in CYP3A genes and the activation of the human pregnane X receptor (PXR) by ligand (RIF) and subsequent transcriptional activation of CYP3A4 gene by the PXR/RXR heterodimer. dNR-1–3, nuclear receptors 1, 2, and 3, respectively; PXR, pregnane X receptor; RXR, retinoid X receptor; RIF, rifampicin; SRC-1, steroid receptor co-activator; XREM, xenobiotic responsive enhancer module.

binding sites (dNR1 and dNR2), neither of which is solely responsible for the activity of XREM. An additional nuclear receptor site, dNR3 located several hundred bases downstream of XREM also appears to have some importance in induction.

Recent work has demonstrated that the nuclear orphan receptor, pregnane X receptor (PXR) is the major determinant of CYP3A gene regulation by xenobiotics. Several lines of evidence support PXR involvement with CYP3A induction. First, both PXR and CYP3A isoforms are predominantly expressed in liver and intestine, with less expression found in lungs and kidneys. Second, PXR binds to human and rat CYP3A promoter regions and can activate expression of CYP3A4 promoter in transfection assays. Third, many of the same inducers of CYP3A isoforms also activate PXR. Fourth, interspecies differences in response to CYP3A inducers have been demonstrated to be due to the ability of these inducers to activate PXR in these species. Fifth, disruption of the mouse PXR gene eliminated induction of CYP3A by PCN and mice "humanized" with the PXR gene were able to respond to rifampicin induction. These observations suggest that many of the significant differences in CYP3A induction profiles between species may be due to differences in the PXR.

Peroxisome proliferators, including hypolipidemic drugs such as clofibrate, phthalate plasticizers, and herbicides bring about the induction of a CYP4A isoform that catalyzes the oxidation of many biologically important fatty acids, including arachidonic acid and other eicosanoids. CYP4A expression is part of a pleiotropic response in the rodent liver, which includes increased liver weight, proliferation of peroxisomes, and the elevation of several peroxisomal enzymes such as catalase. Peroxisome proliferators are often epigenetic carcinogens in rodents, but since the effect is primarily seen in rodents, its significance for other species such as humans is unclear. The receptor protein peroxisome proliferator-activated receptor-α PPARα was first cloned in 1990. PPARα knockout mice exposed to chemicals that normally induce CYP4A as well as peroxisome proliferation do not exhibit these characteristics, demonstrating the essential nature of PPARα for these responses. Like PXR, PPARα, which is constitutively nuclear, also binds to DNA as a PPARα/RXR heterodimer in response to peroxisome proliferating chemicals.

CYP2E1 catalyzes metabolism of several low molecular weight xenobiotics including drugs (e.g., acetaminophen), solvents (e.g., ethanol and carbon tetrachloride), and procarcinogens (e.g., *N*-nitrosodimethylamine). Induction of CYP2E1 can occur as a result of exposure to several xenobiotics including ethanol, acetone, and imidazole, or alternatively, as a result of physiological conditions such as starvation and diabetes. Its induction by either fasting or diabetes is believed to be due to the high levels of ketones likely to be present in either of these conditions. It might also be noted that although CYP2E1 is in the same family as 2B1 and 2B2, it is not induced by phenobarbital-type inducers. In contrast to many other inducible CYPs, CYP2E1 induction is not accompanied by high levels of CYP2E1 mRNA, suggesting that regulation is by means of post-transcriptional mechanisms.

The regulation of CYP2E1 gene expression involves several mechanisms that do not primarily include increased transcription. Recent studies demonstrated that rapid increases in CYP2E1 protein levels following birth are due to stabilization of preexisting proteins by ketone bodies released at birth. Rats treated with ethanol or acetone can have three- to sixfold increases in CYP2E1 protein in the absence of increased CYP2E1 mRNA. Other studies have demonstrated that substrates including ethanol, imidazole, and acetone had little effects on CYP2E1 transcript content and that these substrates

tend to prevent protein degradation. Thus increased protein expression in response to these substrates may be due to protein stabilization (e.g., decreased turnover), as a result of the inhibition of ubiquitin-mediated proteolysis. The ubiquitination process normally tags proteins with a chain of multiple ubiquitin moieties that can be detected as smears at the tops of SDS gels. The ubiquitin tags allow for the selective degradation of associated proteins by a cytosolic 26S protease, known as the proteasome. In recent studies an antibody prepared against a putative ubiquitination-target site on the CYP2E1 protein quenched ubiquitination in a concentration-dependent manner. The same antibody also prevented catalysis of chlorzoxazone. These results provide a plausible mechanistic explanation for the observation that substrate binding protects the CYP2E1 protein from ubiquitin-dependent proteolysis.

In other observations, diabetes is known to increase CYP2E1 expression at both the mRNA and protein levels in both chemically induced and spontaneous diabetic rats. Elevation of mRNA levels as a result of diabetes has been attributed to mRNA stabilization, which can be reversed by daily insulin treatment. Recent research has shown that insulin destabilizes CYP2E1 mRNA by binding to a 16-bp sequence within the 5′ coding sequence of CYP2E1. The mechanism for regulation by this means is still uncertain, although other genes have also been reported with similar destabilizing sequences within their coding sequences. Many other possibilities for mRNA stabilization/destabilization exist within the 5′ and 3′ untranslated regions of the DNA that are being explored.

Effects of Induction. The effects of inducers are usually the opposite of those of inhibitors; thus their effects can be demonstrated by much the same methods, that is, by their effects on pharmacological or toxicological properties in vivo or by the effects on enzymes in vitro following prior treatment of the animal with the inducer. In vivo effects are frequently reported; the most common ones are the reduction of the hexobarbital sleeping time or zoxazolamine paralysis time. These effects have been reported for numerous inducers and can be quite dramatic. For example, in the rat, the paralysis time resulting from a high dose of zoxazolamine can be reduced from 11 hours to 17 minutes by treatment of the animal with benzo(a)pyrene 24 hours before the administration of zoxazolamine.

The induction of monooxygenase activity may also protect an animal from the effect of carcinogens by increasing the rate of detoxication. This has been demonstrated in the rat with a number of carcinogens including benzo(a)pyrene, *N*-2-fluorenylacetamide, and aflatoxin B_1. Effects on carcinogenesis may be expected to be complex because some carcinogens are both activated and detoxified by monooxygenase enzymes, while epoxide hydrolase, which can also be involved in both activation and detoxication, may also be induced. For example, the toxicity of the carcinogen 2-naphthylamine, the hepatotoxic alkaloid monocrotaline, and the cytotoxin cyclophosphamide are all increased by phenobarbital induction—an effect mediated by the increased population of reactive intermediates.

Organochlorine insecticides are also well-known inducers. Treatment of rats with either DDT or chlordane, for example, will decrease hexobarbital sleeping time and offer protection from the toxic effect of warfarin. Persons exposed to DDT and lindane metabolized antipyrine twice as fast as a group not exposed, whereas those exposed to DDT alone had a reduced half-life for phenylbutazone and increased excretion of 6-hydroxycortisol.

Effects on xenobiotic metabolism in vivo are also widely known in both humans and animals. Cigarette smoke, as well as several of its constituent polycyclic hydrocarbons, is a potent inducer of aryl hydrocarbon hydroxylase in the placenta, liver, and other organs. The average content of CYP1A1 in liver biopsies from smokers was approximately fourfold higher than that from nonsmokers. Hepatic activity of CYP1A1 as measured by phenacetin *O*-deethylation, was also increased from 54 pmol/min/mg of protein in nonsmokers to 230 nmol/min/mg of protein in smokers. Examination of the term placentas of smoking human mothers revealed a marked stimulation of aryl hydrocarbon hydroxylase and related activities—remarkable in an organ that, in the uninduced state, is almost inactive toward foreign chemicals. These in vitro differences in metabolism are also observed in vivo, as smokers have been demonstrated to have increased clearance rates for several drugs metabolized principally by CYP1A1 including theophylline, caffeine, phenacetin, fluvoxamine, clozapine, and olanzapine.

Induction of Xenobiotic-Metabolizing Enzymes Other Than Monooxygenases. Although less well studied, xenobiotic-metabolizing enzymes other than those of the P450 system are also known to be induced, frequently by the same inducers that induce the oxidases. These include glutathione *S*-transferases, epoxide hydrolase, and UDP glucuronyltransferase. The selective induction of one pathway over another can greatly affect the metabolism of a xenobiotic.

9.5.3 Biphasic Effects: Inhibition and Induction

Many inhibitors of mammalian monooxygenase activity can also act as inducers. Inhibition of microsomal monooxygenase activity is fairly rapid and involves a direct interaction with the cytochrome, whereas induction is a slower process. Therefore, following a single injection of a suitable compound, an initial decrease due to inhibition would be followed by an inductive phase. As the compound and its metabolites are eliminated, the levels would be expected to return to control values. Some of the best examples of such compounds are the methylenedioxyphenyl synergists, such as piperonyl butoxide. Because P450 combined with methylenedioxyphenyl compounds in an inhibitory complex cannot interact with CO, the cytochrome P450 titer, as determined by the method of Omura and Sato (dependent on CO-binding to reduced cytochrome), would appear to follow the same curve.

It is apparent from extensive reviews of the induction of monooxygenase activity by xenobiotics that many compounds other than methylenedioxyphenyl compounds have the same effect. It may be that any synergist that functions by inhibiting microsomal monooxygenase activity could also induce this activity on longer exposure, resulting in a biphasic curve as described previously for methylenedioxyphenyl compounds. This curve has been demonstrated for NIA 16824 (2-methylpropyl-2-propynyl phenylphosphonate) and WL 19255 (5,6-dichloro-1,2,3-benzothiadiazole), although the results were less marked with R05-8019 [2,(2,4,5-trichlorophenyl)-propynyl ether] and MGK 264 [*N*-(2-ethylhexyl)-5-norbornene-2,3-dicarboximide].

9.6 ENVIRONMENTAL EFFECTS

Because light, temperature, and other in vitro effects on xenobiotic metabolizing enzymes are not different from their effects on other enzymes or enzyme systems,

we are not concerned with them at present. This section deals with the effects of environmental factors on the intact animal as they relate to in vivo metabolism of foreign compounds.

Temperature. Although it might be expected that variations in ambient temperature would not affect the metabolism of xenobiotics in animals with homeothermic control, this is not the case. Temperature variations can be a form of stress and thereby produce changes mediated by hormonal interactions. Such effects of stress require an intact pituitary-adrenal axis and are eliminated by either hypothysectomy or adrenalectomy. There appear to be two basic types of temperature effects on toxicity: either an increase in toxicity at both high and low temperature or an increase in toxicity with an increase in temperature. For example, both warming and cooling increases the toxicity of caffeine to mice, whereas the toxicity of D-amphetamine is lower at reduced temperatures and shows a regular increase with increases in temperature.

In many studies it is unclear whether the effects of temperature are mediated through metabolism of the toxicant or via some other physiological mechanism. In other cases, however, temperature clearly affects metabolism. For example, in cold-stressed rats there is an increase in the metabolism of 2-naphthylamine to 2-amino-1-naphthol.

Ionizing Radiation. In general, ionizing radiation reduces the rate of metabolism of xenobiotics both in vivo and in enzyme preparations subsequently isolated. This has occurred in hydroxylation of steroids, in the development of desulfuration activity toward azinphosmethyl in young rats, and in glucuronide formation in mice. Pseudocholinesterase activity is reduced by ionizing radiation in the ileum of both rats and mice.

Light. Because many enzymes, including some of those involved with xenobiotic metabolism, show a diurnal pattern that can be keyed to the light cycle, light cycles rather than light intensity would be expected to affect these enzymes. In the case of hydroxyindole-*O*-methyltransferase in the pineal gland, there is a diurnal rhythm with greatest activity at night; continuous darkness causes maintenance of the high level. Cytochrome P450 and the microsomal monooxygenase system show a diurnal rhythm in both the rat and the mouse, with greatest activity occurring at the beginning of the dark phase.

Moisture. No moisture effect has been shown in vertebrates, but in insects it was noted that housefly larvae reared on diets containing 40% moisture had four times more activity for the epoxidation of heptachlor than did larvae reared in a similar medium saturated with water.

Altitude. Altitude can either increase or decrease toxicity. It has been suggested that these effects are related to the metabolism of toxicants rather than to physiological mechanisms involving the receptor system, but in most examples this has not been demonstrated clearly. Examples of altitude effects include the observations that at altitudes of $\geq$ 5000 ft, the lethality of digitalis or strychnine to mice is decreased, whereas that of *D*-amphetamine is increased.

Other Stress Factors. Noise has been shown to affect the rate of metabolism of 2-napthylamine, causing a slight increase in the rat. This increase is additive with that caused by cold stress.

9.7 GENERAL SUMMARY AND CONCLUSIONS

It is apparent from the material presented in this chapter and the previous chapters related to metabolism that the metabolism of xenobiotics is complex, involving many enzymes; that it is susceptible to a large number of modifying factors, both physiological and exogenous; and that the toxicological implications of metabolism are important. Despite the complexity, summary statements of considerable importance can be abstracted:

- Phase I metabolism generally introduces a functional group into a xenobiotic, which enables conjugation to an endogenous metabolite to occur during phase II metabolism.
- The conjugates produced by phase II metabolism are considerably more water soluble than either the parent compound or the phase I metabolite(s) and hence are more excretable.
- During the course of metabolism, and particularly during phase I reactions, reactive intermediates that are much more toxic than the parent compound may be produced. Thus xenobiotic metabolism may be either a detoxication or an activation process.
- Because the number of enzymes involved in phase I and phase II reactions is large and many different sites on organic molecules are susceptible to metabolic attack, the number of potential metabolites and intermediates that can be derived from a single substrate is frequently very large.
- Because both qualitative and quantitative differences exist among species, strains, individual organs, and cell types, a particular toxicant may have different effects in different circumstances.
- Because exogenous chemicals can be inducers and/or inhibitors of the xenobiotic-metabolizing enzymes of which they are substrates; such chemicals may interact to bring about toxic sequelae different from those that might be expected from any of them administered alone.
- Because endogenous factors also affect the enzymes of xenobiotic metabolism, the toxic sequelae to be expected from a particular toxicant will vary with developmental stage, nutritional statue, health or physiological status, stress or environment.
- It has become increasingly clear that most enzymes involved in xenobiotic metabolism occur as several isozymes, which coexist within the same individual and, frequently, within the same subcellular organelle. An understanding of the biochemistry and molecular genetics of these isozymes may lead to an understanding of the variation among species, individuals, organs, sexes, developmental stages, and so on.

SUGGESTED READING

Anderson, K. E., and A. Kappas. Dietary regulation of cytochrome P450. *An. Rev. Nutr.* **11**: 141–167, 1991.

Banerjee, A., T. A. Kocarek, and R. F. Novak. Identification of a ubiquitination-target/substrate-interaction domain of cytochrome P-450 (CYP) 2E1. *Drug Metabol. Disp.* **28**: 118–124, 2000.

Conney, A. H. Induction of drug-metabolizing enzymes: A path to the discovery of multiple cytochromes P450. *An. Rev. Pharmacol. Toxicol.* **43**: 1–30, 2003.

Denison, M. S., and J. P. Whitlock, Jr. Minireview: Xenobiotic-inducible transcription of cytochrome P450 genes. *J. Biol. Chem.* **270**: 18175–18178, 1995.

Goodwin, B., M. R. Redinbo, and S. A. Kliewer. Regulation of CYP3A gene transcription by the pregnane X receptor. *An. Rev. Pharmacol. Toxicol.* **42**: 1–23, 2002.

Guengerich, F. P. Cytochrome P-450 3A4: Regulation and role in drug metabolism. *An. Rev. Pharmacol. Toxicol.* **39**: 1–17, 1999.

Hodgson, E., and J. A. Goldstein. Metabolism of toxicants: Phase I reactions and pharmacogenetics. In *Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson, R. C. Smart, eds., New York: Wiley, 2001.

Kakizaki, S., Y. Yamamoto, A. Ueda, R. Moore, T. Sueyoshi, and M. Negishi. Phenobarbital induction of drug/steroid-metabolizing enzymes and nuclear receptor CAR. *Biochim. Biophys. Acta* **1619**: 239–242, 2003.

Kocarek, T. A., R. C. Zanger, and R. F. Novak. Post-transcriptional regulation of rat CYP2E1 expression: Role of CYP2E1 mRNA untranslated regions in control of translational efficiency and message stability. *Arch. Biochem. Biophys.* **376**: 180–190, 2000.

Lieber, C. S. Cytochrome P-4502E1: Its physiological and pathological role. *Physiol. Rev.* **77**: 517–544, 1997.

Lin, J. H., and A. Y. H. Lu. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *An. Rev. Pharmacol. Toxicol.* **41**: 535–567, 2001.

Newton, D. J., R. W. Wang, and A. Y. H. Lu. Cytochrome P450 inhibitors: Evaluation of specificities in the in vitro metabolism of therapeutic agents by human liver microsomes. *Drug Metab. Disp.* **23**: 154–158, 1995.

Okey A. B. Enzyme induction in the cytochrome P-450 system. *Pharmac. Ther.* **45**: 241–298, 1990.

Ronis, M. J. J., and H. C. Cunny. Physiological factors affecting xenobiotic metabolism. In *Introduction to Biochemical Toxicology*, 3rd ed., E. Hodgson, and R. C. Smart, eds. New York: Wiley, 2001.

Rose, R. L., and E. Hodgson. Adaptation to toxicants. In *Introduction to Biochemical Toxicology*, 3rd ed. E. Hodgson, and R. C. Smart eds. New York: Wiley, 2001.

Sueyoshi, T., and M. Negishi. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *An. Rev. Pharmacol. Toxicol.* **41**: 123–143, 2001.

Smith, C. A. D., G. Smith, and C. R. Wolf. Genetic polymorphisms in xenobiotic metabolism. *Eur. J. Cancer* **30A**: 1935–1941, 1994.

Whitlock, J. P., Jr. Induction of cytochrome P4501A1. *An. Rev. Pharmacol. Toxicol.* **39**: 103–125, 1999.