Anesthesiology
V 39, No 2, Aug 1973

Hepatic Microsomal Enzyme Induction

*Burnell R. Brown, Jr., M.D., Ph.D.**

MAMMALS have proven remarkably resistant to toxic environmental chemicals throughout the ages, an adaptability which is being challenged in modern times because of the enormous amount and variety of pollutants produced by an industrialized society. Untold thousands of tons of products of combustion, industrial wastes, insecticides, carcinogens, and other toxic materials are pumped into the environment daily. In addition, our drug-oriented culture consumes enormous quantities of tranquilizers, analgesics, sedatives, stimulants, and narcotics. The intensity and duration of action of such xenobiotics in biologic systems often depend on the rate of enzymatic metabolism by the host. Man and other mammals have developed a remarkable system capable of metabolizing a wide variety of structurally unrelated pollutants, toxic agents, and drugs. This system is located within the endoplasmic reticulum fraction of many tissues—predominately in the liver—and is termed the "microsomal mixed-function oxidase (or hydroxylase) system." The *raison d'etre* of this system is twofold. First, the microsomal oxidase system biodegrades xenobiotics, thereby usually abolishing, or at least reducing, pharmacologic action. In some instances this action reduces toxicity. Second, it converts lipophilic chemicals into polar compounds, which are soluble in water and which can be conjugated and transported for elimination by the kidneys. Generally, pollutants and drugs are quite lipid-soluble. If it were not for the ability of the hepatic microsomal oxidase system to convert these compounds into materials readily eliminated by the renal route, half-lives of many of them would be measured in years.

* Associate Professor of Anesthesiology and Pharmacology, University of Arizona College of Medicine, Tucson, Arizona 85721.

Supported in part by Research Grant 1 RO1 AM16715-01 from the National Institutes of Health, and a Grant-In-Aid from Ayerst Laboratories, Inc.

The purpose of this review is to describe this enzyme system, with particular reference to the compensatory mechanism available to mammals when chronically exposed to excess quantities of xenobiotics: hepatic microsomal enzyme induction. The implication of this effect as regards the practice of anesthesia and the clinical situation will be particularly stressed.

The Hepatic Microsomal Oxidase System

When liver is homogenized and subjected to ultracentrifugal forces, the endoplasmic reticulum may be effectively isolated from other organelles.[1] The endoplasmic reticulum is converted, by the artifact of separation, from membrane sheets and tubular aggregates into small round spherules, termed "microsomes."[2] The oxidative metabolism of many drugs and steroid hormones is catalyzed by enzymes located within the microsomal fraction of the liver, as well as other tissues. These reactions have the simultaneous requirement for reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen.[3, 4, 5] Mason [6] termed these enzymes "mixed function oxidases," since one atom of molecular oxygen is incorporated into the organic product, and the other atom is concomitantly reduced to water. The overall reaction described by Mason is illustrated in figure 1. Although by 1960 it was well established that the hepatic microsome was the pivotal organelle of drug oxidations, the nature of the electron-transport chain had not been elucidated. Klingenberg [7] and Garfinkel [8] demonstrated that mammalian hepatic microsomes contained a unique carbon monoxide-binding pigment. This pigment has an intense absorption band at 450 nm when reduced and complexed with CO. Omura and Sato [9] characterized the hemoprotein nature of this pigment and were the first to call it "cytochrome P-450." Cooper *et al.*[10] established that NADPH ultimately reduces

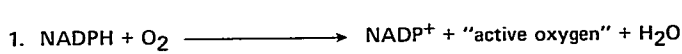

FIG. 1. The classic mixed-function oxidase scheme of drug metabolism as proposed by Mason.[6] It is now realized that oxygen is "activated" by cytochrome P-450 only after complexing with drug.

cytochrome P-450 and that this cytochrome is the terminal oxidase of the mixed-function oxidase system functional in drug and steroid hydroxylation reactions. The role of cytochrome P-450 in drug metabolism has recently been reviewed in an excellent article by Gillette *et al.*[11] There is strong evidence that reduction of cytochrome P-450 by NADPH is mediated by a flavoprotein enzyme intermediate, NADPH–cytochrome c reductase.[12, 13] NADPH-cytochrome c reductase is frequently equated with NADPH–cytochrome P-450 reductase, even though there is no cytochrome c in microsomes. Since microsomes contain electron-transport systems oriented to NADH as well as to NADPH,[14] mention must be made of the role of this coenzyme in drug oxidations. Like cytochrome P-450, cytochrome b_5 is found in significant concentrations in hepatic microsomes. NADH is the major source of reducing equivalents for cytochrome b_5.[15] Hildebrandt and Estabrook[16] postulated that cytochrome b_5 mediates the transfer of an electron from NADH to the oxygenated complex of ferrous cytochrome P-450. A simplified scheme of multiple electron-transport reactions of the microsomal enzyme system is depicted in figure 2. There are reservations to the postulated role of cytochrome b_5 in electron transfer from NADH to cytochrome P-450, since antibodies to NADH-cytochrome b_5 reductase were found incapable of inhibiting drug oxidative metabolism in the presence of either NADH or NADPH.[11]

The unique feature of cytochrome P-450 is the currently-held view that it combines with a wide variety of structurally unrelated compounds. Customarily, enzymes are considered quite substrate-specific; the cytochrome P-450 system is capable of metabolizing widely differing substances. Substrates and inhibitors combine with oxidized cytochrome P-450, causing significant changes in the absorbance spectra of microsomal suspensions.[18] Two types of spectral changes are observed upon addition of various substances to microsomal suspensions. Type I substances are those producing a trough at 420 nm and a peak at 385 nm as determined by ultraviolet difference spectrophotometry: hexobarbital is the classic Type I substance. Type II compounds produce a trough at 390 nm and a peak at 430 nm as determined by ultraviolet difference spectrophotometry: aniline is the classic Type II compound. It has been proposed that these spectral changes represent the complexing of drug and cytochrome P-450 in a functional manner analogous to formation of an enzyme–substrate complex.[18] Evidence for this concept is controversial, however. Mannering presents rather convincing arguments that Type I and II spectral changes are not functionally significant.[19] Under one set of experimental conditions the rate-limiting step of the microsomal electron transfer chain is the reduction of the ferric cytochrome P-450 substrate complex, a function ascribed to NADPH–cytochrome c reductase.[20] However, under other conditions the rate-limiting step can be assigned to the reduction of the oxygenated complex of ferrous cytochrome P-450 or to the dissociation of the hydroxylated product of the reaction.[21] Although high concentrations of drug substrates will inhibit drug metabolism, NADPH oxidation does not appear to be impaired in this circumstance; substrate inhibition in this case represents an uncoupling of NADPH oxidation and drug metabolism.[22]

The reduction of cytochrome P-450 enzymes is clearly dependent on the phospholipid matrix of the endoplasmic reticulum in which they are imbedded. Hepatic endoplasmic reticulum is rich in saturated and unsaturated fatty acid esters of phosphatidylcholine and phosphatidylethanolamine. If the lipid matrix of microsomes is extracted or disrupted by

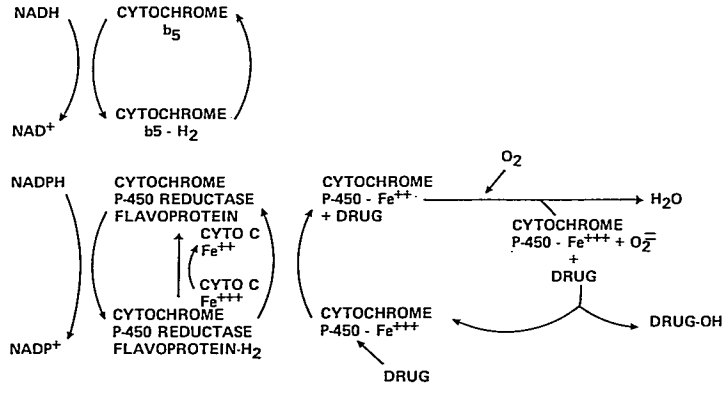

FIG. 2. Proposed electron-transfer system of the hepatic mixed-function oxidase system (modified from Shenkman [17]). In this scheme, drugs combine with oxidized cytochrome P-450. Only after reduction of cytochrome P-450 by reducing equivalents from NADPH (and perhaps NADH) does oxygen attach to the complex, oxidizing both cytochrome P-450 and the drug.

detergents, the ability of the enzymes to metabolize drugs is impaired; the subsequent addition of phosphatidylethanolamine is able to restore activity of microsomal enzymes that have been lipid-extracted and solubilized.[23]

General Features of Enzyme Induction

More than 200 drugs, carcinogens, and other chemicals have been shown to stimulate hepatic microsomal drug-metabolizing enzymes.[24] Table 1 lists some of the more common of these drugs. This effect was first noted by Brown *et al.*,[25] while studying dietary influences on metabolism of aminoazo dyes. Conney and others demonstrated that injections of small amounts of 3-methylcholanthrene and 3,4-benzpyrene increased the rate of metabolism of several drugs.[26, 27] It was Remmer [28, 29, 30] who discovered the inducing effects of barbiturates, while studying mechanisms of barbiturate tolerance. From that time, research in this area has expanded logarithmically.

When an animal is given an inducing agent such as phenobarbital over a period of several days, alterations in the microsomal drug-metabolizing system can be readily demonstrated. Morphologically, marked proliferation of the hepatic endoplasmic reticulum is observable by electron microscopy.[31, 32] In addition, there is an apparent increase in microsomal protein per gram of liver concomitant with the development of drug tolerance in animals. For example, three days of phenobarbital pretreatment reduces both sleeping time with and plasma half-life of pentobarbital (Nembutal) in animals more than 50 per cent.[33] In fact, barbiturate sleeping times are frequently used as an index of enzyme induction in experimental animals. Concomitant with these histologic and pharmacologic effects, *in-vitro* drug metabolizing activity for a wide variety of substrates is enhanced. The enhancement appears to be the result of increased enzyme content rather than a basic change in the nature of the enzyme microsomal oxidative complex. Michaelis constants and inhibitor constants for the overall reaction of drug metabolism are similar in induced and non-induced microsomal enzyme systems, indicating that a quantitative rather than a qualitative change is produced by the inductive process.[34] Hepatic microsomal enzyme induction is basically an increase in drug-metabolizing enzyme content stimulated by chronic administration of a foreign compound.

There are two generic types of inducing agents.[24] One group, typified by phenobarbital, increases the rates of a host of reactions, including oxidation, glucuronidation, and reduction of many substrates. The other group, primarily certain aromatic polycyclic hydrocarbons, with 3-methylcholanthrene (3-MC) as the prototype, stimulates far fewer reactions. There are several other major differences bebetween phenobarbital induction and 3-MC induction. Whereas phenobarbital causes threefold to fourfold increases in microsomal cytochrome P-450 content and NADPH–cytochrome P-450 reductase, and a slight increase in cytochrome b_5, 3-MC, on the other hand, stimulates formation of a new hemoprotein (or new variant), which Mannering and colleagues call "P_1-450."[35, 36] Kuntzman *et al.*,[37] as well as Hildebrandt *et al.*,[38] call this hemoprotein "P-448" since it is distinct from cytochrome P-450 spectrally as well as in substrate specificity, ethyl isocyanide difference spectra, and drug-complexing properties. The times required for induction by phenobarbital and 3-MC are different. The maximal increase in microsomal enzymic activity with phenobarbital is not reached for three days, whereas with the injection of 3-MC, enzymic activity is doubled within three to six hours and maximal increase is reached in 24 hours.[38, 39] These spectrally distinct forms of micosomal cytochrome pigments also appear to differ in drug-metabolizing activity. P-448 is far more potent in 3,4-benzpyrene hydroxylation than is P-450.[40]

Mechanisms of Enzyme Induction

There are several possibilities to account for the mechanism of quantitative increase in drug-metabolizing enzymes by the induction process: 1) Direct interaction of the drug with DNA, stimulating DNA-directed synthesis of messenger RNA, which in turn enhances enzyme production. 2) Interaction of the drug with genetic repressors. 3) Interaction of the drug with hepatic endoplasmic reticulum, thereby enhancing translation of messenger RNA or ribosomes. 4) Prevention of a feedback inhibition of enzyme biosynthesis. 5) Inhibition of enzyme degradation.

TABLE 1. Partial List of Drugs Capable of Producing Microsomal Enzyme Induction

Hypnotics
Barbiturates
Glutethimide (Doriden)
Ethanol
Chloral hydrate
Tranquilizers
Chlorpromazine (Thorazine)
Promazine (Sparine)
Meprobamate (Equanil)
Chlordiazepoxide (Librium)
Anticonvulsants
Diphenylhydantoin (Dilantin)
Methylphenylethylhydantoin (Mesantoin)
Antihistaminics
Diphenhydramine (Benadryl)
Steroids
Cortisone
Prednisolone
Norethynodrel (Enovid)
Methyltestosterone
Anesthetics
Diethyl ether
Halothane (Fluothane)
Methoxyflurane (Penthrane)
Insecticides
DDT
Chlordane
o,p'-DDD

Since there is strong evidence that the induction phenomenon represents enzymic protein synthesis stimulation, several types of blocking agents have been employed to amplify the mechanism of change in enzyme content. Ethionine, which blocks protein synthesis by decreasing hepatic high-energy phosphate levels, prevents phenobarbital-stimulated induction of drug metabolism.[39] Puromycin and actinomycin D, blockers of RNA translation and transcription, respectively, likewise prevent the induction phenomenon.[41, 42] Enzyme inducers may accelerate DNA-directed synthesis of RNA molecules that function as templates for the synthesis of drug-metabolizing enzymes at the ribosomal level.[24]

Hormonal, dietary, species, and age factors have significant effects on drug metabolism

and the induction phenomenon. Adult male animals appear to metabolize certain drugs such as hexobarbital at a significantly greater rate than do females of the same age.[43] Other studies [44] have related drug-metabolizing activity to androgenic hormones. Certain drugs, such as aniline, possess a sex-independent metabolism. Starvation *per se* has variable effects on drug metabolism. In male rats starvation impairs sex-dependent enzymes which metabolize aminopyrine and hexobarbital, but enhances the sex-independent hydroxylation of aniline. By contrast, starvation induces metabolism of aminopyrine, hexobarbital, and aniline in female animals.[45] Thus, starvation may interfere with the stimulating effects of androgenic hormones. Fasting animals will respond to inducing agents faster than nonfasting animals, presumably because fasting decreases the rate of breakdown of cytochrome P-450.[46] Gillen and Nebert [47] have demonstrated that phenobarbital will induce the cytochrome P-450-dependent oxidase system of fetal liver in tissue culture. Young male and adult female animals have a more marked response to inducing agents than do adult males. This is probably because higher levels of drug-metabolizing enzymes are normally present in the adult male.[24] Adrenalectomy and thyroidectomy do not influence the course of drug induction in animals, suggesting that the induction process is not hormone dependent.[48]

Miscellaneous Effects of Drug Induction

Since there are many similarities between drug- and steroid-hydroxylating enzymes in hepatic microsomes,[49] induction with phenobarbital stimulates hepatic microsomes to hydroxylate androgens, estrogens, progesterones, and glucocorticoids at an accelerated rate.[24] Although 6β-hydroxylation of cortisol is a rather minor pathway of steroid metabolism in man, it is specifically induced by phenobarbital. Indeed, urinary 6β-hydroxycortisone in relation to 17-hydroxycorticosteroids has been suggested as an index of induction in man.[24] Further, barbiturates stimulate the enzymatic conjugation of bilirubin by hepatic glucuronyltransferase enzymes.[50]

Implications of Enzyme Induction in Man

The drugs listed in table 1, frequently employed in man, are known to stimulate microsomal enzymic activity. Multiple-drug regimens are often used in man with little regard to the influence one drug may simultaneously have on another. A classic case of human induction is observed with the simultaneous administration of bishydroxycoumarin (Dicumarol) and phenobarbital during treatment of patients with ischemic heart disease. Phenobarbital, 30 mg, three times per day, significantly increases the dosage of bishydroxycoumarin needed for optimal anticoagulation [51] because of increased metabolism of the prothrombin antagonist. When phenobarbital is withdrawn from patients on such combined therapy, the dosage requirement of the anticoagulant is decreased.[52] There has been at least one fatal case involving a patient simultaneously receiving chloral hydrate and bishydroxycoumarin.[53] When the chloral hydrate (the inducing agent) was discontinued, prothrombin time increased and hemorrhage resulted. Other examples of such drug interaction in man have been reported, for example, chronic phenobarbital therapy reduces plasma levels of diphenylhydantoin in epileptic patients,[54] and administration of barbiturates and glutethimide (Doriden) accelerates the metabolism of Dipyrone.[55]

Treatment with enzyme-inducing agents has been employed therapeutically for several clinical problems. Phenobarbital has been used in neonates threatened with kernicterus (deposition of unconjugated bilirubin in the central nervous systems). Phenobarbital is able to stimulate hepatic glucuronyltransferase enzymes involved in bilirubin glucuronide conjugation, enzymes which are normally deficient in the newborn. The conjugated bilirubin does not pass the blood–brain barrier and hence is innocuous to the central nervous system. Phenobarbital thus may reduce complications of fetal hyperbilirubinemia.[56, 57] o,p'-DDD and diphenylhydantoin have been used to induce cortisol metabolism and hence ameliorate symptoms of patients who have adrenal cortical hyperplasia (Cushing's syndrome).

TABLE 2. Demonstration that Normal Human Liver Possesses an Oxidative Microsomal Drug-metabolizing Chain*

Patient's Age (years), Sex	Cause of Death	Autopsy (Hours Postmortem)	Aminopyrine Demethylase†	NADPH–Cytochrome Reductase†	NADPH–Cytochrome P-450 Reductase†	Cytochrome P-450‡
44, M	Stab-wound, chest	3	N.D.	58	2.6	0.39
26, F	Broken neck	1	3.4	91	N.D.	0.57
16, M	Head injury	3	0.9	20	N.D.	0.20
22, M	Head injury	2	0.14	7	N.D.	0.18
26, F	Stab wound, neck	1	2.1	24	N.D.	0.33
26, F	Head injury	5	N.D.	4	0.33	0.19
25, M	Gunshot wound, thorax	6	0.2	10	0.40	0.29
45, M	Myocardial infarction	2	1.2	47	1.2	0.45
20, M	Transection of aorta	3	0.67	9	0.7	0.20
49, M	Gunshot wound, thorax	4	1.0	10	0.86	0.25
31, F	Heart failure	2	1.6	29	1.5	0.17
28, M	Multiple injuries	3	0.65	14	0.66	0.11

* The range of values is wide, although none of these patients had a history of previous drug use. Drug metabolism as determined by aminopyrine demethylation correlates best with the level of NADPH cytochrome c reductase activity. (Data from Nelson, Raj, Belfi *et al.*,[59] used with permission of the authors.)

† Nanomoles/min/mg liver microsomal protein; N.D. = not determined.

‡ Nanomoles/mg liver microsomal protein.

Local anesthetics can be considered drugs whose metabolism may be increased by induction. Heinonen *et al.*[58] reported that the rate of disappearance of lidocaine (Xylocaine) from plasma of epileptic patients receiving anticonvulsant therapy continuously is greater than that in normal persons. Recently, studies have been undertaken to evaluate the concentrations of microsomal oxidative enzymes in samples of human liver.[59] As shown in table 2, there is great variation in the amounts of flavoprotein reductase and cytochrome P-450 found. Although patient histories do not permit evaluation of exposures to inducing agents, the large differences in enzyme content undoubtedly reflect potential differences in capacity to metabolize drugs and other toxic agents.

Enzyme Induction and Inhalation Anesthetics

Two aspects of anesthetics and hepatic microsomal enzyme induction are worthy of discussion. On one side is the effect of prior drug therapy on metabolism of the anesthetics, and on the other is the effect chronic contact with anesthetics has on the microsomal enzyme system. The inhalation anesthetics are metabolized by the NADPH–O_2-dependent microsomal enzyme system. Van Dyke[60] demonstrated that phenobarbital pretreatment of animals induced methoxyflurane (Penthrane)–ether cleavage in addition to increasing the rates of dechlorination of halothane (Fluothane) and methoxyflurane. Phenobarbital pretreatment in rats increases the rate of production of oxalic acid following methoxyflurane anesthesia.[61] Fluroxene (Fluoromar) metabolism may be stimulated by phenobarbital and 3-MC.[62]

The biotransformation and accelerated metabolism of the inhalation anesthetics by induction may be of more than casual academic concern. It is quite plausible that organ toxicity occasionally produced in man by these agents may be related to such metabolism.[63, 64] The mechanism of this metabolism-linked toxicity could theoretically be ascribed either to production of toxic metabolites, to free-radical formation, or to combination of metabolites with protein to form a sensitizing haptene (or haptenes). Any of these mechanisms would be more operative in the presence of induced microsomal enzymes. Evidence indicates that the nephrotoxicity associated with

methoxyflurane anesthesia correlates with free fluoride levels formed during metabolism of the anesthetic.[65] Since metabolism of methoxyflurane is inducible by agents such as phenobarbital,[61] use of the anesthetic for patients receiving continuous drug therapy is theoretically hazardous, even if it is employed in low concentrations. Scholler [66] demonstrated that phenobarbital pretreatment significantly enhances chloroform hepatotoxicity in rats. Since this damage could be decreased by protective antioxidants, the implication is that free radicals formed during chloroform metabolism are the proximate vectors of this particular hepatotoxicity. This free-radical mechanism of halogenated hydrocarbon toxicity correlates well with the mechanism proposed by Recknagel and Ghoshal [67] for carbon tetrachloride and that described by Van Dyke for chloroform.[68] Brown [69] demonstrated that halothane anesthesia in phenobarbital-pretreated animals causes peroxidative breakdown of unsaturated lipids of hepatic microsomes. This action is inhibited by antioxidants and by the microsomal enzyme inhibitor, SKF 525-A. Of further interest in this regard is the case of a patient receiving large doses of phenobarbital and diphenylhydantoin (Dilantin) who had massive fatal hepatic failure following fluroxene anesthesia.[70]

Chronic exposure to subanesthetic concentrations of anesthetic vapors is capable of inducing the drug-metabolizing hepatic microsomal enzyme system. Berman and Bochantin [71] were the first to demonstrate that exposure to low concentrations of methoxyflurane produced enzyme induction, manifested by enhanced *in-vitro* metabolism of aminopyrine and reduced hexobarbital sleeping time. Later, Linde and Berman [72] amplified this experiment by showing that all inhalation anesthetics except cyclopropane and nitrous oxide stimulate drug metabolism. The ethers were found to be the most potent in this regard. Brown and Sagalyn [73] found that such anesthetic stimulation is the result of a phenobarbital-like induction, *i.e.*, microsomal cytochrome P-450 and NADPH–cytochrome c reductase rapidly increase in quantity and activity, followed by a slower marked rise in cytochrome b_5 content.

The clinical implications of chronic inhalation of subanesthetic concentrations of vapors are not at all clear. Animal studies indicate that halothane is capable of inducing its own metabolism.[74] Trace amounts of anesthetic gases and vapors can be found throughout operating suites,[75, 76] and anesthetics are found in measurable amounts in exhaled air and blood of anesthesia personnel.[77, 78, 79] Cascorbi *et al.*[80] established that anesthetists have a faster rate of halothane biotransformation than do personnel not working in the operating room environment. This observation apparently confirms that halothane can induce its own metabolism in man. Animal experiments reveal that chronic exposure to one anesthetic can induce the metabolism of other anesthetics.[60] Therefore, by extrapolation, anesthetic cross-induction may exist in man, although this is unconfirmed. There is extreme dependence of the induction process on both the anesthetic agent and its concentration. Exposure of adult rats to 3,000 ppm methoxyflurane for ten days causes weight loss, fatty infiltration of the liver, and decreased activity of microsomal enzymes. Exposure to methoxyflurane, 300 ppm, for the same interval produces enzyme induction.[73] Diethyl ether, on the other hand, continues to produce marked enzyme induction without evidence of toxicity even in concentrations as high as 10,000 ppm.

The significance of the effects on personnel of operating room pollution by anesthetics has not been clearly established. Certainly such an environment is conducive to hepatic microsomal enzyme induction. There seems to be no doubt that female operating room personnel have an increased rate of spontaneous abortion.[81] However, the relation of this observation to anesthetic metabolism is unknown. "Sensitization" of anesthetists to hepatic damage by halothane has been reported.[82, 83] Unfortunately, these cases were not studied for induced enzymic activity as evidenced by higher-than-normal output of halothane metabolites. Resolution of such important questions must await further investigation.

Summary

The presence of an enzyme complex associated with the endoplasmic reticulum of many cells whose function is to activate oxygen for the biotransformation of a broad spectrum of endogenous and exogenous compounds affords

the opportunity to rationalize many unexplained drug reactions observed in patients. The complexity of antagonism of substrates, induction of the hydroxylating enzyme system, and variations in patterns of compounds metabolized suggests the need for better understanding and evaluation of the molecular events associated with metabolic transformations. The present report is only a superficial survey of some of the facets to be considered; as our knowledge increases, the rudimentary nature of these beginnings will be obvious.

The author acknowledges the excellent review and critique of this manuscript by Ronald W. Estabrook, Ph.D., Virginia Lazenby O'Hara Professor and Chairman, Department of Biochemistry, Southwestern Medical School, Dallas, Texas.

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