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INDUCTION OF DRUG-METABOLIZING ENZYMES: A Path to the Discovery of Multiple Cytochromes P450*

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■ **Abstract** This article provides a personal account of the discovery of the induced synthesis of drug-metabolizing enzymes and of subsequent research that led to the discovery of multiple cytochromes P450 with different catalytic activities. The manuscript also emphasizes the role of environmental factors (in addition to genetic polymorphisms) in explaining person-to-person and day-to-day differences in rates and pathways of drug metabolism that occur in the human population.

EARLY DAYS

I was a depression baby born in 1930 in Chicago where my father was a pharmacist managing a Liggett's drugstore. In 1937, my father opened an independent pharmacy in Winnetka, Illinois, and we moved into a small house on the less expensive west side of Winnetka where my next door neighbor was Donald Rumsfeld (current Secretary of Defense). My early childhood was marked by much good fortune—loving and hardworking parents with strong ethical values, a pharmacy named Conney's Pharmacy (which still retains the name today) that was a center of our family life, and an outstanding school system that was (and still is) among the best in our nation. Graduates from Winnetka's New Trier High School included such notables as Jack Steinberger (Nobel Laureate, physics), Bruce Alberts (President, National Academy of Sciences, U.S.A.), Donald Rumsfeld (Secretary of Defense), Ralph Bellamy (actor), and Archibald McLeish (poet).

*This manuscript is dedicated to the memory of my graduate school mentors James A. Miller and Elizabeth C. Miller and to the memory of my friends and former colleagues Alvito Alvares, James Gillette, and Shu-jing (Caroline) Wei.

I started working in my father's pharmacy at age 10 for 10 cents an hour and all the ice cream that I could eat. I enjoyed my interactions with the people that came into the store, and I learned much from my father about the use of drugs for the treatment of diseases. My early years were marked by a love of my academic studies, music (both jazz and classical), and competitive sports (table tennis and baseball). I played flute and piccolo in a number of concert bands and orchestras during my early years and also later in college where I was a member of the concert band and the marching band that performed at football games.

COLLEGE DAYS

In 1948, I entered the School of Pharmacy at the University of Wisconsin-Madison with the intention of pursuing a career in pharmacy. The School of Pharmacy and the basic science departments at the University had an exceptionally strong research-oriented faculty, and they provided me with a strong education in the basic sciences and the practice of pharmacy (including instructions on how to make suppositories during the heat of summer). The faculty in the School of Pharmacy provided their students with opportunities for doing research, and I took a part-time job as a research assistant helping Professor Louis Busse in studies on the properties of powders that would be helpful in preparing tablets. My first "independent" research project was with Professor Takeru Higuchi who was a leader in physical pharmacy. He had me work on the development of a silica gel chromatography system that would allow the specific analysis of chloramphenicol.

After completion of pharmacy school, I passed the Pharmacy State Board Examination in Illinois and became a registered pharmacist. Although I could have started a career in pharmacy in my father's drugstore, I chose to pursue further education in preparation for a career in research after also considering the possibility of entering medical school. I inquired about opportunities as a graduate student in a number of biochemistry-oriented departments on the Madison campus. Again, I had good fortune in meeting with Dr. Harold Rusch, director of the McArdle Laboratory for Cancer Research. Dr. Rusch spent a great deal of time with me describing the oncology graduate program and the many interesting research projects that were ongoing at McArdle. After this meeting, I was convinced that I wanted to enter the oncology Ph.D. program at McArdle, and Dr. Rusch introduced me to Drs. James and Elizabeth Miller who discussed their research on chemical carcinogenesis and carcinogen metabolism with me and accepted me as their graduate student. There were no laboratory rotations in the early 1950s, and a simple introduction by Dr. Rusch and an agreement between the student and professor was all that was needed to start the graduate student-mentor relationship.

MY FAILURE AS A SYNTHETIC CHEMIST

I joined the Miller laboratory in September, 1952, and my first project was to synthesize 1-hydroxy-2-aminonaphthalene, a suspected carcinogenic metabolite of β-naphthylamine (a bladder carcinogen). After many attempts to synthesize

and purify the β-naphthylamine derivative during a two-month period failed to yield pure product and also resulted in two explosions, the Millers realized that I did not have much future as a synthetic chemist; I believe they were also worried about the destruction of their laboratory. These concerns led to a change in my research project. My failure as a synthetic chemist was extremely fortunate because it led me into a new field—the induced synthesis of microsomal drug-metabolizing enzymes—which was to become my major area of research for the next several decades.

THE JOYS OF DISCOVERY: ENZYME INDUCTION BY POLYCYCLIC AROMATIC HYDROCARBONS

After my failure as a synthetic chemist, the Millers and I discussed earlier research by H.L. Richardson and his colleagues indicating that administration of 3-methylcholanthrene inhibited the hepatocarcinogenic activity of 3′-methyl-4-dimethylaminoazobenzene in rats (1). We discussed the possibility that treatment of rats with the protective hydrocarbon might alter the metabolism of carcinogenic aminoazo dyes, and in late 1952 or early 1953 I started studies on the effects of treating rats with 3-methylcholanthrene and other polycyclic aromatic hydrocarbons on the hepatic N-demethylation and azo-link reduction of aminoazo dyes—metabolic pathways that resulted in noncarcinogenic products. Almost immediately, I experienced the joys of discovery by finding that treatment of rats with a single i.p. injection of 3-methylcholanthrene caused a rapid and many-fold increase in azo dye N-demethylase activity [M.S. thesis (2)]. A stimulatory effect of dietary 3-methylcholanthrene on azo dye N-demethylase activity was also observed during these early studies (2). While I was studying the effects of 3-methylcholanthrene on hepatic azo dye N-demethylase activity in rats, Raymond Brown (another Miller graduate student) found that livers from mice fed a Friskies dog chow diet for two weeks had about twice as much hepatic azo dye N-demethylase activity as the livers of mice fed a purified diet (3). Brown then focused his attention on constituents in the crude diet that could increase azo dye N-demethylase activity when fed in the purified diet (3).

My first full-length publication provided strong evidence that treatment of rats with a single i.p. injection of 3-methylcholanthrene induced the synthesis of hepatic aminoazo dye N-demethylase and azo-link reductase (4). The induction of hepatic azo dye N-demethylase and azo-link reductase activities by 3-methylcholanthrene is shown in Figure 1. These studies on the induced synthesis of azo dye-metabolizing enzymes were early examples of enzyme induction in mammals. Curiously, my first full-length publication started on page 450 of *Cancer Research* (Vol. 16) (4). The coincidence of this paper starting on page 450 and the later development of the field of multiple cytochromes P450 was striking. Our early research indicated that polycyclic aromatic hydrocarbons that inhibited aminoazo dye-induced liver cancer (1, 5) were potent inducers of azo dye N-demethylase activity (2, 4), whereas other polycyclic hydrocarbons that did not influence azo

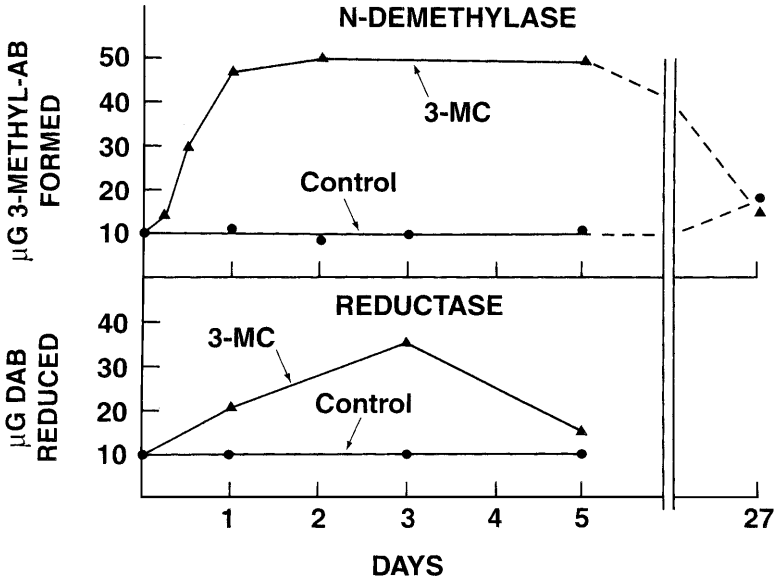

Figure 1 Induction of hepatic aminoazo dye N-demethylase and reductase activities. Rats (50 g) were injected once with 1 mg of 3-methylcholanthrene (3-MC). N-Demethylase activity was determined in fortified liver homogenates by measuring the metabolism of 3-methyl-4-monomethylaminoazobenzene to 3-methyl-4-aminoazobenzene (3-methyl-AB). Reductase activity was determined by measuring the reduction of the azo linkage of 4-dimethylaminoazobenzene (DAB). Demethylase activity is expressed as μg of 3-methyl-AB formed per 50 mg of liver per 30 min. Reductase activity is expressed as μg of DAB reduced per 30 mg of liver per 30 min. Each point is the average of the activities for two or three rats. Taken from Ref. (4).

dye-induced carcinogenesis (5) had little or no effect on azo dye metabolism (2, 4). Our research on the induction of azo dye-metabolizing enzymes provided a mechanistic explanation for the inhibitory effects of polycyclic hydrocarbons on azo dye carcinogenesis and was an early example of mechanisms of cancer chemoprevention. These studies also placed into perspective the meaning of safety and benefit/risk ratio for the fields of pharmacology and cancer chemoprevention. If I were a rat in an environment of carcinogenic aminoazo dyes that would with great certainty cause liver cancer and all that was available for protection was 3-methylcholanthrene (a poor carcinogen when administered orally), I would ingest the hydrocarbon because it would save my life. Clearly, safer chemopreventive agents would be more desirable, and having a mechanistic understanding of chemoprevention led to a search for safer and more effective chemopreventive agents.

In 1955, the Millers and I initiated research to determine whether polycyclic aromatic hydrocarbons that stimulated azo dye N-demethylase activity could also

stimulate their own metabolism. In 1957, we reported that treatment of rats with benzo[a]pyrene (BP), 3-methylcholanthrene, or several other polycyclic hydrocarbons induced the synthesis of hepatic BP hydroxylase (6). In later studies after leaving Madison, my colleagues and I found that induction of BP hydroxylase was paralleled by increased in vivo metabolism of BP and other polycyclic aromatic hydrocarbons. The increased level of BP hydroxylase activity in BP-pretreated rats was reflected in vivo by decreased blood and tissue concentrations of this carcinogen and enhanced biliary excretion of its metabolites (7, 8). The stimulatory effect of BP on its own metabolism was illustrated by a decreased tissue concentration of this compound that occurred when it was administered chronically. At 24 h after a single oral dose of 1 mg of [^{3}H]BP to adult rats, the concentration of the hydrocarbon in fat was 249 ng/g, whereas 24 h after seven daily doses of [^{3}H]BP, its concentration in fat was only 24 ng/g (7).

In 1966–1967, we demonstrated that treatment of rats with certain polycyclic hydrocarbons or aromatic azo derivatives stimulated the in vitro and in vivo metabolism of 7,12-dimethylbenz[a]anthracene (9, 10), which provided a mechanistic explanation for earlier research indicating an inhibitory effect of these compounds on 7,12-dimethylbenz[a]anthracene-induced mammary cancer and adrenal toxicity in rats (11–15). These early studies on mechanisms of inhibition of azo dye and polycyclic hydrocarbon carcinogenesis as well as subsequent studies suggested that induction of carcinogen detoxifying enzymes may be a useful strategy for cancer chemoprevention [reviewed in Refs. (16–18)].

ENZYME INDUCTION BY DRUGS

After receiving my Ph.D. degree, I worked in my father's drug store for a few months as a pharmacist before pursuing a full-time career in research. I was intrigued by the possibility that polycyclic aromatic hydrocarbons may induce the synthesis of drug-metabolizing enzymes and also by the possibility that drugs might induce the synthesis of drug-metabolizing enzymes. The last experiment that I did before leaving Madison in 1956 indicated that treatment of rats with aminopyrine (an analgesic drug metabolized by N-demethylation) stimulated hepatic aminoazo dye N-demethylase activity. This study (which was never published) was an early indication of a stimulatory effect of a therapeutically useful drug on hepatic drug metabolism. In 1957, I met with Dr. Bernard Brodie, whose Laboratory of Chemical Pharmacology in the Heart Institute at the National Institutes of Health was at the forefront of drug metabolism research in the 1950s, and I discussed with him the possibility that drugs may induce increased levels of microsomal drug-metabolizing enzymes. I wanted to obtain a postdoctoral position in Brodie's laboratory to study the pharmacological significance of microsomal enzyme induction. Although Brodie was not able to take me into his laboratory, he introduced me to Dr. John Burns who was interested in the stimulatory effects of drugs on ascorbic acid synthesis, and we wondered whether the drugs that stimulated ascorbic acid synthesis would also enhance drug metabolism. John Burns

obtained a $6000/year fellowship stipend from McNeil Laboratories for me to study the fate of zoxazolamine (Flexin) and chlorzoxazone (Paraflex), which were muscle relaxant drugs produced by McNeil Laboratories.

In 1957, I moved with my wife and infant son from the drugstore in Winnetka to Bethesda, Maryland, and started working with John Burns at the National Institutes of Health. I isolated and identified metabolites of zoxazolamine and chlorzoxazone from my urine (19, 20), and I also studied microsomal enzyme induction part time. We found that treatment of rats with several drugs and polycyclic aromatic hydrocarbons stimulated both ascorbic acid synthesis and hepatic aminoazo dye N-demethylase activity (21). The reasons for this intriguing relationship and the mechanisms responsible for the stimulatory effect of drugs on ascorbic acid synthesis are still unknown. We then found that treatment of rats with phenobarbital, barbital, aminopyrine, phenylbutazone, orphenadrine, or benzo[a]pyrene caused a marked increase in the activity of liver microsomal enzymes that metabolized several drugs (22). Evidence was presented in our early studies that administration of phenobarbital induced the synthesis of drug-metabolizing enzymes and also stimulated liver microsomal protein synthesis (22, 23). A young Alfred Gilman provided considerable help with these studies during a summer internship prior to his interest in G proteins (23). Administration of several drugs individually enhanced the ability of liver microsomes to metabolize the same or a closely related compound. Thus, treatment of rats with phenylbutazone, aminopyrine, benzo[a]pyrene, or phenobarbital increased the ability of liver microsomes to metabolize the compound administered or a closely related compound (22). At the time we were studying the effects of drugs and polycyclic aromatic hydrocarbons as inducers of microsomal monooxygenases at the National Institutes of Health, Drs. Herbert Remmer and Ryuichi Kato, working independently, also demonstrated a stimulatory effect of barbiturates and other drugs on drug metabolism (24–27).

SELECTIVE ENZYME INDUCTION BY BENZO[A]PYRENE: EVIDENCE FOR MULTIPLE DRUG-METABOLIZING ENZYMES

In 1959, we reported that treatment of rats with benzo[a]pyrene had a selective stimulatory effect on the hydroxylation and N-demethylation of some foreign compounds but not others. Treatment of rats with benzo[a]pyrene had a large stimulatory effect on the hydroxylation of benzo[a]pyrene, acetanilide, and zoxazolamine, but there was little or no effect on the hydroxylation of chlorzoxazone (Table 1) (28). Similarly, treatment of rats with benzo[a]pyrene markedly stimulated the N-demethylation of 3-methyl-4-monomethylaminoazobenzene (3-Me-MAB) but had a much smaller stimulatory effect on the N-demethylation of N-methyl-aniline, and this treatment inhibited the N-demethylation of diphenhydramine (Benadryl®) and meperidine (Table 1) (28). These studies suggested a family of monooxygenases with individual members that were under separate regulatory control. In additional studies, we found that treatment of rats with benzo[a]pyrene stimulated

TABLE 1 Selective induction of liver microsomal monooxygenase activities by benzo[a]pyrene

		Metabolism (nmol per gm liver/hr)		
Reaction	**Substrate**	**Control**	**Benzo[a]pyrene**	**Activity ratio**
Hydroxylation	Benzo[a]pyrene	0.42	5.1	12.0
	Acetanilide	0.48	3.1	6.5
	Zoxazolamine	1.3	4.9	3.8
	Quinoline	0.20	0.41	2.0
	Chlorzoxazone	2.2	3.0	1.4
N-Demethylation	3-Me-MAB	1.9	10.0	5.3
	N-Methyl-aniline	0.41	0.82	2.0
	Benadryl	0.46	0.32	0.70
	Meperidine	0.38	0.12	0.32

Immature male rats were injected once with corn oil or with 1 mg of benzo[a]pyrene in corn oil, and the animals were sacrificed 24 h later. 3-Me-MAB is 3-methyl-4-monomethylaminoazobenzene. Taken from Ref. (28).

the hydroxylation of the muscle relaxant drug zoxazolamine to an inactive product, stimulated the in vivo metabolism of zoxazolamine, and shortened its duration of action from 730 min to 17 min (22). In contrast to these observations, treatment of rats with benzo[a]pyrene did not stimulate the liver microsomal metabolism of hexobarbital or shorten its duration of action (22). Although benzo[a]pyrene had a selective stimulatory effect on the metabolism of only certain drugs, administration of phenobarbital or the administration of several other drugs stimulated the metabolism of many drugs (22). Selectivity for enzyme induction by polycyclic aromatic hydrocarbons such as benzo[a]pyrene and 3-methylcholanthrene and the broadness of inducing activity for phenobarbital towards many substrates gave rise to the notion during the 1960s of two kinds of enzyme inducers for xenobiotic metabolism—polycyclic aromatic hydrocarbons (such as benzo[a]pyrene and 3-methylcholanthrene) that were selective inducers and phenobarbital-like drugs that stimulated the metabolism of many drugs.

In 1970, I visited Hans Selye in Montreal to give a Claude Bernard Lecture, and we discussed Selye's observation that treatment of rats with certain steroids inhibited the toxicity of a large number of foreign chemicals. Pregnenolone-16α-carbonitrile was one of Selye's most active compounds, and I suggested the possibility that this steroid was an inducer of microsomal detoxifying enzymes. In 1972, we identified pregnenolone-16α-carbonitrile as a new type of inducer of xenobiotic-metabolizing enzymes in liver microsomes (29). Treatment of rats with pregnenolone-16α-carbonitrile induced a different profile of xenobiotic-metabolizing enzymes than occurred after treatment of rats with either phenobarbital or 3-methylcholanthrene (29). The cytochrome(s) that were induced by pregnenolone-16α-carbonitrile were later identified as members of the CYP3A family, a major cytochrome P450 family in human liver. Later studies indicated that

treatment of rats with 3-methylcholanthrene, phenobarbital, pregnenolone-16α-carbonitrile, ethanol, dioxin, polychlorinated biphenyls, isosafrole, or clofibrate each induced a different profile of xenobiotic metabolism and a different profile of cytochromes P450. In additional studies, Takemori & Mannering showed that addition of 10^{-5} M SKF 525-A to a mouse liver 9000 $\times$ g supernatant fraction inhibited the demethylation of morphine and codeine by about 50%, but the N-demethylation of 3-methyl-4-monomethylaminoazobenzene was unaffected (30). The selective effects of microsomal enzyme inducers and inhibitors on the oxidative metabolism of several drugs suggested multiple enzyme systems for the metabolism of these compounds, and stronger evidence for this concept would come from studies on factors that regulated the metabolism of a single substrate that was hydroxylated in multiple positions.

SPECIFICITY IN THE REGULATION OF 6β-, 7α-, AND 16α-HYDROXYLATION OF TESTOSTERONE: FURTHER EVIDENCE FOR MULTIPLE MONOOXYGENASES

In early studies, we found that treatment of rats with phenobarbital or certain other inducers of xenobiotic metabolism also increased the level of liver microsomal enzymes that metabolized testosterone, $^{4}\Delta$-androstene-3,17-dione, estradiol, estrone, progesterone, and deoxycorticosterone, and this treatment decreased the action of these steroids (31–35). During the course of this research, we developed paper chromatography methods that allowed us to measure the metabolism of ^{14}C-labeled testosterone to 6β-, 7α-, and 16α-hydroxytestosterone and to study factors that regulated these reactions. We found that the three hydroxylation reactions were selectively modulated during the development of rats with age (first 13 weeks of life), during enzyme induction, after castration, during the storage of frozen microsomes, and after the addition of chlorthion or carbon monoxide (CO) (36).

Different patterns for the development of the 6β-, 7α-, and 16α-hydroxylation of testosterone were observed during the first 13 weeks of life in male rats (Figure 2) (36). The 16α-hydroxylation of testosterone was low at birth and remained low for four weeks, but increased markedly during the next several weeks. In contrast to the 16α-hydroxylation activity, 6β-hydroxylation activity increased during the first week of life, remained relatively constant during the next six weeks, and increased moderately during the next three weeks. The 7α-hydroxylation of testosterone was low at birth and increased by one week. This activity remained constant for an additional three weeks and then decreased during the following three weeks.

Storage of frozen rat liver microsomes for 20 or 48 days at -15°C resulted in a progressive decrease in enzyme activity for the 6β- and 16α-hydroxylation of testosterone, but 7α-hydroxylation activity was stable (36). The in vitro addition of 10^{-4} M chlorthion almost completely inhibited the 16α-hydroxylation of testosterone by rat liver microsomes but only inhibited the 6β- and 7α-hydroxylation reaction by 31 and 14 percent, respectively (36, 37).

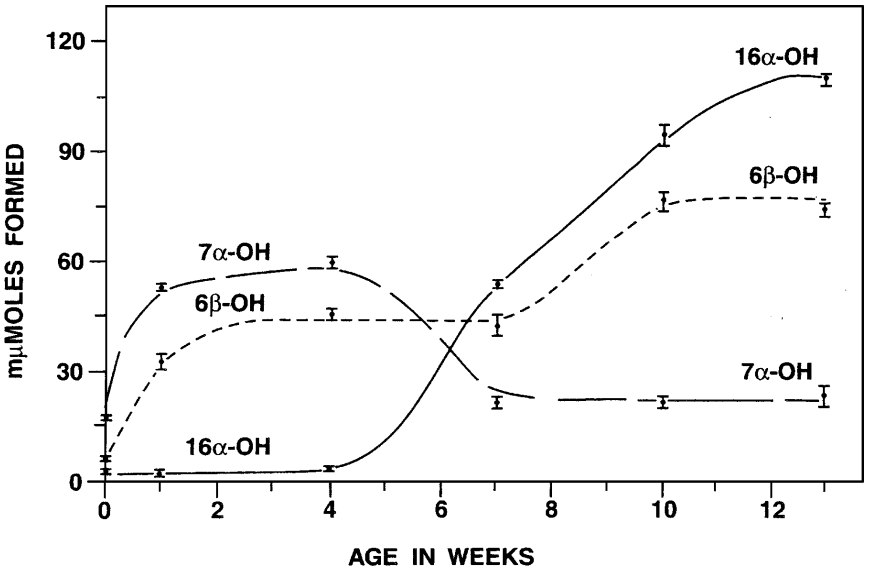

Figure 2 Effect of age on the 6β-, 7α-, and 16α-hydroxylation of testosterone by rat liver microsomes. Liver microsomes equivalent to 333 mg of wet-weight liver from male rats were incubated with 700 mμmoles of testosterone-4-C^{14} in a final volume of 5.7 ml for 7.5 min at 37°C in the presence of an NADPH-generating system. Formation of 6β-hydroxytestosterone (6β-OH), 7α-hydroxytestosterone (7α-OH), and 16α-hydroxytestosterone (16α-OH) was measured. Each value represents the average $\pm$ SE of three to six values where each value was obtained with the pooled livers from three to six rats. Taken from Ref. (36).

Treatment of immature male rats with phenobarbital for three days increased the 6β-, 7α-, and 16α-hydroxylation of testosterone by liver microsomes to different degrees (36). The 16α-hydroxylation reaction was stimulated several-fold, whereas the 6β- and 7α-hydroxylations were stimulated to a smaller extent. In contrast to these results, the administration of 3-methylcholanthrene had little or no stimulatory effect on the 6β- or 16α-hydroxylation of testosterone by liver microsomes but caused a significant increase in the 7α-hydroxylation reaction.

Early studies by Klingenberg (38), Garfinkel (39), and Omura & Sato (40, 41) demonstrated the presence of a unique liver microsomal pigment with a carbon monoxide difference spectrum with a peak at 450 nm (after reduction of the hemoprotein with dithionite). This hemoprotein was named cytochrome P450 by Omura & Sato (40, 41). In 1963 and 1965, Estabrook, Cooper, Rosenthal, and their colleagues demonstrated the functional role of cytochrome P450 for the oxidative metabolism of steroids and drugs—a key discovery (42, 43), and there was considerable debate during the middle to late 1960s about the number of liver microsomal cytochromes P450 that participated in the oxidative metabolism of drugs and

steroids. Some thought there was only a single cytochrome P450 that could exist in multiple interchangeable forms, whereas our laboratory favored the concept of multiple cytochromes P450 that were under separate regulatory control. Alvares and his colleagues in my laboratory presented evidence for the presence of at least two CO-binding cytochromes in liver microsomes (44–46). Whereas treatment of rats with phenobarbital increased the concentration of a CO-binding pigment with a reduced CO difference spectrum maximum at 450 nm, treatment of rats with 3-methylcholanthrene increased the concentration of a CO-binding pigment with a reduced CO difference spectrum maximum at 448 nm (44–46). Because this effect of 3-methylcholanthrene on the reduced CO difference spectrum maximum was prevented by treating the rats with ethionine or actinomycin D, we believed that it represented the synthesis of a previously unidentified cytochrome P450 (44–46). Other spectral evidence for the induction of different microsomal cytochromes by phenobarbital and 3-methylcholanthrene was presented by Sladek & Mannering (47).

Carbon monoxide inhibited the 6β-, 7α-, and 16α-hydroxylation of testosterone by rat liver microsomes to different extents. A CO/O_2 ratio of 0.5 inhibited the 7α-, 6β-, and 16α-hydroxylation reactions by 14%, 25%, and 36%, respectively, and the ratio of CO/O_2 needed for 50% inhibition of testosterone hydroxylation in the 16α-, 6β-, and 7α-positions was 0.93, 1.54, and 2.36, respectively (36, 48). Studies on the photochemical action spectrum revealed that CO inhibition of the three hydroxylation reactions was maximally reversed by monochromatic light at 450 nm, but there were differences in the shape of the photochemical reactivation spectra for the 6β-, 7α-, and 16α-hydroxylation reactions (36, 48). The data from our laboratory summarized above and at the First International Symposium on Microsomes and Drug Oxidation in 1968 pointed to multiple cytochromes P450 with different catalytic activities that were under separate regulatory control (36, 45, 46), and we indicated that the actual number of cytochromes that participate in the multiple hydroxylation reactions must await the solubilization and purification of the microsomal system (36). The use of different inducers of liver microsomal monooxygenases caused selective increases in the concentration of specific cytochromes P450 in liver microsomes that greatly facilitated the isolation and purification of these hemoproteins.

SOLUBILIZATION AND PURIFICATION OF MULTIPLE CYTOCHROMES P450

In 1957, we reported on the use of the detergent deoxycholate for the solubilization of aminoazo dye N-demethylase, and we also reported an inhibitory effect of CO on aminoazo dye N-demethylase activity (49). In a key study in 1968, Lu & Coon described the deoxycholate-dependent solubilization and resolution of a liver microsomal fatty acid ω-hydroxylation system into three components by column chromatography, and they were able to reconstitute catalytic activity by combining the three fractions (50). The three fractions were identified as cytochrome P450,

NADPH cytochrome P450 reductase, and a lipid fraction or phosphatidylcholine (51, 52). The reconstituted hydroxylation system was then shown to metabolize a large number of drugs, carcinogens, and steroid hormones. These studies paved the way for subsequent studies on the purification and characterization of cytochrome P450.

In 1970, Anthony Lu joined my laboratory and we initiated studies on the purification and catalytic activities of cytochrome P450 obtained from liver microsomes of control rats, phenobarbital-treated rats, and 3-methylcholanthrene-treated rats. We anticipated finding different cytochromes P450 with different catalytic activities in liver microsomes from animals treated with the different inducers, and we found that the reconstituted hydroxylation enzyme system from rats treated with phenobarbital exhibited high catalytic activity for benzphetamine N-demethylation in vitro but had very low activity for benzo[a]pyrene hydroxylation (Table 2, Experiment 1) (53). Replacement of the cytochrome P450 fraction by the cytochrome P448 fraction from rats treated with 3-methylcholanthrene resulted in a marked decrease in benzphetamine N-demethylation and a large increase in benzo[a]pyrene hydroxylation (Table 2, Experiment 1) (53). Conversely, the reconstituted system from 3-methylcholanthrene-treated rats showed high benzo[a]pyrene hydroxylase

TABLE 2 Effect of different P450 fractions on drug metabolism in reconsitituted systems

	Experiment 1		
	PB P450	**3-MC P448**	
Substrate	**nmoles metabolized**		
Benzo[a]pyrene	0.07	1.13	
Benzphetamine	3.23	0.97	
	Experiment 2		
	Control P450	**PB P450**	**3-MC P448**
Substrate	**nmole metabolite formed**		
Pentobarbital	0.10	1.00	0.06
Benzo[a]pyrene	0.33	0.23	1.29
Chlorcyclizine	1.02	2.26	1.58

Rats were treated with vehicle (control), phenobarbital (PB), or 3-methylcholanthrene (3-MC). Cytochrome P450, lipid, and reductase fractions were prepared and reconstituted. The reductase and lipid fractions were prepared from PB-treated rats. No hydroxylation activity was detected when hemoprotein was omitted from the reaction mixture. In Experiment 1, benzo[a]pyrene metabolism was measured by formation of fluorescent phenolic metabolites, and benzphetamine metabolism was measured by the rate of benzphetamine-dependent NADPH oxidation. In Experiment 2, the metabolism of pentobarbital, benzo[a]pyrene, and chlorcyclizine was measured by product formation. Experiment 1 was taken from Ref. (53) and Experiment 2 was taken from Ref. (55).

activity, which was greatly decreased when the cytochrome P448 fraction was replaced by the cytochrome P450 fraction from rats treated with phenobarbital (Table 2, Experiment 1) (53). The reductase and lipid fractions obtained from phenobarbital- or 3-methylcholanthrene-treated rats were interchangeable in supporting benzo[a]pyrene and benzphetamine metabolism. This study and subsequent studies in our laboratory indicated that the substrate specificity for the hydroxylation of drugs and steroids resided in the cytochrome P450 or P448 fractions rather than in the other components (53, 54). In additional studies, we found that the cytochrome P450 fraction from liver microsomes of control, phenobarbital-treated, or 3-methylcholanthrene-treated rats each had its own distinct substrate specificity when reconstituted with reductase and lipid (Table 2, Experiment 2) (55). These studies confirmed our prediction of the presence of multiple cytochromes P450 with different catalytic activities in rat liver microsomes. Work on the purification and properties of the multiple cytochromes P450 in rabbit liver by Minor Coon and Eric Johnson; in rat and human liver by Fred Guengerich; and in rat liver by Anthony Lu, Wayne Levin, and Ronald Kuntzman continued for the next several years and greatly advanced our understanding of the properties of these enzymes. The use of phenobarbital, 3-methylcholanthrene, pregnenolone-16α-carbonitrile (or dexamethasone), polychlorinated biphenyls, dioxin, isosafrole, isoniazid (or ethanol), and clofibrate as selective inducers to increase the levels of different liver microsomal cytochromes P450 greatly facilitated their purification, isolation, and characterization.

In 1978, Anthony Lu left my laboratory to head a drug metabolism group at Merck, and Wayne Levin and his colleagues continued studies on the purification and characterization of the multiple cytochromes P450 in rat liver microsomes. Levin and his colleagues purified to homogeneity 11 different cytochromes P450 from rat liver microsomes (56–61), and they characterized these proteins with respect to their minimum molecular weights, electrophoretic properties, spectral properties, chromatographic properties, immunological properties, primary structures, and catalytic activity profiles with a large number of xenobiotic and steroid substrates. In 1979, Levin and his colleagues demonstrated different partial amino acid sequences for different forms of cytochrome P450 (cytochromes P450a, P450b, and P450c; CYP2A1, CYP2B1, and CYP1A1, respectively) (62). This report on the partial structures of three purified cytochromes P450 demonstrated that different forms of cytochrome P450 possessed different primary structures and that these different forms of cytochrome P450 were separate gene products. In an additional early study, Haugen & Coon showed different amino acid compositions and a different COOH-terminal amino acid for cytochromes $P450_{LM2}$ and $P450_{LM4}$ from rabbit liver microsomes (63).

Paul Thomas, Wayne Levin, and their colleagues utilized their highly purified cytochromes P450 to pioneer in the development and use of monospecific antibodies (polyclonal and monoclonal) for studies on the structure, function, and regulation of multiple cytochromes P450. This research provided early reports on the immunoquantitation of specific cytochrome P450 isozymes and early reports

on the effects of age, sex, and inducers on the levels of these proteins in microsomes (64–66). By 1980, the presence of multiple liver microsomal cytochromes P450 as separate gene products that were under separate regulatory control was well established (67).

MULTIPLE MONOOXYGENASES IN HUMAN LIVER: STUDIES WITH ACTIVATOR FLAVONOIDS

An approach for determining the presence of multiple monooxygenases in rat liver microsomes was the use of chemicals that selectively affected certain monooxygenase activities but not others. For example, 7,8-benzoflavone (α-naphthoflavone) markedly inhibited the hydroxylation of benzo[a]pyrene by liver microsomes from 3-methylcholanthrene treated rats or by a cytochrome P448-dependent reconstituted enzyme system (68, 69), but there was no effect or only a small stimulatory effect of 7,8-benzoflavone on benzo[a]pyrene metabolism in livers from untreated rats (68).

In the mid-1970s, we initiated studies with 7,8-benzoflavone to determine whether there were multiple monooxygenases that metabolize xenobiotics in human liver and also to determine whether livers from different individuals had different profiles of the monooxygenases. Although we expected to find inhibitory effects of 7,8-benzoflavone on xenobiotic metabolism by human liver, we were surprised to observe a dramatic stimulatory effect of added 7,8-benzoflavone (50–100 μM) on the hydroxylation of benzo[a]pyrene by human liver homogenates or microsomes (up to an 11-fold increase in catalytic activity) (70). 7,8-Benzoflavone also increased the rates of hydroxylation of zoxazolamine and antipyrine by human liver (70) and stimulated the metabolic activation of benzo[a]pyrene 7,8-dihydrodiol and aflatoxin B_1 to mutagens (71, 72). In contrast to these results, addition of 7,8-benzoflavone to homogenates of human liver had little or no effect on the rates of oxidative metabolism of 7-ethoxycoumarin, coumarin, or hexobarbital (70). The selective stimulatory effect of 7,8-benzoflavone on the metabolism of some substrates but not others indicated the presence of multiple monooxygenases in human liver. Marked individuality for the activating effects of 7,8-benzoflavone was observed in different liver samples, and we pointed out that individuality for activation may result both from the presence of multiple monooxygenases in varying amounts and proportions in the different liver samples and from a selective effect of 7,8-benzoflavone on certain of the monooxygenases (70). Although 7,8-benzoflavone is a synthetic flavonoid, examples of naturally occurring flavonoids that are activators of monooxygenases in human liver include flavone, tangeretin, and nobiletin (72). Several-fold activation of benzo[a]pyrene metabolism by 7,8-benzoflavone was observed in liver microsomes from rabbits, hamsters, and humans, but little or no activation was observed in liver microsomes from rats or guinea pigs (73).

In mechanistic studies with cholate-solubilized human or rabbit liver microsomes, 7,8-benzoflavone decreased the Km for cytochrome P450 reductase,

increased the Vmax for benzo[a]pyrene hydroxylation, and stimulated the NADPH-dependent reduction of cytochrome P450 either in the presence or absence of benzo[a]pyrene (73). The results suggested that 7,8-benzoflavone stimulated the hydroxylation of benzo[a]pyrene in liver microsomes at least in part by enhancing the interaction between cytochrome P450 and cytochrome P450 reductase (73).

In two partially purified cytochrome P450 fractions (isolated by column chromatography) from rabbit liver microsomes, flavone had a specific stimulatory effect on one of the reconstituted partially purified cytochrome P450 systems, but an inhibitory effect on the other reconstituted cytochrome P450 system (73). In studies with highly purified cytochrome P450 isozymes from rabbit liver, benzo[a]pyrene metabolism was stimulated more than fivefold by the addition of flavone to a reconstituted monooxygenase system containing cytochromes $P450_{LM3c}$ or cytochrome $P450_{LM4}$ (74). In contrast, an inhibitory effect of flavone was observed when cytochromes $P450_{LM2}$, $P450_{LM3b}$, or $P450_{LM6}$ was used in the reconstituted system (74). Addition of 7,8-benzoflavone (50–100 μM) had a strong stimulatory effect on cytochrome $P450_{LM3c}$-mediated benzo[a]pyrene hydroxylation, but $P450_{LM6}$-mediated benzo[a]pyrene hydroxylation was strongly inhibited (74). Our results demonstrated that the activating and inhibiting effects of 7,8-benzoflavone on benzo[a]pyrene metabolism depended on the type of cytochrome P450 used in the reconstituted monooxygenase system (74). Later studies demonstrated that the activator flavonoids were potent activators of human CYP3A4 (75, 76). Other examples of activation of CYP3A4-dependent reactions included 7,8-benzoflavone-mediated stimulation of the 6β- and 16α-hydroxylation of progesterone, the 10,11-epoxidation of carbamazepine, and the 1′-hydroxylation of midazolam (77–79). Studies on the activation of xenobiotic metabolism by flavonoids were recently reviewed (80).

IN VIVO ACTIVATION OF DRUG METABOLISM

We investigated whether the activator flavonoids could activate the metabolism of drugs in vivo. In preparation for in vivo activation studies, we evaluated the effects of several naturally occurring and synthetic flavonoids on the metabolism of zoxazolamine to 6-hydroxyzoxazolamine in a five-day-old rat model. Flavone, nobiletin, tangeretin, and 7,8-benzoflavone (50–250 μM) stimulated the hydroxylation of zoxazolamine by liver microsomes obtained from five-day-old rats (81, 82). Evidence was obtained indicating that flavone decreased the apparent Km for zoxazolamine at high but not at low substrate concentrations, and the Vmax value for zoxazolamine hydroxylation was increased (82). The i.p. injection of 5 μmol of flavone together with or 90 min after a 3 μmol dose of zoxazolamine immediately stimulated the total body metabolism of zoxazolamine to 6-hydroxyzoxazolamine in five-day-old rats (81, 82). Although an i.p. injection of 5 μmol of flavone caused an immediate three- to fivefold stimulation in the in vivo metabolism of 740–3000 nmol of zoxazolamine, flavone had little or no stimulatory effect when a

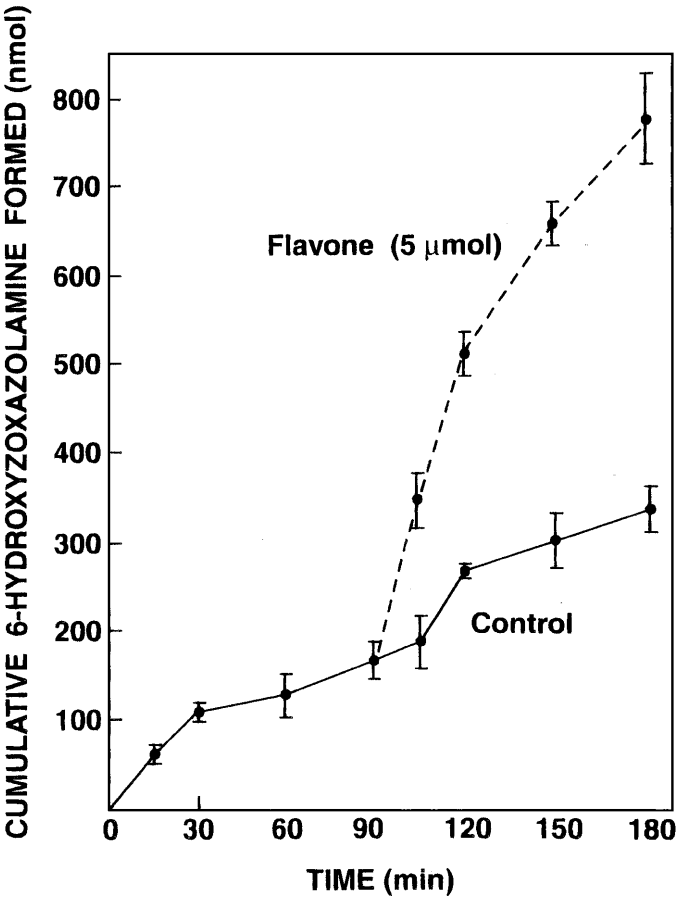

Figure 3 In vivo activation of zoxazolamine metabolism by flavone. Neonatal rats were injected with 3 μmol of [4,6-^{3}H]zoxazolamine followed 90 min later with an injection of 5 μmol of flavone or vehicle. Total body homogenates were made at the indicated times, and ^3H_2O was measured and expressed as 6-hydroxyzoxazolamine formed. Each point represents the average $\pm$ SE from four animals. Taken from Ref. (82).

much lower 74 nmol dose of zoxazolamine was administered (82). The immediate activating effect of flavone on the in vivo metabolism of zoxazolamine is shown in Figure 3.

A second example of in vivo activation of drug metabolism was recently reported by Wei Tang and his associates (83). The in vitro addition of quinidine to monkey liver microsomes or hepatocytes stimulated the cytochrome P450 3A4-mediated metabolism of diclofenac to 5-hydroxydiclofenac (83). Although quinidine had little or no effect on the Km for diclofenac metabolism by monkey

liver microsomes, the Vmax was increased 2.5-fold in the presence of quinidine. The intravenous infusion of diclofenac alone or together with quinidine in rhesus monkeys indicated that quinidine rapidly stimulated the clearance of diclofenac presumably by the activation of cytochrome P450 3A4 (83). There are now many examples of drugs that activate xenobiotic metabolism in vitro, and possible mechanisms have been described (73, 80, 82). However, the possibility of in vivo activation of foreign compound metabolism by these drugs has not been well explored. Enzyme induction and enzyme inhibition are well-recognized major mechanisms of drug-drug interactions in humans. The possibility of drug-induced activation of drug-metabolizing enzymes in humans is another potential mechanism of drug-drug interactions that is worthy of further investigation.

INDUCTION AND INHIBITION OF DRUG METABOLISM IN HUMANS

Effects of Drugs on Drug Metabolism

Many drugs are selective inducers of drug metabolism in humans and examples include phenobarbital (inducer of the CYP2B and 3A families), rifampicin (inducer of the CYP3A and 2C families), clotrimazole (inducer of CYP3A4), omeprazole (inducer of the CYP1A family), phenytoin (inducer of the CYP2C family), and ethanol (inducer of CYP2E1). Recent reports indicate that self-medication with the herbal antidepressant St. John's wort enhances the metabolism of the HIV protease inhibitor indinavir, the immunosuppressant cyclosporin, and oral contraceptives that are metabolized by CYP3A4 (84–88). The stimulatory effect of St. John's wort on drug metabolism explains the rejection of heart transplants in two patients treated with cyclosporin who also self-medicated with St. John's wort (88). Treatment of primary human hepatocytes with an extract of St. John's wort or with hyperforin (a major antidepressant constituent of St. John's wort) induces the expression of CYP3A4 (89). Hyperforin was shown to stimulate drug metabolism by functioning as a ligand for the pregnane X receptor (PXR) that regulates the expression of CYP3A4 (89). These studies with St. John's wort point out potential hazards of interactions between herbal remedies and prescription drugs.

Examples of drugs that inhibit drug metabolism in humans include bishydroxycoumarin, chloramphenicol, phenyramidol, sulfaphenazole (inhibitor of CYP2C9), cimetidine (inhibitor of several P450s), ketoconazole (inhibitor of the CYP3A family), itraconazole (inhibitor of the CYP3A family), and quinidine (inhibitor of CYP2D6). The antihypertensive drug mibefradil (potent inhibitor of the CYP3A family) is an example of a drug that was removed from the market shortly after its introduction because of serious interactions with statins and other drugs. Drug-drug interactions and interactions between drugs and herbal remedies are important problems during drug therapy and many clinically important examples have been described.

Effects of Cigarette Smoking on Drug Metabolism

Polycyclic aromatic hydrocarbons are selective inducers of cytochromes P4501A1 and 1A2 in the rat, and these hydrocarbons are ubiquitous environmental contaminants formed as products of incomplete combustion (90) that very likely contribute to interindividual differences in the metabolism of xenobiotics in humans. Because cigarette smoke contains substantial amounts of polycyclic aromatic hydrocarbons, we studied the effects of cigarette smoking on xenobiotic metabolism in experimental animals and humans. Investigations on the effects of cigarette smoking on the oxidative metabolism of drugs, carcinogens, and steroid hormones in human placenta at full term revealed that cigarette smoking markedly stimulated benzo[a]pyrene hydroxylase, aminoazo dye N-demethylase, zoxazolamine hydroxylase, and estradiol 15α-hydroxylase activities (91–94), and CYP1A1 levels were also elevated (95). Cigarette smoking had a smaller stimulatory effect on the O-dealkylation of 7-ethoxycoumarin (96) and did not change the hydroxylation of estradiol in the 2-position (94) or the oxidative aromatization of Δ^4-androstene-3,17-dione to estradiol and estrone (97). These results indicated the presence in human placenta of several monoxygenases that are under different regulatory control. Among the subjects who smoked 15 to 20 cigarettes per day, placental benzo[a]pyrene hydroxylase activity varied over a 70-fold range (97). The expression and regulation of drug metabolism in human placenta was recently reviewed (98).

Studies in rats identified pyridine and acetone as major constituents of cigarette smoke that synergistically induce hepatic and extrahepatic xenobiotic metabolism and increase the levels of CYP1A1 and CYP1A2 in liver and CYP1A1 in the lung (99). Research with human lung explants revealed induction of CYP1A transcripts by pyridine, 2-hydroxypyridine (a metabolite of pyridine), and acetone in some but not all samples (100). Additional studies on the relative inducing activities of polycyclic aromatic hydrocarbons, pyridine, and acetone alone or in combination are needed. It is likely that the stimulatory effect of cigarette smoking on human drug metabolism results from exposure to a complex mixture of inducers in tobacco smoke.

Because the metabolism of phenacetin to its major metabolite, acetaminophen, occurs by a polycyclic aromatic hydrocarbon-inducible enzyme system in rat liver and intestine (CYP1A family) (101), we investigated the effect of cigarette smoking on the in vivo metabolism of phenacetin in humans. We found that cigarette smoking lowered the plasma levels of orally administered phenacetin without changing its plasma half-life or the plasma levels of total acetaminophen (102, 103). The ratio of the concentration of total acetaminophen in plasma to that of phenacetin in plasma was markedly increased in cigarette smokers, which suggests that cigarette smoking stimulated the metabolism of phenacetin to acetaminophen in the gastrointestinal tract and/or during its first pass through the liver. Although cigarette smoking stimulated the metabolism of phenacetin in most subjects studied, some cigarette smokers did not have enhanced phenacetin metabolism.

Additional studies revealed that cigarette smokers have shorter plasma half-lives of antipyrine (104), theophylline (105), and caffeine (106) than do nonsmokers, but cigarette smoking did not stimulate the metabolism of phenytoin, meperidine, or nortriptyline, indicating selective effects of smoking on the induction of some but not other monooxygenases. The clinical significance of the effects of cigarette smoking on the metabolism and action of drugs was recently reviewed (107, 108).

Effects of Diet on Drug Metabolism

Studies in collaboration with Drs. Attallah Kappas, Alvito Alvares, and Karl Anderson at Rockefeller University and with Dr. Eugene Pantuck at Columbia University showed that several dietary factors influenced the metabolism of drugs in humans. Charcoal-broiled beef, a food that contains high concentrations of polycyclic aromatic hydrocarbons, is eaten by large numbers of people. Feeding a charcoal-broiled beef diet for several days enhanced the CYP1A1- and CYP1A2-dependent oxidative metabolism of phenacetin, theophylline, and antipyrine, but the conjugation of acetaminophen was not altered (109–111). In these studies, feeding charcoal-broiled beef for four days markedly lowered the plasma levels of orally administered phenacetin and increased the ratio of acetaminophen to phenacetin in the plasma. Marked interindividual differences occurred in the plasma concentrations of phenacetin among the nine subjects who had been fed the control diet, and there were also large individual differences in the responsiveness of the subjects to the charcoal-broiled beef diet. Switching from the control diet to a charcoal-broiled beef diet resulted in a decreased area under the plasma concentration of phenacetin versus time curve for seven of the nine subjects. The two subjects who did not respond to charcoal-broiled beef feeding had very low plasma concentrations of phenacetin while on the control diet. The reason(s) for these low plasma concentrations in two of the subjects throughout the study is unknown, but a low concentration of phenacetin in plasma may have resulted from genetic and/or environmental factors. One of the two subjects worked as a carpenter and may have been exposed to volatile oil inducers of drug metabolism that are present in certain soft woods.

Increasing the ratio of protein to carbohydrate in the diet stimulated the oxidative metabolism of antipyrine and theophylline in humans, but changing the ratio of fat to carbohydrate had no effect (112–114). Although the average half-lives of antipyrine and theophylline increased 63% and 46%, respectively, when six subjects were shifted from a high-protein, low-carbohydrate diet to an isocaloric high-carbohydrate, low-protein diet, there was considerable individuality in response to this alteration. The increase in antipyrine half-lives among the six subjects studied ranged from no change in one subject to 111% in another subject. The increase in theophylline half-lives ranged from 14% to 71% in the different subjects. Other studies have shown that switching people from their home diet to a semisynthetic diet caused a 57% decrease in the cytochrome P450-dependent oxidative

dealkylation of 7-ethoxycoumarin in the intestinal mucosa, but NADPH-cytochrome c reductase and 1-naphthol glucuronyltransferase activities were not affected (115). These results indicate that certain cytochrome P450-dependent oxidations in the intestinal mucosa are very sensitive to dietary changes.

Ingestion of cabbage and brussels sprouts for 10 days enhanced the oxidative metabolism of phenacetin and antipyrine and stimulated the glucuronidation of acetaminophen in humans (116, 117). The glucuronidation of oxazapam, however, was not enhanced (117), indicating selectivity of cabbage and brussels sprouts administration for the induction of glucuronidation of different drugs. In an additional study, feeding a brussels sprouts and broccoli–containing diet for 12 days enhanced the metabolism of caffeine (CYP1A2 probe) and decreased the urinary excretion of unchanged 2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo-(4,5b)-pyridine (PhIP) ingested in a cooked meat meal, suggesting that the vegetable diet may have enhanced the metabolism of these heterocyclic amines (118).

Ingestion of watercress, which contains high levels of phenethyl isothiocyanate, increased the urinary levels of 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (NNAL) and its O-glucuronide [metabolically inactivated metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] in smokers (119). This treatment also increased the glucuronidation of cotinine and *trans*-3′-hydroxycotinine in smokers, and the glucuronidation of *trans*-3′-hydroxycotinine correlated with the glucuronidation of NNAL (120). In other studies, ingestion of watercress inhibited the oxidative metabolism of acetaminophen and chlorzoxazone, suggesting an inhibitory effect on the activity of CYP2E1 (121, 122). Watercress administration, however, had no effect on acetaminophen glucuronidation (121). These results indicate that ingestion of watercress has a selective stimulatory effect on some but not all UDP-glucuronosyltransferase enzymes and that ingestion of watercress also inhibits CYP2E1. It is apparent from these studies that the effects of watercress ingestion on xenobiotic metabolism are complex.

A single glass of grapefruit juice increased the oral bioavailability of felodipine, nifedipine, and several other drugs that are metabolized by CYP3A4 presumably by inhibiting the first pass metabolism of these drugs in the gastrointestinal tract and/or liver (123, 124). It was shown that administration of grapefruit juice three times a day for six days increased the area under the plasma concentration–time curve for felodipine and caused a 62% decrease in the concentration of CYP3A4 in the small bowel epithelium without influencing the concentration of CYP1A1 or CYP2D6 in the small bowel or the hepatic CYP3A4 activity as measured by the [^{14}C-N-methyl] erythromycin breath test (124).

Alcoholics, when sober, show a tolerance for drugs to which they are highly sensitive when inebriated. The tolerance to drugs that is observed in alcoholics when they are sober may be explained in part by an induction of CYP2E1 and other cytochromes P450 in human liver after chronic ingestion of ethanol and by the enhanced rates of drug metabolism measured in vivo in alcoholics (125). In contrast to the stimulatory effect of chronic ingestion of alcohol on drug metabolism, the

administration of large amounts of ethanol immediately before administration of meprobamate or pentobarbital increased the plasma half-lives of these two drugs by two- to four-fold (125). This inhibitory effect of acute ethanol administration on human drug metabolism in vivo helps explain the dangerous and synergistic central depression that has been observed when ethanol and a sedative or hypnotic drug are ingested together.

The results of our studies on enzyme induction in humans indicated marked person-to-person differences in the response of individuals to enzyme-inducing substances. Some individuals were markedly induced whereas others were refractory. In addition, the extent of activation of xenobiotic metabolism in human liver by 7,8-benzoflavone was also variable and depended on the liver sample studied. Studies in twins indicated that the magnitude of the stimulatory effect of phenobarbital administration on the in vivo metabolism of antipyrine varied in different twin pairs, but much greater concordance was observed among monozygotic twins than among dizygotic twins (126), indicating that variability for induction of drug metabolism in different individuals is under genetic control. Examples and possible explanations for variability in the induction and inhibition of drug metabolism was recently reviewed by Lin & Lu (127).

INTRAINDIVIDUAL VARIABILITY IN DRUG METABOLISM

Both genetic and environmental factors control the rates and pathways of xenobiotic metabolism in humans. Early studies indicated that interindividual variations in the oxidative metabolism of certain drugs in the absence of known inducers or inhibitors are greater in dizygotic than in monozygotic twins (128–130). The importance of genetic factors in the regulation of human drug metabolism has also been emphasized by molecular genetics studies. There are now many studies demonstrating polymorphisms in genes that code for specific drug-metabolizing enzymes, and mutations in these genes can lead to impaired drug metabolism and altered drug action in patients (131). In addition, there are examples of gene duplication or multiple copies of a gene that codes for a drug-metabolizing enzyme, and these individuals metabolize the drug more rapidly than the general population (131). Although studies in subjects with genetic mutations and multiple gene copies help explain person-to-person differences in drug metabolism, environmental factors also have an important impact in explaining person-to-person differences in rates of drug metabolism.

Variability in the in vivo metabolism of a prototype drug that occurs when it is given to an individual on several occasions is an approach that we have used for assessing the influence of environment and life style on the metabolism of xenobiotics in humans (132, 133). In these studies, we found that the amount of day-to-day variation in the oxidative metabolism of three prototype drugs (phenylbutazone, antipyrine, and phenacetin) by seven healthy volunteers who

TABLE 3 Intraindividual variations in the metabolism of phenacetin as measured by AUC values

Subject	Area under phenacetin plasma concentration-time curve, 0→7 h, AUC (μg · min · ml^{-1})						% Difference min–max*
	10/21	12/2	1/13	2/24	4/6	Mean AUC	
A	250	174	156	261	115	191	127
B	40	19	31	122	19	46	542
C	266	421	768	358	234	409	228
D	480	267	414	457	1110	546	316
E	390	71	515	610	257	369	759
F	35	41	122	237	124	112	577
G	461	501	336	250	160	342	213

Phenacetin (900 mg) was administered orally between 8 and 9 a.m. to seven healthy volunteers on five occasions at approximately six week intervals. Breakfast was withheld for 2 h after each dose of drug. Plasma concentrations of phenacetin were measured, and areas under the plasma concentration-time curves (AUCs) were determined. Taken from Ref. (133).

*The percent difference between the maximum and minimum AUC of each subject $= \frac{\max}{\min} - 1 \times 100$.

were not taking any medications but who were allowed to pursue their normal life styles and to eat unrestricted diets depended on both the drug and the subject studied (132, 133). Substantial intraindividual variations in the areas under the phenacetin plasma concentration–time curves (AUCs) were observed in subjects given an oral 900 mg dose of phenacetin—a drug that undergoes extensive first pass metabolism (Table 3). When phenacetin was administered to seven individuals before breakfast on five occasions at approximately six week intervals, the percent difference in the areas under the plasma concentration of phenacetin-time curves (from the minimum to the maximum AUC) on the five occasions varied over a 127% range for subject A and over a 759% range for subject E (Table 3). Intraindividual differences for the metabolism of caffeine and dextromethorphan were recently evaluated and some individuals exhibited substantial intraindividual differences when they were studied on several occasions (134, 135). Kalow has also emphasized the use of studies on intraindividual variations in drug metabolism as an approach for assessing the role of environmental and life style factors in regulating drug metabolism (136–138).

It is important to point out that the use of intraindividual differences in drug metabolism as an approach for studies on the role of environment on drug metabolism tends to underestimate the role of environment in regulating human drug metabolism because the presence of a potent environmental modifier of drug metabolism would remain undetected unless the degree of exposure to the modifier changed during the study. Many of us lead a rather routine life, eat similar foods from day-to-day, and don't vary our life-styles dramatically from day to day. Others, however, may have a more variable life style that could influence the metabolism and therapeutic or toxic action of drugs during therapy. Environmental

factors that influence the metabolism of xenobiotics include ingestion of medicinal agents or herbal remedies, cigarette smoking, alcohol ingestion, dietary factors, viral infections, and exposure to environmental chemicals that influence the levels and activities of the multiple cytochrome P450 enzymes.

CONCLUDING REMARKS

Our studies started 50 years ago by asking why 3-methylcholanthrene administration inhibited the formation of liver cancer by carcinogenic aminoazo dyes in rats? In answering that question, we found that 3-methylcholanthrene induced the synthesis of liver microsomal enzymes that metabolized the dyes to noncarcinogenic products. These studies then led to further research indicating that polycyclic aromatic hydrocarbons had selective inducing effects on the oxidative metabolism of some foreign compounds but not others, indicating a family of xenobiotic-metabolizing monooxygenases with members that were under different regulatory control. In addition, treatment of animals with different microsomal enzyme inducers resulted in different profiles of catalytic activity for the metabolism of foreign compounds. Selective modulation of the liver microsomal 6β-, 7α-, and 16α-hydroxylation of testosterone was observed during the development of the animals with age and by the use of enzyme inducers and CO as an inhibitor. These results and the selective induction of liver microsomal CO-binding hemoproteins with different spectral properties in animals treated with phenobarbital or 3-methylcholanthrene indicated that multiple cytochromes P450 catalyzed the hydroxylation of testosterone in the 6β-, 7α-, and 16α-positions. Treatment of animals with different inducers of the monooxygenases selectively increased the levels of certain cytochromes P450 that facilitated their purification and characterization. Solubilization, purification, and characterization of the microsomal monooxygenase system confirmed the presence of multiple cytochromes P450 with different amino acid sequences and different but often overlapping substrate specificities. The number of cytochromes P450 identified in rats and humans has grown markedly in recent years and is now estimated to approach 60 in each species. The pharmacological significance of the induction of cytochromes P450 for the action of drugs and other foreign compounds depends on which ones are induced and the action and profile of metabolites that are produced by the induced cytochromes P450.

Modulation of the cytochrome P450 enzymes at the portals of entry of chemicals into the body (gastrointestinal tract, lung, and skin) and in extrahepatic target cells at or near drug receptors are particularly important areas for further research. Changes in the level or activity of a drug-metabolizing enzyme in the gastrointestinal tract may influence the amount of drug that reaches the systemic circulation. Changes in the level or activity of a drug-metabolizing enzyme at or near a receptor may change the drug's pharmacological activity without a corresponding change in the blood level.

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