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PHARMACOLOGICAL IMPLICATIONS OF MICROSOMAL ENZYME INDUCTION

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I. INTRODUCTION

The duration and intensity of action of many drugs are largely determined by the speed at which they are metabolized in the body by enzymes in liver microsomes. The activities of these enzymes can be altered by dietary and nutritional factors, hormonal changes in the body, and the ingestion of foreign chemicals. Studies during the past decade have shown that the activities of drug metabolizing enzymes in liver microsomes are markedly increased when animals are treated with various hormones, drugs, insecticides, and carcinogens. This increase in activity appears to represent an increased concentration of enzyme protein and is referred to as "enzyme induction." The induction of liver microsomal enzymes is important pharmacologically, for it leads to an accelerated biotransformation of drugs *in vivo* and so alters the duration and intensity of drug action in animals and man. Recent studies suggest that steroid hormones

and other normal body constituents are also substrates of drug-metabolizing enzymes in liver microsomes. Accordingly, treatment of animals with inducers of microsomal enzymes enhances the hydroxylation of steroid hormones and other normal body constituents by liver microsomes. This review describes some of the implications of drug-induced increases in the liver microsomal metabolism of drugs and normal body constituents.

The stimulatory effect of foreign compounds on liver microsomal enzyme activity was first observed by Brown, Miller, and Miller (29), who studied dietary factors that influence the activity of hepatic aminoazo dye N-demethylase. Studies by Conney, the Millers, and their associates revealed that in rats the injection of small amounts of polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene, 3,4-benzpyrene, and 1, 2, 5, 6-dibenzanthracene, rapidly induce several-fold increases in the activities of liver microsomal enzymes that (1) reduce the azo linkage and N-demethylate aminoazo dyes (74), (2) hydroxylate 3,4-benzpyrene (75) and several drugs (62), (3) ring-hydroxylate 2-acetylaminofluorene (86), and (4) bind aminoazo dyes in covalent linkage to protein (130). Evidence was presented in these early studies that polycyclic hydrocarbons increase enzyme activity by inducing the synthesis of more enzyme protein (74, 75). The stimulating effect of barbiturates and other drugs on liver microsomal drug metabolism was discovered independently by Remmer and his associates while investigating mechanisms of barbiturate tolerance (280, 286, 287, 291) and in our laboratory during investigations on the effects of barbiturates and several other unrelated drugs on ascorbic acid biosynthesis and drug metabolism (54, 56, 60, 73).

Research on enzyme induction in liver microsomes has expanded rapidly during recent years and several reviews have appeared that emphasize various aspects of this problem (33, 35, 38, 53, 54, 58, 59, 78, 79, 112, 127, 128, 138, 282, 283, 294).

II. CHARACTERISTICS OF THE ENZYME INDUCERS

More than 200 drugs, insecticides, carcinogens, and other chemicals are known to stimulate the activity of drug-metabolizing enzymes in liver microsomes. Examples are shown in table 1. The characteristic pharmacological actions of these compounds on the organism are extremely diverse, and there is no apparent relationship between either their actions or structure and their ability to induce enzymes. It is of interest that most of the inducers are soluble in lipid at a physiological pH.

The quantity of inducer necessary to have an appreciable effect on the enzyme varies considerably. Phenobarbital given to lactating rabbits increased the levels of drug-metabolizing enzymes in their nurslings at doses that did not affect the behavior of the offspring (119). Treatment of immature rats with as little as 0.5 mg of phenobarbital per kg intraperitoneally twice daily for several days is sufficient to significantly stimulate liver microsomal drug metabolism and decrease the anesthetic action of hexobarbital (72); however, greater responses are observed with higher doses of phenobarbital.

TABLE 1

Compounds studied as potential stimulators of drug metabolism

Pharmacological Action	Drug Tested	Effect	Reference
Hypnotics and sedatives	Barbiturates	+	56, 60, 209, 282, 283, 286, 287, 289, 292, 327
	Glutethimide (Doriden)	+	198, 199, 209, 283
	Chlorobutanol (Chloretone)	+	56, 198, 203, 209
	Urethane	+	122, 198, 283
	Carbromal (Adalin)	+	283
	Pyridione (Persedon)	+	283
	Methyprylone (Noludar)	+	283
	Ethanol	±	198, 209, 282
	Chloral hydrate	±	92, 198, 209, 282
	Ethinamate (Valmid)	0	282
	Hydroxydione (Viadril)	0	282
	Thalidomide	0	284
	Paraldehyde	0	198, 209
Anesthetic gases	Nitrous oxide	+	282
	Methoxyflurane	+	350
	Halothane	±	284, 350
	Ether	±	198, 209, 282
Central nervous system stimulators	Nikethamide (Coramine)	+	20, 198, 209, 283, 327
	Bemegride	+	283
	Pentylenetetrazol (Metrazol)	0	282, 283
	Amphetamine	0	198, 203, 209
Anticonvulsants	Methylphenylethylhydantoin (Mesantoin)	+	283
	Diphenylhydantoin (Dilantin)	+	65, 209
	Paramethadione (Paradione)	+	283
	Trimethadione (Tridione)	0	283
Tranquilizers	Phenaglycodol (Ultran)	+	198, 199, 209
	Meprobamate	+	101, 198, 209, 270
	Chlordiazepoxide (Librium)	+	170
Antipsychotics	Chlorpromazine	+	198, 209, 357
	Triflupromazine	+	198, 209
	Promazine	+	357
Hypoglycemic agents and related sulfonamides	Tolbutamide (Orinase)	+	282, 283
	Carbutamide	+	283
	Sulfaethidole	0	283
	Sulfanilamide	0	283
Anti-inflammatory agents	Phenylbutazone	+	37, 60, 91, 203
Muscle relaxants	Orphenadrine	+	60
	Carisoprodol	+	198, 209
	Zoxazolamine	±	60, 198, 203

TABLE 1—*Continued*

Pharmacological Action	Drug Tested	Effect	Reference
Analgesics	Aminopyrine	+	60
	Aspirin	0	38, 203
	Morphine and Levorphan	Decrease	9, 50, 236
Antihistaminics	Chlorcyclizine	+	37, 73
	Diphenhydramine	+	203
Alkaloids	Nicotine	+	369, 378
	Cotinine	+	378
Insecticides	Chlordane, DDT, Hexachlorocyclohexane, Dieldrin, Aldrin, Heptachlorepoxide	+	85, 116, 136, 157, 158, 213
	Pyrethrums	0	116
Steroid hormones and related substances	4-Androstene-3,17-dione	+	18
	Testosterone	+	18, 180, 200, 255, 276
	4-Chlorotestosterone	+	200
	19-Nortestosterone	+	18, 258
	Methyltestosterone	+	18
	4-Chloro-19-nortestosterone acetate	+	18, 258
	4-Chloro-17α-methyl-19-nortestosterone	+	258
	17α-ethyl-19-nortestosterone (Norethandrolone)	+	258
	Cortisone	+	279
	Prednisolone	+	279, 281
	Norethynodrel	+	192
	Progesterone	±	104, 192, 248, 255
	Estradiol	Decrease	180, 255, 276
	Cholesterol	0	29
	Oxidized cholesterol	+	29
	Oxidized dihydrocholesterol	+	29
	Oxidized ergosterol	+	29
Carcinogenic polycyclic aromatic hydrocarbons	3-Methylcholanthrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene	+	5, 60, 62, 74, 75, 86, 129, 360

The accidental finding that the spraying of animal rooms with halogenated hydrocarbon insecticides alters the action of drugs led to the discovery that these insecticides (see table I) stimulate drug-metabolizing enzymes (155, 158). Studies of drug metabolism were disrupted for several weeks, even though no more insecticide was applied. Prolonged feeding of a diet containing 5 ppm of DDT in rats results in elevated levels of liver microsomal enzymes that metabolize drugs (157). As little as 10 μg of DDT (plus its metabolite DDE) per gram

of fat is associated with a decreased action of pentobarbital (134, 316). Two days after the intraperitoneal administration of DDT, the duration of sleep produced by a dose of pentobarbital was decreased by 25% with 1 mg of DDT per kg and by 50% with 2 mg of DDT per kg, and the corresponding concentrations of DDT in the fat were 9.4 μg/g and 15.5 μg/g (134, 316). After a single intraperitoneal injection of DDT (200 mg/kg), rats metabolize hexobarbital more rapidly than normal for 65 to 90 days; and when dieldrin is the inducer, the effect lasts 20 days (136). Treatment of dogs with 5 mg of chlordane per kg orally 3 times a week for 5 to 6 weeks stimulates the metabolism of phenylbutazone, antipyrine, and bishydroxycoumarin (38, 79, 364); the increased metabolism of phenylbutazone lasts for 4 months after the last dose. The long action is attributed to the storage of these insecticides in body fat.

Organophosphate insecticides are unlike the halogenated hydrocarbons in that they inhibit, rather than stimulate, the hydroxylation of drugs and steroids by liver microsomes, when given chronically (304, 366, 368).

Inducers are of at least two types, exemplified by phenobarbital and 3-methylcholanthrene. Many compounds are like phenobarbital in stimulating varied pathways of metabolism by liver microsomes, including oxidation and reduction reactions, glucuronide formation, and de-esterification (table 2). In contrast, polycyclic aromatic hydrocarbons, typified by 3-methylcholanthrene and 3,4-benzpyrene, stimulate a more limited group of reactions (table 2). The differences between phenobarbital and polycyclic hydrocarbons have helped in the identification of several hydroxylating enzyme systems in liver microsomes (60, 62, 342). In rats or mice, 3,4-benzpyrene stimulates the 2-hydroxylation of biphenyl but not its 4-hydroxylation, whereas phenobarbital causes a large increase in the 4-hydroxylation but only a small increase in the 2-hydroxylation (88). These findings suggest that separate enzyme systems catalyze the hydroxylation of biphenyl in the 2- and 4-positions and that the syntheses of the two enzyme systems are under separate control.

When rats were given doses of the inducers that were maximal for their characteristic enzyme inductions, the liver microsomes were more active in metabolizing drugs when both 3,4-benzpyrene and phenobarbital had been given than when either had been given separately (138, 139). On the other hand, the activity was about the same when the inducers used were 3,4-benzpyrene and 3-methylcholanthrene, together or separately. Similar studies suggest that anabolic steroids and phenobarbital induce drug-metabolizing activity by different mechanisms (138, 139) and that chlordane stimulates drug metabolism by the same mechanism as phenobarbital and not that of 3-methylcholanthrene (156, 254). The N-demethylation of 3-methyl-4-monomethylaminoazobenzene by liver microsomes from normal or phenobarbital-treated rats was inhibited by SKF 525-A (β-diethylaminoethyl-diphenylpropylacetate), whereas that from rats treated with 3-methylcholanthrene was not inhibited (324). The authors suggested that the enzyme induced by the latter compound differs from the normal and phenobarbital-induced enzymes.

The two types of inducers differ in the course and intensity of induction. On

TABLE 2

Effects of phenobarbital and polycyclic hydrocarbons on microsomal pathways of drug metabolism

Animals were treated with phenobarbital or polycyclic hydrocarbons such as 3-methylcholanthrene or 3,4-benzpyrene.

Pathway Studied	Substrate	Enhanced Enzyme Activity after Treatment with	
		Polycyclic hydrocarbons	Phenobarbital
Aromatic hydroxylation	3,4-Benzpyrene	+ (75)	+ (60)
	Acetanilid	+ (62)	
	Zoxazolamine	+ (62)	+ (60)
	Chlorzoxazone	± (62)	
Aliphatic hydroxylation	Hexobarbital	0 (60)	+ (60, 286, 287)
	Pentobarbital	0 (246)	+ (73)
	Testosterone and other steroids	± (67)	+ (67)
N-Hydroxylation	N-Ethylaniline	+ (193)	
	Aniline	± (193)	
	2-Acetylaminofluorene	+ (234)	
O-Dealkylation	Acetophenetidin	+ (76)	+ (30, 138)
	p-Nitroanisole	+ (256)	+ (256)
N-Dealkylation	3-Methyl-4-monomethylaminoazobenzene	+ (60, 74)	+ (56, 60)
	Aminopyrine	0 (60)	+ (60)
	Meperidine	0 (62)	+ (291)
	N-Methylbarbital	0 (162, 226)	+ (162, 226)
S-Dealkylation	Methylthiopurine	+ (162)	
Sulfoxidation	Chlorpromazine	0 (62)	+ (305)
Dehalogenation	Methoxyflurane	0 (350)	+ (350)
	Halothane	0 (350)	+ (350)
Nitro group reduction	*p*-Nitrobenzoic acid		+ (84, 153)
	Chloramphenicol		+ (292, 294)
Azo link reduction	4-Dimethylaminoazobenzene	+ (74)	
	Neoprontosil		+ (165)
De-esterification	Procaine		+ (292)
Glucuronidation	*o*-Aminophenol	+ (6, 7, 180)	
	Sulfadimethoxine		+ (285)
	Bilirubin	+ (6, 7)	+ (48, 89, 376, 377)
	Salicylamide		+ (377)

daily administration of phenobarbital to rats, the maximal increase of enzyme activity (3- to 10-fold) is not reached for at least 3 days (60). A single intraperitoneal injection of dieldrin (40 mg/kg) or DDT (200 mg/kg) in rats elevates the metabolism of hexobarbital and acetophenetidin, tested *in vitro*, to a maximum 2 to 3 times control values in 5 to 10 days (136). After the intraperitoneal injection of polycyclic hydrocarbons, the enzymatic activity is more than doubled within 3 to 6 hours and maximal increases (5- to 10-fold) are observed after 24 hours (74, 75). The stimulating effect of 3-methylcholanthrene on the N-demethylation of 3-methyl-4-monomethylaminoazobenzene by liver microsomes is shown in figure 1.

Among more than 60 polycyclic aromatic hydrocarbons studied with N-demethylation of an aminoazo dye as a test system, the optimal size for enzyme induction ranged from 75 to 150 Å^2, and coplanar hydrocarbons were more potent than noncoplanar (5). These requirements suggest the importance of steric fit between the hydrocarbon and the biological structure upon which it acts. The potency of a polycyclic hydrocarbon as an enzyme inducer is not always correlated with its carcinogenicity. The carcinogen, 3,4-benzphenanthrene, and several of its carcinogenic derivatives do not induce hepatic enzymes, but the noncarcinogens 2'- and 3'-methyl-1,2-benzanthracene do so. Both 1, 2, 3, 4-dibenzanthracene and 1, 2, 5, 6-dibenzanthracene induce aminoazo dye N-demethylase but only the latter has carcinogenicity (5, 74). It would be of interest to compare the activity of polycyclic hydrocarbons as skin carcinogens with their activity as inducers of N-demethylases and hydroxylases in skin.

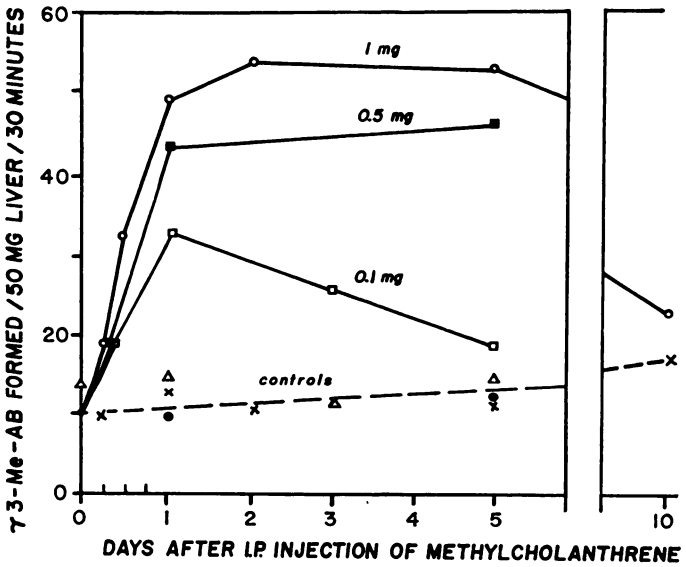


FIG. 1. Activity of hepatic aminoazo dye N-demethylase at various times after a single intraperitoneal injection of 3-methylcholanthrene into 50 g male rats (74). Activity is expressed as μg of 3-methyl-4-aminoazobenzene (3-methyl-AB) formed from 3-methyl-4-monomethylaminoazobenzene.

III. CONSEQUENCES OF ENZYME INDUCTION FOR THE ACTION OF DRUGS

When the enzyme that acts on a drug is induced, the drug is metabolized more rapidly. It disappears more quickly, and the metabolite is formed more quickly. The consequences for the organism depend upon the relative activity of the drug and its metabolite. When the metabolite has little effect of its own, enzyme induction speeds the termination of action of the drug by accelerating its inactivation. Examples are zoxazolamine (60), meprobamate (57, 209), carisoprodol (57, 199), diphenylhydantoin (91), and several barbiturates (60, 73, 198, 282, 286, 287). When the metabolite has the same effect as the drug and is of comparable or greater potency, or when the effects seen on administration are actually those of the metabolite, enzyme induction may intensify the effects by accelerating the production of the metabolite. Examples are tremorine (215), the dimethoxy ester of benzotriazine dithiophosphoric acid (Guthion) (255), and octamethylpyrophosphoramide (Schradan) (196). When the drug is not appreciably metabolized by the induced enzymes, one would expect no change in the duration or intensity of action; and this is the case with barbital in the rat (39, 73).

The magnitude of the change in drug action that may occur after enzyme induction is illustrated by data obtained in rats with zoxazolamine (60). A high dose paralyzed the rats for more than 11 hours, but after treatment with phenobarbital for 4 days the same dose paralyzed rats for only 102 minutes, and after treatment with 3,4-benzpyrene 24 hours before the test, the same dose of zoxazolamine paralyzed the rats for only 17 minutes. The biological half life of zoxazolamine is 9 hours in control rats, 48 minutes after phenobarbital, and 10 minutes after 3,4-benzpyrene. The stimulation of zoxazolamine hydroxylase activity by any of a series of polycyclic aromatic hydrocarbons paralleled their effectiveness in decreasing the muscle-relaxing action of zoxazolamine (5).

Enzyme induction alters not only the duration, but also the intensity of drug action. Whereas all control rats were killed by a dose of 150 mg of zoxazolamine per kg, none died when given a single injection of 3-methylcholanthrene 24 hours before this dose of zoxazolamine (38, 53). Similarly, enzyme induction decreases the acute toxicity of meprobamate (38, 53), pentobarbital (346), strychnine (196), bishydroxycoumarin (90), warfarin (178), phenylbutazone (364), lidocaine, mepivicaine, and cinatest (159).

The opposite effect is illustrated by Schradan. A certain dose killed 6% of control rats, but 80% of the rats treated with enzyme inducers (phenaglycadol, thiopental) (196). By inducing the enzyme that metabolizes Guthion, 3-methylcholanthrene increases the lethal effect of Guthion, whereas inhibition of this enzyme with SKF-525 A decreases the lethal effect (255). Welch and Coon (363) reported that treatment of mice with phenobarbital or chlorcyclizine markedly decreased the acute lethal effect of malathion, parathion, and EPN [O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate]. The authors suggested that phenobarbital and chlorcyclizine increase the levels of certain esterases in the liver and plasma that, by combining with the cholinesterase inhibitors, prevent them from reacting with the more important acetylcholinesterase. More recently, DuBois and Kinoshita (105) suggested that nikethamide and phenobarbital decrease the lethal effect of EPN by accelerating its hepatic metabolism.

The halogenated hydrocarbon insecticides can markedly influence the acute toxicity and other effects of drugs. Treatment of immature rats with DDT or chlordane for several days increased the LD50 of warfarin more than 10-fold, and the protection of rats was associated with decreased plasma levels of warfarin and enhanced activity of liver microsomal enzymes that metabolize this drug (178). Treatment of rodents with halogenated hydrocarbon insecticides increase the activity of enzymes in liver microsomes that oxidatively metabolize drugs such as hexobarbital, aminopyrine, and chlorpromazine (116, 155, 157, 158) and protects the animals from the lethal effects of bishydroxycoumarin (90).

Inducers of enzymes can deplete the body of substances like insecticides, which accumulate in body fat and are metabolized by hepatic microsomal enzymes. DDT given to rats previously fed dieldrin or heptachlor markedly decreases the storage of the latter compounds in the fat (338–340). DDT, 50 ppm, in the feed also causes a 15-fold reduction in the amount of dieldrin in the fat of rats simultaneously fed 1 ppm of dieldrin. The quicker depletion of dieldrin continues for weeks after discontinuing the DDT. Similar effects occur in the rat, swine, and sheep, but not in the chicken (340), and the same effect can be produced by other stimulators of liver microsomal enzymes, such as phenobarbital, tolbutamide, aminopyrine, heptabarbital, phenylbutazone, and methoxychlor (93, 339, 341). Treatment of rats with phenobarbital markedly accelerates the elimination of the metabolites of hexachlorocyclohexane (214).

Repeated administration of a drug often results in the induction of enzymes that metabolize the drug. Chronic treatment with the drug accelerates its metabolism, lowers its blood level, and decreases its effect. Examples of drugs that stimulate their own metabolism are listed in table 3. After a single dose of Citrus

TABLE 3

Drugs that stimulate their own metabolism during chronic administration

Drug	Species	Reference
Phenylbutazone	Dog, rat	38, 60
Chlorcyclizine	Dog	38
Probenecid	Dog	38
Tolbutamide	Dog	282, 298
Hexobarbital	Dog, rat	283, 290
Pentobarbital	Rat, rabbit	283, 289, 290, 299
Phenobarbital	Dog, rat	112, 296, 297
Aminopyrine	Rat	60
Meprobamate	Man, rat	101, 198, 209, 270
Glutethimide	Man	312
Chlorpromazine	Rat	110
Chlordiazepoxide	Rat	170
DDT	Rat	251
Citrus red no. 2	Rat	277
Methoxyflurane	Rat	350
3,4-Benzpyrene	Rat	75
9,10-Dimethyl-1,2-benzanthracene	Rat	70, 356
Benzene	Rabbit	329

Red No. 2 (2,5-dimethoxyphenyl-azo-2-naphthol), a high concentration of this dye occurred in the fat of rats, but when it had been given daily for 5 days, the concentration in fat was reduced by a factor of 15, and after 7 daily doses no dye could be detected in the fat (277). This change is attributed to accelerated metabolism of the dye, since closely related compounds stimulate liver enzyme activity (70). After the first day of administration of a high dose of phenylbutazone, rats developed gastrointestinal lesions and had high plasma levels of the drug, but after daily administration of phenylbutazone for two weeks the lesions were absent and the plasma levels were low (40, 363a).

When the responses to a drug diminish upon repeated administration of the drug, the phenomenon is called "tolerance." Remmer showed that tolerance to several barbiturates is related to their accelerated metabolism in the liver (283, 289, 290, 296, 297, 299). In many instances, little or none of the tolerance can be attributed to the induction of enzymes. This is true of tolerance to barbital in the rat, since this compound is not appreciably metabolized (39) and its distribution is the same in tolerant as in normal rats (109). Although phenobarbital stimulates its own metabolism when high doses are administered chronically to dogs (296, 297), Butler *et al.* (45a), demonstrated that tolerance to this drug in dogs and people was not related to enhanced metabolism, but occurred presumably by an adaptation of the central nervous system. The tolerance to morphine or meperidine cannot be explained by enhanced liver microsomal metabolism (9), nor can the tolerance to ethanol be explained by increased alcohol dehydrogenase activity in the soluble fraction of liver (151). Other aspects of tolerance have been discussed by Axelrod (9) and Shuster (320).

Enzyme induction can seriously affect the results of tests meant to evaluate the metabolism or the acute or chronic toxicity of drugs. Care should be taken to exclude halogenated hydrocarbon insecticides from animals used in such studies. Pyrethrum is suitable for use in laboratory animal quarters, since it does not stimulate drug metabolism (116). When mice previously housed in plastic cages with corncob bedding were placed in cages with red cedar chips, the duration of action of barbiturates was decreased (113). This observation suggests the possible presence of inducers of liver microsomal enzymes in the red cedar chips. Scientific advisors to the World Health Organization have discussed the importance of enzyme induction in chronic toxicity tests (374). The administration of a drug for a short time may provide more information about its toxicity than its administration for weeks or months; and in the cases in which enzyme induction occurs, it is not yet clear what is the best protocol to follow.

New drugs are tested for effects on the fetus by feeding subtoxic doses to male and female rats for a preliminary period of 2 months and then through two pregnancy cycles. This test is intended to detect drugs that affect spermatogenesis, fertility, or implantation, or that cause teratologic effects. An erroneous interpretation of the results can occur when the drug studied stimulates its own metabolism (34). Chlorcyclizine, for example, on chronic administration stimulates its own metabolism and also that of its metabolite, norchlorcyclizine (37, 38). When pregnant rats were given chlorcyclizine during days 10 to 15 of gesta-

tion, the fetuses had a high concentraion of norchlorcyclizine and a high incidence of cleft palates, but if the pregnant rats were treated with the same dose of chlorcyclizine from the 1st to the 15th day of gestation, fetal concentrations of norchlorcyclizine were low and no cleft palates occurred (210). These results stress the need for further work to design adequate protocols for teratologic studies when enzyme induction occurs.

Several examples are now known in which enzyme induction blocks chemical carcinogenesis. Treatment of rats with 3-methylcholanthrene or certain other polycyclic hydrocarbons stimulates the liver microsomal metabolism of aminoazo dyes and 2-acetylaminofluorene to noncarcinogenic metabolites (74, 86). These results explain why the hydrocarbons inhibit the ability of aminoazo dyes and 2-acetylaminofluorene to cause liver cancer, mammary cancer, and ear duct cancer (245, 247, 249, 300). The administration of 9, 10-dimethyl-1,2-benzanthracene causes adrenal damage and mammary cancer in rats (173, 175), and both effects are inhibited by various polycyclic aromatic hydrocarbons and aromatic azo derivatives (94, 171, 172, 174, 176) that stimulate the activity of hepatic enzymes metabolizing 9,10-dimethyl-1,2-benzanthracene (70, 185). A report indicating that phenobarbital inhibits the carcinogenicity of 3,4-benzpyrene (212) may also be explained by the stimulatory effect of phenobarbital on the hydroxylation of 3,4-benzpyrene to noncarcinogenic metabolites (60). Recently, it was reported that treatment of rats with 3-methylcholanthrene or 1,2-benzanthracene prevented the formation of ear duct tumors by 4-dimethylaminostilbene (343), but the effect of these polycyclic hydrocarbons on 4-dimethylaminostilbene metabolism is unknown. The ability of one chemical to block cancer formation by another in animals raises the possibility that cancer formation by environmental carcinogens in man might be blocked by suitable nontoxic enzyme inducers (section XIII). This concept has led Wattenberg to search for nontoxic chemicals that induce the synthesis of benzpyrene hydroxylase activity in liver, lung and gastrointestinal tract (354, 355, 357). In some instances, however, carcinogens are formed by the drug-metabolizing enzymes, as in the case of dimethylnitrosamine (235a).

IV. TESTS FOR ENZYME INDUCTION

In order to find out whether a compound induces drug-metabolizing enzymes, it is ultimately necessary to prepare the liver or its fractions from treated animals and assay enzyme activity with suitable drugs as substrates. Simpler tests have been devised to identify compounds worthy of more detailed study, or to explore enzyme induction in man. They involve measurement of the duration of action of standard drugs or the amount of an easily measured drug or metabolite in the blood or urine. Hexobarbital and zoxazolamine are useful test drugs since their duration of action in the body is regulated largely by the levels of liver microsomal enzymes that inactivate them and since most of the inducers (table 1) decrease the duration of action of one or both of them. It is important to study both test drugs, since both are metabolized more rapidly after treatment of the animal with the compounds of the phenobarbital type (section II), whereas com-

pounds of the 3-methylcholanthrene type accelerate the metabolism of zoxazolamine but not hexobarbital (5, 60). We inject the suspected inducer twice daily for 4 days into 50-g male rats, and on the 5th day we administer a dose of hexobarbital or zoxazolamine and measure the duration of sleep or paralysis (60).

The rate of metabolism of a barbiturate in rats or dogs has been used as a test for the induction of enzymes (282, 297). In dogs, phenylbutazone remains in the plasma at convenient levels 7 hours after an intraperitoneal injection of 25 mg/kg, and some of the inducers of liver microsomal enzymes given chronically cause the plasma level of phenylbutazone to be much lower at this interval (37, 38, 91). Antipyrine is distributed through body water and does not bind appreciably to plasma proteins, and the rate of its metabolism in dogs, measured by determining blood levels, is accelerated after chronic treatment with representative inducers of the phenobarbital type (79, 90, 365). In man, or in the dog or rat, Remmer and his associates (283, 297, 322) have given Dipyrone and measured the urinary excretion of its metabolite 4-aminoantipyrine as an indicator of enzyme induction.

Since drugs that stimulate drug metabolism by liver microsomes also stimulate the hydroxylation of steroids in animals (65, 67, 78) and man (16, 42, 218, 370) (sections XI, XII), the measurement of the urinary excretion of the metabolite of cortisol, 6β-hydroxycortisol, in relation to the total 17-hydroxycorticosteroids (which are not changed by the inducers), may be a useful index of the induction of liver microsomal hydroxylases in man.

In rats, the urinary excretion of ascorbic acid may be used as an index of enzyme induction, since compounds that increase the activity of microsomal enzymes stimulate the metabolism of glucose and galactose *via* the glucuronic acid pathway through D-glucuronic acid and L-gulonic acid to ascorbic acid (34, 54, 56) (section XI). There is a parallel acceleration of the synthesis of D-glucaric acid, and treatment of patients with typical inducers causes them to excrete more D-glucaric acid in the urine (1). This may prove to be useful as a test for enzyme induction in man.

Examination of the hepatic parenchymal cell under the electron microscope reveals proliferation of the smooth-surfaced endoplasmic reticulum in animals treated with the liver microsomal enzyme inducers phenobarbital, tolbutamide, nikethamide, chlordane, and DDT (120, 267, 293, 294). Chemicals like 3-methylcholanthrene, which stimulate a smaller number of microsomal pathways, have a smaller effect on the smooth-surfaced endoplasmic reticulum (120). Electron microscopic examination of the smooth-surfaced endoplasmic reticulum may be a useful index of drug-induced changes in liver microsomal enzyme levels.

V. MECHANISM OF INDUCTION OF MICROSOMAL ENZYMES BY POLYCYCLIC HYDROCARBONS AND DRUGS

Although several hormones can alter the activity of drug-metabolizing enzymes (section X), the stimulation of TPNH-dependent enzymes in liver microsomes by polycyclic hydrocarbons or drugs does not require the hormones of the testis or ovary, or of the adrenal, pituitary, or thyroid glands. 3-Methylchol-

anthrene still increases aminoazo dye N-demethylase activity in hypophysectomized, adrenalectomized, or ovariectomized rats (74, 319). Phenobarbital and chlorcyclizine still increase the levels of enzymes that oxidize barbiturates in hypophysectomized or adrenalectomized-castrated rats (73), and phenobarbital stimulates the liver microsomal metabolism of aminopyrine and hexobarbital in thyroidectomized rats (138, 264). Whereas thalamic lesions in rats do not influence the induction of aminoazo dye N-demethylase by 3-methylcholanthrene, hypothalamic lesions partially inhibit this response (319).

Addition of phenobarbital, chlordane, 3-methylcholanthrene, or 3, 4-benzpyrene to liver homogenates does not increase the activity of drug-metabolizing enzymes (60, 74, 75, 156). *In vivo* the increase in enzyme activity does not appear for several hours (60, 74, 75, 282), and a similar course of induction can be shown in the isolated perfused liver. Juchau *et al.* (191) found that polycyclic hydrocarbons perfused through isolated rat livers for 6 hours enhanced the activity of benzpyrene hydroxylase. A similar direct effect of polycylic hydrocarbons on benzpyrene hydroxylase occurs in lung organ culture (359).

Attempts to demonstrate that polycyclic hydrocarbons or phenobarbital enhance drug-metabolizing activity by increasing the level of a possible endogenous activator or cofactor or by decreasing the level of an inhibitor have been unsuccessful (60, 74, 201). Liver homogenate from animals treated with phenobarbital or 3-methylcholanthrene does not alter the activity of drug-metabolizing enzymes in control liver homogenate when the two homogenates are mixed.

A. Effect of drugs on the kinetics of drug metabolism by liver microsomes

The stimulatory effect of phenobarbital or polycyclic hydrocarbons on liver microsomal enzyme activity cannot be explained on the basis of an altered affinity of the enzyme for the substrate. Phenobarbital administration increases the maximal velocity of ethylmorphine N-demethylation, chlorpromazine sulfoxidation, and hexobarbital oxidation but does not influence the Michaelis constants or the susceptibility of the enzyme systems to various inhibitors (305). Phenobarbital and 3,4-benzpyrene increase the maximal velocity of *p*-nitroanisole O-demethylation without influencing the Michaelis constant of the O-demethylase (256). Similarly, the Michaelis constant for aminopyrine N-demethylase (138) or for solubilized procaine esterase from liver microsomes (282) was not altered by administration of phenobarbital.

B. Inhibition of enzyme induction by inhibitors of protein synthesis

Evidence that drug-induced increases in microsomal enzyme activity represent an induction of more enzyme protein came from the finding that certain inhibitors of the synthesis of protein or DNA-dependent RNA prevent drug-induced increases in microsomal enzyme activity. The induction of drug-metabolizing enzyme activity by 3-methylcholanthrene or phenobarbital is blocked by ethionine (60, 74, 75, 124, 196), puromycin (63, 129, 264), or actinomycin D (dactinomycin) (53, 129, 264, 266). These inhibitors block protein synthesis by

different mechanisms. Ethionine blocks protein synthesis by decreasing the ATP levels in liver (351), puromycin blocks the transfer of soluble RNA-bound amino acid into microsomal protein (379), and actinomycin D, by binding to DNA, blocks the DNA-directed synthesis of nuclear RNA required for protein synthesis (278). The inhibition of protein synthesis in liver caused by ethionine can be prevented *in vivo* by the administration of either methionine or ATP. In a similar manner, ATP (351) or methionine (60, 74, 75) prevent the inhibitory effect of ethionine on the induction of drug-metabolizing enzymes. It is not known why the effects of ethionine are blocked by either ATP or methionine, but these results suggest that adequate levels of S-adenosyl-methionine may be important for the synthesis of microsomal enzymes in the liver. The ability of actinomycin D to block the induction of drug-metabolizing enzymes by 3-methylcholanthrene or phenobarbital suggests that these enzyme inducers may accelerate the DNA-directed synthesis of RNA molecules that function as templates for the synthesis of drug-metabolizing enzymes on ribosomes. When puromycin, ethionine, or actinomycin D was administered several hours after 3-methylcholanthrene, these blocking agents prevented further increases in the level of aminoazo dye N-demethylase, and enzyme activity was maintained at a partially elevated level (53, 63, 74). The results with actinomycin D suggest that 3-methylcholanthrene may increase the formation of a short-lived messenger RNA that is required for the synthesis of aminoazo dye N-demethylase.

Jervell *et al.* (187) recently found that whereas the induction of azo dye reductase activity by 3-methylcholanthrene was inhibited by ethionine, puromycin, or actinomycin D, the induction of this enzyme by starvation was blocked by ethionine or puromycin, but not by actinomycin D. The authors suggested that although the 3-methylcholanthrene induction of azo dye reductase activity may be caused by enhanced DNA-directed RNA synthesis, the induction of this enzyme by starvation occurs *via* another mechanism, perhaps at the level of messenger RNA translation on the ribosomes. It is of interest that the induction of aminoazo dye reductase by starvation was blocked by the administration of glucose or fructose (187). The carbohydrate repression of enzyme synthesis in microorganisms has been known for a long time, but repression of mammalian enzyme synthesis by carbohydrates is a recent discovery by Pitot and Peräino (268, 271), who showed that glucose and fructose repress the formation of threonine dehydrase and ornithine δ-transaminase in the liver. A similar carbohydrate repression of the induction of δ-aminolevulinic acid synthetase has also been described (349).

C. Effect of drugs on the synthesis and degradation of microsomal enzymes

Mammalian enzyme levels can be increased by enhancing the rate of enzyme synthesis or by inhibiting the rate of enzyme degradation. The increase in hepatic tryptophan pyrrolase activity in rats treated with tryptophan results from the stabilization of tryptophan pyrrolase, which normally has a short half-life of about 2 hours *in vivo* (13, 309, 310, 311). Cortisol, on the other hand, accelerates the synthesis of tryptophan pyrrolase without preventing its degradation. The

level of arginase in rat liver is also regulated by changes in the rate of either synthesis or degradation of this enzyme (308). The stabilization of arginase, which normally has a half life of 4 to 5 days, results in a 2-fold increase in arginase in 5 days. The possibility that drugs, carcinogens, and insecticides may increase the levels of liver microsomal enzymes by stabilizing these enzymes, as well as by accelerating their rate of synthesis, is now being studied in several laboratories.

Jick and Shuster (188) suggested that treatment of mice with phenobarbital increased liver microsomal TPNH-cytochrome c reductase levels by increasing its rate of synthesis and by decreasing its rate of breakdown. This enzyme was chosen for study because its level in liver microsomes is increased by treatment with phenobarbital and because it can be purified. In order to measure the turnover of TPNH-cytochrome c reductase, tritiated L-leucine was injected into mice 24 hours before phenobarbital treatment for 3 days. The half time for loss of radioactivity from TPNH-cytochrome c reductase *in vivo* averaged 2.8 days in control mice, but there was no loss of radioactivity from the enzyme in phenobarbital-treated animals. Evidence that phenobarbital stimulated the synthesis of TPNH-cytochrome c reductase came from the finding that the incorporation of tritiated L-leucine into microsomal TPNH-cytochrome c reductase over a 90-minute interval was increased 2- to 3-fold in mice treated for 2 or more days with phenobarbital (188). Studies by Shuster and Jick (321) suggested that phenobarbital increased the total liver microsomal protein in rats by increasing the rate of synthesis and by decreasing the breakdown of microsomal protein. A similar inhibitory effect of phenobarbital on liver microsomal phospholipid degradation was recently proposed by Holtzman and Gillette to explain the accumulation of liver microsomal phospholipid in fasted rats treated with phenobarbital (169).

The molecular basis for liver microsomal enzyme induction by structurally unrelated drugs, carcinogenic hydrocarbons, insecticides, and hormones is unknown. The enzyme inducer could stimulate the formation of more enzyme by interacting directly with DNA in such a way as to stimulate the DNA-directed synthesis of specific messenger RNA. The inducer could also interact with repressors synthesized by a regulator gene (182, 183) or with other regulators of gene function such as histones (3, 43). It is also possible that enzyme inducers may interact with the endoplasmic reticulum so as to enhance the translation of messenger RNA on the ribosomes. Alternatively, the inducer could interact with the finished end product of gene activity, the microsomal drug-metabolizing enzyme itself. This interaction might lead to increased enzyme levels by inhibiting the degradation of the microsomal enzyme *in vivo* or by preventing a feed-back inhibition of enzyme synthesis. Nearly all the drugs listed in table 1 as enzyme inducers are lipid-soluble molecules, capable of penetrating the microsomes, and many of these compounds have been shown to exert a biphasic effect on drug metabolism. Thus, drug metabolism is inhibited during the first 6 hours after drug administration and then is stimulated after 12 hours (20, 202, 203, 282, 283, 306, 317, 318). Compounds that exert a biphasic effect on drug metabolism or duration of drug action include chlorcyclizine, glutethimide, phenagly-

cadol, nikethamide, tolbutamide, trimethadione, mesantoin, N-methyl-3-piperidyl diphenyl carbamate (MPDC), and β-diethylaminoethyl diphenylpropyl acetate (SKF 525-A). Phenobarbital (203) and 3-methylcholanthrene (74) have little or no inhibitory effect on drug metabolism in rats shortly after their administration. Both compounds are lipid soluble, however, and recent studies have shown that the injection of phenobarbital into rats results in a rapid binding of this drug to liver microsomes (112).

D. Stimulatory effect of drugs on liver growth and the synthesis of protein in liver microsomes

Phenobarbital not only elevates drug-metabolizing activity in liver microsomes, but also exerts a marked anabolic effect on the liver, which results in a 20 to 40% increase in microsomal protein per g of liver (60, 63, 292). In contrast, polycyclic hydrocarbons such as 3-methylcholanthrene cause little or no increase in the amount of microsomal protein per g of liver, but do stimulate liver growth and the synthesis of total liver protein (5, 63, 74). These studies suggested a relationship between the ability of polycyclic hydrocarbons to stimulate liver growth and their ability to induce microsomal enzyme formation (5). Besides phenobarbital and polycyclic hydrocarbons, chemicals that stimulate liver growth and increase liver microsomal enzyme levels include chlorcyclizine (314, 363), nikethamide (21, 373), chlorpromazine (323), phenylbutazone (77, 323), benzydamine (323), chlordane (85, 156), aldrin (216), and hexachlorocyclohexane (315). Chloroform, halothane and penthrane are examples of chemicals that increase liver growth without enhancing liver microsomal enzyme activity (216). Golberg (144) recently studied ten 2-tertbutylphenol derivatives and found that their ability to stimulate liver growth was not correlated with their ability to stimulate the activity of aminopyrine N-demethylase or hexobarbital oxidase. An extensive study of the effects of several barbiturates and other drugs on liver growth in the mouse was described by Kunz and his associates (216). Histological studies and DNA analyses showed that phenobarbital (41, 63, 164), nikethamide (21, 373), chlorcyclizine (314) and hexachlorocyclohexane (315) increase liver growth in rats by stimulating liver mitosis and cell division. Although Herdson and his associates reported that treatment of rats with phenobarbital did not increase the size of liver parenchymal cells (164), Preis and his associates found that treatment of mice with phenobarbital caused a marked increase in the size of the liver cell (274). Drugs stimulate the growth not only of normal liver, but also of regenerating liver. Diphenylhydantoin (326), phenylbutazone (135), nikethamide (135) and acenaphthalene (135) were recently shown to stimulate liver regeneration in the partially hepatectomized rat. It is also of interest that phenobarbital and several other stimulators of liver protein synthesis accelerate bromsulfophthalein (BSP) clearance in rats (123, 323) and that phenobarbital accelerates bile flow (301). The physiological significance of enhanced liver growth and enhanced liver function in animals treated with various drugs has not yet been determined, and it is not clear whether these effects are harmful or beneficial. The ability of drugs such as phenobarbital,

nikethamide, and diphenylhydantoin to enhance liver growth and liver regeneration without obvious toxicity raises the question of whether such drugs would be useful in persons with poor liver function (section XIII).

E. Drug-induced increases in incorporation of amino acids into liver microsomal protein

Studies by Gelboin and Sokoloff (131) with 3-methycholanthrene and phenobarbital and by Von der Decken and Hultin (353) with 3-methylcholanthrene showed that the stimulatory effect of these chemicals on the synthesis of microsomal enzymes and protein *in vivo* is paralleled by increased incorporation of amino acids into microsomal protein *in vitro*. Kato and his associates (208) showed that treatment of rats with phenobarbital stimulates the incorporation of C^{14}-leucine into microsomal protein *in vivo* but has no effect on the incorporation of this amino acid into protein in other subcellular fractions. The maximal stimulation of the amino acid incorporating system in liver by phenobarbital is greater than that produced by 3-methylcholanthrene (131); these results are in accord with the observation that phenobarbital can stimulate more synthesis of microsomal protein *in vivo* than 3-methycholanthrene (63). Gelboin and Sokoloff showed that 3-methylcholanthrene administration stimulated the incorporation of soluble RNA-bound amino acids into liver protein *in vitro*. This finding suggested that the hydrocarbon was acting, not on reactions leading to activated amino acid, but on steps between transfer RNA and the formation of protein on the ribosomes (131). These investigators suggested that the increase in amino acid incorporation induced by 3-methylcholanthrene was due at least in part to an increase in the number of active microsomal incorporation sites and an apparent increase in the messenger-RNA content of the microsomes (126, 132). After removal of endogenous messenger-RNA by incubation of the microsomes, L-C^{14}-phenylalanine incorporation into microsomal protein was completely dependent on added polyuridylic acid, and, in the presence of saturating amounts of polyuridylic acid, microsomes from rats treated with 3-methylcholanthrene incorported more L-C^{14}-phenylalanine than control microsomes (126).

Treatment of rats with phenobarbital increased by 108 to 266% the incorporation of arginine, leucine, lysine, phenylalanine and valine into liver microsomal protein *in vitro* (206). This effect could not be attributed to changes in cofactor level, amino acid concentration or factors present in the cell sap. After removal of endogenous messenger-RNA by incubation of microsomes, the microsomes from phenobarbital-treated rats displayed greater L-C^{14}-phenylalanine-incorporating activity in the presence of either subsaturating or saturating amounts of polyuridylic acid (206, 207). Thus, the microsomes from phenobarbital-treated rats devoid of their greater endogenous messenger-RNA content are more sensitive than control microsomes to the addition of an exogenous messenger-RNA such as polyuridylic acid. The greater phenylalanine-incorporating activity of phenobarbital microsomes that contain endogenous messenger RNA and the greater sensitivity added to polyuridylic acid are characteristic of microsomes from phenobarbital-treated rats, but do not occur with ribosomes

from the same animals. Thus, phenobarbital-induced increases in microsomal amino acid incorporation are not directly related to alterations in the ribosomes. The results suggest that phenobarbital alters a component in the membranes of the endoplasmic reticulum that is important for the translation of polyuridylic acid on the ribosomes. These effects of phenobarbital treatment are similar to those obtained in regenerating liver by Campbell *et al.* (46), who found that microsomes but not ribosomes isolated from regenerating liver incorporate amino acid more rapidly than those from normal liver. Henshaw *et al.* (163) suggested that endoplasmic membranes play a role in the protein-synthesizing machinery of microsomes, since ribosomes attached to membranes produced more protein than free ribosomes. Studies by Kato and his associates (206) suggested that a portion of the greater amino acid incorporating activity of microsomes from phenobarbital-treated rats may be due to a phenobarbital-induced shift to a greater proportion of membrane-bound ribosomes.

F. Drug-induced increases in ribonucleic acid synthesis

Studies by Gelboin and his associates (133) and by Bresnick (23) suggested that 3-methylcholanthrene and phenobarbital may alter gene activity and enhance DNA-directed RNA synthesis. Treatment of rats with 3-methylcholanthrene (23, 133) or phenobarbital (133) stimulates rat liver DNA-dependent RNA polymerase. However, DNA isolated from the livers of drug-treated or control rats show identical template activity when measured with purified RNA polymerase from *Micrococcus lysodeikticus*.

Loeb and Gelboin (232, 233) reported that treatment of rats with 3-methylcholanthrene increased the content of messenger-RNA in isolated liver nuclei. The nuclear RNA from treated rats was more active than equivalent amounts of RNA from normal rats in directing the incorporation of L-phenylalanine-C^{14} into protein in the cell-free *Escherichia coli* system of Matthaei and Nirenberg (241). 3-Methylcholanthrene treatment, however, did not alter the ultracentrifugation sedimentation profile of nuclear RNA (24, 233). In other studies, treatment of rats with 3-methylcholanthrene stimulated the incorporation of orotic acid into nuclear RNA (233). This effect was unequal in the various fractions of RNA analyzed and was largest in the 18S to 23S region (233). Bresnick and his associates (24) failed to confirm a stimulatory effect of 3-methylcholanthrene on orotic acid incorporation into nuclear or total liver RNA, but this effect has been observed by others (166, 187). Bresnick recently showed that 3-methylcholanthrene administration did not alter the polysome distribution in rat liver (25).

G. Proliferation of membranes of the hepatic endoplasmic reticulum in animals treated with drugs

Remmer and Merker (293, 294) and Fouts and Rogers (120) found that treatment of animals with phenobarbital, tolbutamide, or chlordane caused a marked proliferation of smooth-surfaced membranes of the endoplasmic reticulum of the liver cell. These drugs exerted little or no effect on the rough-surfaced endo-

plasmic reticulum, which contains ribosomes. A similar stimulatory effect of DDT on the proliferation of smooth-surfaced endoplasmic reticulum was observed by Ortega (267). The stimulatory effect of phenobarbital on the proliferation of smooth-surfaced endoplasmic membranes has been confirmed and extended in several laboratories (41, 164, 264, 265). In contrast, treatment of animals with 3-methylcholanthrene or 3,4-benzpyrene had little or no effect on the proliferation of smooth membranes of the endoplasmic reticulum (120).

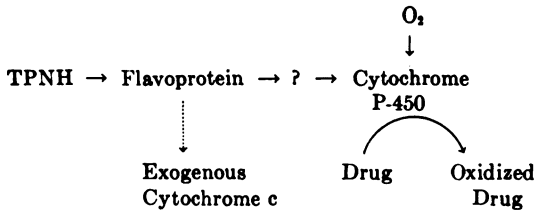
Studies by Fouts indicated that drug-metabolizing enzymes in liver microsomes are concentrated in the smooth membranes of the endoplasmic reticulum (115), but Orrenius and Ernster (266), using different methodology, suggested that aminopyrine N-demethylase was equally distributed between the smooth and rough-surfaced endoplasmic reticulum of the rat. More recent studies by Gram *et al.* (121, 147) showed that the degree of concentration of several drug-metabolizing enzymes in the smooth membranes of the endoplasmic reticulum depends on the animal species studied and the method used for the preparation of smooth membranes. Under all conditions studied, the metabolism of several drug substrates was concentrated in the smooth membrane fraction, but some drugs were metabolized at the same rate by rough and smooth-surfaced membranes. Remmer and Merker (292) showed that treatment of rabbits with phenobarbital for several days increased drug-metabolizing enzymes, protein, and lipid in both the rough-surfaced and the smooth-surfaced endoplasmic reticulum, but the increases were greater in the smooth-surfaced endoplasmic reticulum. Orrenius and Ernster (266) confirmed these results and reported that the administration of phenobarbital first gave rise to increased levels of aminopyrine N-demethylase, TPNH-cytochrome c reductase, and cytochrome P-450 in the rough-surfaced vesicle fraction, which was examined 6 hours after a single phenobarbital injection (112, 262). Between 6 and 12 hours, the increase began to level off in the rough vesicle fraction, and enzyme levels increased at a progressive rate in the smooth vesicle fraction. Repeated administration of phenobarbital resulted in the accumulation of smooth-surfaced membranes highly active in drug oxidation (262, 292). Immunochemical and electrophoretic studies with extracts from rat submicrosomal membranes revealed that treatment of the rats with phenobarbital increased the amount of electrophoretically different antigens, some of which possess esterase activity (235). The typical electrophoretic mobilities of the antigens make them suitable for further studies on the properties of these antigens and on the effects of phenobarbital on their synthesis and degradation.

Orrenius and Ernster found that phenobarbital markedly increased the rate of incorporation of inorganic phosphate into phospholipid of both smooth and rough vesicle fractions of the microsomes, and they suggested that enhanced phospholipid synthesis was an early step in the phenobarbital-induced proliferation of smooth-surfaced endoplasmic reticulum (112, 262, 264, 265). Holtzman and Gillette (169), however, failed to confirm these studies and found that phenobarbital increased hepatic microsomal phospholipid by inhibiting phospholipid catabolism (169).

The high levels of membranes and phospholipid induced by phenobarbital treatment persist in the cytoplasm of rat hepatocytes for 15 days after the last of a series of 5 daily phenobarbital injections, but aminopyrine demethylase activity in liver microsomes returns to control values by 5 days after the last phenobarbital injection (265). A second series of injections of phenobarbital, at a time when the animals exhibit normal drug-metabolizing activity but an excess of liver endoplasmic membranes, increases the aminopyrine demethylase activity more slowly than does the initial treatment with phenobarbital (265).

VI. EFFECT OF DRUGS ON ELECTRON TRANSPORT SYSTEMS IN LIVER MICROSOMES

The mechanism of drug oxidation by liver microsomes is not yet clear, but it is believed that the electron transport scheme is as follows:



The evidence for this pathway has been reviewed recently (112, 141, 240, 261).

Cytochrome P-450 is a cytochrome in liver microsomes that functions as an oxygen-activating enzyme for drug oxidations (81, 261). Presumably, reduced cytochrome P-450 reacts with oxygen to form "activated oxygen," which can be transferred to a drug or steroid substrate. Cytochrome P-450 binds carbon monoxide, and this explains an observation made 10 years ago that carbon monoxide inhibits the oxidative N-demethylation of aminoazo dyes by liver microsomes (55). More recently, it was shown that carbon monoxide inhibits the metabolism of a variety of drugs by liver microsomes (55, 81, 141, 194, 263). The elegant studies of Cooper *et al.* (81) showed that the liver microsomal oxidation of codeine, 4-monomethylaminoantipyrine, or acetanilide was inhibited by carbon monoxide, and the inhibition was reversed by oxygen or by monochromatic light at 450 mμ, which liberates carbon monoxide from binding sites on cytochrome P-450. More recent studies have shown that the addition of various drug substrates to liver microsomes causes spectral changes suggesting an interaction of the drugs with the cytochrome P-450 (179, 295). The two types of spectral changes that were observed when drugs were added to microsomes suggested the presence of two forms of cytochrome P-450. Interestingly, treatment of rats with phenobarbital or other enzyme inducers increased the magnitude of the spectral changes (295). An interesting study by Sladek and Mannering (325) recently provided evidence for two forms of cytochrome P-450, and these investigators showed that 3-methylcholanthrene caused a selective increase in the microsomal concentration of one of the forms.

Gillette *et al.* (142) reported the oxidation of TPNH to TPN by liver micro-

somes in the absence of an exogenous electron acceptor such as cytochrome c or drug and postulated that this enzyme system (TPNH oxidase) is involved in drug oxidations. The fact that TPNH oxidase is inhibited by carbon monoxide (141, 335) supports this hypothesis.

The first suggestion that stimulators of drug metabolism could alter microsomal electron transport came from the observation that treatment of rats with phenobarbital, chlorcyclizine, phenylbutazone, or orphenadrine markedly stimulated TPNH oxidase activity in liver microsomes but had little or no effect on DPNH metabolism (59, 73). The stimulating effect of phenobarbital on TPNH oxidase in liver microsomes was confirmed in several laboratories (197, 292, 305), but Ernster and Orrenius (112) did not find it. Treatment of rats with chlordane (156) or any of several testosterone derivatives (18), or treatment of squirrel monkeys with chlordane (85) also stimulated TPNH oxidase activity in liver microsomes. 3-Methylcholanthrene, which stimulates the metabolism of some drugs without influencing the metabolism of others (section II), had only a slight stimulating effect on TPNH oxidase activity (59).

Treatment of rats with phenobarbital or certain other drugs that stimulate liver microsomal enzyme activity also increases the amount of red pigment in the microsomes and the concentration of cytochrome b_5 (66), but the rate of drug metabolism rises and falls with the concentration of cytochrome P-450, and the cytochrome b_5 rises more slowly than the activity of drug-metabolizing enzymes (294) or the increase in cytochrome b_5 may be absent (266). Several cytochromes (b, c, c_1, and a) in the mitochondria are not increased (313). The parallel rise and fall of cytochrome P-450 with drug metabolism observed by Remmer and Merker (294) suggests that phenobarbital increases drug metabolism by increasing the amount of cytochrome P-450 in the microsomes. In relation to the synthesis of microsomal cytochromes, it is of interest that several barbiturates and other drugs induce the formation of δ-aminolevulinic acid synthetase in liver mitochondria and so bring about an increased synthesis of porphyrins (148–150). Perhaps the drug-induced synthesis of cytochrome P-450 in liver microsomes is related to an effect of the drug on δ-aminolevulinic acid synthetase in the mitochrondria.

Treatment of rats with phenobarbital or certain other drugs that enhance drug metabolism and increase the levels of cytochrome P-450 in liver microsomes also increases the levels of TPNH-cytochrome c reductase (112, 197, 266). Orrenius and Ernster (112, 266) found 3-methylcholanthrene to have this effect, but neither Von der Decken and Hultin (353) nor Hernandez *et al.* (165) could confirm its stimulation of TPNH-cytochrome c reductase.

The ability of several stimulators of liver microsomal drug metabolism to increase the concentrations of TPNH oxidase, TPNH-cytochrome c reductase, and cytochrome P-450 in liver microsomes suggests that the induction of these electron transport systems may play a role in the stimulatory effect of drugs on drug metabolism. This scheme is incomplete, however, for it cannot explain the selective effect of enzyme inducers that stimulate some liver microsomal hydroxylations without influencing others (section II). The specificity of polycyclic

hydrocarbons as enzyme inducers raises the possibility that several proteins may function in the transport and positioning of drug and steroid substrates on cytochrome P-450 and that drugs may selectively induce the synthesis of some of these proteins without influencing others. Alternatively, several hypothetical cytochromes, sensitive or insensitive to carbon monoxide, may activate oxygen for drug oxidations, and the adminstration of drugs *in vivo* may induce some of these cytochromes but not others. The sulfoxidation of diaminodiphenyl sulfide and the N-hydroxylation of aniline and N-ethylaniline by liver microsomes are not inhibited by carbon monoxide (141, 194). Hence cytochrome P-450 is not required for the microsomal oxidation of all drugs. These results emphasize our ignorance of the properties and number of electron transport systems involved in drug oxidations and the specific steps that are enhanced by drug administration.

VII. ENZYME INDUCTION IN HEPATOMAS

The transformation of a normal liver cell into a cancer cell is accompanied by a decrease in the activity of several drug-metabolizing enzymes in the microsomes (2, 27, 55, 59, 154, 257, 328, 348). Low levels of drug-metabolizing enzymes are also found in other situations of rapid liver growth such as newborn (117, 189) and regenerating liver (118, 255, 352). Not only the more grossly deranged tumors, but also hepatomas that resemble normal liver histologically and biochemically, possess little or no drug-metabolizing activity (2, 59, 154). This circumstance suggested that the depletion of these TPNH-dependent enzymes, or the smooth-surfaced endoplasmic reticulum from which they are derived, may play a role in the formation or growth of hepatomas (59). If this is true, it is important to know the physiological substrates of the drug-metabolizing enzymes, whether these enzymes play a role in controlling cell growth, and whether these enzymes are inducible in the hepatoma.

The evidence suggests that hepatomas resembling normal liver are likely to possess inducible drug-metabolizing enzymes. Administration of 3-methylcholanthrene did not induce the formation of azo dye N-demethylase in a hepatoma that was produced by the feeding of an aminoazo dye (55), but it did so in the less deranged Morris hepatoma 5123 (59). Phenobarbital stimulated the metabolism of several drugs in hepatomas deviating little from normal structure and function, but in the more grossly deranged Novikoff hepatoma it stimulated only the metabolism of hexobarbital (154). If drug-metabolizing enzymes are involved in controlling growth, their induction could result in inhibition of tumor growth, and indeed phenobarbital slowed the growth of the Morris 7800 hepatoma (303).

VIII. ENZYME INDUCTION IN ANIMALS OF DIFFERENT SPECIES, STRAIN, AND AGE

Polycyclic hydrocarbons increase drug metabolism in the rat, mouse, guinea pig, hamster, cotton rat, and cat (86, 362); phenobarbital stimulates drug metabolism in the rat, mouse, guinea pig, rabbit, dog, and man (38, 44, 60, 65, 84, 90,

287, 292, 297); and chlordane stimulates drug metabolism in the rat, mouse, rabbit, dog, and squirrel monkey (38, 85, 119, 157, 158). Phenylbutazone and DDT stimulate drug-metabolizing enzymes in the rainbow trout (32). It is of considerable interest that DDT stimulates drug metabolism (157), as well as the hydroxylation of estradiol by liver microsomes (220) in the rat, but has no effect in the mouse (*cf.* section II). DDT decreases the storage of dieldrin in the rat, swine, and sheep, but not in the chicken (340). Pretreatment of rats, rabbits, or mice with the enzyme inducer, nikethamide, reduces the effect of pentobarbital, but this effect does not occur in guinea pigs or chickens, or in the cold-blooded chameleon, caiman, or frog (22). Studies in the *Triatoma infestans* nymph indicate that 3-methylcholanthrene slightly increases the metabolism of DDT to polar metabolites (250).

Studies on the hydroxylation of testosterone by liver microsomes revealed an interesting example of species variations in enzyme induction. Whereas treatment of rats with phenylbutazone stimulated the 6β-, 7α-, and 16α-hydroxylation of testosterone, treatment of dogs with phenylbutazone had no effect on the 7α-hydroxylation of testosterone, a process normally low or absent in this species, but did markedly stimulate the liver microsomal 6β- and 16α-hydroxylation reactions (77). These results suggest that the synthesis of the 7α-hydroxylase is regulated by a different enzyme-forming system from that which synthesizes the 6β- and 16α-hydroxylases, and that the system that synthesizes the 7α-hydroxylase is absent in the dog. The above results indicate that caution is needed before extrapolating data from one animal species to another.

The genetic make-up of the individuals of a population within a given species may be important in determining the occurrence or magnitude of enzyme induction. We have observed that an occasional rabbit, guinea pig, or mongrel dog is refractory to the effects of liver microsomal enzyme stimulators, and people also vary in responsiveness. Whereas phenobarbital decreased the anticoagulant activity of bishydroxycoumarin by lowering its plasma level in most persons studied, an occasional subject did not respond to phenobarbital (90). These results suggest that genetic variation within a species is important in determining whether a given individual will respond to enzyme inducers. Cram *et al.* (84) recently explored this problem with six strains of rabbit. Phenobarbital caused large increases in the hepatic metabolism of hexobarbital and aminopyrine in the cottontail rabbit, but had a much smaller effect on hexobarbital metabolism and no effect at all on aminopyrine metabolism in the English rabbit. Phenobarbital treatment stimulated benzpyrene hydroxylation in only two of the strains studied; it stimulated the metabolism of hexobarbital in all six strains, but the degree of stimulation varied from 2-fold to 26-fold in the different strains.

The liver microsomes of newborn animals have little or no ability to metabolize drugs such as hexobarbital, aminopyrine, acetophenetidine, acetanilide, *l*-amphetamine, and chlorpromazine (117, 189). This is paralleled *in vivo* by a low rate of drug metabolism and a prolonged duration of drug action. Newborn mice treated with 10 mg of hexobarbital per kg sleep for longer than 6 hours, whereas adult mice treated with 10 times this dose regain righting reflexes in less that 1

hour. The development of drug-metabolizing enzymes with advancing age parallels drug metabolism *in vivo* and results in a decreased duration of drug action.

Treatment of newborn rabbits with phenobarbital or chlordane enhances the activity of liver enzymes that metabolize drugs such as hexobarbital, aminopyrine, and *p*-nitrobenzoic acid (119, 153). Treatment of rabbits with phenobarbital during the final week of pregnancy increases the activity of these systems in the newborn, but drug-metabolizing enzymes cannot be stimulated before the last 4 days of fetal life. This suggests a defective enzyme-forming mechanism before this time. Treatment of pregnant rabbits with chlordane also stimulates the activity of drug-metabolizing enzymes in the newborn, and the administration of phenobarbital or chlordane to lactating mothers increases the levels of drug-metabolizing enzymes in the nurslings (119). The dose of phenobarbital administered to the mother in these experiments did not cause sedation in the offspring.

Treatment of newborn rats with 3,4-benzpyrene stimulates *o*-aminophenol glucuronyl transferase in liver microsomes, but no such stimulation is observed in newborn rats when their mothers had been treated with 3,4-benzpyrene during pregnancy (180). Treatment of pregnant rats with chloroquine or chlorcyclizine stimulates hepatic bilirubin glucuronide formation in the newborn (6,7), and treatment of pregnant mice with barbital for 6 days increases the activity of the bilirubin-conjugating enzyme in the liver of the newborn (48). Treatment with phenobarbital decreased the levels of free bilirubin in 2 infants with congenital hyperbilirubinemia (see section XIII).

The stimulating effect of foreign compounds is usually more marked in the immature male and adult female rat than in the adult male rat, perhaps because of higher levels of drug-metabolizing enzymes normally present in the adult male rat (*cf.* section X). An exception is the effect of 3-methylcholanthrene on the metabolism of 2-acetylaminofluorene. Although liver microsomes of the immature and adult male rat metabolize 2-acetylaminofluorene at the same rate, 3-methylcholanthrene induces a 10-fold increase in hepatic hydroxylation of 2-acetylaminofluorene in the imature rat, but only a 2-fold increase in the adult male rat (86). It is not known whether the baby rat is intrinsically more responsive to a given dose of 3-methylcholanthrene than the adult rat, or whether the greater response of the baby rat to enzyme induction may be caused by a slow metabolism of the enzyme inducer to inactive metabolites.

IX. ENZYME INDUCTION IN NONHEPATIC TISSUES

Although oxidative drug-metabolizing enzymes are localized primarily in liver microsomes, low levels of these enzymes are also present in nonhepatic tissues such as the lung, gastrointestinal tract, and kidney (82, 100, 108, 129, 143, 146, 356, 357, 360). The inducibility of benzpyrene hydroxylase in liver (75) led Wattenberg and his associates (356, 357, 360) to investigate the effects of polycyclic hydrocarbons on benzpyrene hydroxylase in nonhepatic tissues. Using extremely sensitive fluorometric and histochemical techniques, these in-

vestigators found TPNH-dependent 3,4-benzpyrene hydroxylase in the liver, kidney, adrenal and small intestine of normal rats. The administration of 3-methylcholanthrene caused large increases in benzpyrene hydroxylase activity in liver, kidney and small intestine and caused the appearance of activity, previously too low to be detected, in the thyroid, lung and testis. Activity was increased in the skin (72) but not in the adrenal gland (95, 356). The benzpyrene hydroxylase activity in the small intestine of normal rats was mainly in the epithelial cells covering the villi (356, 360); cells in the glandular crypts, the stroma and muscle had no detectable activity, and no activity was detected in the esophagus, stomach, cecum or large intestine of the normal rat. After oral treatment of rats with 1,2-benzanthracene, there was considerable benzpyrene hydroxylase activity in the squamous epithelium of the forestomach, in the duodenal mucosa, throughout the small intestine, and in the surface epithelium of the cecum and colon. Similar increases in benzpyrene hydroxylase activity of the gastrointestinal tract were achieved by treating animals with 3-methylcholanthrene, 3,4-benzpyrene or 1, 2, 5, 6-dibenzanthracene. Dietary factors also regulate the level of benzpyrene hydroxylase in the gastrointestinal tract since a 15-fold decrease in benzpyrene hydroxylase activity in duodenal mucosa was observed in rats starved for 72 hours or fed a fat-free diet (360). The presence of inducible benzpyrene hydroxylase in lung, intestine and kidney was also observed by Gelboin and Blackburn (129); they found several-fold increases in benzpyrene hydroxylase activity in nonhepatic tissues within 7 hours after a single intraperitoneal injection of 3-methylcholanthrene. Since the increases in enzyme activity were blocked by puromycin or actinomycin D, an induced synthesis of more enzyme protein is postulated.

Wattenberg and Leong (357) investigated over 100 chemicals for activity as inducers of benzpyrene hydroxylase in liver and small intestine. Several phenothiazine derivatives (chlorpromazine, promazine, phenothiazine, pyrathiazine and thioridazine) induced benzpyrene hydroxylase activity in the gastrointestinal tract and liver. Phenothiazine increased benzpyrene hydroxylase activity in the liver, gastrointestinal tract, kidney, lung, spleen and thymus. No detectable activity was present in the hearts or brains of control or phenothiazine-treated rats. Although benzpyrene hydroxylase activity was present in adrenals from control rats, this activity was not increased by treatment with phenothiazine. Heterocyclic compounds that induce benzpyrene hydroxylase activity in lung include phenothiazine, 2,5-bis-(4-pyridyl)-1,3,4-thiadiazole, 2,5-diphenyl-1,3,4-oxadiazole, dibenzothiophene and flavone (358). Phenobarbital and several other stimulators of drug metabolism in the liver were among the compounds that had little or no effect on benzpyrene hydroxylase activity in the gastrointestinal tract (360). Although treatment of rats for several days with phenobarbital markedly stimulated the hepatic oxidation of pentobarbital, no enhanced metabolism of pentobarbital was observed in lung homogenates (217). Other studies indicated that phenobarbital stimulated neither nitro reductase in kidney nor aminopyrine N-demethylase in kidney, heart, spleen, brain, muscle or lung (138).

Several nonhepatic pathways of drug metabolism are stimulated by treatment

of animals with appropriate inducers. Application of 3,4-benzpyrene to the skin of rodents increased glucuronide synthesis (108) and 3,4-benzpyrene hydroxylation (72) by skin homogenate. 3-Methylcholanthrene injections induced the formation of aminoazo dye N-demethylase in lung and kidney (143) and menadione reductase in the lung (171). The presence of inducible N-demethylase, hydroxylase and glucuronyl transferase in nonhepatic tissues suggests that these enzymes, as well as their hepatic counterparts, may play a role in the biotransformation of drugs and other foreign chemicals, and that changes in the low activity of these enzymes at or near a receptor may alter the action of drugs and other chemicals that have escaped metabolic conversion by the liver. The possible role of this effect in man is discussed in section XIII.

X. HORMONAL REGULATION OF DRUG METABOLISM

Androgens, estrogens, progestational steroids, glucocorticoids, anabolic steroids, norepinephrine, insulin, and thyroid hormone influence drug action by altering the activity of drug-metabolizing enzymes in liver microsomes. Some of these effects are described below.

A. Sex hormones

It has been known for many years that the duration and intensity of drug action is often greater in the adult female rat than in the adult male (167, 168, 272). Quinn *et al.* (276) provided an explanation for this difference with respect to hexobarbital by finding that the enzyme that metabolizes this drug is less active in females than in males. At birth, neither the male nor the female has much drug-metabolizing enzyme activity (section VIII), and up to the age of 4 weeks the response of the two sexes is identical, but between 5 and 6 weeks an increased activity of the hexobarbital-metabolizing enzyme in males is manifested by an abrupt and lasting decrease in the action of this drug. Murphy and DuBois (255) found a similar course of development in young rats for the enzyme in liver that catalyzes the oxidation of the dimethoxy ester of benzotriazine dithiophosphoric acid (Guthion, DBD) and ethyl *p*-nitrophenyl thionobenzenephosphonate (EPN) to anticholinesterase agents.

It was suggested that a balance between the male and female sex hormones is important in determining the activity of drug-metabolizing enzymes, and that this balance is upset in the male rat at puberty. In young males the low enzyme activity in the liver with respect to DBD and EPN is increased by prolonged administration of testosterone, and in adult males the high activity is decreased by castration (255). Testosterone given to female rats increases the activity of liver microsomal systems that metabolize hexobarbital (276), DBD and EPN (255), O, O-diethyl-O-(4-methylthio-*m*-tolyl) phosphorothioate (DMP) (104), *o*-aminophenol (180), and morphine, methadone, and meperidine (8). In adult males the opposite effect is brought about by estrogens (180, 255, 276).

Prolonged administration of progesterone to male rats decreases the high activity of their livers in oxidizing DBD or EPN (255), but it does not alter enzyme activity with respect to hexobarbital (192). Juchau and Fouts (192) studied the

metabolism in male rats not only of hexobarbital but also of zoxazolamine, which, along with aniline, is exceptional in that no sex difference in its hydroxylation exists (204). Two hours after administration of progesterone or norethynodrel, the enzyme activity was inhibited (*cf.* section VC), but chronic administration of norethynodrel enhanced the liver microsomal metabolism of hexobarbital and zoxazolamine, and chronic progesterone treatment did not alter enzyme activity. The chronic administration of Enovid (combination of norethynodrel and the estrogen, mestranol) inhibited hexobarbital metabolism. Miya *et al.* (248), recently reported that chronic treatment of ovarectomized rats with progesterone increased the hepatic N-oxidation, demethylation, and sulfoxidation of chlorpromazine and decreased the hypothermic action of this drug. DuBois and Kinoshita (104) showed that pretreatment of female rats with progesterone decreased the anticholinesterase activity of O,O-diethyl-O-(4-methylthio-*m*-tolyl) phosphorothioate (DMP) possibly by stimulating the activity of a hepatic microsomal enzyme that detoxifies it. Other steroids that inhibited the anticholinesterase activity of DMP when administered chronically to female rats were testosterone, methyltestosterone, estradiol, diethylstilbestrol, deoxycorticosterone, and cortisone.

In contrast to a sex difference in the rat, no such difference was observed for the metabolism or action of hexobarbital in guinea pigs, rabbits, cats, and dogs (168, 276), but the results in mice are conflicting. In one study (276) no sex difference was found in this species with hexobarbital and little change in its rate of disappearance occurred when sex hormones were administered. In another study, however, in contrast to the situation in rats, male Swiss-Webster mice were affected by pentobarbital for a longer time than females, and the duration of action was decreased by treatment of the males with stilbestrol and increased by treatment of the females with testosterone (372). Chronic treatment of male mice with either testosterone or methyltestosterone prolonged the action of hexobarbital, and testosterone accordingly decreased the activity of the corresponding enzyme system (258). On the other hand, hexobarbital's duration of action was shortened and its metabolism *in vitro* was stimulated in the male mouse by chronic treatment with any of several 19-nortestosterone derivatives (19-nortestosterone, norethandrolone, and 4-chloro-17α-methyl-19-nortestosterone).

In the female rat, Booth and Gillette (18) recently found that the stimulation of drug-metabolizing enzymes by testosterone derivatives parallels their anabolic activity more closely than their androgenic activity. Thus, 19-nortestosterone and 4-chloro-19-nortestosterone acetate, which are anabolic steroids with only slight androgenic activity, stimulate enzyme systems in the rat that metabolize hexobarbital, demethylate monomethyl-4-aminoantipyrine, and hydroxylate naphthalene. The activities of these enzyme systems are also increased in rats treated with testosterone propionate, methyltestosterone, and Δ^4-androstene-3, 17-dione. The anabolic steroid, 4-chlorotestosterone, similarly shortens the duration of paralysis due to carisoprodol by accelerating its metabolism (200).

Sex differences also occur for the hydroxylation of sex hormones by the liver

microsomes of rats (52, 160, 221, 223, 231). The hydroxylase activities for testosterone, estradiol, and progesterone are higher in adult males than in females. Recent studies indicate that the metabolism of testosterone by liver microsomes in the mouse differs from that in the rat (68). Further comparative studies on the metabolism of sex hormones in the rat and mouse may be helpful in explaining why the effect of testosterone on microsomal enzyme activity in the mouse differs from that in the rat.

B. Other hormones

Remmer (279, 281) reported that *adrenalectomy* of either male or female rats decreases the activity of liver microsomal enzymes that N-demethylate monomethyl-4-aminoantipyrine and that oxidize hexobarbital, but Gillette and Kato (139, 205) found that adrenalectomy decreases hexobarbital and aminopyrine metabolism, tested *in vitro*, in male but not in female rats. Administration of prednisolone to adrenalectomized male rats for several days restored the activity of these enzymes to control values, and administration of prednisolone or cortisone to intact female rats shortened the duration of hexobarbital action and accelerated its metabolism by the liver (279, 281). Bousquet, Miya, and their associates (19, 102, 103) showed that subjecting rats to hindleg ligation for 2.5 hours shortened the duration of the response to hexobarbital, pentobarbital, meprobamate, and zoxazolamine, but not to barbital and phenobarbital, whose effects are terminated primarily by renal excretion. The plasma levels of hexobarbital, pentobarbital, or meprobamate in these rats were decreased, and this effect did not occur when the ligation was performed in hypophysectomized or adrenalectomized animals. Pentobarbital blood levels were lowered by corticosterone but not ACTH in adrenalectomized rats, and by both corticosterone and ACTH in hypophysectomized animals. Treatment of rats with actinomycin D blocked the ability of stress to lower pentobarbital blood levels, and in liver perfusion studies, the metabolism of pentobarbital was stimulated in stressed animals and this stimulation was blocked by actinomycin D (section VB). These findings suggest a regulatory function for the pituitary-adrenal system in the control of drug metabolism.

Dixon *et al.* (99) found that the acute or chronic administration of *norepinephrine* to male rats inhibited the metabolism of hexobarbital, aminopyrine, and aniline, tested *in vitro*, and lowered hepatic glycogen concentrations. After stopping norepinephrine treatment, hepatic glycogen rapidly returned to normal, but drug-metabolizing enzyme activity remained depressed for several days. Kato and Gillette (205) found that epinephrine decreased the liver microsomal metabolism of hexobarbital and aminopyrine in the male rat, but this effect did not occur in the female rat.

Chronic treatment of male rats with *thyroxin* shortened the duration of action of a subsequent injection of zoxazolamine by accelerating its metabolism, but the metabolism of zoxazolamine *in vitro* was not altered (61). This discrepancy was explained by finding that thyroxin stimulated liver growth and the activity of TPNH-generating systems in the soluble fraction of liver. In contrast, pretreat-

ment of male rats with thyroxin prolonged the duration of action of hexobarbital by decreasing the activity of the hexobarbital-metabolizing enzyme system in liver microsomes (61). Similarly, thyroxin administration decreased the activity of the enzyme system in liver microsomes that N-demethylates morphine (51). Kato and Gillette (205) recently found that thyroxin treatment inhibited the liver microsomal metabolism of aminopyrine and hexobarbital in the male rat, but not in the female, whereas thyroxin stimulated aniline hydroxylase activity in both male and female rats. Treatment of mice with thyroid hormone increased the actions of thiopental and pentobarbital by inhibiting the metabolism of these drugs *in vivo* (273). Thyroidectomy, in rats, greatly prolonged the action of pentobarbital and markedly inhibited its metabolism *in vivo* (273). The authors suggested that decreased body temperature in thyroidectomized rats plays a role in inhibiting the metabolism of pentobarbital, since thyroidectomy does not influence the activity of liver microsomal enzymes that metabolize several drugs (138, 264).

Dixon *et al.* (98), reported that the administration of alloxan to adult male rats prolonged the effects of hexobarbital and decreased the activity of liver microsomal enzymes that metabolize hexobarbital, chlorpromazine, and codeine. Treatment of these diabetic rats with *insulin* rapidly decreased the effect of hexobarbital and increased drug-metabolizing enzyme activity to normal. Although alloxan diabetes decreased the metabolism of aminopyrine and hexobarbital by the microsomes of male rats, aminopyrine metabolism was stimulated and hexobarbital metabolism not affected in the female (140, 205). These investigators also found that alloxan diabetes stimulated aniline hydroxylase activity in both the male and female rat.

XI. STIMULATORY EFFECT OF DRUGS ON THE METABOLISM OF NORMAL BODY CONSTITUENTS

TPNH-dependent enzymes in liver microsomes metabolize not only drugs, but also a variety of normal body substrates: steroid hormones (67, 114, 221, 253), fatty acids (239, 336), tyramine and other sympathomimetic amines (10, 227), thyroxin (333, 375), anthranilic acid (195), N-acetyltyramine (181), N-acetylserotonin (181), methylated purines (161, 242, 243), and various indoles such as tryptamine and indoleacetic acid (186). Similarly, the biosynthesis of cholesterol requires TPNH-dependent enzymes in liver microsomes (17, 31, 260, 307, 344).

The similarities between drug and steroid hydroxylases in liver microsomes (table 4) suggested that drugs and steroids are substrates for the same hydroxylating enzymes (221, 223). The inhibitory effect of carbon monoxide on liver microsomal steroid hydroxylation suggested that cytochrome P-450 (section VI) is involved in this reaction (69). Further evidence for this concept came from finding that the inhibitory effect of carbon monoxide on the 6β-, 7α- and 16α-hydroxylation of testosterone by liver microsomes is prevented by monochromatic light at 450 mμ (69). The low Michaelis constants for the hydroxylation of testosterone, progesterone, and estradiol by liver microsomes also supported the concept that

TABLE 4

Similarities between hepatic hydroxylases that metabolize drugs and steroids

1. Localized in liver microsomes; require TPNH and oxygen for activity.
2. Higher activity in adult male rats than in adult female rats.
3. Little or no sex difference in enzyme activity in mice.
4. Higher activity in male rats than in male mice.
5. Higher activity in adult male rats than in immature male rats.
6. Inhibition *in vitro* by the addition of SKF 525A or chlorthion.
7. Activity is inhibited by carbon monoxide.
8. Activity is increased after treatment of rats with drugs or halogenated hydrocarbon insecticides.
9. Steroid hormones are competitive inhibitors of drug-metabolizing enzymes.

steroids are normal body substrates for oxidative drug-metabolizing enzymes (222). As might be predicted from these low Michaelis constants, steroids are potent competitive inhibitors of drug-metabolizing enzymes in liver microsomes (345).

The many similarities between the hepatic hydroxylation of drugs and steroids prompted detailed studies on the effects of liver microsomal enzyme inducers on the metabolism of steroid hormones. Treatment of experimental animals with phenobarbital increases the hydroxylation by liver microsomes of testosterone (67), Δ^4-androstene-3,17-dione (67), androsterone (125), estradiol-17β (221), estrone (230), progesterone (64,223), deoxycorticosterone (64), and cortisol, cortisone, and corticosterone (65, 77). However, phenobarbital does not increase the A-ring reduction of Δ^4-3-ketosteroids by liver microsomes (294). Several structurally unrelated drugs and insecticides that stimulate drug-metabolizing enzyme activity also stimulate steroid hydroxylases in liver microsomes. Examples of such compounds include diphenylhydantoin, chlorcyclizine, norchlorcyclizine, orphenadrine, phenylbutazone, chlordane, DDT, and *o,p'*-DDD. In contrast, 3-methylcholanthrene (*cf.* section II) has little or no stimulatory effect on the hydroxylation of testosterone, Δ^4-androstene-3,17-dione, estradiol-17β, cortisol, or cortisone (67, 77, 221). Chronic treatment of rats with phenobarbital, phenylbutazone, or DDT stimulated the liver microsomal hydroxylation of testosterone in the 16α-position to a greater extent than in the 6β- or 7α-position (77, 366). In dogs, treatment with phenylbutazone markedly increased both the hepatic 6β- and 16α-hydroxylation of testosterone but had no effect on the 7α-hydroxylation reaction, a pathway of little or no magnitude in the dog (77). These results suggest that separate enzyme systems catalyze the hydroxylation of testosterone in different positions.

The accelerated hydroxylation of steroid hormones by liver microsomal enzymes is paralleled *in vivo* by an accelerated metabolism and an altered physiological action of steroids. The increased activity of progesterone hydroxylase induced by phenobarbital is associated with a decrease in the anesthetic action of large doses of progesterone and a lower concentration of progesterone and its metabolites in the brain and total body (64, 219). Phenobarbital treatment also decreases the anesthetic action of deoxycorticosterone, androsterone, and Δ^4-

androstene-3,17-dione, while accelerating their metabolism by liver microsomes (64). Inducers of steroid-hydroxylating enzymes bring about an inhibition of the action of estradiol or estrone on the uterus and stimulate the metabolism of small amounts of these estrogens *in vitro* and *in vivo* (229, 367). For instance, 40 minutes after the intraperitoneal injection of 100 ng of tritiated estrone into immature female rats, the total body concentration of estrone was 21 ng in control rats and 4.3 ng in rats that had been treated with 37 mg of phenobarbital per kg twice daily for 4 days. The metabolism of estradiol-17β was less affected than that of estrone. Phenobarbital decreased the concentrations of these estrogens in the uterus and decreased their effect on the size of the uterus (table 5). Inducers of enzymes also inhibit the effect of gonadotropin on ovulation (28), inhibit the effect of estrogen on vaginal cornification and ovulation (152), and decrease fertility (4, 220, 244), but the role of accelerated metabolism in these phenomena is not yet clear. The same is true of the decreased plasma concentration of corticosterone produced by chronic treatment of rats with barbital (228). Although treatment of rats with diphenylhydantoin for 2 days does not prevent the usual rise in plasma corticosterone 30 minutes after a stress, the duration of the rise is reduced, as measured by lower plasma levels 60 minutes after the stress in drug-treated animals (97). In monkeys (15) and guinea pigs (224) inducers of enzymes stimulate the metabolism of cortisol to 6β-hydroxycortisol and increase the urinary excretion of this metabolite, and the latter phenomenon also occurs in man (section XII).

Treatment of golden hamsters with phenobarbital caused a 4-fold increase in the conversion of acetate to cholesterol in liver slices, but *in vivo* there was no accumulation of cholesterol in either the liver or the plasma (190).

Pretreatment of rats with phenobarbital stimulates the liver microsomal demethylation of 6-dimethylaminopurine, 6-methylaminopurine, puromycin, and puromycin aminonucleoside (242, 243). The effect on 6-dimethylaminopurine and 6-methylaminopurine is of particular interest, since these methylated purines are constituents of liver ribonucleic acid (14, 106).

As discussed in section VI, several drugs that increase drug-metabolizing

TABLE 5

Effect of phenobarbital pretreatment on the uterine response to estrogens

In immature female rats, 37 mg of phenobarbital per kg was injected intraperitoneally twice daily for 4 days. On the fifth day, 0.1 μg of tritiated estradiol or 0.3 μg of tritiated estrone was injected intraperitoneally. The rats were killed 4 hours later, the uteri weighed, and radioactivity in the uteri determined.

Pretreatment	Estradiol	Uterine Wet Weight (mg)	Picogram Equivalents of Estradiol in the Uterus	Estrone	Uterine Wet Weight (mg)	Picogram Equivalents of Estrone in the Uterus
Control	−	19.7 ± 0.7		−	19.5 ± 0.6	
	+	29.9 ± 2.0	86.1 ± 6.4	+	30.8 ± 1.8	42.8 ± 1.4
Phenobarbital	−	20.1 ± 0.5		−	18.7 ± 0.2	
	+	24.6 ± 0.7	50.0 ± 4.4	+	19.7 ± 0.5	13.0 ± 0.4

enzyme activity in liver microsomes also increase the levels of microsomal TPNH oxidase (59, 73, 197), cytochrome c reductase (112, 197), cytochrome b_5 (66, 294, 313), cytochrome P-450 (197, 266, 294), as well as an enzyme in liver mitochondria that is important for heme synthesis, namely, δ-aminolevulinic acid synthetase (149). Although phenobarbital increases the levels of microsomal cytochromes, it does not increase the levels of mitochondrial cytochromes such as cytochromes b, c, c_1 or a (313).

Barbiturates and other inducers of liver microsomal enzymes stimulate the metabolism of bilirubin in experimental animals (7, 48, 301), and man (89, 376, 377) (section XIII). Schmid *et al.* (313) recently found that treatment of rats with phenobarbital enhances several-fold the incorporation of glycine-2-C^{14} into bilirubin and stimulates the overall rate of excretion of bile pigment. They also reported that phenobarbital slightly increased the activity of hepatic tryptophan pyrrolase, but did not affect that of hepatic catalase. Phenobarbital and other stimulators of drug-metabolizing enzymes increase the activity of glucose-6-phosphate dehydrogenase (26), 6-phosphogluconate dehydrogenase (26), and uridine diphosphate glucose dehydrogenase (54, 347) in the soluble fraction of liver homogenate. The stimulating effect of drugs on these dehydrogenase reactions may enhance the availability of reduced pyridine nucleotides. In rat liver microsomes, phenobarbital decreases the activity of glucose-6-phosphatase, ATPase, and DPNH cytochrome c reductase, but does not change IDPase activity (112), whereas in the liver microsomes of rabbits it has little or no effect on ATPase levels or TPN nucleotidase, but causes a slight increase in glucose-6-phosphatase activity (292). Treatment of rats with barbiturates and other foreign chemicals that stimulate drug-metabolizing enzymes in liver microsomes enhances the metabolism of glucose and galactose *via* the glucuronic acid pathway to D-glucuronic, L-gulonic, and L-ascorbic acids (36, 54). More recently, Marsh and Reid (238) found that barbital stimulates the urinary excretion of D-glucaric acid, which is also formed through the glucuronic acid pathway.

XII. ENZYME INDUCTION IN MAN

The stimulatory effects of drugs and other foreign chemicals on drug and steroid metabolism described above in animals have been reproduced in a few instances in man. These will be reviewed below. Almost every aspect of enzyme induction is potentially important to human welfare. In therapy, man is given all the drugs in table 1 that induce enzymes in animals, and many of the substances he encounters in his environment are known to stimulate the activity of liver microsomal enzymes in experimental animals: polycyclic aromatic hydrocarbons (5, 74, 75), halogenated hydrocarbon insecticides (79, 157, 158), urea herbicides (211), food preservatives (87, 137), and dyes (70, 277). The differences between species are such (section VIII) that one cannot predict that all the compounds shown in animals to induce these enzymes will induce them also in man. In the case of environmental agents, there is doubtless a degree of exposure too small to permit the enzyme induction. In the rat, a concentration of DDT (plus its metabolite DDE) in the fat as low as 10 μg/g is associated with a de-

creased action of pentobarbital (section II), and many people have this concentration of DDT in their fat (107, 275). It is important to find out whether this and other substances induce microsomal enzymes in man, how much of each it takes, and what the consequences are.

Whenever the liver microsomal enzymes are induced in man, one can expect the changes in the duration and intensity of drug effects described, for animals, in section III. It is unfortunate that patients often are given several drugs at the same time without proper consideration of the possibility that one drug may interact with another. Adding or subtracting a drug can have serious consequences for the metabolism and action of other drugs being given. Anticoagulant therapy in man is attended by risks of this kind. Treatment of rats with phenobarbital markedly stimulates the enzymatic metabolism of bishydroxycoumarin (Dicumarol) by liver microsomes, and phenobarbital stimulates bishydroxycoumarin metabolism *in vivo* in dog and man (90). When a patient treated chronically with 75 mg of bishydroxycoumarin per day was given 65 mg of phenobarbital daily for 4 weeks in addition to the bishydroxycoumarin, there was a substantial decrease in the plasma level of bishydroxycoumarin and a decrease in its anticoagulant action (a shortening of prothrombin time). Upon discontinuing phenobarbital, the plasma level of bishydroxycoumarin and the prothrombin time returned to their original values (90). In 49 adult hospitalized patients, the administration of 30 mg of phenobarbital three times daily decreased the anticoagulant response to bishydroxycoumarin (83). Phenobarbital significantly increased the dosage of bishydroxycoumarin required for anticoagulant maintenance in 8 patients with a history of ischemic heart disease, and when the phenobarbital was withdrawn, the dosage of bishydroxycoumarin required for adequate therapy decreased (145). Heptabarbital decreased the plasma levels of certain coumarins in man, and it was suggested that this might account for the inhibitory action of heptabarbital on the anticoagulant activity of the coumarins (96). The daily administration of 2 mg of phenobarbital per kg to patients antagonized the anticoagulant response to warfarin (302); in rats phenobarbital accelerates the metabolism of warfarin *in vivo* by stimulating the liver microsomal hydroxylation of the anticoagulant in the 6-, 7-, and 8-positions (177). Simultaneous therapy in man with griseofulvin and warfarin decreased the anticoagulant effect of warfarin, and it was restored when griseofulvin was discontinued (47). Although the mechanism of this drug interaction is unknown, it is possible that griseofulvin may stimulate the metabolism of warfarin. Recently, a fatal hemorrhage was reported in a patient who had received chloral hydrate and bishydroxycoumarin in combination: when medication with chloral hydrate was stopped but bishydroxycoumarin was continued, the prothrombin time increased and hemorrhage occurred (92). Later studies showed that chloral hydrate can stimulate the metabolism of bishydroxycoumarin in experimental animals (92). These results indicate that combined therapy with a coumarin anticoagulant and a stimulator of drug metabolism can be hazardous if the enzyme stimulator is withdrawn and therapy with the anticoagulant is continued without an appropriate decrease in dose.

Phenobarbital stimulates the metabolism of several other drugs in man. It

increases the rate of metabolism of diphenylhydantoin (Dilantin) in both dogs and mice (91), and it also reduces the plasma levels of diphenylhydantoin in epileptic patients (90). In rats phenobarbital stimulates the metabolism of griseofulvin (45), and this effect probably also occurs in man since low blood levels of griseofulvin are observed in patients receiving phenobarbital (44). Administration of various barbiturates or glutethimide (Doriden) to man accelerates the metabolism of the aminopyrine derivative, Dipyrone (283); treatment of people with phenobarbital stimulates the metabolism of digitoxin to digoxin (184); and phenylbutazone, which, in rats, stimulates the liver microsomal metabolism of aminopyrine (60), accelerates the metabolism of aminopyrine in man (49).

Drug interactions in man are not limited to a stimulating effect on drug metabolism, since some examples are known in which one drug can inhibit the metabolism of another drug (*cf.* section V C). For instance, Weiner and his associates (361) found that the plasma level of bishydroxycoumarin was elevated and the anticoagulant response to the drug prolonged in people treated with oxyphenbutazone. These investigators reported that the anabolic steroid, methandrostenolone, slowed the metabolism of oxyphenbutazone in man. In both experimental animals and man, Solomon (330) has shown that phenyramidol potentiates the anticoagulant action of bishydroxycoumarin by inhibiting its metabolism.

Chronic administration of a drug to experimental animals often enhances the drug's own metabolism (section III), and this effect can explain some cases of drug tolerance in animals. Certain drugs have also been shown to stimulate their own metabolism when given chronically to man. Patients tolerant to glutethimide (Doriden) metabolize the drug more rapidly than normal persons (312). Earlier observations indicated that in rats glutethimide is a stimulator of drug-metabolizing enzyme activity in liver microsomes (283). Chronic administration of meprobamate causes an increase in the metabolism of meprobamate in man (101) as in rats (209, 270).

It will be important to find out which normal body substrates, such as those listed at the beginning of section XI, are metabolized more quickly as a result of enzyme induction in man, and whether there are changes in the pattern of metabolites. Recent studies have shown that phenobarbital stimulates bilirubin metabolism in children (89, 376, 377), and the significance of this effect is discussed in section XIII. The role of sex hormones (including antifertility medications) in regulating the metabolism of drugs, and the effects of drugs on the metabolism and physiological role of sex hormones and other steroids (sections X and XI) have yet to be worked out in man. Some compounds that stimulate the hydroxylation of steroids by liver microsomes in animals also enhance their hydroxylation in man. Treatment of people with phenobarbital (42), diphenylhydantoin (370), phenylbutazone (218), or *o, p'*-DDD (16, 331, 332) markedly stimulates the metabolism of cortisol to 6β-hydroxycortisol. The first three drugs can stimulate the activity of an enzyme system in guinea pig liver microsomes that hydroxylates cortisol in the 6β position (65, 218), and in rats *o,p'*-DDD stimulates the activity of microsomal enzymes that oxidize drugs (337) and metabolize cortisol to polar metabolites (225). The stimulatory effect of *o,p'*-DDD on cor-

tisol hydroxylation in man suggests that this compound may not decrease urinary 17-hydroxycorticoids by a direct action on the adrenal gland, as previously believed, but that the drug causes an accelerated metabolism of cortisol to highly polar 17-hydroxycorticoid metabolites that are poorly extractable into chloroform. The stimulatory effect of *o,p'*-DDD or other drugs on the extra-adrenal hydroxylation of cortisol could lead to the mistaken conclusion that a fall in chloroform-extractable 17-hydroxycorticoids in the urine reflects a decrease in adrenocortical production of cortisol.

Evidence for a stimulatory effect of drugs on the metabolism of carbohydrates *via* the glucuronic acid pathway in man came from studies by Enklewitz and Lasker (111). These investigators found that aminopyrine and antipyrine, drugs that stimulate L-ascorbic acid synthesis in the rat, elevate the urinary excretion of L-xylulose in persons with essential pentosuria. D-Glucaric acid, which is also formed through the glucuronic acid pathway, was recently identified in mammalian urine (237), and Marsh and Reid (238) presented evidence that treatment of rats with barbital enhances the urinary excretion of D-glucaric acid. A similar effect was observed in the guinea pig (1). Aarts (1) found that treatment of people with phenobarbital or aminopyrine stimulates their urinary excretion of D-glucaric acid, and he suggested that the urinary excretion of D-glucaric acid might be a useful test for drug-induced alterations in the glucuronic acid pathway in man.

XIII. POSSIBLE THERAPEUTIC APPLICATIONS OF ENZYME INDUCTION

Many human illnesses are expressions of specific genetic derangements in the synthesis or activity of enzymes that are important for normal body metabolism (334). Such hereditary enzymic defects include galactosemia, phenylketonuria, glycogen storage disease, and congenital nonhemolytic jaundice. The molecular bases for defective enzyme-forming systems in persons with inborn errors of metabolism are unknown. Molecular abnormalities could include a faulty DNA, impaired translation of DNA to appropriate RNAs, or an impaired translation of RNA on the ribosomes. If the low enzyme activity in patients with inborn errors of metabolism is caused by a defective DNA molecule, the induction of enzyme synthesis would be unlikely; but if the enzyme defect is caused by an abnormal control of DNA expression or enzyme synthesis, it might be possible to treat such persons with suitable enzyme inducers. Studies in recent years have provided a few examples in which enzymatic defects in man were partially corrected by the administration of appropriate chemicals. Pesch *et al.* (269) reported that treatment of galactosemic subjects with progesterone enhances the metabolism of galactose. More recently, Moses and his associates (252) stimulated hepatic glucose-6-phosphatase activity with triamcinolone in a person with glycogen storage disease. Unfortunately, there was no evidence that progesterone treatment was of therapeutic value in galactosemia or that triamcinolone was of therapeutic value in glycogen storage disease.

Treatment of mice with barbiturates stimulates the enzymatic glucuronidation of bilirubin by liver microsomes (48) (section XI). This observation suggested

that barbiturates might have therapeutic value in diseases of hyperbilirubinemia in man. Yaffe and his associates (376, 377) and Crigler and Gold (89) recently tested this idea in two infants with congenital nonhemolytic jaundice. Treatment of the infants with 15 mg of phenobarbital 2 to 3 times daily lowered the free bilirubin concentration, and the jaundice disappeared. Parallel studies on the metabolism of salicylamide in these children showed a defective capacity to conjugate glucuronide; this became normal after treatment with phenobarbital. When the treatment was stopped, serum bilirubin concentrations rose to their original high levels, and restitution of therapy again decreased serum bilirubin levels and the jaundice disappeared. It would be of interest to know whether careful treatment of pregnant women with phenobarbital during the last 2 weeks of pregnancy would enhance the metabolism of drugs and bilirubin in the newborn. This effect might be of value in preventing hyperbilirubinemia associated with erythroblastosis fetalis and in preventing the anoxia that sometimes occurs in the newborn when women in labor are treated with narcotics and barbiturates. However, caution is required in such studies, and further work should be done to determine the possible hazards of treatment of pregnant women or newborn infants with liver microsomal enzyme inducers. For instance, it is not known whether liver microsomal enzyme stimulators that enhance steroid hydroxylase activity alter normal hormonal balance in the expectant mother or newborn. Furthermore, some drugs such as sulfonamides and salicylates are extensively bound to plasma protein and may cause kernicterus by displacing bilirubin from binding sites on plasma protein in the newborn (259).

Recent observations suggest that induction of liver microsomal enzymes may have therapeutic value in diseases marked by overproduction of steroid hormones. Treatment with diphenylhydantoin stimulates the liver microsomal 6β-hydroxylation of cortisol in guinea pigs (65) and enhances the urinary excretion of 6β-hydroxycortisol in man (370). Werk *et al.* (371) administered diphenylhydantoin chronically to two patients with nontumorous Cushing's syndrome and observed biochemical and clinical amelioration of its manifestations. Treatment of the two subjects with diphenylhydantoin (300 to 400 mg/day) for 3 months lowered the plasma 17-hydroxycorticoids from 42 and 38 μg% to 12 and 15 μg% in the two subjects. The rate of cortisol secretion and the concentration of cortisol-binding globulin were not significantly affected. Treatment of rats with DDT or other halogenated hydrocarbon insecticides enhances the activity of oxidative enzymes in liver microsomes that metabolize drugs (136, 157, 158, 337). More recently, DDT (366) and *o,p'*-DDD (225) have been shown to enhance the hepatic transformation of several steroids to polar hydroxylated compounds. Bledsoe *et al.* (16) found that daily treatment of people with 6 to 9 g of *o,p'*-DDD stimulated the urinary excretion of 6β-hydroxycortisol and decreased the chloroform-extractable 17-hydroxycorticoids in urine. These results were confirmed by Southern *et al.* (331, 332), and these authors also found that the administration of *o,p'*-DDD resulted in a remission in the clinical and biochemical features of Cushing's syndrome before any effect of the drug on the rate of cortisol secretion.

Treatment of rats with barbiturates, aminopyrine, phenylbutazone or DDT decreases the storage of dieldrin in body fat (93, 338, 339, 341) (section III). These results suggest the possibility that suitable enzyme inducers may have value in lowering pesticide residues in livestock and in man, and that enzyme inducers may be useful therapeutically in persons who have received excessive doses of dieldrin or other halogenated hydrocarbon insecticides.

The fact that enzyme inducers can increase protein production, cause growth, and accelerate regeneration in the livers of experimental animals (section V D) suggests that the administration of inducers might be useful in patients with impaired function of this organ. On the other hand, the growth of a hepatoma in an experimental animal was slowed by the administration of phenobarbital (303) (section VII).

Treatment of experimental animals with inducers of liver microsomal enzymes stimulates the enzymatic detoxification of several chemical carcinogens such as 3,4-benzpyrene, 9,10-dimethyl-1,2-benzanthracene, 2-acetylaminofluorene and aminoazo dyes (70, 74, 75, 86, 185) (section III). The formation of cancer by several of these carcinogens is blocked when rats are treated with inducers of microsomal enzymes (174, 176, 247, 343). These observations suggest the theoretical possibility of using suitable enzyme inducers prophylactically to protect people from environmental carcinogens. This concept was recently reviewed by Wattenberg (355), who has sought inducers of benzpyrene hydroxylase activity in liver, lung, and gastrointestinal tract (354, 357). Drug-metabolizing enzymes in the gastrointestinal tract, lung, and skin (section IX) may be of particular importance for the metabolism of drugs administered orally and for the detoxification of carcinogens and other chemicals that we encounter in our environment. Since the metabolic products of benzpyrene are less carcinogenic than the parent molecule (12, 80), persons with high levels of benzpyrene hydroxylase in the lung, gastrointestinal tract, and skin may be less susceptible and those with low levels may be more susceptible to the carcinogenic action of polycyclic hydrocarbons ingested in various smoked or cooked foods, or breathed in cigarette smoke or polluted city air. It would also seem likely that genetic differences in the inducibility of enzymes in the lung, gastrointestinal tract, and skin that detoxify hydrocarbons may play an important role in the susceptibility of different persons to the carcinogenic effect of polycyclic hydrocarbons in our environment.

XIV. SUMMARY AND CONCLUDING REMARKS

In increasingly large numbers, drugs, pesticides, herbicides, food additives, and environmental carcinogenic hydrocarbons are being found to stimulate their own metabolism or the metabolism of other compounds. The evidence suggests that foreign chemicals exert this action by increasing the amount of drug-metabolizing enzymes in liver microsomes.Treatment of animals or man with suitable inducers of liver microsomal enzymes accelerates drug metabolism *in vivo* and alters the duration and intensity of drug action. For instance, barbiturates decrease the anticoagulant activity of coumarin anticoagulants by accelerating their metabolism. This effect requires that the dosage of coumarins be raised to

obtain an adequate anticoagulant response, and serious toxicity can result after combined therapy with a coumarin anticoagulant and a stimulator of drug metabolism when the enzyme stimulator is withdrawn and the anticoagulant is continued without an appropriate decrease in dose.

The stimulatory effect of drugs on their own metabolism often allows the organism to detoxify drugs more rapidly. This effect has considerable importance when it causes drugs to become less toxic and less effective during prolonged administration. However, if a metabolite has more activity than the parent drug, enzyme induction can enhance the drug's action. Enzyme induction may also be important during chronic exposure to environmental carcinogens, such as 3,4-benzpyrene. The ability of 3,4-benzpyrene to stimulate its own metabolism in liver, lung, gastrointestinal tract and skin represents an important mechanism for the detoxification of this substance.

Inducers of microsomal enzymes stimulate the metabolism or synthesis of several normal body substrates such as steroid hormones, pyridine nucleotides, cytochromes, and bilirubin. Evidence has accumulated that steroids are normal body substrates of drug-metabolizing enzymes in liver microsomes. Accordingly, treatment of rats with phenobarbital enhances the hydroxylation of androgens, estrogens, glucocorticoids, and progestational steroids by liver microsomes. This effect is paralleled *in vivo* by enhanced metabolism of steroids to polar metabolites and by a decreased action of steroids such as estradiol, estrone, and progesterone.

Recent studies suggest that inducers of liver microsomal enzymes enhance the hydroxylation of steroids in man. Phenobarbital, diphenylhydantoin, and phenylbutazone are examples of drugs that stimulate cortisol hydroxylase activity in guinea pig liver microsomes and enhance the urinary excretion of 6β-hydroxycortisol in man. Further research is needed to learn whether the stimulatory action of drugs on the metabolism of normal body constituents is harmful or whether it restores a homeostasis that was upset by drug administration. It is of considerable interest that certain inducers of liver microsomal enzymes have recently been used therapeutically for the treatment of hyperbilirubinemia in jaundiced children and for the treatment of Cushing's syndrome. Considerable further work is required to evaluate more completely the effects of liver microsomal enzyme inducers on the metabolism of bilirubin, cortisol, and other normal body constituents in experimental animals and man.

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