

interconvertible forms of a single hemoprotein. One view, based on indirect measurements as cited in Mannering (35), is that cytochrome P-450 and cytochrome P₁-450 are similar but separate entities, each of which can exist in two interconvertible forms.

Direct comparison of cytochrome P-450 and cytochrome P₁-450 was made possible through solubilization and partial purification of the microsomal hemoproteins from phenobarbital and 3-MC treated rats (unpublished observations of Fujita and Mannering as cited in Mannering (35)). The absolute spectrum of soluble purified cytochrome P₁-450 is shown in Figure 6, and some properties of cytochromes P-450 and P₁-450 in Table 2. The absolute spectra of the two hemoproteins are very much alike, but there are differences. The Soret peaks at 448 m μ and 450 m μ (reduced + CO) shown by cytochrome P₁-450 and cytochrome P-450, respectively, accord with what was expected from spectral studies employing microsomes. The Soret peak at 414 m μ rather than at 418 m μ (reduced hemoprotein) also distinguishes cytochrome P₁-450 from cytochrome P-450.

Particularly to be noted is the absence of a peak at about 395 m μ . Putatively, a peak at 395 m μ characterizes the form of the P-450 hemoprotein that results when PAHs are administered (20, 53). The most likely explanation for the peak at 395 m μ is that 3,4-benzpyrene, a type I compound (53), or a metabolite, binds with hemoprotein to produce a type I spectrum. The PAH or its metabolite binds more avidly than most type I compounds and is not lost during preparation of the microsomes. However, the loss of 3-MC or its metabolite occurs when the hemoprotein is solubilized.

Further evidence for the existence of two molecular species of P-450 hemoprotein was obtained by comparing the cytochrome P-420 derived from cytochromes P-450 and P₁-450 (56). When hepatic microsomes from untreated rats were incubated under nitrogen at 4°C for 24 hours with 0.07% steapsin, about 25 percent of the P-450 hemoprotein was solubilized as P-420 hemoprotein. After desalting and concentrating the clear solution to about one-fourth its volume, an aggregate of cytochrome P-420 was formed consisting of microtubules with globular substructures (56). Microsomes from rats that had received 3-MC, when treated in the same manner, also yielded aggregates; but only small numbers of the tubular structures were seen, their presence possibly due to the existence of some residual cytochrome P-450 in the microsomes. Aggregates of cytochrome P-420 showed both type I and type II binding with drugs, but aggregates of cytochrome P₁-420 bound only with type II compounds. On the basis of heme content, the molar absorptivity of cytochrome P-420 was determined to be 110 mM⁻¹cm⁻¹, whereas that of cytochrome P₁-420 was 134 mM⁻¹cm⁻¹. Disc electrophoresis of aggregates solubilized with 8 M urea disclosed differences in the ionic mobilities of the two P-420 hemoproteins.

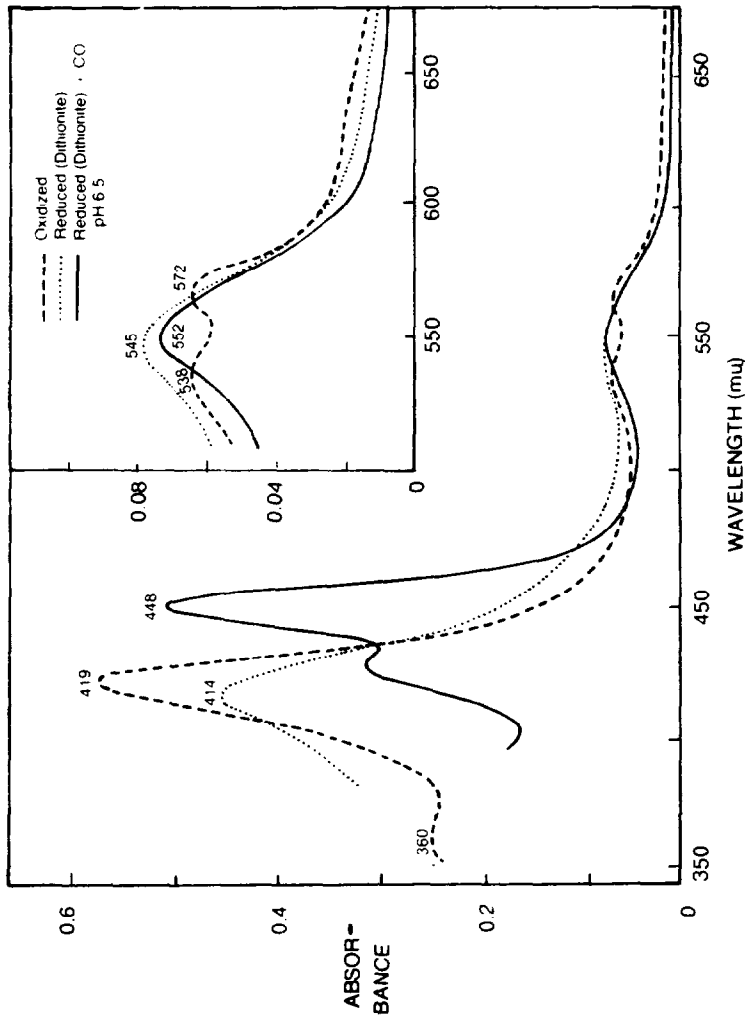


FIGURE 6.—Absolute spectra of solubilized microsomal P-450 hemoprotein (cytochrome P₁-450) from livers of rats treated with 3-MC (Fujita and Mannering, unpublished results). The hemoprotein was solubilized by treating microsomes with Triton N-101 and fractionating the supernatant on a DEAE cellulose column. The preparation was free of cytochrome b₅, but contained a small amount of P-420 hemoprotein. Table 2 summarizes the spectral properties of solubilized cytochromes P-450 and P₁-450

SOURCE: Mannering, G. (35).

In summary, the preponderance of evidence leads to the following conclusions:

TABLE 2.—Absorption peaks and molar extinction coefficients of absolute spectra of soluble cytochromes P-450 and P₁-450^a

Conditions	Cytochrome P-450 ^b		Cytochrome P ₁ 450 ^c	
	max (mμ)	(mM ⁻¹ cm ⁻¹)	max (mμ)	(mM ⁻¹ cm ⁻¹)
Oxidized	360	49.2	360	45.7
	Soret 418	104.2	419	120.3
	537	12.9	537	13.5
Reduced	568	12.3	568	13.4
	Soret 418	86.0	414	90.1
	545	14.9	545	16.4
Reduced + CO	423	65.8	423	60.0
	Soret 450	89.1	448	108.0
	548	13.9	551	15.4

^aThe hemoprotein were solubilized by treating microsomes with Triton N-101 and fractionating the supernatant on a DEAE cellulose column (Fujita and Mannering, unpublished observations). The preparations were free of cytochrome b₅, but they contained small amounts of P-420. The absolute spectrum of cytochrome P₁-450 is shown in Figure 7.

^bThe preparation contained 3.24 mμ moles of P-450 hemoprotein/mg of protein, an increase of 4.3-fold over that contained in the microsomes from which the preparation was obtained. Recovery of hemoprotein was 15.5%.

^cThe preparation contained 4.42 mμ moles of P-450 hemoprotein/mg of protein, an increase of 3.5-fold over that contained in the microsomes from which the preparation was obtained. Recovery of hemoprotein was 13.9%.

SOURCE: Mannering, G. (35).

1. The administration of polycyclic aromatic hydrocarbons (PAHs) causes the biosynthesis of cytochrome P₁-450, a molecular species of cytochrome P-450 not normally detectable in appreciable amounts of microsomes from untreated or phenobarbital-treated animals. This does not exclude the possibility that small amounts of cytochrome P₁-450 may be found in untreated animals; in fact, this can be expected to be the case. PAHs or other substances capable of inducing the synthesis of cytochrome P₁-450 may be present in the diet or atmosphere or may be produced by the intestinal flora. Early recognition of an exogenous inductive effect on the metabolism of a foreign substance was made by Brown, et al. (4) and by Reif, et al. (52) who observed that rancid diets contained oxidized steroids which stimulated the N-demethylation of aminoazo dyes.

2. Both cytochrome P-450 and cytochrome P₁-450 exist in their own interconvertible forms.

3. Cytochrome P₁-450 does not form as a result of the combination of native cytochrome P-450 with PAHs or their metabolites.

Mechanisms of Induction of Drug Metabolism Enzymes

Gelboin (13) has discussed mechanisms of induction of drug metabolism enzymes. Significant highlights of this discussion are as follows:

1. The stimulatory effect of PAHs and drugs on certain liver microsomal enzymes appears not to be mediated through the endocrine

system, as the stimulation of at least the aryl hydrocarbon hydroxylase (AHH) is observed in adrenalectomized and hypophysectomized rats.

2. The inducer acts directly on the target tissue.

3. The half-life of induced AHH activity is 3.3 ± 1.2 hours.

4. Results of studies in cell culture have suggested the following sequence of events in microsomal enzyme induction:

a. Upon addition of the inducer to the culture medium, it is rapidly incorporated, within several minutes, into the cell. This has been shown by the use of radioactive inducer and fluorescence microscopy (Miller and Gelboin, unpublished observations cited in Gelboin (13)). After incorporation, there appears to be a rapid interaction between inducer and receptor site which is followed by a period of RNA synthesis. This stage of enzyme induction involving RNA synthesis is sensitive to actinomycin-D inhibition. This early RNA synthesis phase is independent of translation, since it occurs in the presence of inhibitors of protein synthesis.

b. Then follows the protein synthesis stage which is sensitive to inhibitors of protein synthesis. This stage can proceed in the absence of the RNA synthesis stage and can occur in the presence of actinomycin-D. It seems to be a polymerization of amino acid into polypeptide chains.

c. The next step appears to be an assembly process of the newly-made polypeptide chains. This is independent of protein synthesis and may persist for up to two hours. This entire process results in the appearance of increased levels of AHH. The specific protein, made and assembled in the microsomes, may be either the hydroxylase or another protein which may activate by an allosteric mechanism an inactive form of the hydroxylase. All of these events appear before there are gross changes in either protein or RNA synthesis. This suggests that the RNA and protein, which are required to be synthesized, are very small percentages of total cell RNA and protein and that many of the gross changes of RNA and protein synthesis may be subsequent to, and parallel, but not directly responsible for, the appearance of the early increases of enzyme level.

Thus, the various studies on the effect of methylcholanthrene (MC) on nuclear RNA metabolism have shown that: (1) MC causes an increase in the uptake of orotic acid into nuclear RNA which suggests increased RNA synthesis; (2) MC increases the amount of RNA in liver cell nuclei; (3) RNA isolated from the liver cell nuclei of MC treated rats has greater stimulatory activity in an *E. coli* phenylalanine-incorporating system; and (4) the administration of MC *in vivo* stimulates RNA polymerase activity of either isolated liver nuclei or isolated chromatin. These effects of MC suggest an alteration in genetic transcription.

TABLE 3.—Summary of effects of methylcholanthrene or phenobarbital on gene-action system

Microsomes	Nucleus
Increases of:	Increases of:
1. Specific enzymes and protein (MC, PB)	1. Orotic acid- ¹⁴ C incorporation into RNA (MC)
2. Amino acid incorporation (MC, PB)	2. RNA/DNA ratio (MC)
a. More mRNA (PB)	3. Messenger RNA content (MC)
b. More sensitive to added mRNA (PB)	4. Stimulation of RNA polymerase (MC, PB)
3. Effects prevented by:	
a. Puromycin (MC, PB)	
b. Actinomycin-D (MC, PB)	
c. Ethionine (MC, PB)	
Inhibitions of:	
1. NADPH cytochrome C reductase degradation (PB)	
2. b ₅ degradation (PB)	
Changes in:	
1. Special properties of P-450 (MC)	
2. Phospholipid metabolism (MC)	
3. Kinetic behavior of hydroxylase (MC)	

SOURCE: Gelboin, H. (13).

Table 3 shows a summary of the effects of MC and phenobarbital (PB) on various aspects of nuclear and microsomal metabolism.

Summary

The pervasiveness of tobacco use in our society and the frequency of altered disposition and pharmacological effects of many common drugs in smokers make it apparent that cigarette smoking should be considered as one of the primary sources of drug interactions in man. Most of the experimental work in man, animals, and tissues involving enzyme systems indicates that the dominant effect of smoking is enhanced drug disposition caused by induction of hepatic microsomal enzymes. The primary causal agents are probably the polynuclear aromatic hydrocarbons which are potent and persistent in tissues. While several of the hepatic microsomal drug-metabolizing enzymes are stimulated in smokers, the selectivity of this enhancement in activity is unpredictable. The effects of cigarette smoke on other potential rate-limiting disposition processes for drugs are largely unexplored.

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Effects on Pharmacokinetics and Pharmacodynamics

The effects of smoking on the action of drugs have become a subject of an increasing number of investigations. Because the number of smokers in our population is significant, it is important to determine whether cigarette smoking alters the pharmacologic effects or the pharmacokinetics of drugs.

The mechanism of these alterations includes: stimulation or inhibition of biotransformation of drugs by the various constituents of tobacco smoke, alteration of physiological processes that control drug disposition, direct interference in the mechanism of drug action and modification of psychopharmacological behavior, such as drug consumption and pain threshold. Cigarette smoking may necessitate modification of drug therapy and alter organ function or responsiveness.

Extensive literature is being assembled on the interaction of tobacco smoke and drugs. Recently, Jusko prepared an excellent review (28) on the role of tobacco smoke in the pharmacokinetics and pharmacology of drugs in man and animals. Much of this discussion merely paraphrases the Jusko review.¹ Conney, et al. (14) have previously reviewed the interaction of smoking and biotransformation of drugs, and Jick (27) has addressed smoking and clinical drug effects.

Studies of tobacco smoking and nicotine have been closely associated for many years. Tobacco in the United States yields about 1.2 mg (range 0.1 to 2.2 mg) of nicotine per cigarette. Chronic nicotine inhalation produces various types of pharmacological stimulation. The assimilation of about 0.5 mg/kg/day of nicotine from tobacco smoke offers the potential for altering drug disposition. The extraction of nicotine from inhaled smoke by habitual smokers is nearly complete (25). The half-life of nicotine has been determined to be about one hour (25). Most studies in animals indicate that nicotine is an enzyme inducer, which will be described later.

The most common effect of tobacco smoke on drugs in man and animal is an increase in biotransformation rate consistent with induction in drug-metabolizing enzymes. The first observation of this type in man was made by Rottenstein, et al. (65), who found that intravenous injection of nicotine did not cause nausea in smokers, but in nonsmokers the same dose produced nausea and vomiting. Beckett and Triggs (6) subsequently reported that, following intravenous administration or inhalation of nicotine, the urinary excretion of nicotine by nonsmokers and smokers was 55 to 70 percent and 25 to 50 percent, respectively. The reduced recovery of nicotine in the smoker group was explained by an increased biotransformation of the nicotine. Nicotine had previously been reported to accelerate the biotransformation of meprobamate in mice (88) and of benzo(a)pyrene (BP) by rat

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Table 4.—Plasma levels of phenacetin in cigarette smokers and nonsmokers at various intervals after the oral administration of 900 mg of phenacetin

Subjects	Hours after phenacetin administration			
	1	2	3.5	5
	Phenacetin concentration in plasma, $\mu\text{g/ml}$			
Nonsmokers	$0.81 \pm 0.20^*$	2.24 ± 0.73	0.39 ± 0.13	0.12 ± 0.04
Smokers	0.33 ± 0.23	0.48 ± 0.28	0.09 ± 0.04	0.02 ± 0.01

*Each value represents the means \pm S.E. for nine subjects.

SOURCE: Pantuck, E.J. (55).

liver microsomes (92). Welch, et al. (87) were the first to demonstrate that inhaled tobacco smoke increased the activity of the enzyme benzo(a)pyrene hydroxylase in rat lung. This study has stimulated studies of tobacco smoke as a source of drug interaction.

Phenacetin

Pantuck, et al. (54, 55) first reported that tobacco smoke could induce the metabolism of a therapeutic agent in man. Oral doses of 900 mg of phenacetin were administered to nonsmokers and smokers (smoked more than 15 cigarettes per day). By measuring the concentration of phenacetin in plasma it was determined that the phenacetin concentrations in the plasma of cigarette smokers were markedly lower than those in the nonsmokers (Table 4), but the average half-life of phenacetin (about 50 minutes) in both groups was not different. The lower plasma levels were not due to altered absorption of phenacetin, as the urinary excretion of its major metabolite, N-acetyl-p-aminophenol (APAP), was identical for both groups. The low plasma concentrations of phenacetin in smokers were thus presumed to be caused by increased metabolism of phenacetin by the enzymes either in the gastrointestinal tract or during the "first pass" through the liver. On a theoretical pharmacokinetic basis, an increased degree of "first pass" metabolism will cause a decrease in the area under the plasma level curve with little change in half-life (21).

Similar results were reported almost simultaneously by Welch, et al. (83) on the effect of cigarettes in rats. These workers demonstrated that the enzyme benzo(a)pyrene (BP) hydroxylase was inducible by 3-methylcholanthrene (3-MC) and caused lower plasma phenacetin levels in rats.

Phenacetin has since been extensively studied as a model drug to investigate various aspects of cigarette smoke-induced changes in biotransformation rate. Welch, et al. (83, 86) and Pantuck, et al. (53) exposed rats to cigarette smoke and observed marked increases in the rate of *in vitro* metabolism of phenacetin in liver, lung, and intestinal

homogenates. Similar effects were found when rats were pretreated with 3-MC or BP. Welch, et al. (86) examined the effects of 3-MC treatment of rats on the bioavailability of phenacetin and APAP in portal and peripheral plasma following oral and intravenous administration. Comparison of the plasma phenacetin concentration in portal blood of the control rats and those treated with 3-MC revealed almost identical plasma concentration of phenacetin. The results indicated that 3-MC treatment had little effect on the passage of phenacetin into the portal circulation, but did influence to a very marked extent the passage of phenacetin from the portal circulation into the general circulation. These results were interpreted by the authors to mean that the dominant effect of 3-MC treatment was induction of hepatic rather than intestinal enzyme activity. On this basis, they concluded that the reduced plasma phenacetin concentrations in smokers probably reflected an increased "first pass" metabolism by the liver. However, Kuntzman, et al. (39) have investigated the stimulation of intestinal BP hydroxylase in rats following exposure to cigarette smoke or exposure to BP. Their data showed that rats exposed to cigarette smoke or to pretreatment with BP enhanced the *in vivo* metabolism of phenacetin and stimulated enzymes in the intestinal mucosa to O-dealkylate phenacetin to APAP. Therefore, the question whether the stimulatory effect of cigarette smoking on the metabolism of phenacetin occurs in the gastrointestinal tract or in an additional first-pass increase in liver metabolism remains unanswered.

Antipyrine

Antipyrine is an analgesic often used as a "marker" for several hepatic microsomal drug-metabolizing systems in man and animals. Vestal, et al. (80) studied the effects of aging and cigarette smoking on the disposition of antipyrine in 307 healthy subjects. Determination of the half-life and metabolic clearance rate (MCR) of antipyrine revealed that young and middle-aged smokers metabolized antipyrine more rapidly than nonsmokers (Table 5). The half-life and the metabolic clearance rate were defined as: $t_{1/2} = 0.693/k_e$ where k_e = overall elimination constant, and $MCR = aVd \times k_e$ where aVd = apparent volume of distribution.

The half-life was 16.5 percent longer and the total clearance (Cl_T) rate was 18.5 percent less in the older subjects than in the younger. By old age (60 to 92 years), there was essentially no difference in the Cl_T between smokers and nonsmokers, although the Cl_T diminished with age in all smoking categories. Similar total clearance values were reported by Wilson, et al. (89) and found to be 46.0 ml/hr/kg in smokers and 36.5 ml/hr/kg in nonsmokers following administration of antipyrine to subjects in the 24- to 45-year age range.

Hart, et al. (23) found enhanced metabolism of antipyrine in cigarette smokers. These investigators found a mean half-life of 12.5

TABLE 5.—Effect of age and cigarette smoking on antipyrine metabolism. Data are from 307 healthy subjects

Age group (yr)	t _{1/2} (hr)	Smoking ^a group	No. of subjects	MCR (ml/hr/kg)
Young (18-39)	12.7 ± 0.50 ^b	Nonsmoker	37	30.6 ± 1.24
		Moderate	27	37.3 ± 2.39
		Heavy	9	42.4 ± 4.24
Middle (40-59)	13.8 ± 0.47	Nonsmoker	102	28.0 ± 0.86
		Moderate	30	37.2 ± 2.27
		Heavy	18	36.8 ± 3.02
Old (60-92)	14.8 ± 0.65	Nonsmoker	67	28.2 ± 1.09
		Moderate	14	29.9 ± 2.8 ^c
		Heavy	3	15, 21, 28

^aNonsmoker: Did not smoke or smoked "once in a while," Moderate: Smoked less than 20 cigarettes/day, Heavy: Smoked more than 20 cigarettes/day.

^bMean ± SEM

SOURCE: Vestal, R.E. (80).

hours in 17 nonsmokers and 10.8 hours in 25 smokers, a smaller but significant difference. To determine whether this difference was due to tobacco consumption, eight smokers were restudied two months after they stopped smoking. The half-life of antipyrine had increased in six of the subjects, with an overall increase of about 23 percent. Welch, et al. (84) reported the mean half-life of antipyrine was 4.2 hours in epileptic patients treated with anti-convulsants for more than two months; whereas the mean half-life was found to be 12.6 hours in normal volunteers, three of whom were smokers. These data suggested that the anti-convulsant, phenytoin, may be a much stronger enzyme inducer than tobacco smoke. However, Kellermann and Luyten-Kellermann (31) found that the half-life of antipyrine was decreased 22 percent in normal subjects following 7 days on orally administered phenobarbital. This shortening of the antipyrine half-life is almost identical in the report by Hart, et al. (23).

Kellerman, et al. (31, 32, 33) measured the half-life of antipyrine and the percent induction of BP hydroxylase by 3-MC in mitogen-stimulated lymphocytes from normal individuals. Resting lymphocytes had relatively little BP hydroxylase activity and the capacity to induce lymphocyte activity *in vitro* correlated with hepatic metabolism of various drugs in the same individual. The antipyrine half-life ranged from 7.7 to 16.2 hours and showed a high inverse correlation coefficient ($r=0.923$) with the BP hydroxylase ratio. This indicated that antipyrine and BP share one or more common determinants that are responsible for the observed interindividual variation in the oxidation rates, and that antipyrine may serve as a useful predictor drug for

evaluating the drug- and carcinogen-metabolizing capacity of different individuals in the human population. The difference in the antipyrine half-life and the metabolic clearance rate between smokers and nonsmokers, however, was not large and, therefore, makes antipyrine an insensitive predictor for smoking effects.

Recently, Ambre, et al. (3) reported the antipyrine total clearance rate in patients with bronchogenic carcinoma, in patients with chronic lung disease, and in normal subjects. The mean antipyrine Cl_T values were 2.98 ± 0.68 , 2.02 ± 0.67 , and 2.14 ± 0.69 liters/hour, respectively. These results could not be reproduced by Tschanz, et al. (74), however. The latter group examined patients with lung cancer and a malignancy-free control group very well matched for age, sex, drug intake, smoking, and drinking habits. Their study took more blood samples than the Ambre study and the mean Cl_T values were determined to be 47.5 ± 0.9 in the cancer group and 55.7 ± 0.7 mg/kg/hr in the malignancy-free groups; this was a reversal of the earlier study. This topic should be investigated further, as an increase in antipyrine Cl_T in cancer patients would suggest a common factor in the observations of bronchogenic carcinoma, enhanced drug disposition, and inducibility of BP hydroxylase. This common factor may be a genetic susceptibility (33) to the multiple effects of exposure to polycyclic aromatic hydrocarbons (PAHs, PNAs).

Theophylline and Other Xanthines

Theophylline

Theophylline is of primary importance as a bronchodilator used to treat acute and chronic asthma or bronchitis. It is generally recognized that the therapeutic index of theophylline is narrow and the disposition rate among patients is widely variable. Jenne, et al. (26), Hunt, et al. (24), and Powell, et al. (63) have investigated the interaction of cigarette smoking and theophylline disposition. These investigators have found that the theophylline half-life ranged from about 4 to 6 hours in smokers to 7 to 9 hours in nonsmokers. Theophylline appears to be metabolized mainly in the liver, because only about 10 percent of the dose is excreted unchanged in the urine. Smokers exhibited a Cl_T of 100 ± 44 ml/min/1.73 m². This value was larger and more variable than 45 ± 13 ml/min/1.73 m² found for nonsmokers. A somewhat surprising finding was that four of the smokers who stopped smoking for three months had relatively little change in the Cl_T (24). This suggested that more than three months is needed for the effects of chronic tobacco use to dissipate. The average theophylline half-life of smokers who discontinued their habit for at least 2 months was intermediate between those of nonsmokers and smoker groups (63). Further studies by Jusko, et al. (29) showed that increased age offset the increased Cl_T of theophylline, as was observed earlier in the case of antipyrine. These investigators found mean Cl_T values for theophylline of 55.3

ml/min/1.73 m² in non/light smokers and 77.5 ml/min/1.73 m² in heavy smokers. When younger smokers (20 to 40 years) were compared to older smokers (40 or more years) the mean Cl_r values were found to be 106 and 61 ml/min/1.73 m², respectively.

The increased biotransformation rate of theophylline in smokers appears to be accompanied by a reduced toxicity during clinical use of this drug. Pfeifer and Greenblatt (62) studied the toxic effects of theophylline in 2,766 patients. The frequency of adverse reactions following administration of theophylline correlated negatively with the daily smoking habit. The data revealed a significant trend, with nonsmokers exhibiting 12.9 percent, light smokers (20 cigarettes/day) 10.8 percent, and heavy smokers (20 or more cigarettes/day) 7.0 percent incidence of adverse reactions to theophylline.

The dosing of patients on theophylline therapy is important because of the frequency of adverse reactions of the drug. The rate of elimination of a drug from the body (total body clearance) can be ascertained from the plasma half-life and apparent volume of distribution (aVd) for that drug. The aVd for theophylline does not appear to be altered in patients with a history of smoking; therefore, the shorter plasma half-life in smokers indicates that they have more rapid total body clearance of theophylline. Thus, when a multiple dose regimen (maintenance dose) is used, the steady-state plasma concentration achieved with a given dose will likely be lower in smokers than in nonsmokers. Although there appears to be considerable overlap in the theophylline clearance values, some heavy smokers may require as much as one and one half to two times the maintenance dose of nonsmokers. These large maintenance doses required by heavy smokers could result in toxicity if the patient discontinues smoking. Because specific information about the recovery of the drug-metabolizing enzymes following cessation of smoking is not available, clinical effects should be carefully monitored.

Lohman and Miech (43) have confirmed the inductive effect of 3-MC on theophylline metabolism by liver slices in rats.

Other Xanthines

Welch, et al. (85) and Parsons and Aldridge (56) reported that the biotransformation of caffeine in the rat was accelerated by PAHs in cigarette smoke. Welch, et al. (85) showed that benzpyrene, benzanthrene, dibenzanthracene, chrysene, and pyrene, which are potent inducers of the cytochrome P-448 system in liver microsomes, caused a marked increase in the plasma clearance of caffeine without altering its volume of distribution. On the other hand, phenanthracene and anthracene, generally considered very weak inducers of the liver microsomal cytochrome system, did not change the plasma clearance of caffeine. Following treatment with BP for three days, the Cl_r of caffeine in rats increased from 50.3 to 125.3 ml/hr. Moreover, the

subsequent elimination rates in rats of the caffeine metabolites, theophylline, paraxanthine, and theobromine, were greatly accelerated. A dose response study with BP indicated that a dose of 1 mg/kg or more of BP for 3 days was required for the enzyme induction in the rat and that 0.1 mg/kg had no significant effect. At the higher doses, BP proved to be a more potent inducer than phenobarbital (equivalent induction at 75 mg/kg). Thus, increased caffeine biotransformation may, in part, explain the tendency for smokers to consume more coffee than nonsmokers.

Other Drugs

Imipramine

The disposition of the tricyclic antidepressant, imipramine, has been reported to be affected by smoking. Perel, et al. (60, 61) gave 29 depressed patients daily doses of 3.5 mg/kg of imipramine and determined the mean steady-state plasma concentration of total imipramine and desmethyl imipramine to be 160 ng/ml in smokers and 290 ng/ml in nonsmokers. A strong correlation was also found between these plasma levels and the half-life of phenylbutazone administered to the same patients. These results implied that the pharmacokinetics of phenylbutazone may also be affected by smoking, but no direct evidence is available.

Glutethimide

The metabolism of glutethimide, a hypnotic, has been reported by Crow, et al. (16) to be altered by smoking. They measured plasma concentrations of glutethimide given at 8-hour intervals after attainment of steady-state. The mean area under the curve (0 to 8 hours after the dose) was determined to be 41 mg/liter-hour for four smokers and 26 mg/liter-hour for four nonsmokers. The half-life of glutethimide was not found to be significantly different between groups. These results suggested that the bioavailability was changed and that either the apparent volume of distribution of glutethimide (aV_G) was smaller or the fraction of drug absorbed was larger in smokers. The latter appeared unlikely because there was no difference in the rate of excretion of 4-hydroxy-2-ethyl-2 phenylglutaramide, an active metabolite, in the urine of smokers and nonsmokers. The presence of other active metabolites is a possible explanation for these results, since smokers also performed relatively poorly in a computer-generated tracking test designed for psychomotor response. The possible mechanism of this interaction is difficult to assess. Bennett (?) has pointed out a lack of firm data on the effects of smoking on most aspects of gastrointestinal secretion and mobility.

Vitamin C

Pelletier, et al. (58, 59) have reported that the vitamin C levels in serum and leukocytes were reduced in smokers. It is not clear whether reduced absorption or enhanced catabolism of the vitamin is the mechanism for the reduction in vitamin C, as studies to measure the bioavailability of vitamin C have not been conducted. The studies carried out by Pelletier, et al. (58) suggest that reduced absorption of vitamin C by smokers may be involved in reduced levels of vitamin C.

Bilirubin

Nyman (52) recently reported the effects of maternal smoking on neonatal hyperbilirubinemia. He observed that the biotransformation of bilirubin was enhanced in newborn infants of smoking mothers. The incidence of cases with serum bilirubin concentrations below 100 μM /liter was significantly higher in smokers than in nonsmokers. On the other hand, Conney, et al. (15) reported earlier that the serum bilirubin levels in newborn of 9 nonsmokers and of 14 smokers showed no difference in the serum bilirubin levels between the two groups of newborns. No differences in the serum bilirubin concentration have been observed between adult smokers and nonsmokers (11).

Substances Interfering with the Assay Procedure

In pharmacokinetic studies, the effect of exogenous chemicals on the data obtained with nonspecific assays is of particular concern. Beckett, et al. (5) found that the higher urinary excretion of amphetamine by smokers was explained by an amine which interfered with the assay. This interfering substance was subsequently identified as nicotine. Caution must be used in tobacco-drug studies, because the complex mixture of chemicals in tobacco smoke could present similar problems in drug assays carried out on biological samples from smokers.

Biotransformation of Drugs

Jusko (28) has compiled a list of drugs which have clearly been shown either to have enhanced biotransformation or to have had no effect on drug disposition in cigarette smokers. This list is given in Table 6. The majority of the studies of smoking and drug effects have investigated the drug disposition and clearance, with emphasis on the alterations in the metabolic rate rather than on the absorption or distribution process. Except for ethanol, all of the drugs in the list are biotransformed by microsomal oxidative pathways. Most interesting is the selectivity in the effects of smoking on drugs which undergo N-demethylation. This effect may be accounted for by differences in rate-limiting steps in the overall elimination of the drug. Other rate-limiting processes are plasma protein binding, metabolism in nonmicrosomal systems, and metabolism in nonhepatic tissue. Diazepam,

**TABLE 6.—Summary of smoking effects on *in vivo*,
biotransformation of drugs in man**

Drug	Major biotransformation pathway Increased metabolic rate in smokers	Reference number
Nicotine	Hydroxylation to N of cyclic amine	(6)
Phenacetin	O-Dealkylation	(54,55)
Antipyrine	Aliphatic hydroxylation	(39,80,85)
Theophylline	N-demethylation, purine oxidation	(24,26,28,63)
Imipramine	N-demethylation	(60,61)
Pentazocine	Allylic hydroxylation	(30)
	Not affected by smoking	
Diazepam	N-demethylation	(37)
Meperidine	N-demethylation	(48)
Phenytoin	Aromatic hydroxylation	(64)
Nortriptyline	N-demethylation	(51)
Warfarin	Aromatic hydroxylation	(50,91)
Ethanol	Alcohol dehydrogenation	(79)

SOURCE: Jusko, W. (28).

phenytoin, and warfarin, which showed no difference in pharmacokinetics in smokers, are highly bound to plasma or protein and, for this reason, exhibit low total clearance rates. The plasma binding and diffusion of free drugs may not be altered significantly by tobacco smoke. Contrarily, meperidine and nortriptyline are drugs which exhibit very high total clearance rates, and hepatic blood flow may be the determining factor which is unaffected by smoking. The only generalization which can be made about these drugs is that the enhanced metabolism induced by tobacco smoking appears to be a selective process with several microsomal pathways being induced or unaffected.

Drug Effects in Man

The uncovering of differences in drug effects related to smoking has been attributed to the comprehensive in-hospital drug monitoring by the Boston Collaborative Drug Surveillance Program. Information has been obtained on drug efficacy and toxicity for all drugs administered to medical patients in this program. In addition to these data, an array of basic patient statistics, such as smoking habits, is obtained prior to admission. Several statistically significant findings that have emerged from this program are described by Jick (27).

TABLE 7.—Mean priming dose and maintenance dose of pentazocine for supplementation of nitrous oxide anesthesia

Group	No. of subjects	Mean (\pm SEM) priming dose (mg/kg)	Mean (\pm SEM) maintenance dose (μ g/kg/72)
Smokers	15	0.91 \pm 0.11	3.8 \pm 0.4
Nonsmokers	26	0.57 \pm 0.13	2.5 \pm 0.5

P = 0.05 P = 0.01

SOURCE: Keeri-Szanto, M. (30).

Pentazocine

A number of clinical reports on the alteration of drug responses in smokers have been published. One of the first was the examination of pentazocine dosage requirements for supplementation of nitrous oxide anesthesia. Keeri-Szanto, et al. (30) found that smokers required larger priming and maintenance doses of pentazocine than did nonsmokers (see Table 7).

These results were correlated to plasma concentration of pentazocine, and the increased priming and maintenance doses were attributed to enhanced drug disposition in smokers. These findings have been confirmed by Vaughan, et al. (77) by examination of urinary pentazocine excretion in smokers and nonsmokers. The researchers determined that smokers metabolize 40 percent more pentazocine than nonsmokers.

Propoxyphene

The first drug to be evaluated in detail with respect to smoking in the Boston Collaborative Drug Surveillance Program was propoxyphene (10). Propoxyphene was rated ineffective by 10.1 percent of 335 nonsmokers, 15 percent of 347 light smokers, and 20.3 percent of 153 heavy smokers.

A summary of other observations of differences in drug effects in smokers and nonsmokers made by the Boston Collaborative Drug Surveillance Program (27) and by Jusko (28) is given in Table 8.

Although the disposition of some drugs (phenacetin, theophylline, and antipyrine) is known to be increased in smokers, the mechanisms of other drug/smoking interactions are not well established. An increased "first pass" metabolism is one possibility. A possible explanation for the reduced clinical effect of propoxyphene in smokers is decreased pain threshold. Seltzer, et al. (69) have found that deep pain tolerance is significantly diminished in white male and female cigarette smokers as compared to nonsmokers. In addition, two surveys (one conducted in the United States and the other in Australia) have

**TABLE 8.—Modification of clinical drug effects by smoking:
observations of the Boston Collaborative Drug
Surveillance Program**

Drug	Diminished effect observed	Non	Incidence related to smoking habit (% patients)		Reference number
			Light	Heavy	
Propoxyphene	Pain/headache efficacy	10.1	15.0	20.3	(9)
Chlorpromazine	Drowsiness	16	11	3	(72)
Diazepam	CNS depression	7.9	7.7	2.8	(10)
Chlordiazepoxide	None (CNS)	9.7	6.1	3.5	(10)
Phenobarbital	None (CNS)	5.9	9.3	4.8	(10)
Warfarin	No modification of anticoagulant needs	- -	- -	- -	(50)
Theophylline	Various adverse reactions	12.9	10.8	7.0	(28)

SOURCE: Jick, H. (27), Jusko, W. (28).

found that smokers tend to consume more analgesics than nonsmokers (19, 68).

Other Drugs

There are a few reported tobacco-drug interactions which do not involve enzyme induction. Vapaatalo, et al. (76) found that cigarette smoking somewhat reduced the diuretic effects of furosemide. This interaction was best explained by an increased secretion of the anti-diuretic hormone caused by nicotine.

Kershbaum, et al. (35) reported that the stimulating effect of smoking on adrenocortical secretion could neutralize the suppressive effect of dexamethasone on plasma corticosteroid concentrations.

Beta-blockers such as propranolol have been used to modify nicotine-stimulated catecholamine effects such as increased pulse rate, blood pressure, and ventilatory function (12, 13, 20, 90, 93). Frankl and Soloff (20) reported that five subjects who received propranolol, followed by smoking, experienced significantly decreased cardiac output, significantly increased blood pressure, and significantly increased calculated systemic peripheral resistance compared to smoking without propranolol.

Absence of Smoking Effect

Alteration in drug disposition or pharmacological action in smokers generally received greater attention than those reports demonstrating no effect of tobacco smoke; it is equally important, however, from a

clinical and pharmacokinetic point of view to identify clearly those drugs which are not influenced by tobacco smoke.

Diazepam

A Boston Collaborative Drug Surveillance Program report (9) on the relationship to cigarette smoking of depression of the central nervous system during chronic diazepam therapy indicated that drug-attributed drowsiness became less common as the exposure to cigarette smoke increased. These findings were explained by the stimulation of diazepam metabolism by one or more of the constituents of cigarette smoke. Klotz, et al. (37) have reinvestigated the effects of age, smoking, and liver disease on diazepam disposition. They determined that an induction of the diazepam disposition would manifest itself by an increase in the plasma clearance or by a reduction in the $t_{1/2}$ of drug, yet no obvious differences between these values in smokers and nonsmokers were seen at any age. The authors concluded that cigarette smoking did not affect the disposition of diazepam and suggested that factors other than inferred changes in metabolism were involved in the greater incidence of side effects of diazepam in nonsmokers. These results suggest that further study of the effects of smoking and diazepam disposition is required.

Phenytoin

Phenytoin is subject to highly variable and dose-dependent elimination in patients, and its low therapeutic ratio requires careful patient monitoring for its use as an anticonvulsant. Rose, et al. (64) found that the only effect of tobacco smoke on disposition of phenytoin was an exacerbation of the inherent variability in its elimination, but the mean total clearance and $t_{1/2}$ values were similar in young, closely matched smokers and nonsmokers. No difference in the volume of distribution or the degree of plasma protein binding of phenytoin was observed between the two groups.

Warfarin

The Boston Collaborative Drug Surveillance Program found no difference in maintenance dosages of warfarin administered to hospitalized patients who were nonsmokers, light smokers, or heavy smokers (49). Similarly, Yacobi, et al. (91) have determined that nonsmokers, as well as smokers and patients taking barbiturates, have similar total clearance and plasma protein binding of warfarin. Recently Bachmann and Tarloff (4) have uncovered a species difference in the susceptibility of warfarin disposition to enzyme induction. They have found that pretreatment with benzo(a)pyrene decreased the duration of hypoprothrombinemia and shortened the $t_{1/2}$ of warfarin rate in rats.

Meperidine

Mather, et al. (47) have investigated the effects of cigarette smoking on meperidine disposition in surgical patients and volunteers. The mean total clearance value was determined to be 26.9 liters/hr/m² for smokers and 28.6 liter/hr/m² for nonsmokers.

Nortriptyline

Norman, et al. (51) dosed a group of 22 smokers and 31 nonsmokers with 150 mg/day of nortriptyline and determined steady-state plasma concentrations. Smokers achieved a mean plasma concentration of nortriptyline concentration of 191 ± 141 ng/ml, but nonsmokers had a level of 169 ± 92 ng/ml. This difference was not determined to be significant. Age, sex, and number of cigarettes smoked had no effect on the plasma nortriptyline concentrations achieved.

Ethanol

Smokers tend to consume more coffee, ethanol, and nonnarcotic analgesics than nonsmokers. Therefore the study by Vestal, et al. (79) on ethanol disposition and aging is of interest. The mean maximum biotransformation capacity (V_{max}) for five cigarette smokers was determined to be 75.9 mg/kg/hr while 45 nonsmokers averaged 74.8 mg/kg/hr (79). It should be noted that ethanol metabolism differs markedly from that of other drug metabolism in that it is primarily oxidized by the cytosolic hepatic enzyme, alcohol dehydrogenase. Further studies on the effects of alcohol metabolism and smoking are needed, because Kopun and Propping (38), in a study using 19 identical and 22 fraternal sets of male twins, showed that regular alcohol consumption and heavy smoking correlated with an increased alcohol elimination rate. The number of individuals used in this study was somewhat limited.

Other Drugs

The rate of phenol red excretion was not altered by smoking after administration of the dye by various routes (42).

Hagedorn and Kostenbauder (22) found that cigarette smoke had no effect on the metabolism of prostaglandin F-2 α in the isolated perfused rabbit lung, but administration of cigarette smoke was found to have a pronounced inhibitory effect on the metabolism of both nicotine and BP in this *in vitro* system (44, 48).

Uotila and Hartiala (75) have reported that the covalent binding of BP was greatly enhanced by 3-methylcholanthrene pretreatment. The amount of polar metabolites in the perfusion fluid of 3-MC treated lung was increased. They suggested that this may indicate induction of pulmonary BP metabolizing enzyme, but additional studies are needed.

Mechanism of Tobacco-Drug Interaction

Tobacco smoke is a complex mixture of noxious materials (66). (See the Chapter on the Constituents of Tobacco Smoke.) The particulate phase consists of water-soluble materials such as nicotine, other alkaloids, and a myriad of organic substances. It also contains fat soluble polycyclic aromatic hydrocarbons (PAHs, PNAs) and more complex organic compounds. At least 48 major components have been identified (70) in the PAH fraction. To date only a few of the components of tobacco smoke have been examined with respect to modifying drug disposition in man or animal or their effects on tissue or enzyme systems.

The incomplete combustion of organic materials in tobacco yields PAH. Akin, et al. (2) separated cigarette smoke into the PAH-enriched fraction which comprised 0.4 percent of the weight of the crude condensate, but accounted for virtually all the carcinogenic potential. It has been estimated that a 20-cigarette-per-day smoker of unfiltered cigarettes would inhale about 0.7 $\mu\text{g}/\text{day}$ of BP while filtered cigarettes would yield about 0.4 $\mu\text{g}/\text{day}$ of BP. It has been reported in a number of studies that BP induces the microsomal enzyme benzpyrene hydroxylase (14, 39, 86). The characteristics of this enzyme system have been reviewed in the metabolism section of this chapter.

Other Pathophysiological Factors of Smoking

Tobacco smoking is associated with a number of pathophysiological changes which may not be directly related to any specific drug interaction, but do offer the potential for contributing to altered drug disposition. Smoking and nicotine have been shown to increase corticosteroid secretion (36). It is also known that chronic administration of steroids will accelerate drug disposition. Nicotine treatment has been shown to cause catecholamine release; this can result in mobilization of free fatty acids from adipose tissue (34). The release of free fatty acids could displace drugs from protein binding sites. Dales, et al. (17) examined serum chemistry levels in over 65,000 cigarette smokers and nonsmokers and found slightly lower serum albumin, uric acid, and creatinine concentration in smokers who were over 30 years old. This lower serum albumin may relate either to altered hepatic function or to changes in drug binding. In a similar study, Lellouch, et al. (40) reported that smokers had lower serum urea and uric acid concentration than nonsmokers. The lower values for creatinine, urea, and uric acid may reflect altered renal or hepatic function in smokers. BP is strongly bound to serum albumin (45) and is therefore capable of displacing ligands from similar protein binding sites.

There may be other physiological, biochemical, and behavioral differences in the smoker group. Smokers are a "self-selected" group which means that the unknown factors that cause individuals to smoke may be of importance in drug disposition. Studies have examined the

differences between smokers and nonsmokers. Seltzer, et al. (67) have reviewed several studies; the consensus was that smokers tend to be more energetic, restless, and extroverted than nonsmokers. On the other hand, smokers tend to possess more neurotic traits including greater psychological tension and more psychosomatic symptoms. In addition, smokers tend to be hospitalized more often than nonsmokers and are, as expected, beset with a higher incidence of specific disease such as hypertension, coronary artery disease, and lung problems. The self-selection biases are difficult to remove from pharmacokinetic studies of the effects of smoking.

In the future, it would be helpful if, after cessation of smoking, careful studies of the reversibility of the smoking effect were conducted. Present studies indicate that the induction of BP hydroxylase is not completely reversed following 2- to 3-month cessation of smoking (24).

Smoking and Drug Consumption

The relationship of smoking and drug disposition is complicated by the typical pattern that cigarette smokers tend to consume other drugs and chemicals more frequently than nonsmokers. Furthermore, smokers tend to ingest more coffee and alcohol than nonsmokers. Ferguson (19) found that smokers consumed more alcohol and non-narcotic analgesics. Weitman, et al. (81) and Seltzer, et al. (69) examined the incidence of various types of drugs used in relation to tobacco smoking. In these studies, it was determined that smoking correlated highly with the use of other drugs. Smokers admitted to taking more cough medicine, aspirin-containing drugs, pain medications, prescription analgesics, barbiturates, sleeping pills, tranquilizers, diuretics, hormones, anemia medicine (iron), amphetamines, antibiotics, stomach medicines, and laxatives than nonsmokers. The only drugs taken by a larger percentage of nonsmokers were those for allergic conditions—antihistamines and asthma medicine. Great care must be used in carrying out pharmacokinetic studies of the effects of smoking. Because most studies do not or cannot control for many of the secondary differences between smoker and nonsmokers, care must be used in the interpretation of the results so that the reported associations between smoking and pharmacological action of drugs are not related to psychosomatic differences, drug ingestion patterns, and therapeutic need (threshold dose) of the two groups.

Studies of the effect of smoking on drug disposition usually attempt to quantitate smoke intake by vague descriptive categories such as nonsmokers/smokers, nonsmokers/light smokers/heavy smokers, or number of cigarettes smoked per day. These measures approximate only the potential exposure of man to the various chemicals in tobacco smoke. Factors such as cigarette brand, filters, degree of inhalation, duration of habit, respiratory rate, pharmacokinetics of the chemical in

man, and so forth, are unknowns in a study of this type. All of these factors sometimes make an investigation of the interaction of tobacco smoking and drugs extremely difficult to assess.

In the future, scientific reports describing the pharmacokinetics or clinical pharmacology of a drug should list and examine the smoking status of the subjects employed in the study. Smoking should be included as a basic characteristic of each subject in the same way as age, race, body weight, and presence and type of disease. Monitoring subjects for intensity of tobacco use might be accomplished by determining of serum or urine thiocyanate (26). This substance possesses a long $t_{1/2}$ (about one week), which allows for an assessment of chronic smoking at a consumption rate which is most likely to affect drug disposition. Thiocyanate is relatively easy to assay and serum concentration has been reported to be proportional to the number of cigarettes smoked (24).

Marijuana

The subject of tobacco smoking and drug interaction needs to consider the interaction of drugs and marijuana smoking. It has been estimated that 13 million people in the United States now smoke marijuana (1).

Animal systems show mixed effects, with marijuana studies reporting induction and inhibition of the microsomal drug-metabolized enzymes. Paton and Pertwee (57) reported that cannabis extract prolonged pentobarbital sleeping time in mice and inhibited the aerobic metabolism of phenazone in mouse liver microsomes preparation. Mitra, et al. (50) found that chronic treatment with Δ^9 -tetrahydrocannabinol (THC) for 21 days (10 mg/kg/day) competitively inhibited N- and O-demethylase activity, but had no inhibitory effect on aniline hydroxylase activities. Siemens, et al. (71) found a prolonged pentobarbital sleeping time and a longer $t_{1/2}$ in rats pretreated with various cannabinoid compositions as well as pure Δ^9 -THC.

Sofia and Barry (72) noted both enzyme inhibition and induction in mice following treatment with Δ^9 -THC. Pretreatment with a single high dose of Δ^9 -THC (20 mg/kg) increased the duration of the loss of the righting reflex after a dose of zoxazolamine and hexobarbital, and enhanced the duration of barbiturate sleeping time. Berman and Bochantin (8) also found that chronic doses of Δ^9 -THC (2.5 or 5.0 mg/kg daily for 4 days) increased liver microsomal dichlorinase activity (enzymes that metabolize methoxyflurane and halothane) in rats. Marcotte, et al. (46) have determined that analysis of the smoke condensate from cigarettes and from marijuana placed in a smoking machine gave 0.32 and 0.44 ng of BP/mg of PAH condensate, and 0.42 and 0.67 ng of 3-MC/mg of PAH condensate, respectively. These investigators found that exposure to the smoke of either marijuana or marijuana placebo (with the cannabinoid removed) maximally stimulated benzpyrene hydroxylase activity in rat lung tissue.