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EFFECTS OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE PRETREATMENT ON THE PLASMA HALF-LIFE AND URINARY EXCRETION PROFILE OF THEOPHYLLINE AND ITS METABOLITES IN RATS*

JOSEPH F. WILLIAMS, SAUL LOWITT and ANDOR SZENTIVANYI

Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, FL 33612, U.S.A.

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Abstract—The effects of the inducers of the hepatic microsomal enzyme system, phenobarbital and 3-methylcholanthrene, on theophylline plasma half-life and on the elimination of theophylline and its metabolites in urine and feces have been examined. The results indicate that induction of the hepatic microsomal drug-metabolizing enzyme system significantly decreases plasma theophylline half-life. In this respect, 3-methylcholanthrene was more effective than phenobarbital. Control theophylline half-life was 3.5 hr. After phenobarbital or 3-methylcholanthrene pretreatment, the theophylline half-life was 2.6 and 0.8 hr respectively. Thin-layer chromatographic analysis of the urine showed three radioactive peaks corresponding to 1,3-dimethyluric acid, 1-methyluric acid and unchanged theophylline. Both inducing agents significantly increased the urinary elimination of 1,3-dimethyluric acid above that seen in control animals throughout the 24-hr collection period, but only 3-methylcholanthrene increased the total amounts of 1-methyluric acid excreted. Urinary elimination of unchanged theophylline was decreased from control values by both agents. A small, but not statistically significant, increase in the fecal elimination of radioactive material was also noted in the animals pretreated with phenobarbital. The results indicate that alteration in hepatic drug-metabolizing activity may markedly affect the *in vivo* biotransformation of theophylline.

Theophylline (1,3-dimethylxanthine) is extensively metabolized in man and animals to monomethylxanthine and mono- and dimethyluric acid derivatives [1–3]. Recently, Lohmann and Miech [4] showed that the hepatic mixed-function oxidase system is involved in the *N*-demethylation and oxidation of theophylline. These investigators reported that hepatic slices from rats pretreated with phenobarbital or 3-methylcholanthrene, inducers of hepatic drug-metabolizing activity, had a significantly increased rate of theophylline metabolism.

Clinical pharmacokinetic studies on the use of theophylline in the management of obstructive lung disease have established a narrow range of 10–20 μg/ml as the plasma theophylline concentration for optimal therapeutic response. However, maintenance of these levels requires individualization of dosage due to the wide variations in plasma theophylline half-life values that exist among patients [5, 6]. These variations in theophylline half-life have been ascribed to differences in the rates of theophylline metabolism [5, 7, 8]. Plasma half-life values might reflect differences in rates of metabolism due to genetic factors or, as shown in rat hepatic slices [4], due to exposure to inducers of the hepatic drug-metabolizing enzyme system. The present study was conducted to examine the effect of pretreatment of rats with phenobarbital or 3-methylcholanthrene on the *in vivo* disposition of theophylline. The results indicate that inducers of the hepatic drug-metabolizing enzyme system significantly alter theophylline plasma half-life values. In addition, the data obtained from examination of urinary metabolite levels suggest that theophylline metabolism may be differentially affected by phenobarbital and 3-methylcholanthrene.

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METHODS

Male Sprague–Dawley rats (200–300 g) were used throughout the study. Rats received four daily intraperitoneal injections of either 80 mg/kg of sodium phenobarbital dissolved in 0.9% NaCl or 20 mg/kg of 3-methylcholanthrene dissolved in corn oil. Control animals received equivalent volumes of 0·9% NaCl or corn oil. During pretreatment, the animals were housed in metal cages and provided free access to food and water. Following the last pretreatment injection, the rats were fasted for 24 hr prior to theophylline administration.

Theophylline half-life was calculated from the rate of plasma theophylline disappearance in animals receiving intravenous injections (tail vein) of 38 mg/kg of theophylline. Animals were anesthetized with ether at various times after theophylline administration and blood samples were obtained by cardiac puncture with heparinized syringes. Plasma theophylline concentration was determined by the procedure of Thithapandha *et al.* [9] which measures only theophylline and none of its metabolites. Aliquots (0.5 to 2.0 ml) of plasma were adjusted to a total volume of 2.0 ml with 0.067 M phosphate buffer, pH 7.4. Diluted plasma samples were shaken for 5 min with 20 ml chloroform, and 15 ml of the chloroform extract was washed with 2.0 ml of the phosphate buffer saturated with sodium chloride. Theo-

phylline was returned to the aqueous phase by shaking with 2.0 ml of 4 N HCl. The acid extract was washed with 1.0 ml of *n*-heptane to remove traces of chloroform. The ultraviolet absorption spectrum of the acid extract between 300 and 240 nm was recorded on a Cary model 15 spectrophotometer. Plasma samples obtained from animals that had not received theophylline were used for preparation of the reference blank and for quantitation of theophylline recovery. Theophylline had an absorption maximum at 263 nm in the acid extract. The theophylline plasma half-life ($T_{1/2}$) and the overall elimination rate constant (k_{el}) were calculated by a least squares method, using the Wang desk computer. Values were calculated from the data obtained in the independent experiments and from the pooled data. For statistical comparison, the pharmacokinetic measures obtained from the data from the separate experiments were tested using Student's *t*-test for independent samples, adopting $P < 0.05$ as the level for significant difference.

The urinary and fecal elimination of theophylline and its metabolites was determined in control, phenobarbital and 3-methylcholanthrene-pretreated rats injected intravenously with 38 mg/kg of theophylline containing 5 μCi [8-^{14}C]theophylline (sp. act., 38 mCi/m-mole). Animals were housed individually in plastic metabolic cages and provided free access to food and water during the 24 hr urinary and fecal collection periods. Urine samples were collected at 2, 4, 8, 12 and 24 hr post-theophylline injection. After each collection interval, the urine volumes were measured and filtered; 10-μl aliquots were added to 10 ml of Aquasol scintillation fluid and the radioactivity was counted in a Nuclear Chicago, Mark II liquid scintillation counter. In addition, aliquots of urine were also spotted on aluminium thin-layer chromatography sheets precoated with silica gel F-254 (Brinkman Instruments, Westbury, NY). Chromatograms were developed in two solvent systems: (a) chloroform–ethanol–concentrated ammonium hydroxide (44:44:12) and (b) *n*-butanol, saturated with 5.8% ammonium hydroxide. A strip (10 × 20 cm) of the chromatogram from the area of urine application was scraped at 0.5-cm intervals and the silica gel eluted with 1.0 ml ethanol–concentrated ammonium hydroxide (8:2). Aliquots (0.5 ml) of the eluate were counted for radioactivity. R_f values for the radioactive areas of the chromatogram were compared with R_f values for the areas of the chromatogram showing ultraviolet absorption, when visualized with an ultraviolet lamp, corresponding to theophylline and metabolite reference compounds. The ultraviolet absorption spectrum of the ethanol–ammonium hydroxide eluate was also compared to that of reference compounds dissolved in the eluting solution. The radioactivity of the 24 hr fecal material was determined according to the procedure of Mahin and Lofberg [10]. Dried fecal material was weighed and pulverized with a mortar and pestle. A 250 mg sample was placed in a glass scintillation vial and 0.2 ml of 60% perchloric acid and 0.4 ml of 30% hydrogen peroxide were added. The vials were capped tightly and shaken in a 60° water bath until the fecal samples dissolved. The vials were then cooled to room temperature and 10 ml of Biofluor scintillation fluid was added. Samples were counted after a 60 min equilibration period in a Beckman scintillation counter. The radioactivity present in the skin and in the rat carcass was determined by homogenization in 200 ml and 1 litre, respectively, of 0.1 N HCl, using a Waring blender. After filtration of the homogenate through gauze, an aliquot (0.5 ml of whole body homogenate or 0.1 ml of the skin homogenate) was solubilized with 2 ml Protosol for 1 hr at 60°. The samples were bleached with 0.5 ml of 30% H_2O_2 for 30 min at 50° and cooled; then 10 ml of Aquasol scintillation fluid was added. Counting efficiency for all samples was determined by sample channels ratio method.

Unlabeled and [8-^{14}C]theophylline were obtained from the Sigma Chemical Co. (St. Louis, MO) and Amersham-Searle (Arlington Heights, IL) respectively. 3-Methylcholanthrene was obtained from the Eastman Kodak Co. (Rochester, NY), 3-Methylxanthine was obtained from the Aldrich Chemical Co. (Milwaukee,

Table 1. Thin-layer chromatographic and ultraviolet absorption characteristics of theophylline and metabolites*

	Thin-layer chromatography				Ultraviolet absorption maximum (nm)	
	R_f in Solvent A		R_f in Solvent B			
Compound	Reference	Radioactive extract	Reference	Radioactive extract	Reference	Radioactive extract
Theophylline	0.72	0.72	0.83	0.82	274	274
1,3-Dimethyluric acid	0.38	0.40	0.42	0.40	297	297
1-Methyluric acid	0.14	0.11	0.22	0.15	295	295
1-Methylxanthine	0.25		0.30			
3-Methylxanthine	0.31		0.37			

* Reference samples and aliquots (10 μl) of urine from animals that had received [8-^{14}C]theophylline intravenously were spotted on silica gel F-254 TLC plates and developed in Solvent A (chloroform–ethanol–concentrated NH_4OH, 44:44:12) and Solvent B (*n*-butanol, saturated with 5.8% NH_4OH).

After the plates dried, areas of u.v. absorption for the reference compounds were visualized with a u.v. lamp and R_f values determined. A 10 × 20 cm strip of the chromatogram from the area of urine application was scraped at 0.5-cm intervals, eluted with 1.0 ml ethanol–concentrated NH_4OH (8:2) and aliquots of the extract were used for radioactivity determination. The u.v. absorption area corresponding to each of the reference compounds was eluted with the ethanol–concentrated NH_4OH solution and aliquots were used for determining the ultraviolet absorption spectrum. R_f values and ultraviolet absorption maxima were determined for the segments of the chromatogram corresponding to areas of peak radioactivity.

WI), whereas 1,3-dimethyluric acid, 1-methyluric acid and 1-methylxanthine were obtained from the Adams Chemical Co. (Round Lake, IL). Protosol, Aquasol and Biofluor were purchased from New England Nuclear (Boston, MA).

RESULTS

The plasma drug concentrations after intravenous injections of theophylline to saline control and phenobarbital-pretreated animals or corn-oil control and 3-methylcholanthrene-treated animals declined exponentially (Fig. 1a and 1b). The theophylline plasma $T_{1/2}$ for the pooled data shown in Fig. 1 was calculated assuming a one-compartment model. The plasma theophylline $T_{1/2}$ for the control groups was 3.5 hr. Pretreatment of the rats with phenobarbital or 3-methylcholanthrene decreased the $T_{1/2}$ values to 2.6 and 0.81 hr respectively. When the data obtained for the separate experiments were analyzed independently and statistically compared, the $T_{1/2}$ values for both groups of induced animals were found to be significantly different from the corresponding control values. Similarly, the estimation of the overall elimination rate constant (k_{el}) was also significantly altered by pretreatment with either phenobarbital or 3-methylcholanthrene. For the saline and corn-oil control groups, the $T_{1/2}$ values (mean ± S.E.) were 3.88 ± 0.41 and 3.93 ± 0.48 hr and the k_{el} values were 0.194 ± 0.029 hr^{-1} and 0.190 ± 0.023 hr^{-1} respectively. Pretreatment of rats with phenobarbital or 3-methylcholanthrene decreased the $T_{1/2}$ values to 2.61 ± 0.14 ($P < 0.05$) and 0·81 ± 0.11 hr ($P < 0.01$) and increased the k_{el} values to 0·264 ± 0.014 ($P < 0.05$) and 0.923 ± 0.102 hr^{-1} ($P < 0.05$) respectively. The apparent volumes of distribution of theophylline were not significantly different among the control (saline, 0.649 ± 0.032 l/kg) and the experimental groups (0.723 ± 0.044 l/kg and

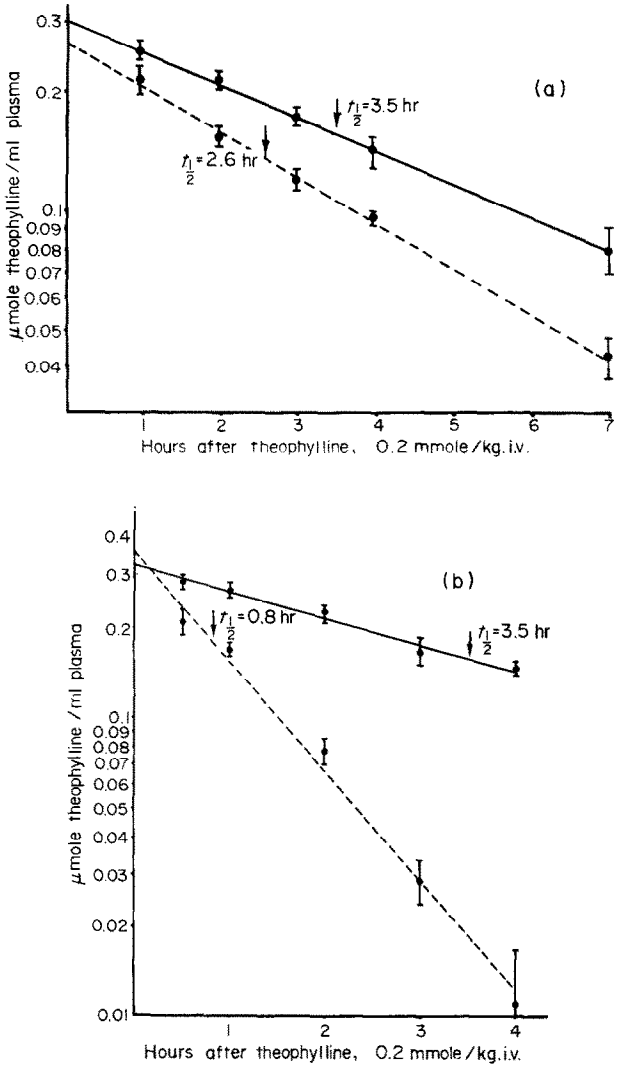

Fig. 1. Plasma disappearance of theophylline (38 mg/kg) administered intravenously to rats that had received (a) 0.9% saline (●——●) or 80 mg/kg of phenobarbital dissolved in 0.9% saline (●– –●) for 4 days; or (b) corn oil (●——●) or 20 mg/kg of 3-methylcholanthrene dissolved in corn oil (●– –●) for 4 days. Each value represents the mean ± S.E. of plasma theophylline concentrations for five animals.

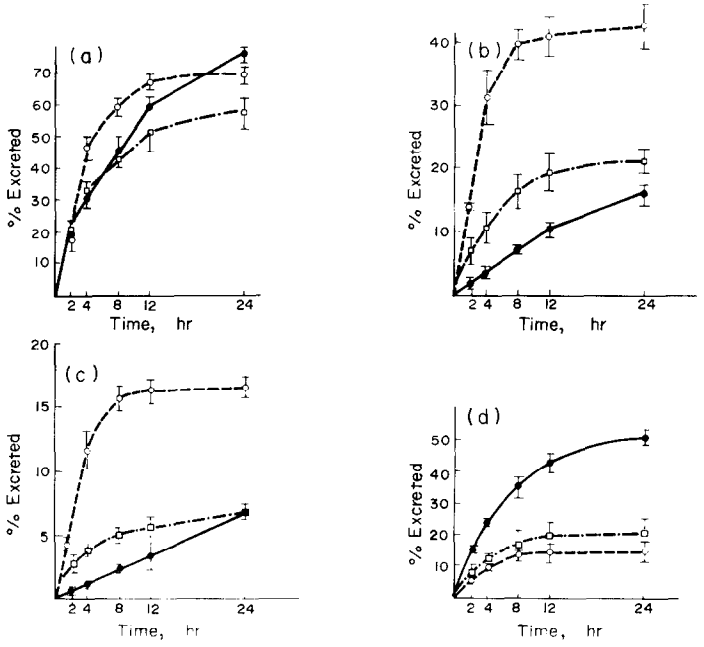

Fig. 2. The 24 hr cumulative urinary excretion of theophylline and its metabolites by control animals (●——●) and by animals pretreated with phenobarbital (□– –□) or 3-methylcholanthrene (○– –○). Each value is the mean ± S.E. for three animals and is expressed as the percentage of injected radioactivity excreted at the various time points for (a) total urine radioactivity and for radioactivity of areas of the thin-layer chromatogram with R_f values corresponding to (b) 1,3-dimethyluric acid, (c) 1-methyluric acid or (d) theophylline.

0.543 ± 0.114 l/kg for the phenobarbital and 3-methylcholanthrene groups respectively).

Thin-layer chromatographic analysis of urine collected from control and experimental animals during the 24 hr period after injection of [8-^{14}C]theophylline showed the presence of three radioactive peaks. Comparison of the R_f values and the ultraviolet absorption spectra for the areas of peak radioactivity with those for reference compounds showed that the radioactive compounds were theophylline, 1,3-dimethyluric acid and 1-methyluric acid (Table 1). The cumulative excretion of theophylline and its two metabolites during the 24-hr experimental period is shown in Fig. 2. Animals pretreated with 3-methylcholanthrene excreted significantly more radioactivity than control animals during the first 12 hr after intravenous theophylline administration (Fig. 2a). However, by the end of the 24 hr collection period, the control animals had a slightly greater total urinary elimination of radioactivity. Although no major difference in urinary elimination of radioactivity was observed between the control and phenobarbital animals during the early collection periods, the total 24 hr cumulative excretion of radioactive material was significantly diminished from control values by phenobarbital pretreatment. Thus, recovery of injected radioactivity in the urine of control, 3-methylcholanthrene or phenobarbital-treated animals was (mean ± S.E.) 73.84 ± 2.12, 67.17 ± 2.54 and 55.97 ± 4.02 per cent ($P < 0.01$) respectively.

The urinary excretion of 1,3-dimethyluric acid, 1-methyluric acid and theophylline by the control and the two experimental groups is depicted in Fig. 2b, c and d respectively. Compared to control animals, pretreatment with either phenobarbital or 3-methylcholanthrene increased the amount of 1,3-dimethyluric acid excreted in the urine (Fig. 2b) and decreased that of theophylline (Fig. 2d). Pretreatment with 3-methylcholanthrene also greatly increased the amount of 1-methyluric acid excreted in the urine (Fig. 2c). Expressed as a percentage (mean ± S.E.) of the injected radioactivity, control animals eliminated 48.79 + 1.47 per cent as unchanged theophylline. Pretreatment of the rats with phenobarbital or 3-methylcholanthrene reduced the amount of unchanged theophylline eliminated in the urine to 20.20 ± 2.68 ($P < 0.05$) or 16.79 ± 2.38 per cent ($P < 0.01$) respectively. The decrease in excretion of parent compound by the induced animals was accompanied by an increase in the amount of radiolabel associated with the methyluric acid metabolites of theophylline. Thus, whereas control animals eliminated 14.90 ± 0.94 per cent associated with 1,3-dimethyluric acid, the values for animals treated with phenobarbital or 3-methylcholanthrene were increased to 20.54 ± 1.72 ($P < 0.05$) or 37.14 ± 3.64 per cent ($P < 0.01$) respectively. Pretreatment with 3-methylcholanthrene increased the amount excreted as 1-methyluric acid from the control level of 6.61 ± 0.49 per cent to 15.27 ± 0.97 per cent ($P < 0.01$). Although phenobarbital pretreatment of the rats increased the elimination of radioactivity associated with the monomethyluric acid derivative during the early urine collection periods, the 24 hr accumulative value of 6.36 ± 0.42 per cent was not significantly different than the control value. Thus, compared to controls, animals induced with 3-methylcholanthrene excreted 3 and 2 times as much 1,3-dimethyluric acid and 1-methyluric acid respectively. While the phenobarbital-treated animals eliminated 1.5 times the con-

trol amount of 1,3-dimethyluric acid, the level of 1-methyluric acid excreted was unchanged.

The elimination of radioactivity in the 24 hr fecal material of the control and treated animals was also determined. The fecal material of control and 3-methylcholanthrene-treated rats contained 3.10 ± 1.2 and 4.5 ± 2.0 per cent of the injected radioactivity. The animals pretreated with phenobarbital eliminated somewhat more (8.3 ± 1.7 per cent) radioactivity in the feces than did either of the other groups of animals; however, the differences in the fecal elimination between these groups were not statistically significant.

In the preceding group of animals, a substantial amount of the administered radioactive label was not accounted for by collection of the urine and feces. It was considered most likely that the unaccounted label was still in the animals, possibly in the bladder or gastrointestinal tract. Therefore, a further series of experiments was conducted using two animals per group. The 24 hr accumulative urinary and fecal elimination of radioactivity was determined as before and, in addition, the radioactivity remaining in the skin and carcass of the animals was evaluated. In these studies, the amount of injected radioactivity recovered in the urine was slightly higher than previously observed for all three groups of animals. Thus, control and 3-methylcholanthrene-treated animals eliminated 87 and 83 per cent and the phenobarbital-treated animals excreted 74 per cent of the injected amount in the urine. Although these values are between 13 and 18 per cent higher than those determined in the previous group of animals, the phenobarbital-treated animals still excreted less in the urine than did either the control or 3-methylcholanthrene group. Fecal elimination of radioactivity by the control, 3-methylcholanthrene or phenobarbital-treated group was 5, 10.8 and 13 per cent respectively. The analysis of the whole body homogenate showed that 5.16, 1.82 and 9.56 per cent of the administered label was present in the control, 3-methylcholanthrene and phenobarbital-treated animals, respectively, at the end of the 24 hr period. The amounts associated with the skin of the control animals averaged 0.9 per cent, with that of the phenobarbital-treated animals, 4.3 per cent, and 1.28 per cent with the skin of the polycyclic hydrocarbon-treated animals. Thus, total recovery of radioactivity was between 95 and 98 per cent for the three groups.

DISCUSSION

The results of the present study of the plasma half-life and the urinary and fecal excretions of theophylline and its metabolites by control rats and animals pretreated with either phenobarbital or 3-methylcholanthrene indicate induction of theophylline metabolism, and are entirely compatible with the reported ability of these agents to induce theophylline metabolism measured *in vitro* in liver slices [4]. The plasma half-life of theophylline was decreased from 3.5 hr for control animals to 2.6 and 0.8 hr for animals that had received phenobarbital or 3-methylcholanthrene respectively.

Chromatographic analysis of the urine at various times after the administration of [8-^{14}C]theophylline revealed that the excreted radioactivity was associated with three compounds identified as 1,3-dimethyluric acid, 1-methyluric acid and unchanged theophylline. These are the same compounds that were reported to be the metabolites produced from theophylline by the hepatic slices in the study referred to above [4].

In terms of total radioactivity, differences in the urinary excretion were noted between the control and experimental groups. Thus, pooling the urinary elimination data from the two separate studies, control and 3-methylcholanthrene-treated animals excreted 77 and 72 per cent of the injected radioactivity, respectively, while the animals treated with phenobarbital eliminated only 63 per cent ($P < 0.05$) of the same. Theophylline and its metabolites have been shown to be secreted into the bile of dogs [11]. The data on the radioactivity recovered in the feces supports the interpretation that biliary secretion is an additional route for elimination of theophylline and/or its metabolites. The increased elimination of radioactivity in the feces of the animals treated with phenobarbital may reflect the previously reported ability of the barbiturate to increase biliary excretion of various compounds [12]. Theophylline itself also increases biliary flow but, since all three groups received theophylline, the augmented fecal elimination is probably not related to this effect of the methylxanthine. Although in the initial studies the 24 hr recovery of radiolabeled material in the urine and feces was low, subsequent studies indicated that the remaining radioactivity was associated with the skin and whole animal. This residual amount probably is present in either the bladder or gastrointestinal tract as unexcreted material.

Pretreatment with phenobarbital or 3-methylcholanthrene significantly increased the amount of theophylline metabolites in the urine, and concomitantly decreased the excretion of unchanged theophylline. In this regard, 3-methylcholanthrene pretreatment was the more effective, increasing the amounts of both 1,3-dimethyluric acid and 1-methyluric acid throughout the entire observation period; although to a lesser extent, phenobarbital also increased the excretion of both metabolites during the early collection periods. However, after 24 hr, the excretion of only 1,3-dimethyluric acid and not 1-methyluric acid, was found to be increased in the phenobarbital-pretreated animals. The reasons for these apparent differences in the effects of the two inducing agents on the urinary elimination of theophylline and its metabolites are not known, but at least two possible explanations can be presently offered. First, 3-methylcholanthrene and phenobarbital may differentially affect the induction of the hepatic microsomal enzymes involved in the conversion of theophylline to the monomethyl and dimethyluric derivatives. The formation of the monomethyluric acid apparently proceeds by two separate enzyme reactions: (1) the *N*-demethylation to 1-methylxanthine and (2) the subsequent conversion of 1-methylxanthine to 1-methyluric acid. Of these two steps, the latter has been shown to be related to the activity of xanthine oxidase [4]. The *N*-demethylase is a microsomal enzyme. Theophylline is not a substrate for xanthine oxidase, and the formation of 1,3-dimethyluric acid has also been shown to be due to a microsomal enzyme [4]. Thus, 3-methylcholanthrene may induce the enzyme responsible for the *N*-demethylation to 1-methylxanthine as well as that catalyzing the oxidation of theophylline to the dimethyluric acid derivative. On the other hand, phenobarbital may induce only the enzyme activity yielding 1,3-dimethyl-

uric acid and not the *N*-demethylase. A second alternative explanation is that in the phenobarbital-treated animals more of the 1-methyluric acid formed may be eliminated by biliary excretion. If this were the case, then the apparent urinary difference between the polycyclic hydrocarbon induction and barbiturate induction of theophylline metabolism would simply be a matter of the degree of effectiveness and the route of elimination utilized. The biliary excretion of theophylline and/or its metabolites also raises a question relative to the possibility of an effect of the entero-hepatic recirculation of drugs on the pharmacokinetic measures. Because of decreased hydrophobicity, the metabolites would probably not be reabsorbed, but secreted theophylline could be taken back into the circulation. Such secretion and reuptake of theophylline may markedly affect the biological half-time. Thus, extensive entero-hepatic recirculation of theophylline, as is likely in the phenobarbital-treated animals, may result in an underestimation of the effect of enzyme induction on the rate of metabolism, as judged by plasma decay of drug. However, the changes in urinary metabolite levels correlate relatively well with the observed effect of the inducing agent on the plasma theophylline half-life, e.g. 3-methylcholanthrene produced a much more marked change than did phenobarbital.

Alterations of theophylline metabolism by inducing agents of the hepatic drug-metabolizing enzyme system may be of great importance in the therapeutic use of this drug in bronchial asthma and chronic obstructive airway disease. The differences in the serum half-lives of theophylline in patients, already noted in the clinical literature [5, 6], may at least partially be attributable to variations in patient exposure to inducing agents, either pharmacological or environmental in nature. Indeed, recent reports of Jenne *et al.* [13], Hunt *et al.* [14] and Powell *et al.* [15] on a decreased theophylline half-life in cigarette smokers versus non-smokers may be related to the effects of components of cigarette smoke which induce the hepatic microsomal enzyme activity in a manner similar to that seen with 3-methylcholanthrene. Comparable clinical studies on the effect of phenobarbital on theophylline disposition have been contradictory. Thus, Piafsky *et al.* [16] reported that phenobarbital administration did not alter theophylline elimination, whereas opposite results were obtained by Landay *et al.* [17]. Changes in theophylline disposition, due either to induction or inhibition [18–20] of the hepatic enzyme system occurring during therapy, may necessitate an adjustment of theophylline dosage to achieve and maintain the desired therapeutic effect. These considerations emphasize the need for further investigation of the role of the hepatic microsomal enzyme system in the metabolism of theophylline.

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