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Effects of oral contraceptives and tobacco use on the metabolic pathways of theophylline

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Summary

The effects of chronic oral contraceptive (OC) use and cigarette smoking on the metabolism of theophylline (THEO) were investigated in 22 healthy female volunteers. Blood and urine samples were collected over 23 h following oral aminophylline dosing. Urine was assayed by HPLC for both unchanged theophylline and its primary metabolites (3-methylxanthine (3-MX), 1-methyluric acid (1-MUA) and 1,3-dimethyluric acid (1,3-DMUA)). The metabolite formation clearances for the subject groups appeared linear except for a Michaelis–Menten pattern in the non-OC users who smoked. Smoking increased metabolite formation clearances for 3-MX (by 55%), 1-MUA (by 49%) and 1,3-DMUA (by 26%). Chronic OC exposure resulted in reductions for 3-MX (by 42%), 1-MUA (by 24%) and 1,3-DMUA (by 19%). Renal clearances of unchanged theophylline were urine flow-dependent and affected secondarily by OC and tobacco use. While 3-MX formation is most sensitive to change, the differential effects from enzyme induction by tobacco and inhibition by OC suggests that each pathway has differing sources of enzymatic regulation.

Introduction

Tobacco and contraceptive steroid use both exert significant effects on drug metabolism (Back et al., 1981; Jusko, 1978). Although their inductive/inhibitory effects have been assessed with respect to theophylline (1,3-dimethylxanthine) plasma clearance (Gardner et al., 1983), their effects on discrete metabolic pathways have not been fully evaluated. Theophylline is extensively metabolized via the hepatic mixed function oxidase system (Cornish and Christman, 1957) to 1,3-dimethyluric acid (1,3-DMUA; 40%), 3-methylxanthine (3-MX; 15%) and 1-methyluric acid (1-MUA; 20%). Two-fold increases in 3-MX and 1-MUA production are evident in smokers, whereas, a 1.6-fold increase in 1,3-DMUA formation was reported in the same subjects (Grygiel and Birkett, 1981). These investigators assumed that the disposition of theophylline was linear, however, others have reported opposite findings (Lesko, 1979; Tang-Liu et al., 1982; Weinberger and Ginchansky, 1977; Monks et al., 1979). Consequently, a more intensive investigation of tobacco's effect(s) on the metabolic formation/excretion patterns is warranted. Similarly, the effects of chronic oral contraceptive (OC) use on these 3 primary pathways have not been investigated.

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We have examined the effects of cigarette smoking and chronic OC exposure on the specific biotransformation pathways involved in theophylline elimination.

Materials and Methods

Subjects

Twenty-two young (19–30 years old) adult females participated in this study. They were part of a larger group of subjects (n = 49) recently examined (Gardner et al., 1983). Of these, 11 did not use OC, whereas 11 used them on a chronic basis (i.e., longer than 6 months). The commercial preparations routinely used varied, but in all cases Ovral (Wyeth Laboratories 0.05 mg ethinyestradiol/0.50 mg norgestrel) was used during the monthly cycle immediately preceding participation. Nine subjects were cigarette smokers. Their average tobacco habit exceeded 20 cigarettes per day, for approximately 9 years. All subjects had normal hepatic/renal function, none were considered to be obese (i.e., total body weight did not exceed estimated ideal body weight (Diem and Lentner, 1970) by more than 15%) and none used excessive amounts of caffeine or alcohol. The non-smokers did not use marihuana, whereas the majority of cigarette smokers used it in moderation (less than one joint per week). A summary of

TABLE 1

CHARACTERISTICS OF SUBJECTS

Characteristics	Non smokers		Smokers	
Group:	I Non-users	II OC users	III Non-users	IV OC users
Number of Subjects	5	8	6	3
Age range, yr.	22–25	19–28	23–30	26–29
Mean age, yr.	22.8	24.3	27.0	27.3
	(1.3)	(3.0)	(2.8)	(1.5)
Total body weight, kg	55.3	61.1	56.6	58.6
	(5.4)	(8.1)	(7.2)	(8.3)
Duration of OC use, yr.	0	4.5	0	6.8
		(2.5)		(2.8)
Tobacco use (pk.day)	0	0	1.3	1.1
			(0.4)	(0.1)
Duration of tobacco use, yr.	0	0	10.2	7.7
			(2.6)	(5.7)
Marihuana use [a]	0	0	0.33	1.0
Alcohol use [a]	1.0	1.0	1.0	0.7
Caffeine use [b]	2.3	1.6	4.0	1.0
	(2.3)	(0.8)	(2.5)	(0.0)
Bilirubin (0.1–1.1 mg%) [c]	0.72	0.33	0.52	0.43
	(0.13)	(0.10)	(0.19)	(0.32)
SGPT (0–43 units/l) [c]	19.4	19.5	13.5	16.0
	(6.8)	(4.4)	(3.7)	(13.7)
SGOT (11–46 units/l) [c]	18.4	16.5	18.5	15.0
	(3.4)	(4.3)	(2.1)	(7.0)
Alkaline phosphatase (34–113 units/l) [c]	55.4	51.6	68.7	54.3
	(8.3)	(14.1)	(17.2)	(14.3)
Serum thiocyanate (mg/l)	2.1	2.1	6.0	8.0
	(1.4)	(1.0)	(1.8)	(2.8)

Standard deviation in parentheses.

[a] Coded as 0 = none, 1 = social, 2 = daily use.

[b] Number of cups of coffee or tea per day.

[c] Normal range in parentheses.

specific demographic/laboratory data are presented in Table 1. The total group was partitioned into 4 disjoint subgroups based upon tobacco and OC use. A two-way analysis of variance (OC–cigarette use) detected significantly higher serum thiocyanate concentration in smokers (non-smokers 2.1 mg/l vs smokers 6.6 mg/l, $P < 0.001$).

Posology and assays

Each subject was fasted overnight and requested to abstain from xanthine-containing foods/beverages for the 24 h period prior to study as well as throughout the study day. An indwelling catheter was inserted into a forearm/hand vein. The patency of this system was maintained using a dilute solution of heparin in normal saline (20 U/ml). Subjects then ingested a single tablet of Ovral followed by 200 ml of distilled water. This protocol was followed for all subjects irrespective of their status as an OC user/non-user. One hour later an oral solution (4 mg/kg) of aminophylline (theophylline-ethylenediamine) was administered with 200 ml of orange juice. Serially collected serum was analyzed for theophylline content as reported previously (Gardner et al., 1983). Quantitative urine samples were collected at 2 h intervals for the first 8 h and during the 8–11 and 11–23 h periods. Total urine volumes were measured and a 10 ml aliquot frozen at −20°C pending analysis (Muir et al., 1980). Serum thiocyanate (SCN) concentrations were determined spectrophotometrically (Pettigrew and Fell, 1972). The samples analyzed were 50:50 mixtures of serum collected prior to Ovral administration and serum collected 30 min later.

Pharmacokinetics

Estimates of plasma clearance were generated using the relationship: CL = Dose × F/AUC. The bioavailabilty factor, F, was assumed to be unity since the absorption of orally administered theophylline is nearly complete (Hendeles et al., 1977). The finite area under the serum concentration vs time profile was estimated using second- and third-order interpolating polynomials (Rocci and Jusko, 1983). Total area (AUC) was generated as the sum of this finite area and the terminal area extrapolated to infinite time. Estimates of the apparent first-order absorption rate constant were generated using a non-linear least squares regression program (Metzler et al., 1974).

The excretion rate of each metabolite in urine ([metabolite concentration × urine volume]/length of the collection interval) served as a reasonable approximation of its rate of formation. This approach is predicated on the assumptions that each metabolite was very rapidly and exclusively excreted in the urine, that each metabolite was not subject to further biotransformation, and that its sole source was the administered theophylline. This approach has been commonly pursued by others (Levy et al., 1972; Tang-Liu et al., 1982). Due to the paucity of data for each individual subject, excretion rate data obtained from members of the same group were merged. The urinary metabolite excretion rate/midpoint theophylline serum concentration data were fitted to both a linear stochastic model with zero intercept as well as to a non-linear function characteristic of a Michaelis–Menten type saturable process. Cubic polynomials to which theophylline serum concentration vs time data had been fitted were used to estimate mid-interval concentration. Data were weighted by the reciprocal of the square root of the theophylline serum concentration. Model selection was based upon statistical analyses of the weighted data. The Akaike information criterion (AIC) numerically expresses the amount of information contained in a set of data, and statistically determines the optimal number of parameters in a model equation. The model generating the minimum AIC was selected. For the data obtained from Groups I, II and IV, estimates of the metabolite formation clearance (Fm × Cl) were generated. In this expression, Fm represents the fraction of the adjusted dose recovered in the urine as the metabolite and CL is the plasma clearance of theophylline. Mean serum concentration vs time profiles were reconstructed for each group using a fourth-order Runge-Kutta numerical technique. Theophylline renal clearance was described as a linear function of time and quantified using bivariate linear regression techniques (weight = square root of time).

The fractional conversion to each metabolite

was calculated as:

Fraction converted

$$= \frac{M_{u(23)}}{\text{Adjusted dose}} = \frac{M_{u(23)}}{D - (V_d \times C_{p(23)})} \quad (1)$$

where $M_{u(23)}$ is the total amount (μmol) of metabolite recovered in the urine during the 23 h interval following dosing, D is the dose (μmol), V_d is the apparent volume of distribution (ml) and $C_{p(23)}$ is the serum concentration of theophylline at 23 h (μmol/ml). V_d was determined by dividing the dose by the product of AUC and the slope of the terminal disposition phase of a log–linear plot of theophylline serum concentration vs time. The fraction excreted as unchanged theophylline was calculated in the same manner. The fraction of the dose accounted for was defined as:

$$\text{Fraction Acct} = \text{3-MX(23)} + \text{1-MUA(23)} + \text{1,3-DMUA(23)} + \text{THEO(23)} / D - (V_d \times C_{p(23)}) \quad (2)$$

Statistics

All assessments of OC–smoking effects were conducted employing two-way analyses of variance. Each main effect was corrected for the influence of the other. The disparate uses of caffeine and marihuana among the 4 groups were not of concern. Although these factors have been shown to alter the plasma clearance of theophylline (Jusko et al., 1978); Monks et al., 1979), the effects exerted on the clearance values for this particular panel of subjects were insignificant. Any effects on the individual biotransformation pathways were assumed to be insignificant as well.

Results

Fig. 1 presents the theophylline plasma clearance data for the 22 subjects in relation to the larger group from which they were extracted. Distributional information for the larger, previously examined, groups (mean and standard deviation) is illustrated. With respect to plasma clearance, the 22 study subjects are representative of their larger groups.

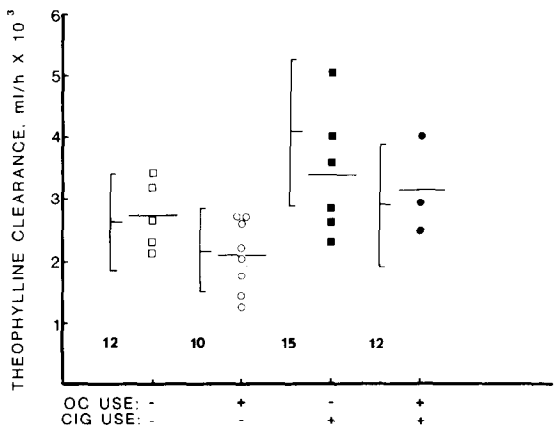

Fig. 1. Theophylline plasma clearance in the 4 subgroups. The symbols represent clearance values obtained from subjects examined in this report with horizontal bars depicting mean values. The mean, S.D. and numbers of subjects for each of the larger groups examined previously (Gardner et al., 1983) are presented to the left and below.

Typical urinary excretion rate profiles for unchanged theophylline and metabolites are depicted in the upper portion of Fig. 2. The lower panel illustrates the time-dependent behavior of the parent compound in serum for the same subject. With the possible exception of 3-methylxanthine, the processes of formation and elimination of these major metabolites appear to be linear. A concentration–time effect was exhibited by some subjects with regard to the urinary excretion of unchanged theophylline. In these cases, the excretion rate of unchanged compound was disproportionately larger at early time points when the concentrations of theophylline in serum were highest. Such behavior is not apparent in subject D.F., however. In general, the decline in the excretion rate of metabolite(s) and theophylline roughly paralleled the decline of parent drug in serum.

Figs. 3–5 depict the relationships between the urinary excretion rates of the primary metabolites of theophylline and the concentration of theophylline in the serum. The combined data for each of the 4 groups are represented by different symbols

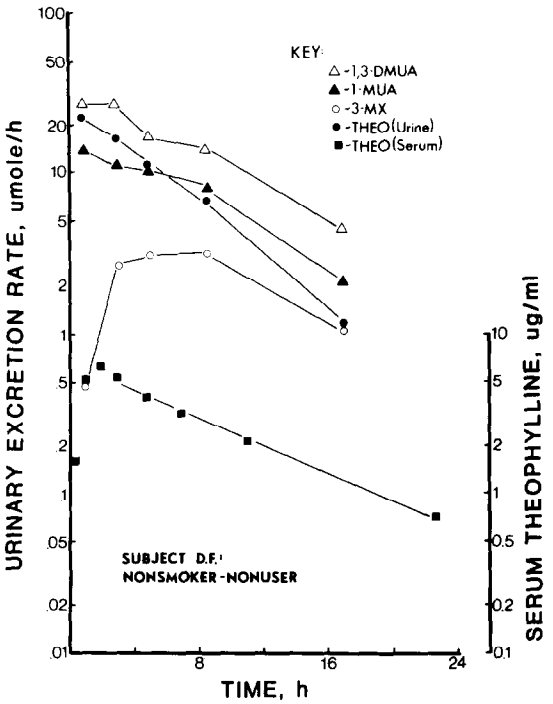

Fig. 2. Typical urinary excretion rates/serum concentration vs time profiles for theophylline and its major metabolites obtained from a single subject in Group I. The upper portion (left axis) presents the urinary excretion rate data while the lower portion (right axis) illustrates theophylline serum concentrations. The key denotes meanings of the symbols used.

as defined in Fig. 1. In all instances, except those involving non-OC users who smoked (Group III), Akaike's information criterion (1976) suggested that the data were more appropriately described by a linear model with zero intercept rather than the non-linear one characteristic of Michaelis–Menten elimination. The non-linear model was deemed superior for all fittings involving data from Group III. Numerical estimates of the fitted parameters for each of the groups are presented in Table 2. For groups I, II, and IV the estimates of formation clearances generated assuming linear disposition were in good agreement with those determined using excretion rate/theophylline serum concentration data ($r = 0.995$; slope = 1.06; intercept = 35.1).

The relationships between the urinary excretion rate of unchanged theophylline and serum theophylline concentration are illustrated in Fig. 6. In

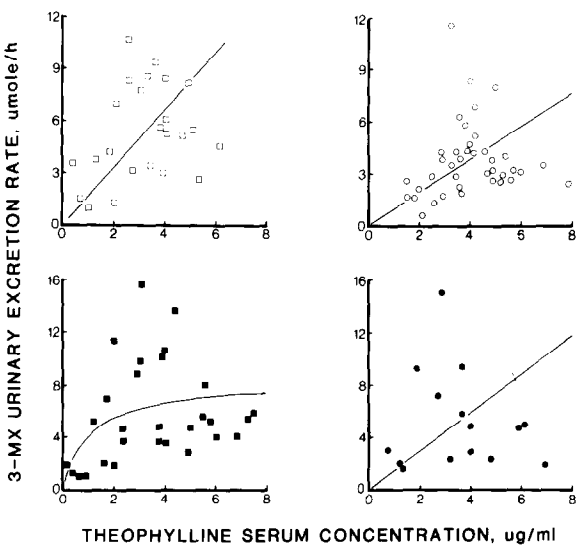

Fig. 3. Relationships between the urinary excretion rate of 3-methylxanthine and serum theophylline concentration in the 4 groups examined. Each figure presents the data obtained from all subjects belonging to that group. The symbols are defined as in Fig. 2. Graphs of the optimal model/least squares fitted functions have been superimposed upon each data set.

each group a non-linear pattern is evident. No model equations were proposed to describe this behavior.

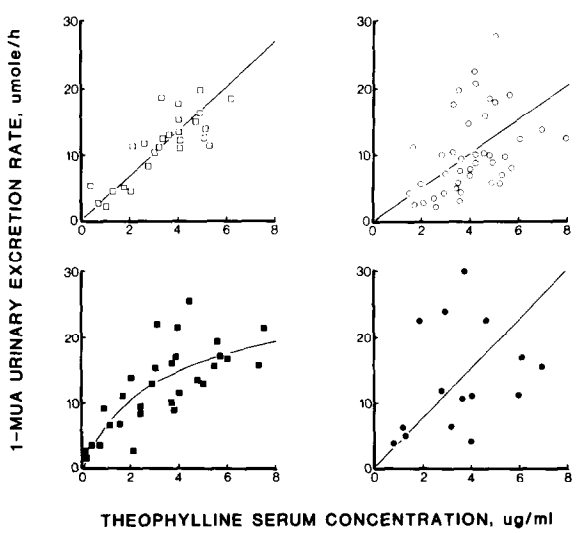

Fig. 4. Relationship between the urinary excretion rate of 1-methyluric acid and serum theophylline concentration in the 4 groups examined. Symbols and panels are defined as in Figs. 2 and 3.

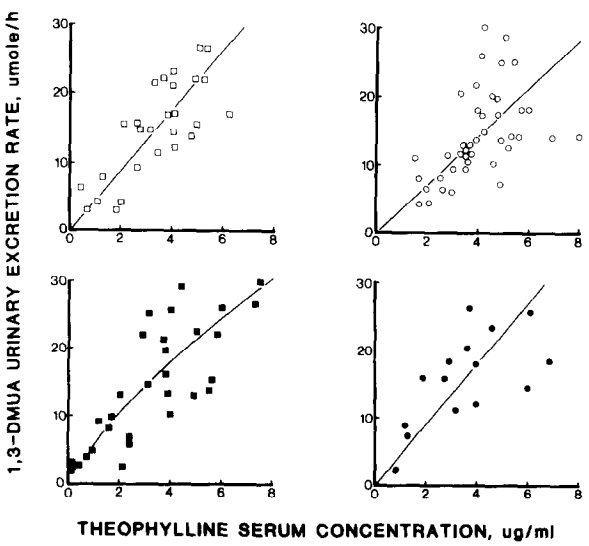

Fig. 5. Relationship between the urinary excretion rate of 1,3-dimethyluric acid and serum theophylline concentration in the 4 groups examined. Symbols and panels are defined as in Figs. 2 and 3.

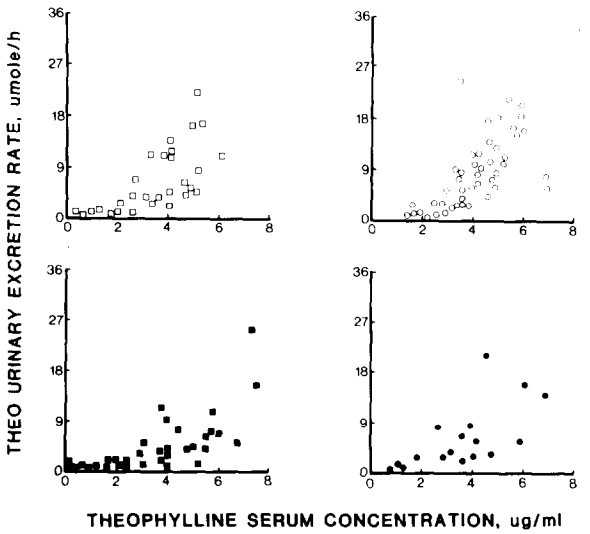

Fig. 6. Relationship between the urinary excretion rate of unchanged theophylline and theophylline concentration in serum for each of the 4 groups. The data from all members of the group are presented on the same graph. The symbols have been defined in Fig. 2.

Simulated mean serum concentrations generally exceeded the experimental mean values (Fig. 7). For each group, the least squares estimates of the formation clearances/renal clearance were incorporated into the differential equation describing the dynamics of the system as a whole. The non-linear behavior of theophylline renal clearance was adequately characterized as a linear function of time. Weighting of the data by the square root of time resulted in fittings with correlation coefficients of 0.84, 0.92, 0.86 and 0.86 for the 4 groups.

No differences in the rate of absorption of theophylline existed among the 4 groups (OC use, $P > 0.6$; smoking, $P > 0.6$).

TABLE 2

APPARENT FORMATION CLEARANCES FOR PRIMARY METABOLITES OF THEOPHYLLINE IN SUBGROUPS PARTITIONED BY ORAL CONTRACEPTIVE AND TOBACCO USE

	Non-smoker		Smoker	
	Non-user	user	Non-user	User
3-MX	275.8 [a]	158.2	8.4 (1.1) [b]	245.4
1-MUA	614.9	466.2	27.4 (3.3)	693.0
1,3-DMUA	864.7	697.1	77.7 (13.1)	878.9

[a] Apparent linear formation clearance, ml/h.

[b] V_{max} (K_m) for non-linear formation pathway, μmol/h (μg/ml).

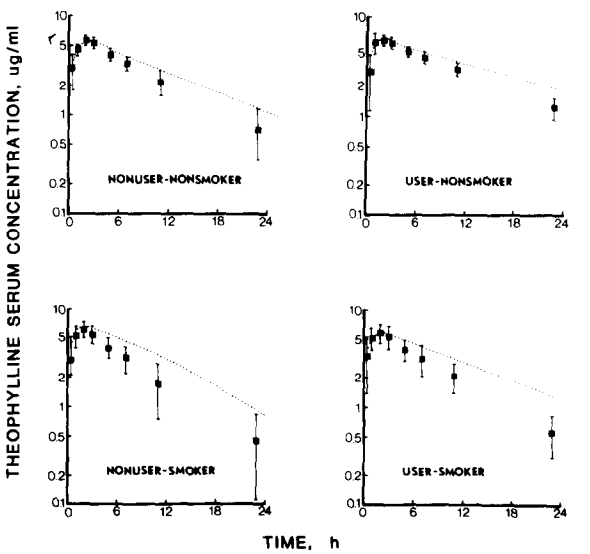

Fig. 7. Serum theophylline concentration vs time plots for each of the groups examined. The mean (S.D.) concentration of the group at each time point is presented. The dotted curves in each panel represent the simulated profiles, using the group estimates of metabolic formation/renal clearances.

TABLE 3

SUMMARY OF EXCRETORY/METABOLIC PROFILES FOR THEOPHYLLINE IN SUBGROUPS PARTITIONED BY ORAL CONTRACEPTIVE AND TOBACCO USE

	Non-smokers		Smokers	
	Non-users	OC users	Non-users	OC users
THEO	11.26 (5.60)	16.11 (6.19)	5.99 (2.29)	8.55 (5.38)
3-MX	10.26 (4.43)	7.91 (1.50)	10.39 (2.63)	9.55 (5.02)
1-MUA	22.45 (3.98)	20.32 (7.86)	21.71 (5.30)	23.82 (8.28)
1,3-DMUA	28.63 (7.30)	30.84 (9.08)	24.47 (6.51)	26.29 (5.43)
TOTAL	72.61 (14.89)	75.18 (18.48)	62.56 (11.18)	68.22 (13.08)

Values are percent of adjusted dose, mean (S.D.).

The fractional recoveries of each metabolite/theophylline are presented in Table 3. No significant alterations were detected. The reduction in the percent of 3-MX recovered in the urine of OC users did approach significance ($0.05 < P < 0.07$). The percent of the adjusted dose excreted unchanged was significantly altered by both the use of OC and tobacco. The average percent excreted unchanged in OC user and non-user groups were 13.2 and 9.25% ($P < 0.02$). Those for smokers and non-smokers averaged 7.5 and 13.8% ($P < 0.02$). The percent of the administered dose accounted for did not vary significantly among the 4 groups.

Discussion

The primary purpose of this investigation was to evaluate the effects of OC use and cigarette smoking on the discrete metabolic pathways responsible for the elimination of theophylline. Proper subject classification was insured by carefully monitoring the daily intake of contraceptive steroids and quantification of serum thiocyanate, the primary detoxification product of inhaled cyanide from cigarette smoke. The serum concentrations of SCN in the smoking group were significantly higher than those of the non-smokers. The average values for each group (2.1 mg/l for non-smokers and 6.6 mg/l for smokers) are in close agreement with those reported by Kagedal et al. (1981). The finding that OC use does not affect serum SCN concentrations suggests that the enzymatic conversion of CN to SCN is not sensitive to chronic OC exposure. This conversion has been shown to occur in various tissues but most prominently in the liver (Himwich and Saunders, 1948).

The effects of smoking and OC use on the biotransformation pathways of theophylline are presented in Figs. 3–5 and summarized in Table 2. A moderate amount of variability in the excretion rates of these metabolites is evident, particularly for 3-MX. The pooling of data undoubtedly exerts a dominant effect in this regard. Intersubject differences in metabolic activity and lack of adjustment for body size may also enhance variability. All biotransformation pathways for all groups except Group III appeared to be linear. Tang-Liu et al. (1982) reported that the 3-MX and 1-MUA pathway were best characterized by non-linear (saturable) models. Conversion to the primary metabolite, 1,3-DMUA, albeit more resistant to saturation, was shown to exhibit non-linear behavior in most instances. Our conflicting results may be attributable to inherent differences in subject populations studied or to the magnitudes of the doses administered. The serum theophylline concentrations attained in our study were generally less than 6 mg/l, whereas those reported by Tang-Liu were twice this. Non-linear behavior may be more perceptible at the higher body loads. No significant dose-dependent changes in clearance were evident when doses of aminophylline were increased from 1.0 to 6.0 mg/kg (Fleetham et al., 1981).

Metabolite formation clearances for Group III are different from those of the other groups. This difference was evident despite achievement of comparable serum theophylline concentrations in

the 4 groups. In a previous communication, we reported that smokers who were not chronic users of OC, but who were acutely exposed to a single dose of contraceptive steroid had significantly lower plasma theophylline clearances. The 6 subjects contained in Group III of this report comprise that subpopulation. Therefore, our previous conclusions regarding theophylline plasma clearances are supported by the present investigation. Interestingly, it appears that all pathways have been affected by this acute exposure. Alteration of absorption kinetics may be misinterpreted as an alteration of clearance for compounds subject to saturable elimination. However, absorption characteristics of theophylline were found to be similar ($P > 0.6$) among the 4 groups.

The apparent formation clearances for the metabolic pathways are presented in Table 2. Since the dispositional behavior is different in Group III, valid comparisons of other groups to this one are difficult. Tobacco and OC use do not exert a significant interactive effect on theophylline plasma clearance (Gardner et al., 1983). Extending these conclusions to each of the metabolic pathways, assessments of OC effects may be made by comparing Groups I and II, while smoking effects would be reflected in differences between Groups II and IV. Use of OC exerts a differential effect on the pathways involved. A 42% reduction in the conversion to 3-MX was noted, while the reductions associated with 1-MUA and 1,3-DMUA were 24 and 19%. Qualitatively similar changes occur with antipyrine metabolism (Teunissen et al., 1982).

Conversion of theophylline to 1-MUA involves the formation of the intermediate metabolite, 1-methylxanthine (1-MX) (Grygiel et al., 1979). Xanthine oxidase rapidly oxidizes 1-MX to form 1-MUA which in turn is excreted in the urine. It has been suggested that the initial demethylation steps involving 3-MX and 1-MX formation are the same enzymes (Grygiel and Birkett, 1980). Our findings do not support this. The degree of inhibition of the 3-MX pathway was considerably greater than that of 1-MUA, and the rate limiting step for the formation of the latter metabolite is believed to be demethylation. Others have reported similar differential effects (Monks et al., 1979).

The routine use of tobacco resulted in an increase in the formation clearances for all metabolic pathways (Table 2). A 55% increase was noted for the conversion of theophylline to 3-MX and increases of 49 and 26% were found for 1-MUA and 1,3-DMUA. These values are considerably smaller than those reported by Grygiel and Birkett (1981). They reported a 2-fold increase in 3-MX and 1-MX formation, whereas the oxidation to 1,3-DMUA was enhanced by a factor of 1.7. These increases support the 78% tobacco-related increase in plasma clearance. Our experiences suggest that an increase of approximately 40% can be expected. The apparent discrepancies between these two investigations may reflect differences in the types of tobacco/smoking habits found in different parts of the world. Increases in all parameters reported by Grygiel and associates consistently differ from ours by a factor of 0.5.

No significant differences in the fractional distribution of metabolites were detected among the groups. The moderate variability in these values (Table 3) could contribute to these findings. However, the urinary recovery of theophylline differed significantly among the 4 groups. Metabolic inhibition allows more of the parent compound to be directly excreted. This effect may be augmented by differences in urine flow (Levy and Koysooko, 1976). The mean urine flow in the OC users was greater than that of non-users over the first 6 study hours (2.69 ml/min in users vs 2.04 ml/min in non-users, $0.05 < P < 0.07$). These differences were corrected for the influences of tobacco use. Such differences in flow should result in relatively small increases in renal clearance (approximately 2.0 ml/min) (Tang-Lui et al., 1983). Since theophylline tended to persist longer in OC users, a prolongation of the diuretic effect may have occurred (Truitt et al., 1950). This is speculative since the fluid intakes of the participants were not stringently controlled. Conversely, cigarette smoking resulted in a significant decrease in the fraction of corrected dose recovered as unchanged drug. In the presence of enhanced tobacco-related metabolic capability, more of the drug is directed along these pathways and less is available for direct excretion. The average urine flow rate in smokers was found to

be significantly less than that of non-smokers (1.46 ml/min in smokers vs 2.32 ml/min in non-smokers, $P < 0.01$). This difference existed over the first 8 h of the study. This effect could be a direct consequence of lower serum concentrations of theophylline.

Simulations of the group data were conducted to assess the reliability of the estimates of formation clearances. As indicated in Fig. 7, the simulated values exceeded the experimentally determined mean concentrations. These findings are consistent with incomplete recovery (70%). Unmonitored clearance pathways, residual body load of primary metabolites at 23 h, urine loss during the collection process, and incomplete absorption of the drug could all contribute to the relatively low recovery.

The non-linear behavior of the simulated profile for Group III does not mimic the experimental values. Despite metabolic pathway saturation, concave decline in serum concentrations of theophylline may not be evident until relatively low concentrations are reached (Tang-Liu et al., 1982). This apparent inconsistency is due to the offsetting influence of enhanced renal clearance at high serum concentration. The slight curvature of the simulated function is likely due to our inability to adequately characterize the time dependency of the renal clearance for this group.

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