Caffeine disposition after oral doses

Caffeine (TMX) disposition was studied in men after 1, 5, and 10 mg/kg in water, as mocha coffee (1.54 mg/kg) and as a soft drink (0.22 mg/kg). TMX and its metabolites were analyzed in plasma and urine by high-pressure liquid chromatography. The design permitted confirmation of most of the partial results in various experimental settings and contributed new data on the metabolic disposition of TMX, with specific reference to the main dimethylxanthine metabolite found in plasma, paraxanthine (1,7-dimethylxanthine). Different analysis methods were compared for the calculated parameters (absorption and elimination rate constants and renal clearance) to assess the consistency of results. The kinetics of TMX and of its dimethylated metabolites in plasma were described with a model that used an analogdigital hybrid computing system. In addition to providing a comprehensive profile of TMX disposition in the healthy adult, the results indicate that TMX exhibits dose-independent kinetics at the levels at which man normally takes TMX.

Maurizio Bonati, M.D., Roberto Latini, M.D., Ferruccio Galletti, M.D., John F. Young, Ph.D., Gianni Tognoni, M.D., and Silvio Garattini, M.D.

Milan, Italy, and Jefferson, Ark.

Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche "Mario Negri," Milan, and Division of Teratogenesis Research, National Center for Toxicological Research, Jefferson

Caffeine (TMX)-containing beverages are consumed daily in nearly all countries. Coffee, tea, carbonated beverages, and cocoa[8, 16] are the main sources of TMX. Moreover, various pharmaceutical products contain TMX in combination with other drugs[30] and it has even been reported in the air of New York City.[13] Daily intake of TMX from all sources in the United States is estimated to be about 210 mg per capita,[15] but there is wide variability between countries, generally depending on social customs. Recently TMX and some of its metabolites have been suggested as risk factors in birth defects,[18] heart disease,[17, 31, 36] benign cystic breast disease,[33] and pancreatic cancer.[19] Despite the long widespread use of TMX from infancy to old age, the profile of its disposition has been more a mosaic of piecemeal information than the result of systematic studies in man. With the notable exception of the study by Axelrod and Reichenthal[5] in 1953, most of the published data on toxic and therapeutic effects in different animals and under different human conditions[2, 3, 7, 9, 14, 22, 23, 27, 28, 32, 35] do not address the relationship of the exposure or dose to possible risk.

Supported in part by a grant from the International Life Sciences Institute, Washington, D. C. and CNR (National Research Council, Rome, Italy) Grant on Clinical Pharmacology and Rare Diseases.

Received for publication Nov. 5, 1981.

Accepted for publication March 12, 1982.

Reprint requests to: Dr. Maurizio Bonati, Laboratory of Clinical Pharmacology, Istituto di Ricerche Farmacologiche, "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

0009-9236/82/070098+09$00.90/0

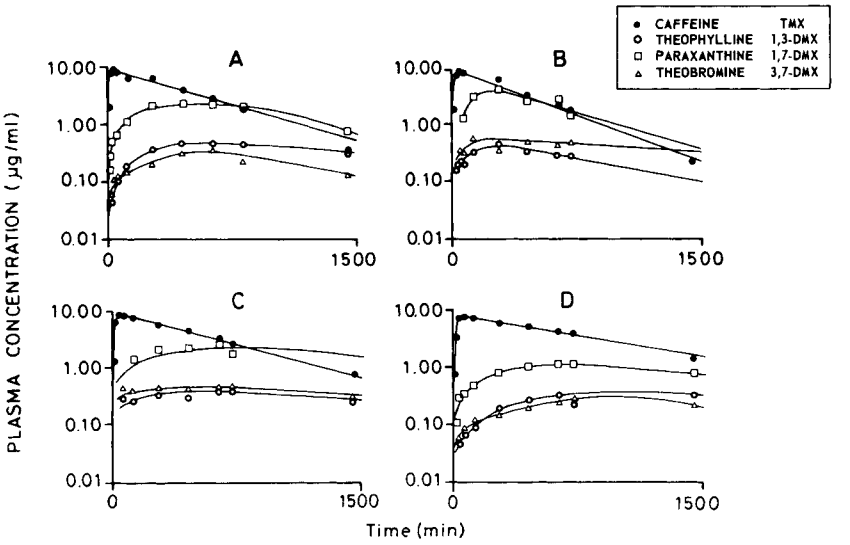

Fig. 1. Plasma concentration-time curves for TMX and the three dimethylxanthines in the four men after a 70-ml oral dose of 350 mg aqueous TMX solution (5 mg/kg).

The demonstration of dose-dependent kinetics in the rat[21] makes it even more urgent to assess TMX disposition after different doses in man. We have addressed this question specifically in the present study of the availability of TMX from normal nutritive sources as well as aqueous solutions at levels that mimic human exposure.

Methods

Our subjects were four healthy men who were 26 to 36 yr old with a mean weight of 70 kg. They abstained from coffee, tea, chocolate, and cola beverages from 10 days before until the end of the study.[41] After overnight fasting each was given the following preparations, containing TMX, in a random sequence, 2 wk apart: 0.22 mg/kg as soft drink (190 ml), 1.54 mg/kg as mocha coffee (50 ml), 1.00 mg/kg as aqueous solution (70 ml at 0.1%), 5.00 mg/kg as aqueous solution (70 ml at 0.5%), and 10.00 mg/kg as aqueous solution (70 ml at 1.0%). Blood sampling followed a similar schedule in all experiments, with more early samples than later ones in an exponential sampling schedule. For a more thorough study of TMX disposition, levels of TMX and its three dimethylxanthine metabolites were determined in blood and urine samples over a 48-hr period after the 5-mg/kg dose.

Analysis

Materials. TMX was supplied by the National Soft Drink Association (NSDA). The International Life Sciences Institute (ILSI) supplied TMX metabolites: 1,3-dimethylxanthine (theophylline; 1,3-DMX), 1,7-dimethylxanthine (paraxanthine; 1,7-DMX), 3,7-dimethylxanthine (theobromine; 3,7-DMX), 1-methylxanthine (1-MX), 3-methylxanthine (3-MX), 7-methylxanthine (7-MX), 1,3,7-trimethyluric acid (TMU), 1,3-dimethyluric acid (1,3-DMU), 1,7-dimethyluric acid (1,7-DMU), 3,7-dimethyluric acid (3,7-DMU), 1-methyluric acid (1-MU), 3-methyluric acid (3-MU), and 7-methyluric acid (7-MU). 4-Amino-(5-formylmethylamino) 1,3-dimethyl uracil (ADMU) was a gift from Drs. Arnaud and Philippossian (Nestlé). Reagents (LiChrosolv, Merck) were ultraviolet grade. A Perkin-Elmer (Norwalk) series 2/2 liquid chromatograph equipped with a LC 55 spectrophotometer was used.

Plasma determinations. To 0.5 ml heparinized plasma, 1.0 ml 0.5% acetic acid and 50 μl of 10 μg/ml theophylline-1-propyl internal standard were added and prepared as described.[6] The samples were shaken for 30 min with 6 ml chloroform:isopropyl alcohol (75:25). After centrifugation the organic phase was transferred and evaporated in a water bath at 60° under a gentle nitrogen stream. The residue was dis-

Table I. *TMX kinetics ($\bar{x} \pm SEM$) in four men after different preparations*

Dose mg/kg	k_{abs}* (hr^{-1})	k_{el}* (hr^{-1})	*Mean elimination half-life (hr)*	*AUC*† *(mg l^{-1}min)*	*aVd*‡ *(l)*	Cl_p§ *(ml/min)*
0.22 (soft drink)	3.15 ± 0.45	0.205 ± 0.073	3.4	170 ± 29	33.7 ± 3.8	104 ± 26
1.00	5.51 ± 1.84	0.165 ± 0.017	4.2	544 ± 64	48.7 ± 2.8	133 ± 14
1.54 (mocha coffee)	5.60 ± 1.75	0.139 ± 0.025	5.0	977 ± 148	53.5 ± 5.7	119 ± 14
5.00	6.31 ± 1.91	0.110 ± 0.016	6.3	5164 ± 691	39.4 ± 1.6	71 ± 9
10.00‖	40.76	0.133	5.2	6815	46.5	103

k_{abs} = absorption constant; k_{el} = elimination rate constant; aVd = apparent volume of distribution; Cl_p = plasma clearance.
*Estimated by the iterative program.
†Calculated by the trapezoidal rule.
‡$aVd = f \cdot D/(AUC \cdot k_{el})$.
§$Cl_p = aVd \cdot k_{el}$.
‖Two subjects only.

solved in 100 μl of the chromatographic mobile phase and about 30 μl was injected into the liquid chromatograph. High-pressure liquid chromatography analysis at 273 nm by the procedures of Sved and Wilson[38] was slightly modified by the use of a higher concentration of methanol buffer (3.3%) and a LiChrosorb Silica-100 column (5-μm particle size, 250 mm × 4 mm, Merck).

Urine determinations. TMX and its metabolites were measured in urine samples by a modified method from Aldridge et al.[1] One milliliter of urine saturated with 400 mg of ammonium sulphate was extracted with 400 μl 8-chlorotheophylline (50 μg/ml aqueous solution) as an internal standard and 20 ml chloroform:isopropyl alcohol extracting solvent mixture. The dissolved residue was injected into the high-pressure system equipped with a reversed-phase column (Hibar RP-8, 5 μm, 250 mm × 4 mm, Merck) and eluted with a linear gradient from 0.5% acetonitrile in 0.5% acetic acid (v/v) in water to 5% acetonitrile within 36 min at a flow rate of 1.7 ml/min. An internal calibration curve of TMX and its metabolites was prepared for each set of about 15 urine or plasma samples.

Data analysis

Iterative analysis. Plasma curves of TMX concentrations-time were analyzed following a one-compartment open model system after oral dosing. Experimental points were fitted by a nonlinear regression iterative program (by Carl Peck, Uniformed Services University) on a HP-85 desk computer (Hewlett-Packard).

Hybrid computer system. The data were fitted by an analog-digital hybrid computer (EAI Pacer 500, Electronic Associates). The model (see Fig. 2) was defined mathematically by a series of differential equations that are solved simultaneously by the analog portion of the system. The control functions, the normalized linear least-square statistical fit of the data, and the documentation were all managed through the digital portion of the hybrid system. The data fit through this operator interactive system directly yielded all of the model associated rate constants and area under the plasma concentration-time curve (AUC) data.[42]

Graphic analysis. AUCs were calculated by the trapezoidal rule and when necessary extrapolated to infinity. For the calculation of apparent volume of distribution [$aVd = f \cdot D/(AUC \cdot k_{el})$] and plasma clearance ($Cl_p = aVd \cdot k_{el}$), the bioavailable fraction (f) of the TMX dose (D) was considered to be equal to 1.0, since our unpublished data in treated animals and data reported here indicated complete absorption of the drug at these doses.

The absorption rate constant was obtained by the method of Wagner and Nelson[40] from the plasma concentration data. The plot of the log of the percentage of TMX still to be absorbed

Table II. *AUC and metabolic rate constants for the dimethylxanthines obtained from the hybrid computer system fit of the plasma concentration-time data from the four subjects after an oral TMX dose of 5 mg/kg*

	AUC (relative concentration-time units)				*Metabolic rate constants (hr^{-1})*		
Subject	*TMX*	*1,3-DMX*	*1,7-DMX*	*3,7-DMX*	*1,3-DMX*	*1,7-DMX*	*3,7-DMX*
A	4205	309	2234	531	0.013	0.104	0.016
B	3794	476	2393	343	0.013	0.154	0.008
C	4924	516	1964	462	0.014	0.092	0.017
D	5901	286	1280	325	0.014	0.046	0.017
Mean	4706	397	1968	415	0.013	0.099	0.015
±SEM	462	58	246	49	0.001	0.022	0.002
t½ (hr)					51.9	7.0	47.6

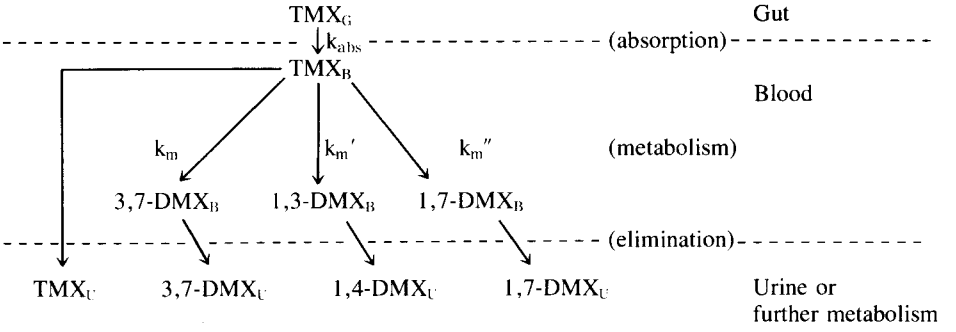

Fig. 2. TMX metabolic pathway model.

against time yielded a straight line, the slope of which was the absorption rate constant.

The elimination rate constant was obtained from the slopes of plots of the urinary data applying two methods: (1) plotting the urinary excretion rate, log Δ_{AU}/Δ_t (amount excreted in urine [Δ_{AU}] over the time interval [Δ_t], versus the time at the midpoint of the urine collection period (''rate method''), and (2) plotting the log of unchanged drug remaining to be excreted versus time (''sigma-minus method'').[26]

Two methods were used to obtain renal clearance values: (1) dividing Δ_{AU}/Δ_t by plasma concentration at the midpoint of the urine collection period to test renal clearance during the time and averaging all values, and (2) plotting Δ_{AU}/Δ_t versus plasma concentrations at the midpoint of the urine collection period, yielding the slope for the renal clearance.

Statistical analysis. All slopes were obtained by linear regression analysis[34] and differences between the kinetic values obtained by the various methods were tested by ANOVA randomized block design.[20]

Results

TMX solutions (5.00 mg/kg oral). The time course of TMX and the three dimethylxanthine plasma concentrations for the four subjects are shown in Fig. 1. Kinetic parameters are summarized in Table I and each subject's values after all five doses are presented in Appendix I. Plasma peak level was reached in 47 ± 5 min after dosing (mean concentration of 8.3 ± 0.1 μg/ml). Values were of the same order in the four subjects, yielding an absorption rate constant of 6.3 ± 1.9 hr^{-1} and an elimination rate constant of 0.11 ± 0.02 hr^{-1}. The TMX plasma level for subject D was maintained at a higher level for a longer period than in the others (Fig. 1, *D*). This is reflected in a lower elimination rate constant, lower plasma clearance value, and higher AUC value than in the others for all doses.

Table III. *Urinary excretion (% of dose) over 48 hr of TMX and its metabolites in four men after an oral TMX dose of 5 mg/kg*

Subject	*Urine volume (l)*	*TMX*	*ADMU*	*1,7-DMX*	*3,7-DMX*	*1,3-DMU*	*1,7-DMU*	*1-MX*	*3-MX*	*7-MX*	*1-MU*	*Total*
A	2.2	0.8	1.3	3.3	2.0	3.3	5.6	10.7	1.9	7.8	52.1	88.8
B	2.9	1.4	0.8	2.3	3.2	2.5	4.5	7.3	2.1	5.2	35.4	64.7
C	2.2	1.8	0.9	6.7	2.1	3.4	11.8	15.9	3.8	10.3	51.1	107.8
D	2.2	1.0	1.3	5.4	2.5	2.6	7.9	17.3	2.4	4.4	35.8	80.6
Mean	2.4	1.2	1.1	4.4	2.4	2.9	7.4	12.8	2.5	6.9	43.6	85.5
±SEM	0.2	0.2	0.1	1.0	0.3	0.2	1.6	2.3	0.4	1.3	4.6	9.0

Table IV. *Elimination rate constants (hr^{-1}) calculated by four methods in four men after an oral TMX dose of 5 mg/kg*

	Urine		*Plasma*	
Subject	*Rate*	*Sigma-minus*	*Iterative*	*Hybrid*
A	0.070	0.077	0.113	0.153
B	0.256	0.274	0.149	0.183
C	0.120	0.125	0.105	0.131
D	0.088	0.176	0.071	0.091
Mean	0.133	0.163	0.110	0.140
±SEM	0.042	0.042	0.016	0.019
t½ (hr)	5.2	4.3	6.3	5.0

The rate of TMX absorption, as calculated by the Wagner-Nelson method,[40] was in good agreement with the iterative program and the hybrid computer method for all subjects. Based on these absorption rate constants, it appears that more than 99% of the administered dose of caffeine is absorbed in about 45 min.

Only the least polar TMX metabolites, the three dimethylxanthines, could be measured in plasma. Comparing the hybrid computer-generated AUCs of the dimethylxanthines, the main plasma metabolite of TMX was 1,7-DMX, accounting for about half the AUC value for TMX; 1,3-DMX and 3,7-DMX were about one-tenth the AUC for TMX (Table II, Fig. 2).

With the hybrid computer system the rate constants for the metabolism of TMX to the three dimethylxanthines were estimated from the plasma concentration-time curves. Since the apparent volume of distribution for the individual dimethylxanthines cannot be determined from this data, the volume for each dimethylxanthine was approximated to be the same as TMX and the plasma concentrations were analyzed without being adjusted. The resulting metabolic rate constants (Table II) supported the AUC data in that 1,7-DMX was formed at about seven times the rate of the other two dimethylxanthines. This relatively larger metabolism-rate ratio than the relative AUC ratio indicated that the further metabolism and elimination of this metabolite was also faster than for the other dimethylxanthines.

About 85% of the TMX dose was recovered in the urine within 48 hr (Table III). The finding that the main excreted metabolites of TMX were 1-MU, 1-MX, 1,7-DMU, 7-MX and 1,7-DMX indicated that its predominant metabolic pathway in man was through 1,7-DMX. Because of the very small amounts of 1,3,7-TMU and 1,3-DMX detected in the urine (less than 0.5% of the dose), they were not included in the Table. 3-MU and 7-MU could not be quantitated because of analytic interference.

With the use of urinary excretion data and applying the graphic "rate" and "sigma-

minus'' methods, the elimination rate constants for each subject were calculated (Table IV). These values were of the same order as those based on the computer-fit blood data.

There was no correlation between renal clearance of TMX and plasma concentrations or urine flow rate with time (Table V), but there was good agreement between the ratios of renal to plasma clearance and the excreted percentage of the dose of TMX (Table VI).

Mocha coffee. The time course of TMX concentration disappearance from plasma was much the same for all subjects who took the aqueous solution, as illustrated in Fig. 1. The kinetic parameters are reported in Table I and Appendix I. The highest concentration (2.0 ± 0.2 μg/ml) developed 52 ± 22 min after dosing. As after the aqueous TMX solution, the values for all kinetic parameters were much the same for all subjects, with subject D again showing a longer half-life and larger AUC, and consequently a lower clearance value.

Soft drink. The plasma profile of TMX after 190 ml of soft drink (0.22 mg/kg TMX) in the same four subjects again followed the same pattern. The various kinetic parameters are reported in Table I and in Appendix I. The time taken to reach peak plasma level (0.4 ± 0.04 μg/ml) was again in the same range for all (38 ± 8 min), but subject D had the slowest elimination characteristics.

The regression for the five different TMX doses versus AUC · elimination rate constant was significant ($P < 0.02$; $r = 0.99$; $F = 290$; Fig. 3). Because of the variability among the subjects in the elimination rate constant and related parameters, the AUC values were multiplied by the estimated constant, as suggested by Wagner,[39] to reduce the individual variability of AUC alone.

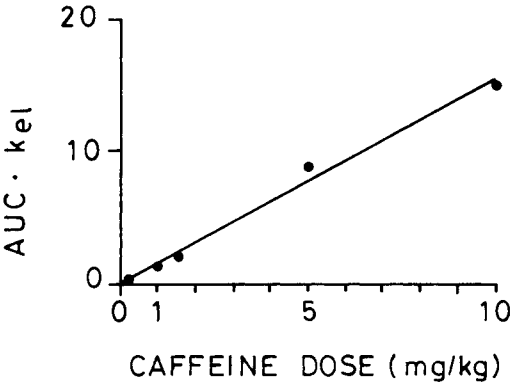

Fig. 3. Relationship between TMX dose and AUC · elimination rate constant after soft drink, mocha coffee, and TMX aqueous solutions in men ($r = 0.99$; $F = 290$, $P < 0.02$; slope = 1.54 ± 0.29; intercept = 0.14 ± 1.45).

Discussion

TMX kinetics in man at different doses and from different sources are very similar and give results in overall agreement with data already reported. Blood concentrations and the time to peak TMX levels correspond closely to reports.[25, 29, 32] After mocha coffee, the TMX blood concentrations were lower than those reported by Marks and Kelly,[25] but in the range of those reported by Sved et al.[37] As Marks and Kelly[25] reported, a lower absorption rate constant for TMX after a soft drink than after mocha coffee and the TMX aqueous solution was found. The lower pH value of the soft drink (2.4, 5.6 for the mocha coffee, and 7.0 for the aqueous solutions), the larger volume of the soft drink (190 ml, 50 to 70 ml for the coffee and aqueous solutions), and lower TMX concentration (0.08 mg/ml for the soft drink and ≥1.0 mg/ml for the other preparations) could explain the tendency for absorption rate to rise with increasing TMX doses (Table I). The similarity between absorption of the TMX aqueous solution (1 and 5 mg/kg) and mocha coffee has also been reported by Czok et al.[12]

Elimination rate constants were as reported by others[10, 28, 29, 36, 37] as were half-life values, which ranged from 1.5 to 9.5 hr for individuals, with intrasubject similarity over the five doses. The close agreement between the metabolic rate constants of TMX conversion to the dimethylxanthine metabolites and the dimethylxanthine plasma AUC values confirms the reliability of the hybrid computer approach used (Table II, Fig. 3), but this approach could not determine the elimination half-lifes for each dimethylxanthine from our data.

No data have been available on 1,7-DMX in man after TMX. Its prevalence as the main metabolite is confirmed by urinary data; 1,7-DMX and its metabolite 1,7-DMU account for 11.8%

Table V. *Plasma concentrations and renal clearance of TMX and urine flow rates in four men after an oral TMX dose of 5 mg/kg*

Time (min)	*Plasma concentration (μg/ml)*	Cl_R *(ml/min)*	*Urine flow rate (ml/min)*
30	7.67 ± 0.29	1.87 ± 1.05	2.36 ± 0.94
120	7.01 ± 0.28	1.27 ± 0.24	2.35 ± 0.93
270	5.80 ± 0.20	0.70 ± 0.11	1.09 ± 0.22
450	4.11 ± 0.45	0.76 ± 0.08	0.98 ± 0.12
630	3.03 ± 0.52	0.92 ± 0.29	1.10 ± 0.15

Cl_R = renal clearance.

Table VI. *Plasma and renal clearance and cumulative urinary amount of TMX in four men after 5 mg/kg TMX*

Subject	Cl_p *(ml/min)*	Cl_R *(ml/min)*	Cl_R/Cl_p *(%)*	*% TMX dose excreted*
A	79.8	0.43	0.5	0.8
B	90.1	1.81	2.0	1.4
C	65.2	1.41	2.1	1.8
D	49.8	0.77	1.5	1.0
Mean	71.2	1.10	1.5	1.2
±SEM	8.8	0.31	0.4	0.2

Abbreviations are as in Tables I and V.

of the TMX dose, 3,7-DMX accounts for only 2.4%, and 1,3-DMU for 2.9% (Table III) of the dose. These data are in the range of the percentages of TMX excretion and of a few metabolites reported by Cornish and Christman[11] and Arnaud et al.,[4] but for 1-MU alone urinary excretion was higher than that reported by them and by Callahan et al.[10]

We confirmed the report of Bonati et al.[7] that the renal clearance of TMX was lower than of theophylline. The reliability of the calculated clearance values was confirmed by the agreement between renal and body clearance ratios and the fraction of TMX excreted unchanged in urine (Table VI). Unlike the data of Levy and Koysooko[24] for theophylline, renal clearance for TMX did not seem to be affected by urinary flow, even though a higher clearance was found early, when the urine flow rate was high, possibly because of the slight diuretic effect of TMX (Table V). No conclusive evidence can be presented because our experiment was not designed to investigate this effect.

In a previous study in rats we reported that TMX kinetics were dose dependent within the range of doses taken by our subjects in this study.[21] From our data it appeared that TMX kinetics in man were linear up to 10 mg/kg, at which level toxic symptoms first appeared (nervousness, lightheadedness, headaches); this is the reason why only two subjects took the highest dose. This observation was supported by the good correlation between dose and AUC values (Fig. 3) and by the similarity of the elimination rate constants after the different doses.

Good agreement was obtained from kinetic parameters determined by graphic or computer-modeled approaches (Table IV), but the metabolic conversion of TMX to the three dimethylxanthines could not have been obtained without the hybrid computer system.

In conclusion, this comprehensive profile of TMX disposition after ingestion of the main forms in which it is used by man confirms most of the information already available; it also supplies new qualitative and quantitative data on the metabolic pathways and excludes the existence of dose-dependent kinetics in man at the levels at which man is normally exposed to caffeine. The latter finding is particularly relevant to the extrapolation of animal toxicologic data to man.

References

1. Aldridge A, Aranda JV, Neims AH: Caffeine metabolism in the newborn. CLIN PHARAMCOL THER **25**:447-453, 1979.
2. Aldridge A, Neims AH: The effects of phenobarbital and β-naphthoflavone on the elimination kinetics and metabolite pattern of caffeine in the beagle dog. Drug Metab Dispos **7**:378-382, 1979.

3. Aranda JV, Turmen T: Methylxanthines in apnea of prematurity. Clin Perinatol **6:**87-108, 1979.
4. Arnaud MJ, Thelin-Doerner A, Ravussin E, Acheson KJ: Study of the demethylation of/1,3,7-Me-^{13}C/caffeine in man using respiratory exchange measurements. Biomed Mass Spectrom **7:**521-524, 1980.
5. Axelrod J, Reichenthal J: The fate of caffeine in man and a method for its estimation in biological material. J Pharmacol Exp Ther **107:**519-523, 1953.
6. Bonati M, Castelli D, Latini R, Garattini S: Comparison of gas-liquid chromatography with nitrogen-phosphorus selective detection and high-performance liquid chromatography methods for caffeine determination in plasma and tissue. J Chromatogr **164:**109-113, 1979.
7. Bonati M, Latini R, Marra G, Assael BM, Parini R: Theophylline metabolism during the first month of life and development. Pediatr Res **15:**304-308, 1981.
8. Bunker ML, McWilliams M: Caffeine content of common beverages. J Am Diet Assoc **74:**28-32, 1979.
9. Burg AW: Physiological disposition of caffeine. Drug Metab Rev **4:**199-228, 1975.
10. Callahan MM, Robertson RS, Lavin MJ, Yesair DW: Human metabolism of caffeine, *in* Federation of American Societies for Experimental Biology. Abstracts of Papers, 63rd Annual Meeting, Dallas, Texas, April 1-10, 1979, abstract 1877.
11. Cornish HH, Christman AA: A study of the metabolism of theobromine, theophylline, and caffeine in man. J Biol Chem **228:**315-323, 1957.
12. Czok G, Schmidt B, Lang K: Distribution of caffeine-8-^{14}C in the rat. Z Ernaehrungswiss **9:**109-117, 1969.
13. Dong M, Hoffman D, Locke DC, Ferrand E: The occurrence of caffeine in the air of New York City. Atmos Environ **11:**651-653, 1977.
14. Eichler O, editor: Kaffee und coffein. Berlin, 1976, Springer-Verlag.
15. Graham DM: Caffeine: Its identity, dietary sources, intake and biological effects. Nutr Rev **36:**97-102, 1978.
16. Greden JF: Coffee, tea and you. The Sciences **19:**6-11, 1979.
17. Heyden S, Tyroler HA, Heiss G, Hames OG, Bartel A: Coffee consumption and mortality. Total mortality, stroke mortality, and coronary heart disease mortality. Arch Intern Med **138:** 1472-1475, 1978.
18. Jacobson MF, Goldman AS, Syme RH: Coffee and birth defects. Lancet **1:**1415-1416, 1981.
19. Jick H, Dinan B: Coffee and pancreatic cancer. Lancet **2:**92, 1981.
20. Kirk RE: Experimental design procedures for the behavioral sciences. Monterey, Calif., 1968, Brooks/Cole Publishing Company.
21. Latini R, Bonati M, Castelli D, Garattini S: Dose-dependent kinetics of caffeine in rats. Toxicol Let **2:**267-270, 1978.
22. Latini R, Bonati M, Marzi E, Garattini S: Urinary excretion of an uracilic metabolite from caffeine by rat, monkey and man. Toxicol Let **7:**267-272, 1981.
23. Latini R, Bonati M, Marzi E, Tacconi MT, Sadurska B, Bizzi A: Caffeine disposition and effects in young and one-year-old rats. J Pharm Pharmacol **32:**596-599, 1980.
24. Levy G, Koysooko R: Renal clearance of theophylline in man. J Clin Pharmacol **16:**329-332, 1976.
25. Marks V, Kelly JF: Absorption of caffeine from tea, coffee, and coca cola. Lancet **1:**827, 1973.
26. Martin BK: Drug urinary excretion data: Some aspects concerning the interpretation. Br J Pharmacol Chemother **29:**181-193, 1967.
27. McCay CM, Lovelace F, Sperling G, Barnes LL, Liu CH, Smith CAH, Saxton JA Jr.: Age changes in relation to the ingestion of milk, water, coffee, and sugar solutions. J Gerontol **7:**161-172, 1952.
28. Patwardhan RV, Desmond PV, Johnson RF, Schenker S: Impaired elimination of caffeine by oral contraceptive steroids. J Lab Clin Med **95:**603-608, 1980.
29. Parsons WD, Neims AH: Effect of smoking on caffeine clearance. CLIN PHARMACOL THER **24:**40-45, 1978.
30. Rall TW: The xanthines, *in* Goodman LS, Gilman A, editors: The pharmacological basis of therapeutics, ed. 6, New York, 1980, Macmillan, Inc., pp. 592-607.
31. Robertson D, Frolich JC, Carr RK, Watson JT, Hollifield JW, Shand DG, Oates JA: Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med **298:** 181-186, 1978.
32. Robertson D, Wade D, Workman R, Woosley RL: Tolerance to the humoral and hemodynamic effects of caffeine in man. J Clin Invest **67:**1111-1117, 1981.
33. Rowner S: Healthtalk: Have you looked into cyclic AMPs?... Coffee, tea and cystic disease. Washington Post, January 18, 1980.
34. Snedecor GW, Cochran WG: Statistical methods, ed. 6, Ames, Iowa, 1967, Iowa State University Press.
35. Soyka LF: Effects of methylxanthines on the fetus. Clin Perinatol **6:**37-51, 1979.
36. Stanton MF, Ahrens RA, Douglass LW: Coffee and cola beverage consumption as heart disease risk factors in men. Experientia **34:**1182-1183, 1978.
37. Sved S, Hossie RD, McGilveray IJ: The human metabolism of caffeine to theophylline. Res

Commun Chem Pathol Pharmacol **13:**185-191, 1976.

38. Sved S, Wilson DL: Simultaneous assay of the methylxanthine metabolites of caffeine in plasma by high performance liquid chromatography. Res Commun Chem Pathol Pharmacol **17:**319-331, 1977.
39. Wagner JF: A method of estimating relative absorption of a drug in a series of clinical studies in which blood levels are measured after single and/or multiple doses. J Pharm Sci **56:**652-653, 1967.
40. Wagner JG, Nelson E: Percent absorbed time plots derived from blood level and/or urinary excretion data. J Pharm Sci **52:**610-611, 1963.
41. Warren RN: Metabolism of xanthine alkaloids in man. J Chromatogr **40:**468-469, 1969.
42. Young JF, White CG, Pierce BA: A hybrid computer system for pharmacokinetic modeling. II. Applications. Proceedings of the 1981 Summer Computer Simulation Conference, pp. 122-129, 1981.

Appendix I. *Individual TMX kinetics for subjects in the study (see Table I for mean values)*

Dose (mg/kg)	*Subject*	k_{abs} (hr^{-1})	k_{el} (hr^{-1})	*AUC (mg/min)*	*aVd (l)*	Cl_p *(ml/min)*
0.22 mg/kg	A	2.21	0.419	85	25.9	181
(soft drink)	B	3.97	0.133	194	35.9	79
	C	3.85	0.169	186	29.5	83
	D	2.56	0.099	215	43.4	72
1.00 mg/kg	A	4.20	0.191	501	43.8	140
	B	10.81	0.179	516	45.4	136
	C	2.32	0.173	431	56.2	162
	D	4.72	0.116	728	49.6	96
1.54 mg/kg	A	7.29	0.203	795	40.8	138
(mocha coffee)	B	1.37	0.151	860	50.8	128
	C	9.39	0.116	834	68.3	132
	D	4.35	0.086	1421	54.1	77
5.00 mg/kg	A	11.94	0.113	4385	42.2	80
	B	5.45	0.149	3884	36.2	90
	C	3.94	0.105	5368	37.3	65
	D	3.91	0.071	7020	41.9	50
10.00 mg/kg	A	44.17	0.140	6579	45.7	106
	B	37.34	0.126	7051	47.3	99

Abbreviations are as in Table I.