(Similar results were obtained from aged samples of betamethasone sodium phosphate formulations.)

To ensure the usefulness of this technique, pure betamethasone sodium phosphate was degraded by heat, alkalinity, and acidity and the decomposed material was chromatographed. The chromatographic system separated all degradation products and possible manufacturing impurities. The decrease in the peak height upon degradation was not compensated for by an increase in the size of the decomposition peak. Therefore, the primary product, presumed to be the 17-ketone (6), was not detected in this system and was probably retained. Constant amounts of reference standard and internal standard were added to varying amounts of placebo and aged placebo. Recoveries were consistently in the 100% range.

The instrument precision was good at the attenuation used. Duplicate assays were performed for the injection formulation over several days, and the results indicate high reproducibility (Table II) and agreed well with the manufacturer label claims. Comparison of these results with the results obtained with a paper chromatographic assay shows the methods to be equivalent (Table III).

This method can be applied to other steroid phosphate formulations (Fig. 3 and Table IV). HPLC shortens analysis time and is accurate and easy to perform. In addition, it can be easily automated with automatic samplers.

REFERENCES

(1) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, pp. 90–92.

(2) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 123–126.

(3) L. Cavatorta, L. Lodi, and R. Rangone, *Farmaco Ed. Prat.*, **25**, 312 (1970).

(4) J. Reiss, *J. Chromatogr.*, **19**, 527 (1965).

(5) W. Beyer and W. Mowzowich, *Ann. N.Y. Acad. Sci.*, **153**, 393 (1968).

(6) D. Guttman and P. Muster, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 553 (1958).

ACKNOWLEDGMENTS

The authors express their appreciation to Mr. S. Gruber and Ms. V. Bole for assistance.

Theophylline Bioavailability following Chronic Dosing of an Elixir and Two Solid Dosage Forms

DANNY D. SHEN ˣ, MARK FIXLEY, and DANIEL L. AZARNOFF

Received May 26, 1977, from the *Departments of Medicine and Pharmacology, Clinical Pharmacology–Toxicology Center, Unive sity of Kansas Medical Center, College of Health Sciences and Hospital, Kansas City, KS 66103.* Accepted for publication October 19, 1977.

Abstract □ Theophylline bioavailability following chronic dosing of an elixir and two commercial tablet formulations (I and II) relative to an acute dose of elixir was evaluated in healthy volunteers. Both tablet formulations contained ephedrine. In addition, Tablet I contained hydroxyzine hydrochloride, and Tablet II contained phenobarbital. The mean area under the serum concentration–time curve (*AUC*) calculated either from time $0 \rightarrow \infty$ for a single dose or over one dosing interval after repetitive doses was the highest after chronic administration of the elixir. The *AUC* after chronic elixir, in fact, was statistically different from the values after acute elixir ($p < 0.05$) and Tablet II ($p < 0.05$). There was, however, a large variation in the elimination half-life among the four theophylline treatments. The mean $t_{1/2}$ was the longest after chronic elixir followed by Tablet I, Tablet II, and acute elixir. The *AUC* values for the four treatments, when corrected for differences in $t_{1/2}$, were no longer significantly different, indicating that the extent of theophylline absorption was essentially the same from all three tested products. The time to peak and the peak serum concentration also did not differ among treatments. The prolongation in $t_{1/2}$ following chronic treatment with the elixir and its subsequent shortening during tablet administration suggest an initial inhibition followed by induction of theophylline metabolism. These changes may be due to the prolonged treatment with theophylline itself or the other drug ingredients in the dosage form.

Keyphrases □ Theophylline—bioavailability of commercial elixir and two tablets compared, chronic dosing in humans □ Bioavailability—theophylline, commercial elixir and two tablets compared, chronic dosing in humans □ Relaxants, smooth muscle—theophylline, bioavailability of commercial elixir and two tablets compared, chronic dosing in humans

Literature reports (1, 2) suggested that incomplete bioavailability of theophylline tablets, singly or in combination with ephedrine and sedatives such as phenobarbital or hydroxyzine, may frequently cause therapeutic failures. Theophylline bioavailability after a single oral dose of an elixir and two different combination tablets was essentially complete as compared to intrave. ous aminophylline (3). However, it is not known whether differences in bioavailability for these theophylline formulations occur when given chronically.

Several factors might affect the serum theophylline concentration when it is administered over an extended period. In humans, theophylline is eliminated largely by oxidative microsomal metabolism (4). Drugs frequently combined with theophylline, such as phenobarbital and hydroxyzine, may alter its pharmacokinetics. Cigarette smoking significantly increased the serum clearance of theophylline (5, 6), presumably due to microsomal enzyme induction by the polycyclic aromatic hydrocarbon constituents in the smoke. Theophylline induced its own metabolism in the rat (7), and a similar action could occur in humans.

The present study was designed to ascertain the chronic oral availability of theophylline from previously studied lots of elixir and combination tablet preparations relative to an acute dose of the elixir. In addition, the effects of long-term administration of theophylline and the other drugs in the combination tablets on the disposition kinetics of theophylline were observed.

EXPERIMENTAL

Subjects—Twelve healthy volunteers between 22 and 40 years of age and weighing between 46.2 and 82.4 kg participated. Of the 12 subjects (five men and seven women), only two were habitual smokers, each averaging fewer than 20 cigarettes/day. Written informed consent, a history, a physical examination, and laboratory tests (complete blood count, urinalysis, long-lead II ECG, serum thyroxine, bilirubin, creatinine, glutamic-oxaloacetic transaminase, and alkaline phosphatase) were

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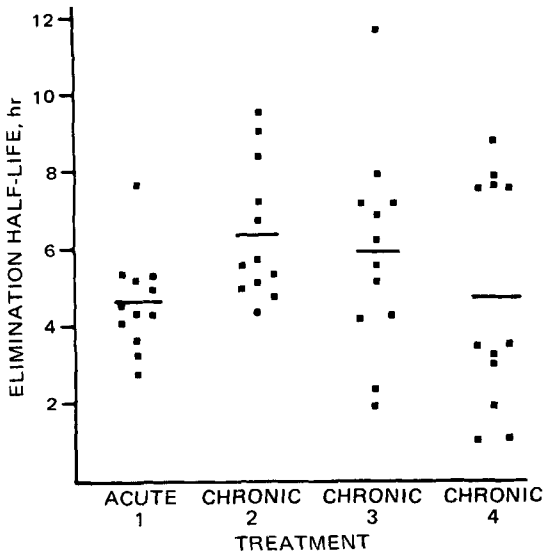

Figure 1—*Comparison of serum theophylline half-life in the 12 volunteers after treatment with an acute dose of the elixir and chronic doses of the elixir and Tablets I and II. The bar represents the mean half-life of each treatment. Key: 1, acute elixir; 2, chronic elixir; 3, chronic Tablet I; and 4, chronic Tablet II.*

obtained before the study, and the results were within normal limits for all volunteers.

Drug Products—Three products containing theophylline were tested: an elixir[1] containing 80 mg of theophylline/15 ml; Tablet I[2], a preparation containing 130 mg of theophylline, 25 mg of ephedrine sulfate, and 10 mg of hydroxyzine hydrochloride; and Tablet II[3], a preparation containing 130 mg of theophylline; 24 mg of ephedrine hydrochloride, and 8 mg of phenobarbital.

Samples of the three preparations were assayed for theophylline content using a previously described high-pressure liquid chromatographic method (8). Theophylline content was within 10% of the label claim for all three dosage forms.

Drug Administration—To minimize any possible alteration of theophylline metabolism by theophylline itself or other drug ingredients in the formulations from influencing the results by a carryover effect, the sequence of administration was not randomized. Accordingly, following a single 130-mg dose of theophylline elixir, the same dosage form was administered for 10 days in a dose of 130 mg at 8-hr intervals. This regimen was followed by an equivalent theophylline dose of Tablets I and II every 8 hr for 10 days each.

A drug-free washout period of at least 21 days followed each 10-day drug administration period. The subjects were forbidden to take other drugs throughout the study and methylxanthine-containing beverages (tea, coffee, cocoa, and cola) on the final 2 days of each treatment period. Compliance was monitored only by verbal questioning.

Blood Sampling—Following the first dose of elixir and on the last day of each 10-day chronic drug administration period, blood samples were drawn at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10, and 12 hr through an indwelling polytef cannula inserted in an antecubital vein. Subjects were required to fast for at least 12 hr before each blood sampling period.

Analysis of Theophylline—Theophylline concentration in the serum samples was measured by the GLC method of Kowblansky *et al.* (9). The method involves flash heater *N*-butylation of theophylline with 8-chlorotheophylline as an internal standard. The precision of this procedure was estimated by including quadruplicate samples of 1 and 5 μg of theophylline/ml standards in each run. The means of the standards were 0.975 and 4.92 μg with coefficients of variations of 15% (n = 17) and 6.3% (n = 52), respectively.

Pharmacokinetic Analysis—A previous study (10) showed that the pharmacokinetics of theophylline following single intravenous bolus administration can be described by a two-compartment model. However, in the present oral study, a clearly discernible distribution phase was not evident in any serum concentration–time course profile. Therefore, the data were analyzed according to a simple linear one-compartment open model (11), using the iterative nonlinear least-squares regression computer program of Metzler (12). Each data point was weighted by the reciprocal of its observed value.

Theophylline absorption from the various dosage forms was characterized by an apparent first-order absorption rate constant, k_a. In some cases, a more satisfactory fit of the observed data was obtained when an absorption lag time, t_0, was introduced into the model. The serum concentration of drug, C, at any time, t, after a *single* oral dose, D, is described by:

$$C = \frac{FD}{V}\left(\frac{k_a}{k_a - K}\right)\left[e^{-K(t-t_0)} - e^{-k_a(t-t_0)}\right] \qquad \text{(Eq. 1)}$$

where F is the fraction of the oral dose absorbed, V is the apparent volume of distribution, and K is the apparent first-order elimination rate constant.

Half-lives, $t_{1/2}$, were calculated by dividing $\log_e 2$ or 0.693 by its rate constant. Based on the estimates of k_a, K, and t_0, the peak concentration, C_{max}, and time to reach the peak, t_p, following a single dose were calculated according to previously established equations (11).

The equation describing the time course of drug concentration in plasma following the last oral dose at steady state is derived from Eq. 1 by multiplying each exponential term by the multiple-dosing function of Benet (13), *i.e.*, $1/(1 - e^{k_i\tau})$, where k_i is the apparent first-order rate constant in each exponential term (*i.e.*, k_a and K) and τ is the dosage interval (*i.e.*, 8 hr). The peak concentration, C^{∞}_{max}, and time to peak, $t_p{}^{\infty}$, following an oral dose at steady state were calculated (11).

The total area under the serum concentration–time curve (AUC) was estimated by the trapezoidal rule. Extrapolation beyond the last recorded serum concentration, C^*, was calculated by C^*/K.

In theory, the entire AUC (*i.e.*, AUC_∞) for a given drug after a single oral dose is equivalent to the AUC over one dosing interval (*i.e.*, AUC_τ), provided that the bioavailability, F, and serum clearance, KV, remain unchanged during repetitive administration. Since theophylline absorption from a single dose of the elixir previously was found to be complete (3), the relative bioavailability of chronic doses of the elixir and Tablets I and II was evaluated by comparing their AUC_τ values with the AUC_∞ after acute elixir.

The average steady-state serum concentration, $\overline{C}_{ss}$, as proposed by Wagner (14) was calculated by dividing AUC_τ by τ. Based on single-dose serum concentration–time data, $\overline{C}_{ss}$ was predicted by dividing AUC_∞ by τ.

Statistical Analysis—An analysis of variance (15) adapted for single-factor experiments with repeated measures on the same individual was used in the statistical evaluation of AUC and $t_{1/2}$. When significant differences between the products were found, a Newman–Keuls test (15) was carried out to find the source of the difference. Homogeneity of variances was examined by Bartlett's test (14).

RESULTS

The means and standard deviations of the various pharmacokinetic parameters estimated by nonlinear least-squares analysis of serum concentration–time data from both the acute and chronic elixir and the two chronic combination tablet studies are summarized in Table I. The pharmacokinetic parameters derived from the acute dose of elixir were not significantly different [Mann–Whitney test (16), $p > 0.05$] from those of a previous single-dose study with the same lot of elixir in another group of normal volunteers (3).

Substantial variations in the elimination half-lives of theophylline were noted following administration of the four different dosage forms. The half-life values are graphically compared in Fig. 1. The mean elimination half-life of theophylline after an acute dose of elixir for the 12 subjects was 4.62 hr (2.78–7.65 hr) and increased to 6.30 hr (4.36–9.49 hr) following chronic administration of elixir. The prolongation in half-life was inversely correlated with the initial half-life (r = 0.625, $p < 0.05$).

The serum half-life after chronic administration of Tablet I had a mean of 5.84 hr and was quite variable (1.88–11.6 hr). In four of the 12 subjects, the elimination half-life remained prolonged; in the remaining subjects, the half-life values tended to return to initial levels. In fact, the theophylline half-life in two subjects in the latter group decreased to below that after the first dose of elixir.

The final course of treatment with Tablet II, which contained 8 mg of phenobarbital/tablet (daily intake of 24 mg of phenobarbital), further decreased the elimination half-life of theophylline in some subjects. The

[1] Elixophyllin, Cooper Laboratories, Parsippany, NJ 07054 (lot number unavailable).
[2] Marax, lot 45340, Roerig Division, Pfizer, New York, N.Y.
[3] Tedral, lot 0459P034A, Warner-Chilcott Division, Warner-Lambert Co., Morris Plains, N.J.

Table I—Comparison of Serum Pharmacokinetic Parameters among Four Different Theophylline Treatments

Parameter	Acute Elixir	Chronic Elixir	Chronic Tablet I	Chronic Tablet II
Peak serum concentration, μg/ml[a–d]	3.84 ± 0.86[e]	7.08 ± 2.52	5.40 ± 1.41	5.14 ± 1.25
Time to peak, hr[a,d]	1.03 ± 0.65	0.79 ± 0.40	1.22 ± 0.72	1.17 ± 0.73
Absorption lag time, hr[f]	0.19 ± 0.30	0.02 ± 0.06	0.25 ± 0.23	0.17 ± 0.28
Elimination half-life, hr[g]	4.62 ± 1.25	6.30 ± 1.81	5.84 ± 2.62	4.69 ± 2.89
		$p < 0.05$		
AUC, μg hr/ml[b,g,h]	29.4 ± 9.7	41.0 ± 14.2	31.2 ± 9.7	28.3 ± 12.1
		$p < 0.05$		
			$p < 0.05$	
(*AUC*)(*K*), μg/ml[b,d,i]	4.50 ± 1.18	4.94 ± 2.72	4.36 ± 1.97	5.76 ± 4.03

[a] Only the parameters from chronically administered formulations were compared by the analysis of variance. [b] A logarithmic transformation was performed prior to the analysis of variance as suggested by Westlake (17). [c] Corrected to a body weight of 70 kg. [d] No significant difference among products by the analysis of variance. [e] Mean ± *SD*. [f] Due to nonnormal distribution, the means cannot be tested for statistical difference. [g] Comparison by Newman–Keuls range test. [h] *AUC* from either time 0 → ∞ or over one dosing interval. [i] *AUC* corrected for variation in the elimination rate constant.

half-lives of three subjects were reduced to less than 2 hr. The mean for the group was 4.69 hr. In three subjects, the theophylline half-life clearly increased after chronic treatment with elixir and remained elevated throughout the study. In the other subjects, however, either chronic treatment with elixir did not result in a definite increase in half-life or further treatment with Tablets I and II tended to normalize that increase.

The magnitude of change in the elimination half-life between treatments was far greater than the intrasubject or intertreatment variation noted in the preceding acute study (3), where the maximum observed change was no more than ±30% in most subjects.

The group variance in elimination half-life gradually increased during the various treatments. For example, the coefficient of variation for the mean of the half-lives increased by more than twofold, from 27% after an acute dose of elixir to 62% after chronic treatment with Tablet II. When tested for homogeneity, the variance of the elimination half-life during administration of Tablet II was significantly different ($p < 0.05$) than that during the other three treatments. Consequently, the half-life data after Tablet II cannot be included in an analysis of variance of the half-life means. The mean theophylline serum elimination half-lives after acute elixir, chronic elixir, and Tablet I were significantly different ($p < 0.05$). According to the Newman–Keuls test, only the half-life of elimination of theophylline after chronic administration of elixir was significantly longer than that after an acute dose of elixir ($p < 0.05$) (Table I).

The time course of mean serum theophylline concentration following the four dosage treatments is depicted in Fig. 2. The extent of variation in the *AUC* with different treatments is illustrated by a comparison of the predicted and observed average serum theophylline concentrations, $\bar{C}_{ss}$, in Fig. 3. On the average, the *AUC* increased approximately 40% after 10 days of treatment with the elixir when compared to that following the first dose (Table I). Despite substantial changes in the half-life, in most of the 12 volunteers the AUC_τ or $\bar{C}_{ss}$ following the tablet formulations was comparable to the AUC_∞ after an acute dose of elixir.

Westlake (17) suggested use of the logarithm of the *AUC* instead of the untransformed *AUC* in an analysis of variance since the intrasubject variation in the serum clearance of the drug can then be treated as an additive effect due to the subject. When analyzed following transformation, a significant difference ($p < 0.05$) between the mean *AUC* for the four treatments was found (Table I). According to the Newman–Keuls test, the *AUC* after chronic administration of elixir was larger than that after both the acute dose of elixir ($p < 0.05$) and chronic administration of Tablet I ($p < 0.05$).

The increase in *AUC* after chronic treatment with elixir may largely be attributed to the increase in the elimination half-life. Wagner (18) suggested that when analyzing *AUC* data from a comparative bioavailability study, a large intersubject variation in elimination can be corrected by multiplying each subject's *AUC* by the first-order elimination rate constant. When the *AUC* was corrected for the treatment variation in elimination rate and further converted by logarithmic transformation, a statistically significant difference between means was no longer observed ($p > 0.05$).

The absorption rate of theophylline from the elixir and the two combination tablets was reasonably rapid. The mean time to peak was 1.03, 0.791, 1.22, and 1.17 hr for acute and chronic elixir and Tablets I and II, respectively (Table I). There was no significant difference in the time to peak for the chronically administered formulations when tested by the analysis of variance. Neither was there any difference in the peak concentration of theophylline. In some subjects, an absorption lag time from approximately 10 min to as much as 1 hr had to be assigned to obtain a good data fit. These lag times may reflect a real delay in absorption because of gastric emptying of the drug.

DISCUSSION

The present attempt to assess the relative steady-state bioavailability of the three oral theophylline preparations was complicated by the

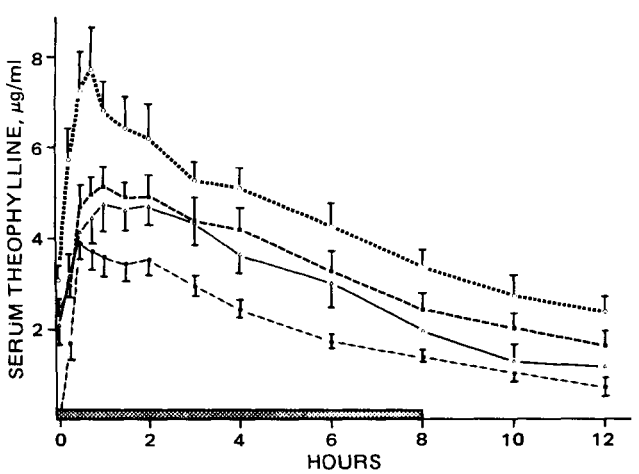

Figure 2—*Serum theophylline concentration after a single administration of 130 mg of theophylline as an elixir (●) and after repetitive administration of the same dose of theophylline in the form of the elixir (○) and commercial Tablets I (■) and II (△). Each point represents the mean and standard error of 12 observations. The individual serum concentrations were normalized to a 70-kg body weight.*

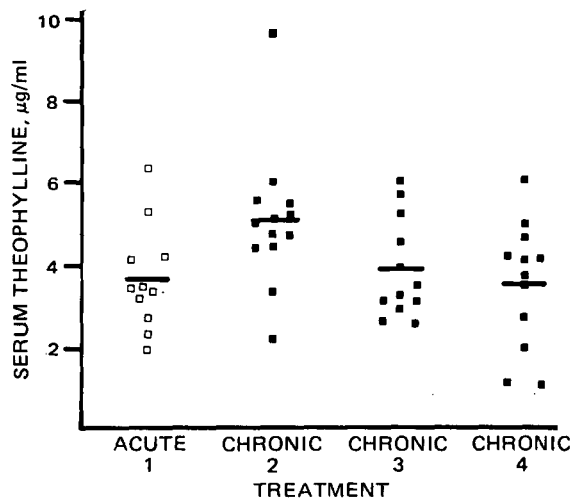

Figure 3—*Average steady-state serum concentration, $\bar{C}_{ss}$, of theophylline during chronic administration of the elixir and commercial Tablets I and II compared to that predicted based on an acute study of the elixir in the 12 volunteers. The bar represents the mean steady-state serum concentration of each treatment. Key: same as Fig. 1.*

marked variation in the elimination half-life of the drug between treatments. However, when individual *AUC* data were corrected for the treatment-dependent change in the elimination half-life, the difference between mean *AUC* values was no longer observed. This result is consistent with the previous single-dose study (3) with the same lots of theophylline products; that study showed that there was no significant difference in relative bioavailability between the elixir and the two theophylline combination tablet formulations. Furthermore, the absorption of theophylline from both the hydroalcoholic solution and tablet preparations was essentially complete when compared to intravenous aminophylline (3).

Although the bioavailability of the three tested products does not appear to be a clinically significant concern in theophylline therapy, the changes in the elimination half-life of the drug observed during the chronic study definitely warrant further investigation. There is no ready explanation for the prolongation in half-life following the 10-day administration of elixir, which was accompanied by an increase in AUC_τ or $\overline{C}_{ss}$. Ingredients in the elixir could have caused an inhibition of theophylline metabolism. The elixir contained 20% (v/v) alcohol and an undisclosed amount of palatable aromatic base (19) for flavoring.

Although inhibition of hepatic microsomal metabolism of drugs has been reported following acute treatment with alcohol (20), the amount of alcohol ingested in each elixir dose (approximately 4 g) was far less than that used in published alcohol–drug interaction studies. Furthermore, long-term administration of alcohol often leads to an opposite effect on drug metabolism, that of microsomal enzyme induction (21). Inhibition of theophylline metabolism because of the alcohol in the elixir preparation is, therefore, an unlikely possibility.

The increase in half-life during repetitive dosing may be a result of nonlinearity in the elimination kinetics of the drug, due perhaps to either self-inhibition or saturation of theophylline metabolism. Although nonlinearity in theophylline elimination kinetics has never been reported, neither has any dose or level dependency study. It may be argued that nonlinearity is an unlikely possibility since similar prolongation in half-life did not occur with other treatments. However, the nonlinearity in theophylline elimination could have been masked by concurrent induction of theophylline metabolism. The mean elimination half-life after both Tablets I and II showed a gradual return to a value comparable to that observed after the initial administration of the elixir. In fact, in a few individuals the elimination half-life was reduced below the baseline value, suggesting that some induction of theophylline metabolism may have occurred.

Theophylline metabolism by rat liver slices was accelerated by pretreatment with phenobarbital and 3-methylcholanthrene (7). On the other hand, pretreatment of male rats with high doses of methylxanthines including theophylline increased hepatic aniline hydroxylase, *p*-nitroanisole demethylase, and aminopyrine demethylase activity (7, 22). Even though a statistically valid comparison of the elimination half-life between treatments with Tablet II and acute elixir cannot be made because of nonhomogeneity of the group variance, the drastic reduction in five of the 12 volunteers strongly suggests that, in certain susceptible individuals, induction of theophylline does occur due to the intake of phenobarbital, hydroxyzine, ephedrine, or a combination thereof. Alternatively, chronic exposure to the drug itself over the three 10-day treatment periods may have resulted in induction.

Concomitant treatment with phenobarbital previously (23) did not cause a statistically significant change in either the half-life or serum clearance of theophylline in 12 male, nonsmoker, healthy volunteers. Induction of theophylline metabolism by smoking, in contrast, is well documented (5, 6) and led to the suggestion (24) that theophylline may be largely metabolized by the cytochrome P-448 system. This system is induced primarily by polycyclic aromatic hydrocarbons and polychlorinated biphenyls.

In a study (4) correlating the urinary excretion of the three major metabolites (3-methylxanthine, 1,3-dimethyluric acid, and 1-methyluric acid) with the serum theophylline concentration, it was concluded that the formation of 3-methylxanthine in humans is the dominant metabolic pathway controlling the serum theophylline concentration. Therefore, it would be of interest to investigate the effect of chronic administration of theophylline on its metabolism, particularly the 1-demethylation to 3-methylxanthine pathway.

In conclusion, since large intersubject variation in the serum theophylline concentration was reported (25) in 83 patients undergoing chronic oral aminophylline therapy, a number of studies attempted to elucidate the underlying factors responsible for this variation (4–6, 23, 24, 26, 27). Although external influences such as differences in dietary and smoking habits are important, individual differences in response to prolonged treatment with theophylline itself or other concurrent drugs may also be a contributing factor.

REFERENCES

(1) H. C. Shirkey, *J. Pediat.*, **76,** 774 (1970).

(2) "Task Force on Prescription Drugs: Final Report," U.S. Department of Health, Education, and Welfare, Washington, D.C., Feb. 7, 1969.

(3) M. Fixley, D. D. Shen, and D. L. Azarnoff, *Am. Rev. Resp. Dis.*, **115,** 955 (1977).

(4) J. W. Jenne, H. T. Nagasawa, and R. D. Thompson, *Clin. Pharmacol. Ther.*, **19,** 375 (1976).

(5) J. Jenne, H. Nagasawa, R. McHugh, F. MacDonald, and E. Wyze, *Life Sci.*, **17,** 195 (1975).

(6) S. N. Hunt, W. J. Jusko, and A. M. Yurchak, *Clin. Pharmacol. Ther.*, **19,** 546 (1976).

(7) S. M. Lohmann and R. P. Miech, *J. Pharmacol. Exp. Ther.*, **196,** 213 (1976).

(8) D. A. Sitar, K. M. Piafsky, R. E. Rangno, and R. I. Ogilvie, *Clin. Chem.*, **21,** 1774 (1975).

(9) J. Kowblansky, B. M. Scheintal, G. D. Cravello, and L. Chafetz, *J. Chromatogr.*, **76,** 467 (1973).

(10) P. A. Mitenko and R. I. Ogilvie, *Clin. Pharmacol. Ther.*, **14,** 509 (1973).

(11) M. Gibaldi and D. Perrier, "Pharmacokinetics," 1st ed., Dekker, New York, N.Y., 1975, p. 33.

(12) C. M. Metzler, Technical Report 7292/69/7293/005, The Upjohn Co., Kalamazoo, Mich., 1969.

(13) L. Z. Benet, *J. Pharm. Sci.*, **61,** 536 (1972).

(14) J. G. Wagner, *Nature*, **207,** 1301 (1965).

(15) B. J. Winer, "Statistical Principles in Experimental Design," 2nd ed., McGraw-Hill, New York, N.Y., 1962, p. 261.

(16) W. J. Dixon and F. J. Massey, Jr., "Introduction to Statistical Analysis," 3rd ed., McGraw-Hill, New York, N.Y., 1969, p. 308.

(17) W. J. Westlake, *J. Pharm. Sci.*, **62,** 1579 (1973).

(18) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Hamilton, Hamilton, Ill., 1975, p. 348.

(19) "Physicians' Desk Reference," 30th ed., Litton Industries, Oradell, N.J., 1976, p. 736.

(20) E. Rubin, H. Gang, P. S. Misra, and C. S. Lieber, *Am. J. Med.*, **49,** 801 (1970).

(21) E. Rubin and C. S. Lieber, *Science*, **172,** 1097 (1971).

(22) H. U. Aeschbacher and H. P. Wurzer, *Toxicol. Appl. Pharmacol.*, **33,** 575 (1975).

(23) K. M. Piafsky, D. S. Sitar, and R. I. Ogilvie, *Clin. Res.*, **23,** 610A (1975).

(24) A. P. Alvares, K. E. Anderson, A. H. Conney, and A. Kappas, *Proc. Natl. Acad. Sci. USA*, **73,** 2501 (1976).

(25) J. W. Jenne, E. Wyze, and F. S. Rood, *Clin. Pharmacol. Ther.*, **13,** 349 (1972).

(26) A. Kappas, K. E. Anderson, A. H. Conney, and A. P. Alvares, *ibid.*, **20,** 643 (1976).

(27) P. G. Welling, L. L. Lyons, W. A. Craig, and G. A. Trochta, *Clin. Pharmacol. Ther.*, **17,** 475 (1975).

ACKNOWLEDGMENTS

Supported by Grant GM 15956 from the U.S. Public Health Service and a grant from Pfizer Laboratories.