Factors Affecting Theophylline Clearances: Age, Tobacco, Marijuana, Cirrhosis, Congestive Heart Failure, Obesity, Oral Contraceptives, Benzodiazepines, Barbiturates, and Ethanol

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Abstract □ Pharmacokinetic, pathophysiologic, and historic data were systematically collected while monitoring theophylline therapy in adult pulmonary patients and from special studies in pediatric patients, normal volunteers, and patients with other diseases. Total body clearances (Cl_T) were estimated by nonlinear computer analysis after infusion dosage or from area under the curve data following single oral or intravenous theophylline doses. The Cl_T values, primarily reflecting the theophylline biotransformation rate, averaged 58 (±30) ml/hr/kg and ranged from 4 to 143 ml/hr/kg. Factors examined for their effect on theophylline Cl_T included age; sex; liver disease; congestive heart failure; obesity; renal function; history of drug, tobacco, marijuana, caffeine, or alcohol use; and pregnancy. The NYBAID (automatic interaction detector) computer program for analysis of variance was employed to determine the order, priority, and combinations of independent variables correlating with Cl_T. The major factors that affected theophylline Cl_T in this population included age, liver disease, smoking status, and congestive heart failure. Age showed strong correlation ($r = -0.49$) with Cl_T. Much additional variability was accounted for in specific subgroups. For example, young adult (20–40 years) marijuana users exhibited highest Cl_T values (83 ± 29 ml/hr/kg) while older patients (>40 years) with liver disease had the lowest metabolism rates (22 ± 10 ml/hr/kg). In particular subject types, sex (in teenagers), oral contraceptives (in smokers), obesity (in young nonsmokers), and barbiturates also affected theophylline disposition. In contrast, several common factors such as chronic theophylline, chronic corticosteroids, caffeine, sex (in adults), and pregnancy did not alter theophylline Cl_T. This unique clinical pharmacokinetic monitoring and statistical approach to drug clearance characterization in patients permits identification of criteria in a format that may be useful for the prediction of drug disposition rates and dosage regimens.

Keyphrases □ Theophylline—metabolism, effect of age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates, ethanol, pulmonary patients □ Smooth muscle relaxants—theophylline, metabolism, effect of age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates, ethanol, pulmonary patients □ Dosage regimen—theophylline, predictive scheme, effect of various patient variables on metabolism

The drug elimination rate as reflected by the total body clearance (Cl_T) and the plasma–tissue distribution as represented by the apparent volume of distribution (V_D) are the primary clinical pharmacokinetic requirements for devising dosage regimens in patients. Reasonable success has been achieved in predicting dosage requirements for drugs whose body clearance is determined largely by renal function where the serum creatinine concentration or creatinine clearance can be used to estimate drug clearance (1, 2). In contrast, numerous genetic, environmental, pathophysiologic, and pharmacokinetic factors that affect biotransformation rates make dosages highly unpredictable for extensively metabolized drugs. Serum concentration monitoring has assisted in the clinical use of many such drugs (3, 4).

Theophylline, an extensively metabolized drug, poses clinical pharmacokinetic difficulties. This xanthine derivative is ~90% biotransformed by microsomal oxidative enzymes with the remaining 10% of the dose excreted unchanged in the urine (5). Numerous studies have demonstrated highly variable half-life and body clearance values in typical adult and pediatric patients (6–9). Many reports indicated that dosage requirements of patients are highly unpredictable and illustrated the need for serum level monitoring (6, 10, 11). The 8–20-mg/liter "window" of serum theophylline concentrations usually produces optimum bronchodilation (6, 12).

Rapid and specific theophylline assays in biological fluids using methods such as high-pressure liquid chromatography (HPLC) (13) have been helpful in patient therapy (10, 11). Serum theophylline concentration measurements are not always possible, however, and the blood collection, analytical, and interpretive procedures take time. It is important, therefore, to develop dosage guidelines for different types of patients to be used before or in the absence of the feedback information from theophylline assays (3).

This report describes common factors that determine theophylline clearance. Pharmacokinetic, pathophysiologic, and historic data were systematically collected while monitoring theophylline therapy in adult pulmonary patients and from special studies in pediatric patients, normal adult volunteers, and subjects with unique characteristics such as obesity or cirrhosis. Pharmacokinetic and statistical methods were utilized to characterize the variability in body theophylline clearances in 200 such persons.

EXPERIMENTAL

Subjects—Body theophylline clearances were determined prospectively in 100 adult inpatients at the Millard Fillmore Hospital. These patients were studied during routine medical management with theophylline administered by intravenous infusion for at least 24 hr. The dosage guidelines (nomogram) and other aspects of the clinical pharmacokinetic monitoring system were described previously (9).

Additional clinical and pharmacokinetic data were obtained from the literature for single-dose aminophylline administration to 15 pediatric patients (7), for normal late-teen or adult subjects (14–17), for 14 obese adults (18), for eight cirrhotic patients (19), and for pregnant women (20). All patients and volunteer subjects were residents of western New York state at the time of the study.

Survey Design—The body clearance, or more precisely the total serum theophylline clearance (Cl_T), was taken as the dependent variable for correlation with various factors likely to affect the drug disposition rate. This parameter was chosen because it could be generated from all

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Table I—Statistical Categories Describing Theophylline Body Clearances

Parameter (Abbreviation)	Division	Group Code	Number of Subjects	Body Clearance, ml/hr/kg IBW, mean (*SD*)
Body clearance	—	All	200	57.9 (29.8)
Age, years	<20	1	23	91.9 (28.1)
	20–39.9	2	79	64.2 (27.2)
	40–59.9	3	37	47.8 (24.8)
	>60	4	61	43.1 (23.2)
Sex	F	2	105	57.0 (24.5)
	M	1	95	58.9 (34.8)
Congestive heart failure (CHF)[a]	None	0	149	64.1 (30.1)
	Mild	1	27	46.5 (21.2)
	Moderate	2	15	34.8 (16.6)
	Severe	3	9	28.0 (13.5)
Obesity[b]	None	0	133	55.5 (28.3)
	Moderate	1	62	62.9 (33.1)
	Severe	2	5	59.7 (21.2)
Creatinine clearance (*CrCl*), ml/min/1.73 m^2	≥100	1	62	60.6 (23.8)
	50–99	2	116	60.4 (32.3)
	20–49	3	20	37.1 (24.3)
	<20	4	2	34.8
Theophylline history	None	0	117	54.3 (28.0)
	User	1	83	62.9 (31.5)
Steroid history	None	0	139	57.0 (29.6)
	User	1	61	59.9 (30.3)
Oral contraceptives (OC)	None	0	186	58.5 (30.4)
	User	1	14	49.8 (18.6)
Barbiturate history (BARBS)	None	0	175	57.9 (29.1)
	User	1	25	57.6 (34.5)
Benzodiazepine history (BENZ)	None	0	175	59.3 (29.7)
	User	1	25	48.2 (29.1)
Phenothiazine history	None	0	196	57.9 (29.7)
	User	1	4	56.2 (36.3)
Tricyclic antidepressant history	None	0	195	58.2 (29.8)
	User	1	5	44.1 (29.6)
Smoking[c] (CIG)	None	0	107	57.0 (30.4)
	Light	1	54	61.1 (29.0)
	Heavy	2	39	55.9 (29.3)
Caffeine use[d]	Slight	0	42	74.0 (30.3)
	Moderate	1	82	49.7 (29.4)
	Heavy	2	76	57.8 (26.4)
Ethanol (EtOH) and cirrhosis	None	0	66	65.1 (30.5)
	Social drinker	1	119	58.3 (27.9)
	Heavy drinker or cirrhotic	2	15	22.4 (10.5)
Marijuana use[e] (MJ)	None	0	177	56.1 (29.5)
	Light	1	9	54.1 (17.5)
	Heavy	2	14	82.9 (28.8)
Pregnancy	No	0	190	57.7 (30.3)
	Yes	1	10	61.5 (17.5)

[a] Based on criteria of Peck *et al.* (27). [b] Moderate obesity = 15–55% overweight; severe obesity = >55% overweight. [c] Light = <1 and heavy = ≥1 pack/day. [d] Slight = ≤2, moderate = 3–5, and heavy = >5 cups of coffee or tea/day; caffeine equivalents from cola were typically "slight." [e] Light = <1 and heavy = ≥2 joints/week.

serum concentration *versus* time data collected and because it is needed to determine maintenance doses (D/T) for achieving a desired steady-state serum concentration (C_p^{ss}):

$$D/T = (Cl_T)(C_p^{ss}) \quad \text{(Eq. 1)}$$

where D is the dose given at time intervals, T. To correct for body size, the Cl_T value was adjusted for lean body weight (18). The lean body weight (IBW) was estimated as the "ideal" weight based on height and bone structure (21). Weight, rather than surface area, was chosen for normalizing the data because of the limited body size range of the subjects, because theophylline clearances are conventionally expressed as milliliters per hour per kilogram (12, 15, 18), and because such Cl_T values are converted easily into a dosage regimen using Eq. 1.

Patient Surveillance—Clinical descriptive data regarding the patients were collected over a 4-year period. The survey approach was described previously (9). Similar information from the normal volunteers was usually obtained in confidence.

The factors gathered as independent variables for this analysis are listed in Table I. Each patient parameter was chosen on the basis of its generally known influence on drug metabolism or because of its potential for affecting theophylline disposition. Only factors capable of being obtained by examination (physical and history) of a patient upon hospital admission or by rapid automated analyzer analysis (serum biochemistry profile) were included. This approach was chosen to yield useful information for designing future dosage regimens for typical patients.

The relationship of age to theophylline clearance was examined because previous studies revealed differences in theophylline disposition between adolescents and adults (7) and because elimination of various drugs is slower in older patients (22–24). Sex was included because of known differences between genders in metabolism of drugs such as antipyrine (23, 25). Congestive heart failure was examined because of the reduction in hepatic clearance that accompanies this disease (26). The severity of congestive heart failure was ranked as absent (0) or graded 1–3, using criteria described by Peck *et al.* (27).

Obesity was examined because physiological changes occur in obese subjects, including increased cardiac output and hepatic blood flow (28). Also, considerable uncertainty has existed regarding use of lean rather than total body weight as a basis for drug dosage regimens (18). Creatinine clearance was included because ~10% of a theophylline dose usually is excreted by the kidneys (5). The creatinine clearance was estimated from the serum creatinine concentration, lean body weight, sex, and age of each patient using the nomogram of Siersbaek-Nielsen *et al.* (29). Creatinine data were not available for the 15 pediatric patients. Normal age-related values were used for these subjects, none of whom had kidney disease.

The patient history was completed by questioning the patient (or guardian) or by consulting the patient's chart or physician. Previous theophylline use was examined because the drug induces its own metabolism in animals (30). Steroid history was included because corticosteroids accelerate the biotransformation of many drugs (31, 32) and have been posed as an interaction factor with theophylline (33). In contrast, oral contraceptives may decrease drug metabolism rates (34) and were noted as a separate variable.

Four other drug history categories were included: barbiturates, because of their well-known enzyme inductive effects (35, 36) and their possible effect on theophylline disposition (37); benzodiazepines, because of their common use in patients and their potential for altering drug biotransformation (38); and phenothiazines and tricyclic antidepressants for similar reasons (39, 40). Other potentially interacting drugs such as erythromycin (41) and troleandomycin (42) were not observed.

Social drug habits studied including smoking because of its well-established effect on drug disposition in general (43) and on theophylline in particular (14, 44, 45). Marijuana use was included for identical reasons (17). Caffeine consumption (number of cups of coffee or tea per day) was quantitated in recognition of the weak inductive effect of caffeine (46) and catechin (47) on drug metabolism in animals and because of the recent observation of an effect of dietary methylxanthine intake on theobromine disposition (48).

Ethanol consumption was included because small quantities of alcohol can stimulate oxidative drug biotransformation pathways, while large amounts may either diminish drug metabolism acutely or cause liver disease (49, 50). In fact, patients with cirrhosis and those consuming large quantities of ethanol were grouped together because of the latter likelihood and the earlier finding of markedly impaired theophylline disposition in cirrhotics (19, 51).

Pregnancy was of interest because the biotransformation of some drugs is enhanced during pregnancy (52) and because asthmatic, pregnant women often experience difficulties in breathing.

Other patient characteristics also were recorded but were not included in this analysis because they either occurred infrequently or not at all. Also, many facts were not recorded because they were not recognized as interaction factors at the time of this study. Acute pulmonary edema (53), thyroid dysfunction (54), low arterial PO_2 (55), acute steroid administration (31), marked dietary changes (56), recent ingestion of charcoaled foods (57), and respiratory viral illness (58) may alter the microsomal oxidation of drugs such as theophylline. Subjects were Caucasian except for two persons from India and Iran and six Negroes. Genetic differences related to race were not examined. Surveillance data included a serum biochemistry profile (SMAC P7), which was largely used to confirm certain disease categories (*e.g.*, liver dysfunction).

Drug Assay—Theophylline concentrations in serum were measured by HPLC (13) in most patients and volunteers and by spectrophotometric assay (59) in the pediatric patients (7) and in some normal adult subjects (15). Good agreement has been found between the two assay methods (13).

Pharmacokinetics—For subjects who received a single oral solution or intravenous dose of theophylline, the body clearance was calculated from:

$$Cl_T = \text{dose/area} \qquad \text{(Eq. 2)}$$

where area represents the area under the serum concentration *versus* time curve extrapolated to time infinity. Because theophylline poses no known GI absorption problems, especially when given in solution (60), and because its low Cl_T should cause little "first-pass" hepatic metabolism, complete systemic bioavailability of oral drug doses was assumed.

Most pulmonary patients received theophylline by continuous intravenous infusion, usually with administration of a loading dose or following previous therapy with the drug. The time course (t) of serum theophylline concentrations (C_p) was characterized by:

$$C_p = (C_p^0)\, e^{(-Cl_T)(t)/V_D} + \frac{k_0}{Cl_T}\left[1 - e^{(-Cl_T)(t)/V_D}\right] \qquad \text{(Eq. 3)}$$

where C_p^0 is the initial serum concentration, V_D is the apparent volume of distribution, and k_0 is the drug infusion rate. Blood samples were generally collected at 1, 12, and 24 hr and often at later times. It was assumed that V_D = 0.45 liter/kg of ideal body weight for each patient, and the 1-hr C_p value was used as C_p^0. The NONLIN computer program (61) generated the least-squares fitted Cl_T value. The suitability of this method for calculating theophylline Cl_T was demonstrated previously (9).

Equation 3 reduces to $C_p^{ss} = k_0/Cl_T$ as the steady state (C_p^{ss}) is approached or attained. This equation is model, volume, and time independent, which accounts for the extremely good Cl_T estimates for most patients who were close to steady state after 24 hr of loading and infusion therapy.

Statistics—Statistical procedures used to assess the relationship of theophylline Cl_T to the selected patient parameters included linear regression analysis (62, 63) and the NYBAID computer program (64). This program quantitates the variance associated with the dependent variable by assessing the order, priority, and combinations of independent variables that allow maximum statistical discrimination between groups. Information fed to the program involved the Cl_T value as a continuous, dependent variable along with 17 independent variables. As shown in Table I, it was necessary to partition each independent variable into coded integers. Selection of the number of subgroups for each variable was based either on obvious discriminating factors (*e.g.*, sex and user/nonuser) or on the ability to quantitate differences based on conventional criteria (*e.g.*, congestive heart failure and smoking).

The largely noncontinuous, nominal, and nonlinear nature of the patient characteristics precluded effective use of multiple linear regression analysis but allowed advantageous employment of the NYBAID program. The program subdivides the Cl_T sample through a series of binary splits into a mutually exclusive series of subgroups with the branching process based on variance analysis techniques. The possibility of interaction between variables at different stages is handled by reintroducing factors whose simple effects already have been reviewed but which may also mediate the effects of factors at a later stage. The linearity and additivity assumptions inherent in conventional multiple regression techniques are not required.

Program control includes reducibility criteria for splitting groups ($p < 0.01$), minimum size of groups before and after splitting (no minima used), and weights (all data were weighted = 1). Subgroups are produced based on a primary test of reduction of the unexplained sums of squares rather than on differences between subgroups as assessed by conventional t tests. Several patients were studied on more than one occasion, and their data were included each time to self-weight such multiple observations and to account for clinical changes occurring between these times.

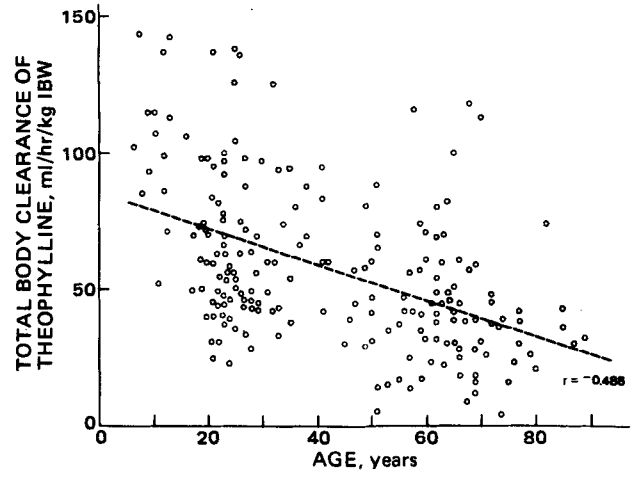

Figure 1—*Total body clearances of theophylline in relation to age of 200 patients and normal volunteers who received oral or intravenous theophylline. The regression line depicts age dependence of theophylline biotransformation in spite of the great variability in clearances.*

RESULTS AND DISCUSSION

Body Clearances—Half of the pharmacokinetic data for this survey were obtained while monitoring theophylline therapy in hospital patients. Dosing guidelines (65) provided an intravenous loading dose (short-term infusion) based on body weight with a dosage reduction if recent theophylline administration had occurred. The maintenance infusion rate was based on body weight and on three levels of anticipated biotransformation capability. An initial serum concentration (C_p^0) was achieved at 1 hr after the loading dose. Serum concentrations thereafter attained or progressed toward a steady state. Least-squares fitting using Eq. 3 generated the designated Cl_T values and their standard deviations (SD). In most cases, the SD was relatively small, indicative of a close fit of the equation to the experimental data (9).

The Cl_T values in relation to the age of 200 subjects are summarized in Fig. 1. The major characteristic of theophylline disposition is the pronounced variability in Cl_T values over all age groups. The Cl_T averaged 58 (SD = 30) ml/hr/kg and ranged from 4 to 143 ml/hr/kg. However, the trend ($r = -0.488$) for Cl_T to decrease with age was apparent and statistically significant ($p < 0.01$). The figure also shows the wide age range of subjects, with a generous distribution of persons within each

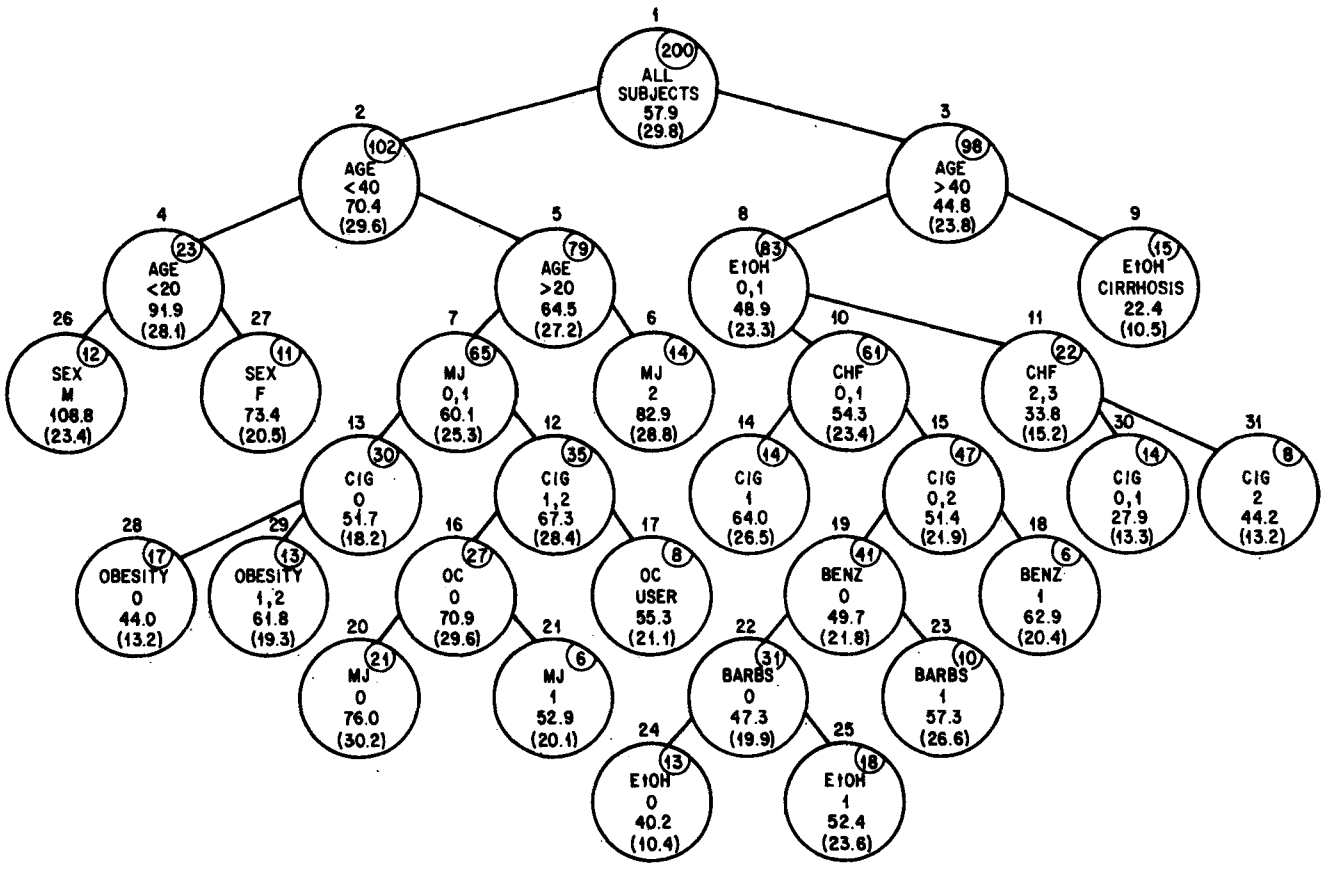

Figure 2—*Cascade of factors determining theophylline total body clearances. The NYBAID statistical computer program was used to seek the order, priority, and combinations of independent variables (Table I) that correlate with theophylline clearances. Each partition represents a statistical difference of* $p < 0.01$, *and the circles list the number of subjects (circled), descriptive factor, group mean, and standard deviation (in parentheses).*

decade past childhood. Because of this finding and the extraction of data from patients actually undergoing therapy, the information is reasonably representative of the typical cross-section of persons requiring theophylline.

Two additional patients with extraordinarily high Cl_T values (195 and 217 ml/hr/kg) were identified and excluded from this and all other statistical analyses. These patients were outside the general characteristics of this population (*i.e.*, were outliers), and inclusion of their variances distorted the analysis. Acute high-dose steroid therapy (rather than chronic steroid use), a factor that accelerates antipyrine metabolism (31), may account for the high Cl_T value in one female patient. The other patient was a male heavy smoker with severe renal impairment and hypertension who had been receiving a variety of drugs including phenobarbital, diazepam, propranolol, reserpine, and methyldopa.

Patient Variables—The NYBAID computer program was employed to examine the patient variables listed in Table I. This table provides the division of variables into subgroups with the number of patients, mean Cl_T, and *SD* for each subgroup. The study population characteristics can be best appreciated from this summary. For example, many patients were over 60 years old, which is typical of an adult hospital patient population. There were nearly equal numbers of male and female subjects. Fifty-one patients had some degree of congestive heart failure, and 15 patients had liver disease (cirrhosis). Only two patients had very poor renal function.

Obesity was a frequent occurrence, although only five patients were grossly overweight (>55%). Many persons were chronically receiving theophylline, corticosteroids, oral contraceptives, and various tranquilizers. Ninety-three subjects were smokers including 45 of the 100 adult patients with pulmonary disease. All marijuana smokers were young, normal adults. Many persons consumed moderate to appreciable quantities of caffeinated drinks and ethanol.

The mean values in Table I are of interest in examining the patient subgroups representing each factor. Parameters that had the strongest influence on theophylline disposition can be identified by inspection. For example, young age, congestive heart failure, liver disease, and marijuana use stand out. On the other hand, because of drug–disease interactions and diverse patient characteristics, the role of many important factors determining theophylline Cl_T can be discerned only after further patient data become available.

Analysis of Variance—*Age*—The cascade of patient variables associated with differences in theophylline Cl_T values among 200 persons is shown in Fig. 2. Complementing the regression analysis, the data were initially partitioned according to age with 40 years as the demarcating age. As will be demonstrated by the types of patients found further down the cascade (a lower Cl_T in cell 10 than in cell 5), this age difference may be a dual effect of aging and of a greater incidence of hepatic dysfunction, cirrhosis, and congestive heart failure among the older group. In the under 40-year group, a second split based on age separated the adolescent/teenage subjects from the young adults.

These data, while partly derived from the prospective study by Ellis *et al.* (7), further confirm the observation that children metabolize theophylline more rapidly than do adults. This enhanced theophylline biotransformation extends down to the 1–4-year range as well. Loughnan *et al.* (66) found a mean Cl_T of 100 (±36) ml/hr/kg in such younger children.

Gender—A new finding in the young subjects, 6–19 years old, is an apparent gender difference in Cl_T. These data (Fig. 3) indicate that young males metabolize theophylline more rapidly than do females. Reexamination of the data of Leung *et al.* (67) for children 6–16 years old lends support to this observation. They listed Cl_T values averaging 89.8 (34.3) ml/hr/kg in 19 males and 58.8 (17.8) ml/hr/kg in 11 females. On the other hand, the original data of Ellis *et al.* (7) and of Loughnan *et al.* (66) suggest no gender differences in theophylline Cl_T. This possible sex effect on theophylline biotransformation does not extend into adults; Powell

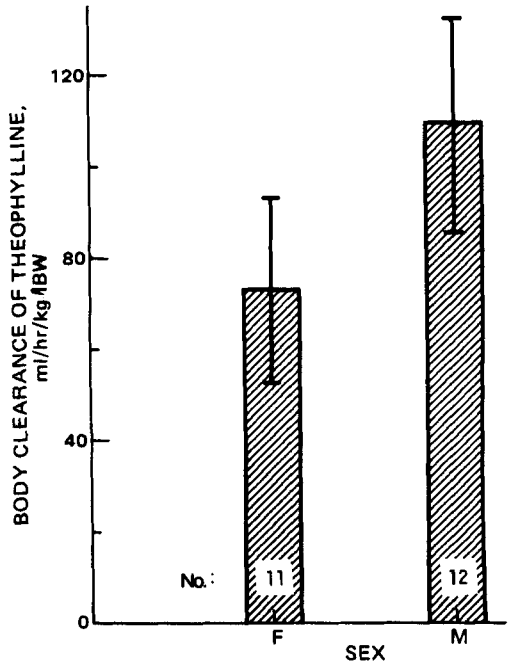

Figure 3—*Effect of sex on total body clearances of theophylline in adolescents and teenagers. This and subsequent graphs show mean values as bars, standard deviations as vertical lines, and numbers of subjects within bars. Subject groups correspond to cells 26 and 27 in Fig. 2.*

et al. (45) previously observed no differences in Cl_T in young adults, and our remaining cascade data (Fig. 2) provided no further gender discrimination.

Smoking—The major factors affecting theophylline disposition in young adults are tobacco and marijuana smoking. This factor emerges largely because an appreciable number of the subjects in the 20–39.9 year class were relatively healthy persons with no overt hepatic or cardiac diseases (17). Use of social drugs (tobacco, alcohol, marijuana, and caffeine), tranquilizers (benzodiazepines), and oral contraceptives was common in this group and provided the only unique characteristics for examination by the NYBAID program. Many asthmatic patients were included in the 79 persons in this age range, and the data probably are highly representative of young adults who need theophylline therapy.

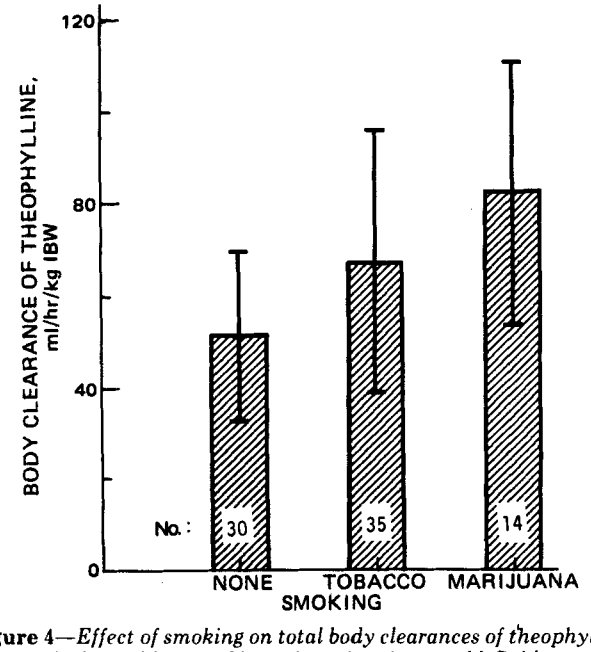

Figure 4—*Effect of smoking on total body clearances of theophylline in normal adult subjects and in patients 20–40 years old. Subject groups correspond to cells 13, 12, and 6, respectively, in Fig. 2.*

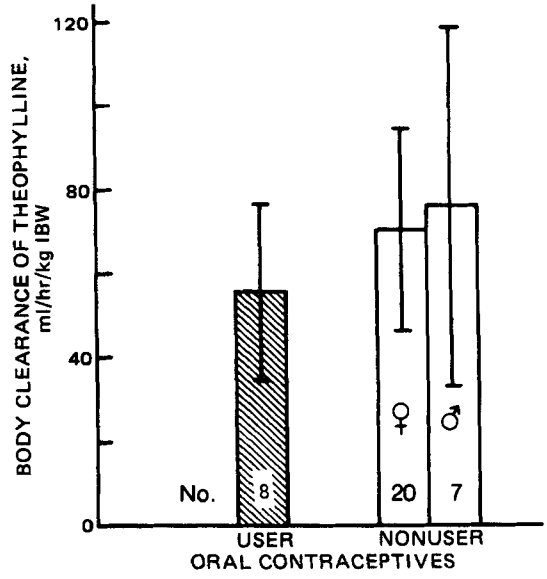

Figure 5—*Effect of oral contraceptives on total body clearances of theophylline in adult smokers. Subject groups correspond to cells 17 and 16, respectively, with the latter partitioned further according to sex.*

Figure 4 shows the differences in Cl_T among young adults in relation to smoking habits. The enhanced theophylline Cl_T in both marijuana and tobacco smokers extends and confirms previous findings (14, 17). This phenomenon is probably due to induction by polycyclic aromatic hydrocarbons present in smoking materials (43) as well as in charcoaled foods (57). The use of marijuana or a combination of marijuana and tobacco produced the largest clearances observed in young adults (83 ± 29 ml/hr/kg). Other effects of smoking on theophylline Cl_T will be described in subsequent sections.

Oral Contraceptives—An interesting finding (Fig. 2) is that the effect of oral contraceptives on theophylline Cl_T was obtained only in smokers. The use of oral contraceptives was associated with a diminished Cl_T (55 ± 21 ml/hr/kg in users *versus* 71 ± 30 ml/hr/kg in nonusers). This result is, in part, consistent with data of Homeida *et al.* (34) who found a lower antipyrine clearance in young women during prolonged oral contraceptive therapy. Since the oral contraceptive nonusers included both males and females, the data were further examined by splitting Group 16 (Fig. 2) according to sex (Fig. 5). This approach confirmed the lack of gender difference in Cl_T at this branch of the cascade. The metabolic interaction of smoking and oral contraceptives is intriguing in view of mounting evidence for other associations of these two factors, such as an increased incidence of circulatory disorders and mortality risk (68, 69).

Liver Disease—The most profound determinant of theophylline Cl_T in persons >40 years of age is the presence of liver impairment, expressed as either clinically diagnosed cirrhosis or inferred from heavy use of ethanol and abnormal serum biochemistry. Patients with the latter two factors (Fig. 6) exhibited a mean Cl_T of only 22.4 (10.5) ml/hr/kg, the smallest grouped value in the entire cascade. This result includes and extends the previous study of eight cirrhotics (19) and further confirms the findings of Piafsky *et al.* (51) who also found extremely low Cl_T values in most patients with cirrhosis. There was an additional difference in Cl_T lower in the cascade between persons who used ethanol socially and those who abstained, as will be described.

Congestive Heart Failure—The presence of congestive heart failure was the second major factor accounting for decreased theophylline clearance in older adults (Figs. 2 and 7). Mild congestive heart failure apparently was without effect, since these patients were grouped with noncongestive heart failure subjects with a mean Cl_T of 54 (23) ml/hr/kg. Patients with moderate and severe congestive heart failure were combined and exhibited a Cl_T of 34 (15) ml/hr/kg, a substantial difference from the remaining patients.

Congestive heart failure may induce hepatic congestion with structural and functional derangements. In addition, the clearance of drugs that are highly dependent on liver blood flow is usually diminished (26). Decreased theophylline clearance was found in nine of the 10 reported patients with left-sided heart failure (53, 70), but the effects of right heart failure are less clear. Fourteen of our patients with congestive heart failure

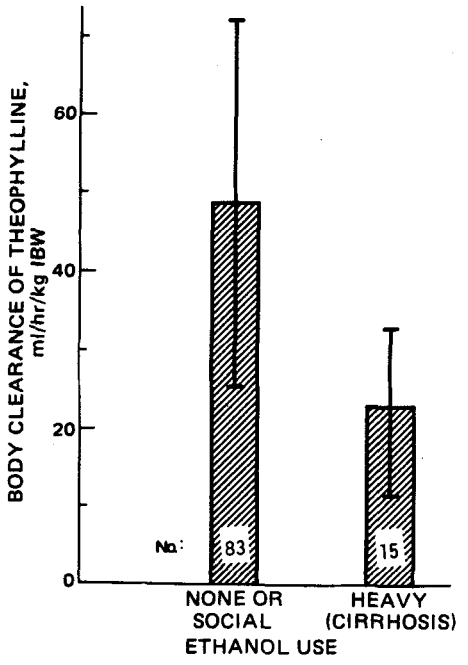

Figure 6—*Effect of heavy ethanol use and/or hepatic cirrhosis on body clearances of theophylline in adult subjects >40 years of age. Subject groups correspond to cells 8 and 9 in Fig. 2.*

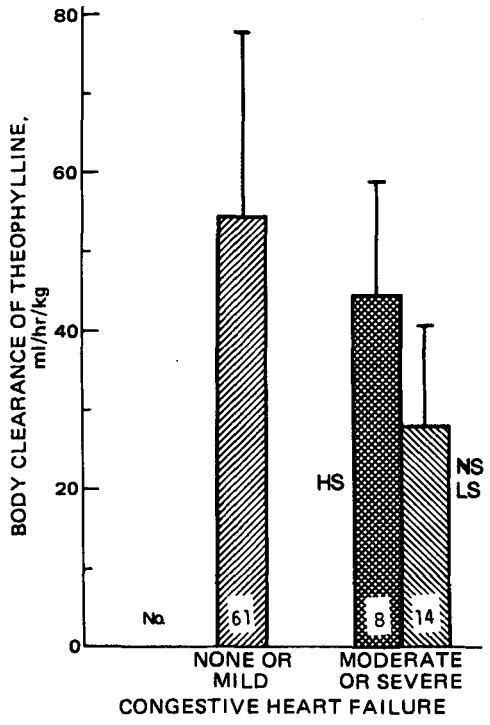

Figure 7—*Effect of congestive heart failure (CHF) and smoking on body clearances of theophylline in older adult patients (>40 years) without liver impairment. Congestive heart failure diminishes theophylline clearance, but heavy smokers (HS) with congestive heart failure exhibit larger clearances than nonsmokers (NS) or light smokers (LS). Subject groups correspond to cells 10, 30, and 31, respectively, in Fig. 2.*

(including two with liver disease) were previously found to exhibit a mean Cl_T of 31.8 (14.8) ml/hr/kg (65). In the overall surveillance, the presence of congestive heart failure was usually indicated by history and physical findings including edema and elevated central venous pressure. The gradation of the disease was characterized according to the criteria of Peck *et al.* (27). However, these groups should be accepted only as approximate since the criteria were applied by the clinical pharmacokineticist in concert with the house staff or consulting physician and were not unbiased scores solicited from a "blinded" cardiologist. Nevertheless, the congestive heart failure diagnosis was obtained without knowledge of the patient's theophylline Cl_T.

An interesting finding was the interaction between congestive heart failure and smoking (Fig. 7). Heavy smoking appeared to offset the diminished Cl_T found in moderate to severe congestive heart failure. This is the first observation of an effect of smoking superimposed on major organ dysfunction. The ability to discern this type of drug–disease interaction is a unique advantage of the NYBAID approach.

Obesity—An effect of obesity on theophylline disposition is seen in the younger subjects (<40 years) on the cascade (Fig. 2). The overweight subjects tended to have larger clearances than the lean persons. Since this finding disagrees somewhat with previous results (18), the data were assessed more rigorously using a continuous regression analysis. Figure 8 depicts the Cl_T values (normalized for ideal body weight in relation to the ratio of total to ideal body weight. Both the correlation coefficient ($r = 0.476$) and the regression slope (0.168) were statistically significant ($p < 0.025$). Thus, a trend for increased Cl_T with obesity occurs in a select group of young adults who are nonsmokers.

In contrast, previous investigation of theophylline disposition in 57 normal and 14 obese subjects yielded essentially identical Cl_T values when normalized for ideal body weight in this way (18). The disparity between the two studies may be explained in noting that the data were previously assessed without separating possible confounding factors. A similar gross evaluation of mean data (Table I) reveals no differences among lean, moderately obese, and severely obese persons. The small increase in Cl_T in uncomplicated obesity is consistent with physiological expectations, because severely obese persons have been shown to have increased cardiac output, resulting in a small increase in hepatic blood flow (28). Moreover, it was previously found (18) that the apparent volume of distribution of theophylline corresponds to total body weight rather than to ideal weight, suggesting that theophylline readily distributes into body fat.

Benzodiazepine, Barbiturate, and Ethanol Use—A splitting of subgroups that is awkward to explain is shown as Groups 14 and 15 in Fig. 2. Older adults without significant liver or cardiac impairment were split on the basis of smoking, but the non- and heavy smokers were merged. This result may be a statistical artifact or suggest a lack of a dose–effect relationship for the role of smoking in older subjects, or unclassified secondary factors such as disease or drug use may overwhelm the primary effect in one subgroup. This anomaly would cast some suspicion on the remaining elements of the cascade, except that they are comprised of expected drug interaction effects.

An observation that emerges in older subjects once the effects of liver disease and congestive heart failure are separated is an effect of chronic benzodiazepine, barbiturate, and ethanol use on theophylline Cl_T. As shown in Figs. 2 and 9, users of these compounds exhibited larger Cl_T values than nonusers. These groups were comprised almost entirely of pulmonary patients (most of the normal volunteers were <40 years old), and these data thus provide the first indication that these enzyme inducers may alter theophylline disposition under conventional clinical conditions.

The barbiturate effect is consistent with recent observations of Landay

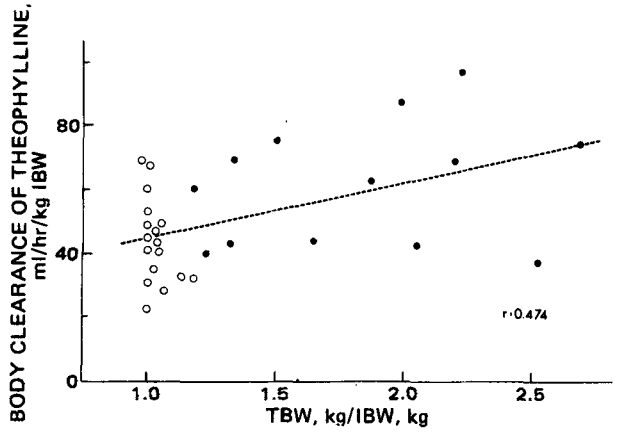

Figure 8—*Effect of obesity on body clearances of theophylline in adult nonsmoking subjects 20–40 years of age. Key: ○, cell 28 in Fig. 2; and ●, cell 29 in Fig. 2.*

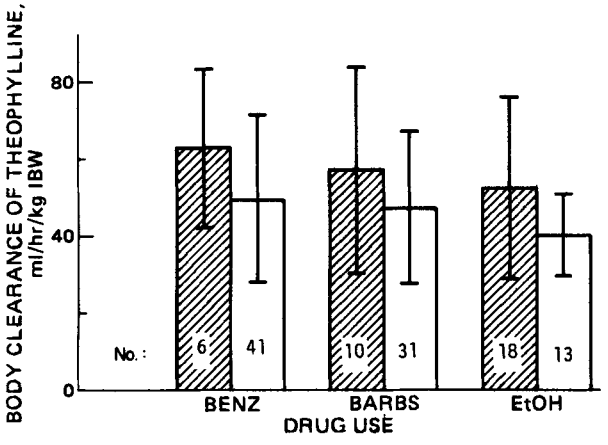

Figure 9—*Effect of use of benzodiazepines (BENZ), barbiturates (BARBS), and social ethanol (EtOH) on body clearances of theophylline in patients >40 years of age without liver or cardiac disease.*

et al. (37) who found a mean increase in theophylline Cl_T of 34% in six healthy subjects following 4 weeks of phenobarbital administration. In contrast, Piafsky *et al.* (71) found no such effect after briefer phenobarbital treatment in healthy adults. Microsomal enzymes for theophylline metabolism in the rat also are weakly susceptible to barbiturate induction, but, as in humans, are more strongly affected by polycyclic aromatic hydrocarbons (30). While theophylline is largely a substrate for the P-448 microsomal enzymes, phenobarbital is capable also of altering liver size and hepatic blood flow and may thereby indirectly affect the apparent theophylline clearance by a nonenzymatic mechanism (36).

The effects of the benzodiazepines and ethanol (social use) are similar to those of the barbiturates. These effects are consistent with known drug interaction mechanisms (38, 50). However, the small number of benzodiazepine users diminishes the credibility of this finding; an additional study is needed to substantiate both effects.

Other Factors—The NYBAID program provided a further breakdown of patient characteristics into many additional groups. However, the analysis was terminated with 31 cells (Fig. 2) because the number of subjects per group was becoming small and statistically less reliable. In fact, the authors of the NYBAID program advise against splitting cells containing fewer than 25 observations unless there is an *a priori* hypothesis to be tested (64). However, these subsequent splittings provided the opportunity to analyze the final groupings with regard to the major parameters listed in Table I. This approach ruled out any underlying effects of the dominating factors affecting theophylline Cl_T in a portion of the subgroup. In addition, a histogram of the residuals in the NYBAID analysis was constructed to assess whether the unaccounted variance may have a nonnormal distribution. The residuals clearly showed a Gaussian distribution, suggesting that a random array of factors (rather than a pharmacogenetic source, for instance) produce the remaining variability in the data.

Several factors existed in numerous patients but failed to emerge as important determinants of theophylline disposition. These factors include sex in adults and chronic use of theophylline, corticosteroids, and caffeine (Table I). Also, no effects of pregnancy were found. It is not appropriate to rule out any effects of phenothiazines, tricyclic antidepressants, severe renal impairment, or oral contraceptives in nonsmokers at this time because too few patients were encountered for these categories.

COMMENTARY

The clinical pharmacokinetic surveillance of patients provides the opportunity to collect data for evaluating basic assumptions about pharmacokinetics and for identifying useful criteria for the dosage regimen design (3, 72). One rationale underlying such an approach to digoxin pharmacokinetics was described by Sheiner *et al.* (73). These investigators included factors such as serum digoxin concentration, digoxin dosage history, body weight, sex, serum creatinine concentration, and thyroid function in developing a computerized system for interpreting serum digoxin concentrations in patients and predicting digoxin dosages. Digoxin is primarily eliminated by renal excretion, which simplifies optimum dosage prediction (2). The characterization of biotransformation rate determinants has remained a more formidable task because of the myriad factors affecting liver function and the lack of a good marker for the hepatic metabolism rate.

Surveillance Limitations—There are several limitations to this analysis. Not all factors that may modify theophylline disposition were included. For example, marked dietary changes (56, 57), respiratory viral infections (58), and immaturity (74, 75) have been identified as affecting theophylline biotransformation and were not included. The Cl_T may change with clinical status (67), possibly increasing with resolution of acute bronchial obstruction; our data usually represented only one to three 24-hr periods (typically at the onset of therapy).

Some diseases that may affect theophylline biotransformation such as thyroid disorders, which were sought, were not encountered frequently enough to be included in the current analysis. Genetic determinants of theophylline disposition (race, twin, or family data) were neither obtained nor considered, partly because of the predominance of Caucasian subjects and partly because genetic data are not likely to be available in newly admitted patients in a manner useful for drug metabolism rate estimation. Finally, the possibility of dose dependence or nonlinearity in theophylline pharmacokinetics could not be tested (76–78). This aspect of theophylline disposition requires administration of at least two dosage levels in a subset of patients. These inaccuracies, coupled with the analytical and curve-fitting errors associated with estimating the Cl_T values and the qualitative nature of most of the factor or disease categories, contribute to the large variances remaining in the metabolic cascade.

If a correlation is made between the 16 peripheral group mean values (using the outer cells as "predicted" clearances) and the individual clearances for the 200 subjects, the resulting correlation coefficient is 0.720. Thus, 51.8% ($r^2 \times 100$) of the variance in the data is accounted for. An enlargement of the number of factors, subgroups, and patients, more precise Cl_T values, and better linear quantitation of the factor subgroups would be required to improve this analysis. However, even this degree of predictability should be helpful for clinical purposes where dosage regimens must be devised to fit a wide therapeutic range (8–20 mg/liter) rather than to attain a specific serum concentration (65).

The factors identified as important in theophylline body clearances are associations found by retrospective statistical analysis which need not imply a cause-and-effect relationship, especially where a pathophysiologic or drug interaction rationale does not exist. Often these factors need further confirmation by prospective examination of cohorts of subjects with the disease or history in question. This approach may be almost impossible for many uncommon factors under the restrictions that the subjects also be patients with pulmonary dysfunction undergoing therapy with theophylline. However, several of the isolated determinants of theophylline disposition—youth (7), tobacco use (14), marijuana (17), cirrhosis (19), congestive heart failure (65), and barbiturate use (36)—have already evolved, and the NYBAID program recovered these factors in a realistic configuration in spite of the confounding effect of being merged with an array of additional data and variables. This analysis confirmed the role of these effects in actual patients, and this observation lends greater credibility to the new associations that emerged on evaluation of theophylline clearances.

Dosage Guidelines—The NYBAID computer program is generally available as a library program. This facilitates its use by other investigators, although it has had little use in the biomedical sciences to date. The grouping of patient variables affecting theophylline Cl_T by the NYBAID analysis is both attractive and practical. The results of the statistical analysis are provided in a format (Fig. 2) directly useful to any practicing clinician since it does not require access to a computer. We only included factors known or presumed to affect the biotransformation of theophylline or other durgs and that could be assessed easily and rapidly from most patients.

Figure 2 can be greatly simplified and adapted for use as a dosing nomogram by clinicians who need only to assign patients to the proper Cl_T categories and aim for an appropriate steady-state serum concentration. The theophylline maintenance dose can be obtained using Eq. 1 with a target serum concentration of 14 mg/liter (6, 12):

$$D/\mathrm{T}\ (\mathrm{mg/hr}) = [Cl_T\ (\mathrm{ml/hr/kg})][\mathrm{IBW}\ (\mathrm{kg})][0.014\ (\mathrm{mg/ml})] \quad \text{(Eq. 4)}$$

where IBW is the lean body weight. If a loading dose is desired, it should be the commonly recommended dose of 5.6 mg of aminophylline/kg (or 4.8 mg of theophylline/kg) administered orally or intravenously over a 20-min interval (78).

Employment of Fig. 2 in construction of a dosing nomogram should be with the reservations that most cells contain appreciable variance, that

many of the drug/disease/history/physiologic associations remain to be proven in a prospective clinical trial, and that many potential factors affecting theophylline disposition remain to be added to the metabolic cascade as described in the previous section.

Pharmacokinetic Monitoring—The advantage of the NYBAID analysis in clinical pharmacokinetics is the ability to examine the interrelationships between patient variables commonly expected to affect the drug biotransformation rate in patients undergoing medical therapy. Pharmacokinetic guidelines for drug therapy are often based on studies in reasonably normal subjects which may only approximate the dosage needs of distressed patients. Most studies of factors altering drug metabolism take place under ideal conditions in groups of subjects who are preselected on the basis of having a specific disease. Medical patients, on the other hand, are encountered with a variety of pathophysiologic problems, their drug and environmental histories are highly diversified, and any expectations of biotransformation rates have been fraught with considerable uncertainty.

This study demonstrates one approach to resolving several clinical pharmacokinetic problems simultaneously. Serum concentration monitoring aids in managing the immediate therapeutic needs of the patient, the appropriate timing in blood specimen collection assists in anticipating the pharmacokinetic behavior of the drug, and the retrospective correlation of a reasonable array of patient variables with the pharmacokinetics confirms or enlarges the knowledge of factors determining drug disposition rates while providing guidelines for future clinical drug use.

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N-Benzoyl Derivatives of Amino Acids and Amino Acid Analogs as Growth Inhibitors in Microbial Antitumor Screen

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Abstract □ Twenty-seven *N*-benzoyl derivatives of amino acids and amino acid analogs were prepared and tested for growth-inhibitory activity in a microbial antitumor screen. Of these, 19 showed some inhibitory capacity, from a modest 13% to a potent 96% at 1 mg/ml. The activities of the "modest" inhibitors were comparable to those of most inhibitory chloroacetyl and trifluoroacetyl derivatives reported earlier. The intermediate inhibitors were as active as *N*-chloroacetyl-β-hydroxy-D-norleucine isomer B, the most active acyl derivative noted previously. The most active compounds in this study were *N*-benzoyl-*p*-chloro-DL-phenylalanine and *N*-benzoyl-*m*-fluoro-DL-phenylalanine, which inhibited the test organism almost completely under the assay conditions.

Keyphrases □ *N*-Benzoyl amino acid derivatives—antineoplastic activity, potential, inhibition of microbial antitumor screen, structure–activity relationships □ Amino acid derivatives—*N*-benzoyl, potential antineoplastic activity, inhibition of microbial antitumor screen, structure–activity relationships □ Antineoplastic agents, potential—*N*-benzoyl amino acid derivatives, inhibition of microbial antitumor screen, structure–activity relationships □ Structure–activity relationships—*N*-benzoyl amino acid derivatives, inhibition of microbial antitumor screen

Studies of possible antimetabolic effects of novel β-hydroxyamino acids produced evidence that an otherwise inert amino acid could, upon *N*-chloroacetylation, actively inhibit growth of a microbial antitumor screen (1). This study was extended to include the *N*-chloroacetyl (2) as well as the *N*-acetyl (2), *N*-propionyl (2), and *N*-trifluoroacetyl (3) derivatives of other amino acids, both natural and unnatural. Both the amino acid and the *N*-acyl moieties of the acyl amino acid were important in imparting the growth-inhibitory characteristic to the compound, since only certain amino acids and certain acyl groups were capable of doing so (2).

One deterrent to using amino acid analogs as antimetabolites in mammalian systems is their high toxicity (4–7). In view of the finding that there is an alteration in biological properties, especially cytotoxicity, upon *N*-acylation (2), more potent inhibitors might be prepared by attachment of acyl groups other than those studied previously. Since a more lipophilic acyl group might increase the mobility of these compounds in a lipoid milieu such as the cellular membranes in mammalian systems, a benzoyl derivative series was prepared. These compounds were tested for growth inhibition in a microbial system selected specifically as an antitumor screen. Although the ultimate objective of these studies is to find compounds for cancer therapy, the immediate aim has been to improve the growth-inhibitory properties of the compounds in the screen described earlier.

The present paper reports the results of these studies.

EXPERIMENTAL

The free amino acids except the β-hydroxynorleucines, which were prepared in this laboratory (8, 9), were obtained commercially and were recrystallized from water–ethanol before use. Some *N*-benzoyl derivatives were obtained commercially and were recrystallized from ethanol–water before use. Others, especially those of amino acid analogs, were prepared by the Schotten–Baumann procedure (10) and were recrystallized from ethanol or ethanol–water. The sources of the amino acids and of the benzoyl derivatives are shown in Table I.

The purity of the amino acids and of the benzoyl derivatives was ascertained by: (*a*) Van Slyke nitrous acid determination of primary amino nitrogen (11), (*b*) optical rotation measurement, where applicable, and (*c*) elemental analysis. In addition, the purity of the free amino acids was checked by paper chromatography in at least four different solvent systems (8), and that of the benzoyl derivatives was checked by melting-point determination (Table I).

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