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Title: BIOAVAILABILITY AND PHARMACOKINETICS OF DYPHYLLINE AND THEOPHYLLINE IN RABBITS

Abstract approved: Redacted for Privacy

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Part I. Theophylline

The bioavailability and pharmacokinetics of theophylline in rabbits were determined following both oral and intravenous administration of the drug. Serum theophylline concentrations were determined using high pressure liquid chromatography. Pharmacokinetic parameters were determined and verified by computer analysis. The overall mean Beta disposition constant was 0.089 hr^{-1} which corresponds to a total body half-life of 7.79 hours. Results are compared with previously published results in humans.

In vitro dissolution studies of a prolonged release product showed rapid dissolution in simulated intestinal fluid and very poor dissolution in simulated gastric fluid. The same product was completely absorbed in rabbits. Rabbits are shown to be useful experimental animals for testing bioavailability of orally administered theophylline.

Pharmacokinetic parameters obtained in rabbits correlate well with those reported in humans.

Part II. Dyphylline

Rabbits were administered intravenous dyphylline and commercially available dyphylline tablets. Serum dyphylline concentrations were determined using high pressure liquid chromatography. Computer analysis of serum concentrations determined pharmacokinetic parameters. Total body elimination half-life of dyphylline in rabbits was 3.89 hours. Published data for dyphylline studies in humans were analyzed by computer fitting the reported serum concentration data and compared to values obtained in this study. Computer analysis and linear regression showed that the reported elimination half-life of dyphylline in humans may be an underestimate of the true value.

Multiple dosing studies in rabbits showed a poor correlation of values obtained in this study with computer predicted values. The loss of hematocrit due to frequent blood sampling is offered as a possible explanation. It is proposed that dyphylline enters or binds to erythrocytes similar to theophylline. Thus the decreased hematocrit should allow for a larger serum concentration value than predicted as was seen in this study.

In vitro dissolution and *in vivo* availability studies showed that the dyphylline tablets studied were rapidly

dissolved, rapidly absorbed, and completely available. Rabbits are shown to be useful experimental animals for bioavailability and pharmacokinetic studies involving dyphylline.

Bioavailability and Pharmacokinetics of Dyphylline and Theophylline in Rabbits

by

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PART I. THEOPHYLLINE

INTRODUCTION

Theophylline (1,3-dimethylxanthine) is an alkaloid closely related to caffeine and theobromine. Its pharmacological actions include mild diuresis, myocardial stimulation, smooth muscle relaxation and bronchodilation [1]. The main clinical use of theophylline has been in the treatment of chronic reversible obstructive airway diseases such as bronchial asthma. It appears to act by competitive inhibition of the enzyme phosphodiesterase, which results in the delayed breakdown of 3'5' cyclic AMP (adenosine monophosphate) leading to an accumulation of this agent in intracellular spaces. This mechanism is thought to be the pathway leading to relaxation of bronchial smooth muscles, either directly or indirectly [2].

Toxic effects of theophylline are related to serum levels, which include insomnia, tremors, anorexia, vomiting, tachycardia, and other undesirable effects [2]. These toxic effects often preclude its use. Prolonged release dosage forms have been marketed for theophylline, but dissolution and absorption studies on these products are not available. Wide variations (3.0 hours to 9.5 hours) have been reported for elimination half life ($t_{1/2}$) values [3] for theophylline.

These variations are usually the result of inter-subject hepatic metabolism differences since theophylline is about 85 percent metabolized [3].

Biopharmaceutic and pharmacokinetic studies can be quite time consuming and expensive when done in human subjects. Considerable time and cost could be saved if an adequate experimental animal could be used instead of humans. Several articles have recently appeared in the literature describing some pharmacokinetic values for theophylline in humans [3,4,5]. With this in mind, the objectives of this study were: (a) to obtain information on the pharmacokinetics of theophylline in rabbits; (b) to determine if the pharmacokinetic parameters for theophylline in rabbits could be correlated with published data obtained from studies in humans, and (c) to determine the in vitro dissolution characteristics of some prolonged release theophylline products and correlate the in vitro data with absorption in rabbits.

EXPERIMENTAL

In Vitro Dissolution Studies--Dissolution studies for an oral product[1] were completed in simulated gastric fluid and simulated intestinal fluid at 37.5°C. The gastric fluid contained 2.0 g NaCl and 18.9 ml 37% HCl brought to a total volume of 1000 ml with distilled water. The intestinal fluid contained 8.05 gm Na_2HPO_4 and 1.65 gm NaH_2PO_4 brought to a total of 1000 ml with distilled water. Both simulated gastric and intestinal fluids were enzyme free. An ultraviolet spectroscopic analysis with a standard Beers law curve was used to quantify the dissolved theophylline. All solutions were allowed to equilibrate at 37.5°C prior to use.

Materials--Analytical grade chemicals were used without further purification. Methanol was U.S.P. grade. All water was distilled prior to use.

Pharmacology--All experimental work was performed using female New Zealand White rabbits except for two rabbits when females were not available. There was no detectable difference between values obtained in the two males when compared to females. Aminophylline[2] was administered intravenously (130 mg/5.2 ml) as a continuous infusion over a period of 20-25 minutes. Oral preparations of theophylline were

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[1] Aerolate SR., 260 mg, Fleming & Co., Fenton, MO.

[2] Aminophylline, 250 mg/10 ml, (lot number 1-998), G.D. Searle & Co., Chicago, IL.

administered as prolonged release products[3], by both single and multiple (every 12 hours) dosing schedules. Food, but not water, was withheld 12 hours prior to dosing with oral preparations. Six milliliter blood samples were collected at predetermined time intervals (see Tables I, III, and IV) *via* a cannula located in the external jugular vein. An equal volume of normal saline was injected as volume replacement. Surgery and placement of the cannula were performed as previously conducted in this laboratory. Silastic cannule were implanted into the rabbit's left external jugular vein while under anesthesia with ethyl ether. In those cases when the cannula was malfunctioning the rabbit was anesthetized with ethyl ether and blood samples were obtained *via* direct cardiac puncture.

Blood samples were centrifuged (550 x G for 20 minutes), the plasma was separated and filtered *via* molecular filtration[4] to remove the protein. An aliquot of the filtrate was combined with glyceryl guaiacolate (Guafenesin) solution (449.6 mg/l) which was the internal standard. The final solution which was analyzed contained 90 percent plasma and 10 percent internal standard solution.

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[3]Aerolate SR., 260 mg, (lot number 6014250), and Aerolate JR., 130 mg (lot number 6014265), Fleming & Co., Fenton, MO.

[4]13 mm Pellicon Molecular Filters (PTGCO 1310, 10,000 nominal molecular weight), Millipore Corp., Bedford, MA.

Analytical Method--Plasma samples containing theophylline were quantified using a high pressure liquid chromatograph[5] (HPLC) with a reverse phase column[6], a UV-visual spectrophotometric detector[7], and a dual pen recorder[8]. The solvent system consisted of methanol (13%) and sodium acid phosphate buffer (0.92% $NaH_2PO_4.H_2O$, pH 5) (87%). The injection volume was 0.4 ml., the solvent flow 3.5 ml per minute, and theophylline was detected at 274 nm. These conditions allowed for satisfactory separation of theophylline and glycerol guiacolate. The amount of theophylline in plasma samples was quantified using a standard curve prepared as indicated below.

Standard Curve--Untreated rabbit plasma was collected and known amounts of theophylline were added (0.75, 1, 2, 5, 10, 20, 30, and 50 µg/ml) as well as internal standard. These samples were anayzed by HPLC for theophylline content. The ratio of the height of the theophylline peak to the height of the internal standard was determined for each of

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[5]Waters Model 201, ALC/GPC equipped with two M-6000 pumps, a Model U-6K injection valve, and a Model 660 programmer, Waters Associates, Inc., Milford, MA.

[6]Corasil C-18, Waters Associates, Inc., Milford, MA.

[7]Varian, Model 635 UV-Visible spectrometer, Palo Alto, CA.

[8]Soltec Co., Encino, CA.

the above drug concentrations. Each standard solution was prepared separately and analyzed four to seven times. Linear regression techniques were used to determine the standard curve. The curve was linear over the range studied (0.75 - 50 μg/ml) and was highly significant ($r = 0.99$).

RESULTS AND DISCUSSION

Data for plasma level (P) determinations of theophylline following intravenous (iv) administration of 130 mg of aminophylline are presented in Table I. Figure 1 shows the corresponding mean serum concentration vs. time curve. The theophylline curve appears to be biphasic and enter a post distribution phase about two hours following administration. The results from the iv administration are compatible with the standard pharmacokinetic two compartment open model (see Figure 2) which also predicts a biphasic shape for a plot of the logarithm of drug concentration in plasma vs. time. The biexponential equation (Eq. 1) [6]:

$$P = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

results from the model and describes the data quite well (weighted mean square error = 0.002) as determined by computer fitting[9] of the data for iv administration (Table 1). The computer generated equation for iv aminophylline is:

$$P = 13.50e^{-2.26t} + 35.93e^{-0.089t}$$

Serum concentration data can be used to generate pharmacokinetic parameters such as elimination half-life ($t_{1/2}$), rate constants for distribution (k_{21}, k_{12}), apparent volume of distribution (Vd), etc. Pharmacokinetic parameters for

[9] SIMPLEX program, Billie Chou, Oregon State University, Computer Center, Corvallis, OR 97331.

Table I - Serum Concentrations of Theophylline (µg/ml) Following Intravenous Administration of 130 mg of Aminophylline

Rabbit Number	Weight (Kg)	Time (hours) 0.25	0.50	0.75	1.0	2.0	3.0	4.0	6.0
48	2.90	46.26	35.88	34.32	32.53	28.46	24.22	19.67	12.14
50	3.00	40.26	38.03	35.38	32.93	31.78	24.38	19.79	10.06
51	2.80	42.24	39.64	a/	23.84	26.96	30.31	24.42	22.77
64	2.50	43.95	40.67	39.50	38.64	-	40.59	33.92	32.93
78	2.20	38.91	37.98	35.95	36.01	29.65	31.04	28.17	31.39
80	2.50	43.87	41.29	41.59	33.33	35.66	22.10	18.18	18.85
Means	2.65	42.58	38.92	37.35	32.88	30.50	28.77	24.02	21.36
± SEM	0.12	1.10	0.82	1.37	2.04	1.51	2.78	2.50	3.90

a/ sample not determined

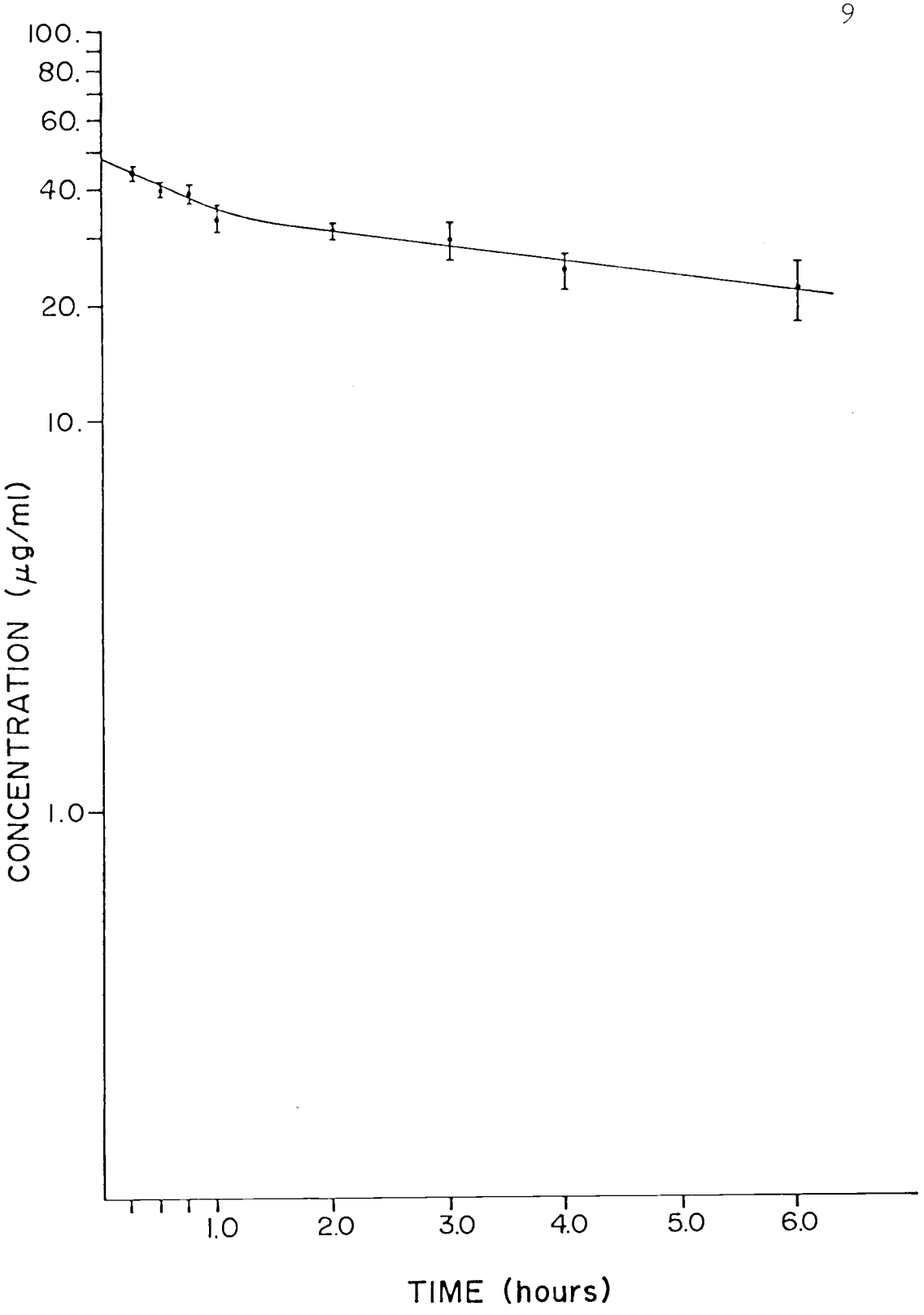

Figure 1 - Mean serum concentration of theophylline vs. time following iv administration of 130 mg of aminophylline. ± SEM

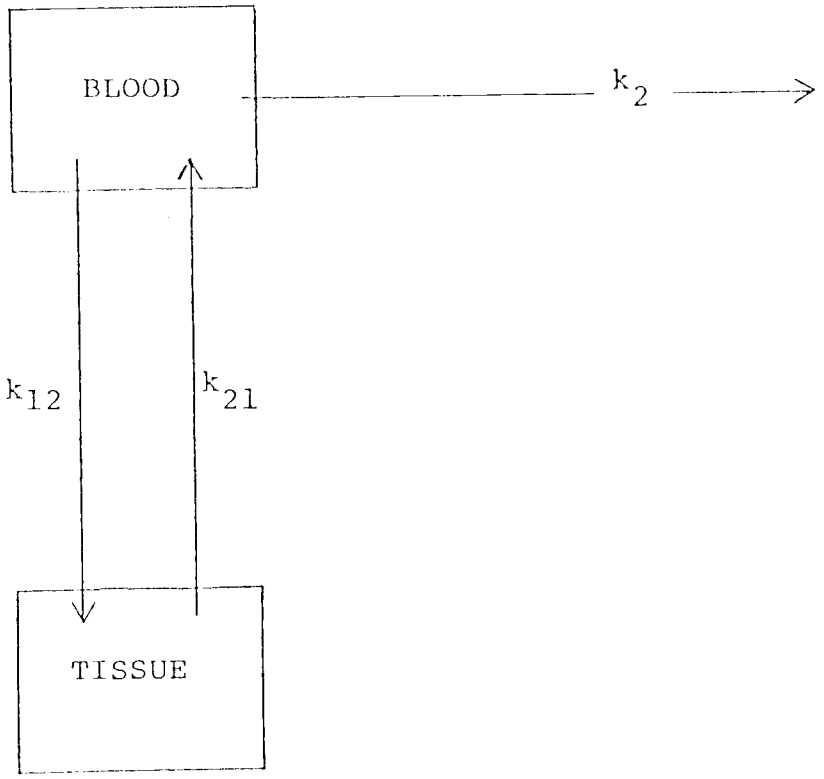

Figure 2 - A two-compartment open model. Rate constants for drug transfer between blood and tissue compartments are represented by k_{12} and k_{21}. k_2 represents the rate constant for removal of drug from blood.

intravenously administered theophylline in the rabbit were determined by computer analysis of the data in Table I and are presented in Table II. Total body elimination $t_{1/2}$ for theophylline in rabbits was found to be 7.79 hours. This corresponds to a β value of 0.089 hr^{-1}. The solid line in Figure 1 was generated by using these parameters and results in an excellent fit with the points that were experimentally determined. Theophylline plasma concentrations following administration by iv injection to rabbits has been shown to be described by two compartment open model pharmacokinetics.

In an attempt to correlate the results from this study with data previously published for human subjects, a comparison of pharmacokinetic parameters was performed. Table II compares pharmacokinetic parameters determined in this study with previously published values for theophylline in humans [4,5,7,8].

The comparisons made in Table II show that a wide variety of micro-constants have been reported for theophylline in humans. It comes as no surprise then that the micro-constants from rabbit data are close to those from at least one human study [8]. It is interesting to note that the mean $t_{1/2}$ of 7.79 hours in rabbits is within the range of 3 to 9.5 hours which has been reported [3]. The similarities among pharmacokinetic parameters in rabbits and humans

Table II - Mean Pharmacokinetic Parameters for Theophylline Obtained from Computer Analysis of Data Presented in Table I and Previously Published Values

	$k_{12}(hr^{-1})$	$k_{21}(hr^{-1})$	$k_2(hr^{-1})$	$\alpha(hr^{-1})$	$\beta(hr^{-1})$	$Vd(l/k_g)$	$Vp(l/k_g)$	$t_{1/2}(hr)$ [a/]	$t_{1/2}(hr)$ [b/]
Present study	0.561	1.667	0.121	2.259	0.089	1.655	0.794	7.79	5.75
Jenne, et al.[3]	a/	-	-	-	0.134*	0.479*	-	5.20*	-
Mitenko and Ogilvie [4]	4.72*	1.99*	0.555	7.11	0.156	0.429*	0.145	4.44	1.25*
Mitenko and Ogilvie [8]	0.713	1.808	0.155	2.57*	0.109*	0.488*	0.344	6.36*	4.47*
Levy and Koysooko [7] c/	2.15	3.44	0.386	5.71	0.231	0.404	0.242*	3.0	1.80
Mitenko and Ogilvie [5]	2.713*	2.942*	0.314	5.81	0.159	0.592*	0.300	4.36	2.21

* Obtained by evaluation of reported data or results.

a/ Half-life for elimination from the body.

b/ Half-life for elimination from blood.

c/ Values reported are for children

indicates that rabbits may be a useful animal model for determining bioavailability from drug products containing theophylline.

In vitro dissolution studies were performed on a commercially available prolonged release product[1] containing 260 mg of theophylline. Results from these studies are presented in Figure 3. The results show that the product provides very little dissolution when tested in a simulated gastric fluid free of enzymes. Dissolution was rapid and complete when the product was tested in simulated intestinal fluid free of enzymes. In addition, Figure 3 shows that pre-treatment with gastric fluid for 90 minutes followed by exposure to intestinal fluid results in a lag time of about 1.5 hours followed by rapid and complete dissolution. This latter dissolution experiment was an attempt to partially simulate natural processes whereby the product would normally enter the stomach first and then, after a period of time, pass into the intestine. These results indicate that dissolution of the product appears to be pH dependent since the pH of the intestine is significantly higher than that of the stomach. Examination of the dissolution data indicates that neither a pure zero order nor a pure first order process is operative for the sustained release product, but rather a combination of the two.

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[1]Aerolate SR., 260 mg, Fleming & Co., Fenton, MO.

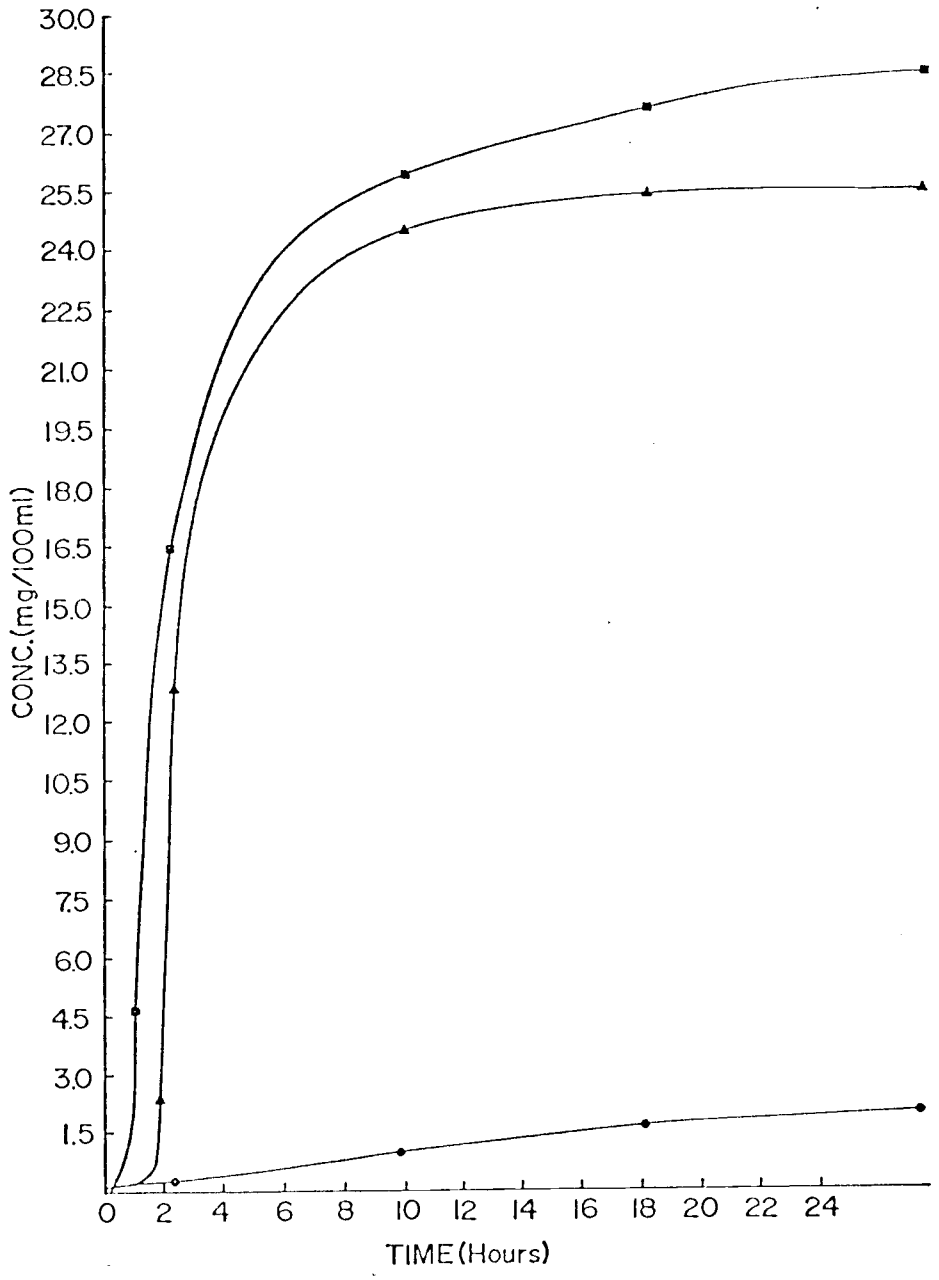


Figure 3. Dissolution of theophylline as a time release oral product.[3] Key: ●, simulated gastric fluid; ■, simulated intestinal fluid; ▲, 90 min. pretreatment with gastric fluid followed by exposure to intestinal fluid

Single dose studies of two oral prolonged release products[1,3] consisted of administering the products to the animals and collecting blood samples at predetermined times. Tables III and IV present the serum concentrations of theophylline following these single dose administrations. Figure 4 provides the corresponding mean serum concentration vs. time curves. We would expect from the dissolution results that the serum theophylline concentration (Figure 4) would begin to climb slowly after administration to about the 1.5 hour mark, then rise rapidly after this time. This would correspond well with the dissolution data (Figure 3) which showed a lag time of about 1.5 hours. Results of the serum concentration data (Tables III and IV) and the serum concentration vs. time curve (Figure 4) reveal that contrary to what was expected, moderately elevated serum levels of theophylline were obtained during the first 1.5 hours. This is probably the result of several factors. The capsules were relatively large in comparison to the animal's oral cavity and administration of the capsules usually resulted in disintegration of the exterior gelatin capsule prior to swallowing. This resulted in the tiny drug containing pellets being liberated prior to the expected time. In addition, the animals had empty stomachs since food had been withheld. The small pellets may have behaved similar to a

[1]Aerolate SR., 260 mg, Fleming & Co., Fenton, MO.

[3]Aerolate SR., 260 mg, (lot number 6014250), and Aerolate JR., 130 mg (lot number 6014265), Fleming & Co., Fenton, MO.

Table III - Serum Levels (µg/ml) Obtained Following Administration of Oral Theophylline, 260 mg, as a Time Rlease Product

Rabbit Number	Weight (Kg)	Time (Hours) 0.25	0.50	1.0	2.0	5.0	8.0
72	2.70	-a/	7.46	7.55	8.56	20.06	17.34
73	2.70	-	0.12	-	4.16	28.49	32.55
77	2.20	3.43	10.22	11.25	16.30	26.15	34.66
85	2.50	0.40	2.27	5.41	14.59	30.95	40.19
86	2.50	3.35	-	8.84	13.33	14.07	16.99
87	2.50	1.79	13.43	19.07	34.02	22.54	45.33
Means	2.52	2.24	6.70	10.43	15.16	23.71	31.18
± SEM	0.75	0.72	2.46	2.36	4.19	2.51	4.79

(continued)

a/ Sample not determined

Table III -(continued)

Rabbit Number	Time (hours)							
	10.0	12.0	14.0	16.0	20.0	24.0	30.0	36.0
72	24.58	31.85	34.23	34.75	51.59	38.70	18.69	22.04
73	37.12	43.43	29.96	36.78	34.05	28.31	11.50	5.09
77	40.32	41.25	32.70	36.15	39.07	40.73	31.31	11.02
85	31.61	35.09	29.47	17.59	13.57	15.61	2.39	1.50
86	40.90	37.73	40.19	46.79	-	27.07	9.90	5.18
87	34.89	29.47	26.69	28.90	14.84	6.75	4.79	3.10
Means	34.90	36.47	32.21	33.49	30.62	26.20	13.10	7.99
± SEM	2.50	2.20	1.92	3.96	7.29	5.36	4.32	3.10

Table IV - Serum Concentration (µg/ml) Following Administation of Oral Theophylline, 130 mg, as a Time Release Product

Rabbit Number	Weight (Kg)	Time (hours) 0.25	0.50	1.0	3.0	5.0	8.0
65	2.50	3.20	9.29	11.74	18.26	a/	18.08
66	2.50	0.73	1.02	6.46	19.58	19.40	28.71
67	2.50	-	0.22	1.67	5.21	7.91	16.95
68	2.40	3.40	6.75	9.54	14.05	19.80	24.05
69	2.40	0.40	2.07	2.31	5.37	8.42	9.94
70	2.50	2.25	9.76	11.10	12.14	13.48	11.28
Means	2.47	2.00	4.85	7.14	12.44	13.80	18.17
± SEM	0.21	0.62	1.74	1.79	2.51	2.56	2.96

(continued)

Table IV - (continued)

Rabbit Number	Time (hours) 10.0	12.0	14.0	16.0	18.0	20.0
65	19.28	21.96	28.04	40.93	27.17	32.12
66	21.38	20.84	16.10	10.10	10.25	9.06
67	19.48	18.88	17.53	20.80	26.79	18.71
68	20.80	26.00	23.63	30.81	26.79	27.16
69	9.22	10.14	6.87	11.08	12.47	10.37
70	17.56	16.38	20.48	19.25	25.19	24.85
Means	17.95	19.03	18.77	22.16	21.44	20.38
± SEM	1.83	2.21	2.96	4.85	3.21	3.81

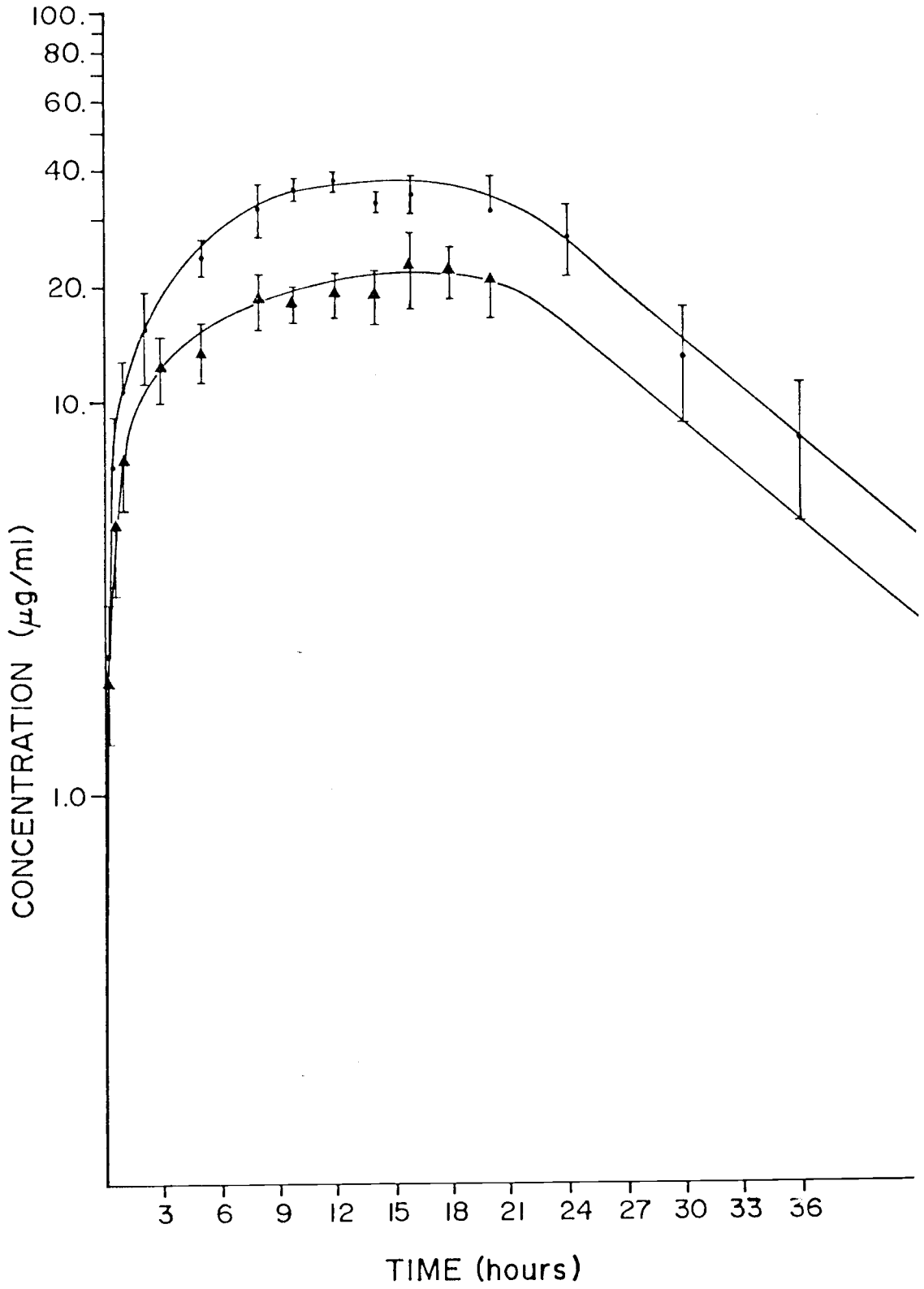


Figure 4 - Mean serum concentration of theophylline vs. time following oral administration of two prolonged release products. Key: ●, 260 mg; ▲, 130 mg. ± SEM

fluid and probably passed through the stomach unhindered. Upon entering the intestinal tract dissolution (due to higher pH) took place and absorption quickly followed. This would explain the results obtained. The 260 mg dose peaks between 10 and 12 hours after administration and remains at high levels until about 24 hours when the concentration begins to drop off at a rapid rate. Similar results occur following the 130 mg dose. This experiment was conducted for a shorter time period than the 260 mg study and the declining phase has been extended beyond the experimental data points as explained below. Absorption of theophylline occurred sooner than expected and sustained serum concentrations were produced following oral administration of the prolonged release products investigated.

The total amount of drug absorbed from the sustained release oral products can be calculated from the area under the mean serum concentration vs. time curves (AUC) from $t = 0$ to $t = \infty$ when compared with the AUC for iv administration of aminophylline (aminophylline is 80 percent theophylline). Comparison assumes 100 percent absorption for the iv dose. Unfortunately, the results of administering the prolonged-action products in the single dose study do not cover a sufficient time period for absolute determination that the absorption phase was complete. However, it is likely that absorption will be complete within 20 hours and was assumed to be complete at the time

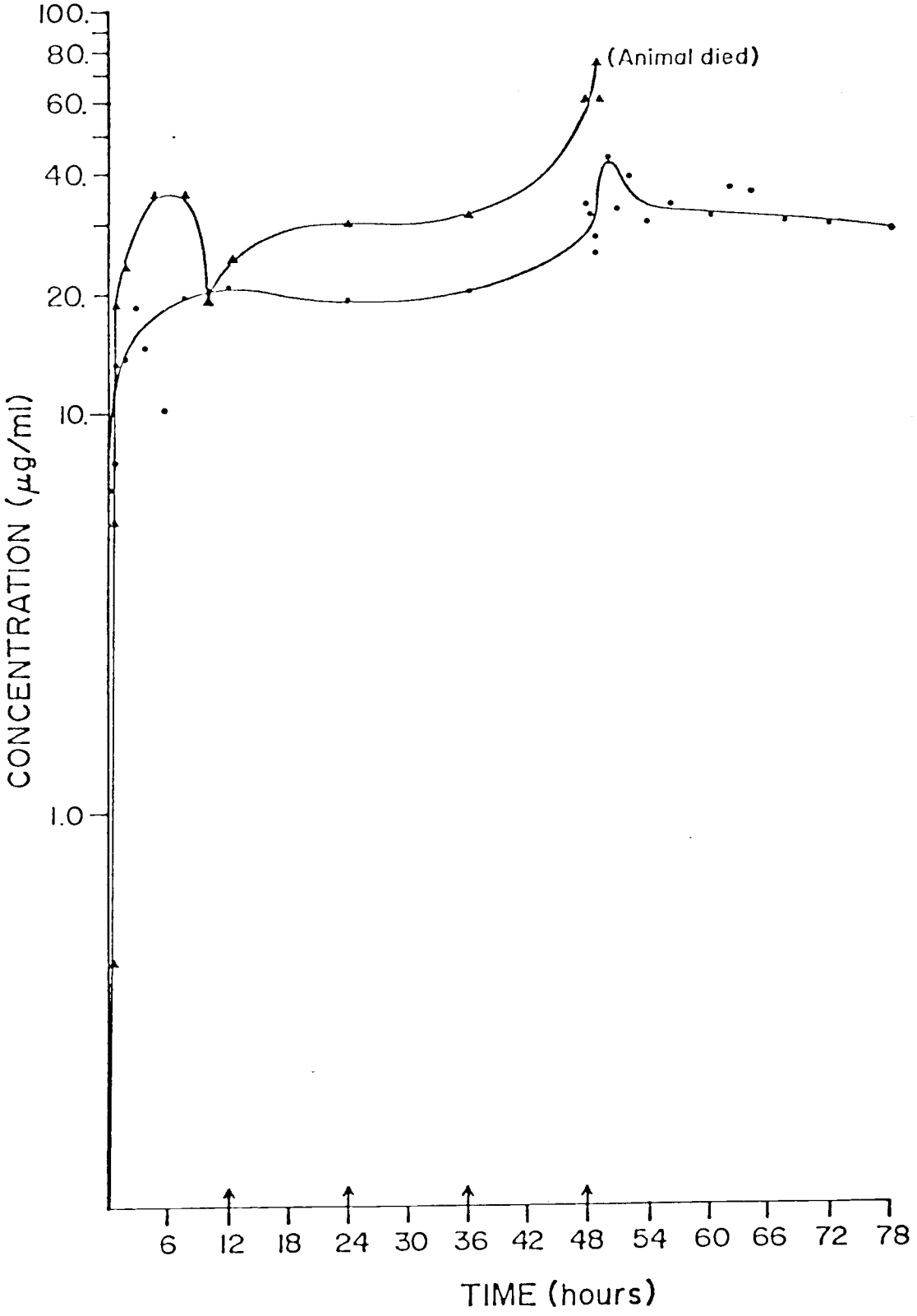


Figure 5 - Serum concentration of theophylline vs. time following multiple dosing of prolonged release products. Key: ▲ , 260 mg; ● 130 mg. ↑, Dose.

the last sample was collected (20 hrs.). It was also assumed that the elimination $t_{1/2}$ following oral administration was the same as obtained following iv dosing and the appropriate line has been extended from the last data points (Figure 4) as an illustration of this assumption. Mean percent absorption from the oral preparations was calculated to be 100 percent for both the 260 mg and 130 mg products. This serves to reinforce the above assumption that absorption was complete within 20 hours.

Theophylline is, by necessity of the disease state being treated (bronchial asthma), usually administered via a multiple dosing schedule. Two animals were, therefore, administered the recommended dose via the recommended [9] dosing schedule (one capsule every 12 hours). The results are presented in Figure 5. Although blood level fluctuations did occur, it is apparent that a prolonged high serum concentration was attained with each agent.

SUMMARY AND CONCLUSIONS

Sustained release oral products in both single and multiple dosing schedules as well as intravenous aminophylline were administered to rabbits. Plasma concentrations of theophylline were measured at predetermined times by high pressure liquid chromatography. In vitro dissolution studies demonstrated little dissolution when tested in simulated gastric fluid. Dissolution was rapid and complete however when performed in simulated intestinal fluid. An overall lag time due to gastric fluid exposure was detected when the product was pretreated with gastric fluid prior to intestinal fluid exposure. This correlates poorly with the corresponding mean serum concentration vs. time curve. The differences noted are explained by consideration of the rabbit's empty stomach and disintegration of the capsules prior to swallowing. It has been shown that prolonged serum concentrations are obtained following multiple dosing with sustained release products.

Pharmacokinetic parameters were obtained by computer fitting of the plasma concentration data. Comparison of some previously published data obtained in humans with the data obtained in the current study with rabbits show similar pharmacokinetic values. It is suggested that rabbits may be useful experimental animals for predicting dosage form bioavailability results and certain pharmacokinetic parameters in humans.

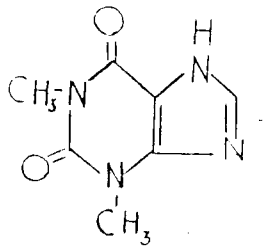
PART II

PHARMACOKINETICS AND BIOAVAILABILITY OF DYPHYLLINE IN RABBITS

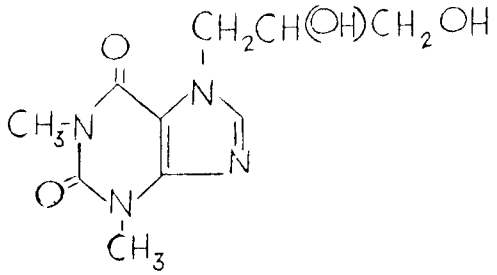
INTRODUCTION

Dyphylline (dihydroxypropyl theophylline) is a member of the family of methylated xanthines. Dyphylline is marketed[1a] for use in the treatment of bronchial asthma. Theophylline is the most popular member of the xanthine family for treating asthma, but the side effects of nausea, vomiting, and gastro-intestinal distress often preclude its use. Dyphylline is reported to be free from these side effects [1a,2a]. The mechanism of action has not been proven for dyphylline although it is assumed to act like other methylated xanthines by inhibition of the enzyme phosphodiesterase and thereby prevent the breakdown of 3'5' cyclic AMP. This would be a direct effect as dyphylline is not metabolized to theophylline [3a].

Comparative structures of dyphylline and theophylline are shown below.



Theophylline



Dyphylline

[1a] Neothylline (Lot Number 2119), Lemmon Pharmacal Co., Sellersville, PA.

Dyphylline is reported to be as water soluble as aminophylline and 40 times as water soluble as theophylline due to the presence of the hydrophilic dihydroxypropyl group [2a]. Dyphylline is stable in gastric fluid, neutral in solution, and is absorbed unchanged from the gastrointestinal tract [2a].

Dyphylline is reported to be effective in the treatment of bronchial asthma when administered in sufficiently large doses [1a,3a]. Simon, et al., reported plasma levels of 10-12 µg/ml and a significant effect on forced expiratory volume (FEV) following single dose administration of dyphylline tablets (15 mg/Kg) to human subjects. Hudson, Tyler, and Petty report that dyphylline was effective in producing significant bronchodilation when administered as 1000 mg but not effective when 500 mg was administered to humans [1a]. The recommended dose for dyphylline is 200 mg administered three to four times daily [4a]. This dose appears to be quite low when considering the studies cited above.

The reported elimination half-life ($t_{1/2}$) for dyphylline is 2.11 hours compared to theophylline which has a reported $t_{1/2}$ of 3 to 9.5 hours [2a]. Additional studies of dyphylline pharmacokinetics are indicated based on the reported effective dose from a single dose study [1a,3a], the recommended dose

of 200 mg three to four times daily [4a], and the reported short $t_{1/2}$ of 2.11 hours [2a]. Biopharmaceutic and pharmacokinetic studies can be quite time consuming and expensive when done with human subjects. At the current time, there has been no reported animal model for measuring bioavailability of dyphylline products. Thus, the objectives of the current study were: (a) to determine the in vitro dissolution properties of a commercially available dyphylline product; (b) to obtain information on the pharmacokinetics and bioavailability of dyphylline in rabbits; and (c) to determine if the bioavailability and pharmacokinetic properties of dyphylline in rabbits could be correlated with published bioavailability and pharmacokinetic properties in humans.

EXPERIMENTAL

The procedures detailed for theophylline (Part I) were essentially the same for dyphylline with the following exceptions:

1. All work was performed using female New Zealand White rabbits.

2. Dyphylline was administered both intravenously (iv) and as oral tablets.[1a]

   Single dose iv injections (200 mg/4ml) of aqueous solutions were given over a 3.0 to 5.0 minute time period. Both single and multiple dose (every 6.0 hours) studies were performed using 200 mg tablets.

Blood samples were collected at predetermined time intervals (see Tables I and II). Dyphylline was quantified using the same analytical method described in Part I.

The standard curve was prepared for dyphylline similar to that of theophylline (Part I). Untreated rabbit plasma was collected and known amounts of dyphylline were added. Linear regression was performed to determine the appropriate line. Results showed that the best standard curve was a combination of two curves. For those standard values analyzed that contained 5.0 µg/ml or less one regression line was used

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[1a] Neothylline (Lot Number 2119), Lemmon Pharmacal Co., Sellersville, PA.

($r = 0.82$) and for those concentrations of dyphylline greater than 5.0 µg/ml a second regression line was used ($r = 0.99$). This method was utilized to insure greater accuracy at low concentrations.

Dissolution studies were performed on 200 mg dyphylline tablets[1a] in simulated gastric and intestinal fluids as described in Part I. Both fluids were enzyme free.

RESULTS AND DISCUSSION

Dyphylline was administered as single dose intravenous injections and as oral tablets (Tables I and II.) The mean serum concentration of dyphylline following intravenous administration (Table I) of 200 mg of aqueous solution was found to be 56.26 μg/ml 15 minutes (0.25 hours) after administration. The mean serum concentration vs. time curve (Figure 1) appears to be described quite well by use of the standard biexponential equation (Eq. 1) for a two compartment open model (Figure 2, Part I)[5a]:

$$P = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

Computer fitting[2a] of the data (Table I) for iv administration of dyphylline shows that indeed the biexponential equation fits the data better than a mono-exponential equation and as well as a higher order exponential equation as determined by the weighted mean square error. Computer generated points provided the solid line in Figure 1 and results in an excellent fit with the experimental points. These results are consistent with the assumption that dyphylline behaves according to two compartment open model kinetics.

The appropriate pharmacokinetic parameters (i.e., $t_{1/2}$, rate constants, apparent volume of distribution, etc.)

[2a] SIMPLEX program, Billie Chou, Oregon State University, Computer Center, Corvallis, OR 97331.

TABLE I - Plasma Levels (ug/ml) of Dyphylline
Following Intravenous Administration of Aqueous Solution (200 mg/4 ml)

Rabbit No.	Weight (kg)	Time (hours) 0.25	0.50	0.75	1.0	2.0	4.0	6.0	9.0	12.0	14.0
62	2.50	67.18	37.09	24.83	20.61	7.84	2.54	1.55	1.47	-[a]	-
12	3.00	-	-	-	13.40	4.00	0.22	-	1.07	-	0.37
10	3.00	58.90	22.44	13.03	10.41	2.79	0.21	0.48	0.32	0.41	0.03
8	2.50	45.55	25.29	14.47	7.25	1.57	0.09	0.70	0.39	-	-
7	2.50	75.72	28.61	-	5.13	4.60	0.51	0.49	-	-	0.03
6	2.50	54.53	26.51	15.42	10.88	2.59	0.41	0.21	0.02	0.18	0.21
5	2.60	35.89	9.24	-	10.35	4.48	-	2.39	1.44	-	0.95
Means	2.66	56.26	24.86	16.94	11.15	3.98	1.48[b]	0.97	0.79	0.30	0.32
± SEM	0.06	5.86	3.72	2.68	1.87	0.76		0.34	0.25	0.12	0.17

[a]Sample not determined.

[b]This value is a qualified estimate based on theoretical expectations and observed data for other dosage forms. The experimental value was grossly deviant due to unavoidably inaccurate results obtained during a period of instrumental malfunction.

TABLE II - Plasma Levels (μg/ml) of Dyphylline

Following Administration of 200 mg Tablets

Rabbit No.	Weight (kg)	Time (hours)								
		0.25	0.50	0.75	1.0	2.0	3.0	4.0	6.0	9.0
40	2.75	8.49	10.24	12.53	16.75	-[a]	11.17	3.08	1.17	-
39	2.50	1.07	-	-	14.08	19.37	10.89	4.34	-	-
38	2.70	0.95	5.11	10.67	15.02	-	5.64	5.51	-	-
35	2.40	1.90	6.29	6.74	4.28	13.23	9.44	4.93	4.54	-
34	2.75	5.09	4.44	4.24	2.71	12.90	6.55	4.31	2.55	-
20	2.50	2.16	27.67	21.95	22.44	13.38	6.85	3.68	2.03	-
13	3.00	9.63	25.50	23.40	16.18	6.23	-	2.55	0.62	-
14	2.80	21.49	22.68	25.50	43.36	11.00	-	2.01	1.49	1.72
18	3.00	1.16	5.32	7.85	11.59	7.31	-	2.84	2.66	0.39
Means	2.71	5.77	13.41	14.11	16.27	11.91	8.42	3.69	2.15	1.05
± SEM	0.07	2.25	3.56	2.94	3.96	1.65	0.97	0.39	0.49	0.67

[a]Sample not determined.

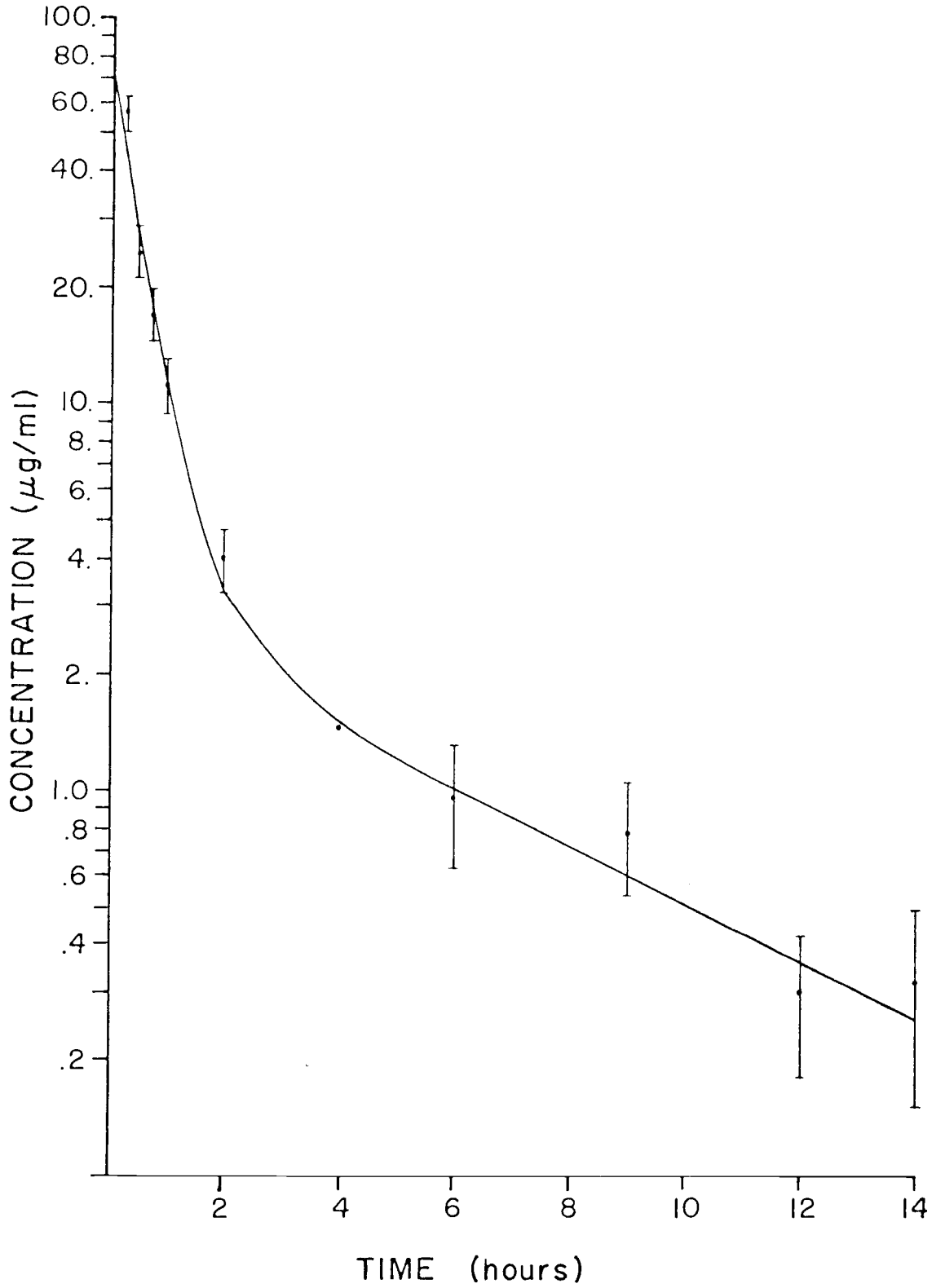

Figure 1 - Mean serum concentration of dyphylline vs. time following iv administration of 200 mg of dyphylline ± SEM. The 4 hour sample was estimated (see Table I).

can be determined by use of serum concentration data. Computer fitting of the data in Table I provided pharmacokinetic parameters for dyphylline as shown in Table III. The total body elimination half-life for dyphylline in rabbits was 3.89 hours which corresponds to a β value of 0.178 hr^{-1}.

In vitro dissolution studies performed on commercially available tablets containing 200 mg of dyphylline (Figure 2) show that total dissolution in both simulated gastric and intestinal fluids was complete in four minutes or less. Such a rapid dissolution rate should lead to rapid absorption of the drug following oral administration, assuming that absorption is a passive diffusion process. It was, therefore, assumed that a two compartment open model with first order absorption would be appropriate to describe plasma concentration curves following tablet administration. Dyphylline was administered (single dose) orally as tablets and the serum concentrations are presented in Table II. The corresponding mean serum concentration vs. time curve (Figure 3) and the data in Table II show that the mean peak serum level following oral administration of tablets was 16.27 µg/ml and occurred about 1.0 hour after administration. The total amount of dyphylline absorbed following oral administration of tablets was determined by comparing the area under the serum concentration vs. time curve (AUC)

Table III - Mean Pharmacokinetic Parameters For Dyphylline Generated by Computer Fitting Rabbit Data Shown in Tables I and II and Previously Published Values

			Parameters				
Subject	Dose	Dosage Form	$K_{12}(hr^{-1})$	$K_{21}(hr^{-1})$	$K_2(hr^{-1})$	$K_a(hr^{-1})$	$\alpha(hr^{-1})$
Rabbits	200mg	iv	0.540	0.256	1.399	-	2.017
Rabbits	200mg	Tablets	0.506	0.250	1.439	0.564	2.017
Humans	5mg/kg	Tablets	0.737	0.546	0.535	0.884	1.640
Humans	10mg/kg	Tablets	0.684	0.395	0.739	0.607	1.640
Humans	10mg/kg	Tablets	0.700	0.416	0.702	1.072	1.640

TABLE III - continued

Subject	Dose	Dosage Form	Parameters				
			$\beta(hr^{-1})$	Vd(l/kg)[a]	Vp(l/kg)[a]	$T_{\frac{1}{2}}$(hr)[b]	$T_{\frac{1}{2}}$(hr)[c]
Rabbits	200mg	iv	0.178	8.24	1.04	3.89	0.50
Rabbits	200mg	Tablets	0.178	8.11	1.01	3.89	0.48
Humans	5mg/kg	Tablets	0.178	0.87	0.29	3.89	1.30
Humans	10mg/kg	Tablets	0.178	1.04	0.25	3.89	0.94
Humans	10mg/kg	Tablets	0.178	1.22	0.31	3.89	0.99

[a]/Assuming 70kg for humans and 2.69kg for rabbits.

[b]/Half-life for elimination from the body.

[c]/Half-life for elimination from blood.

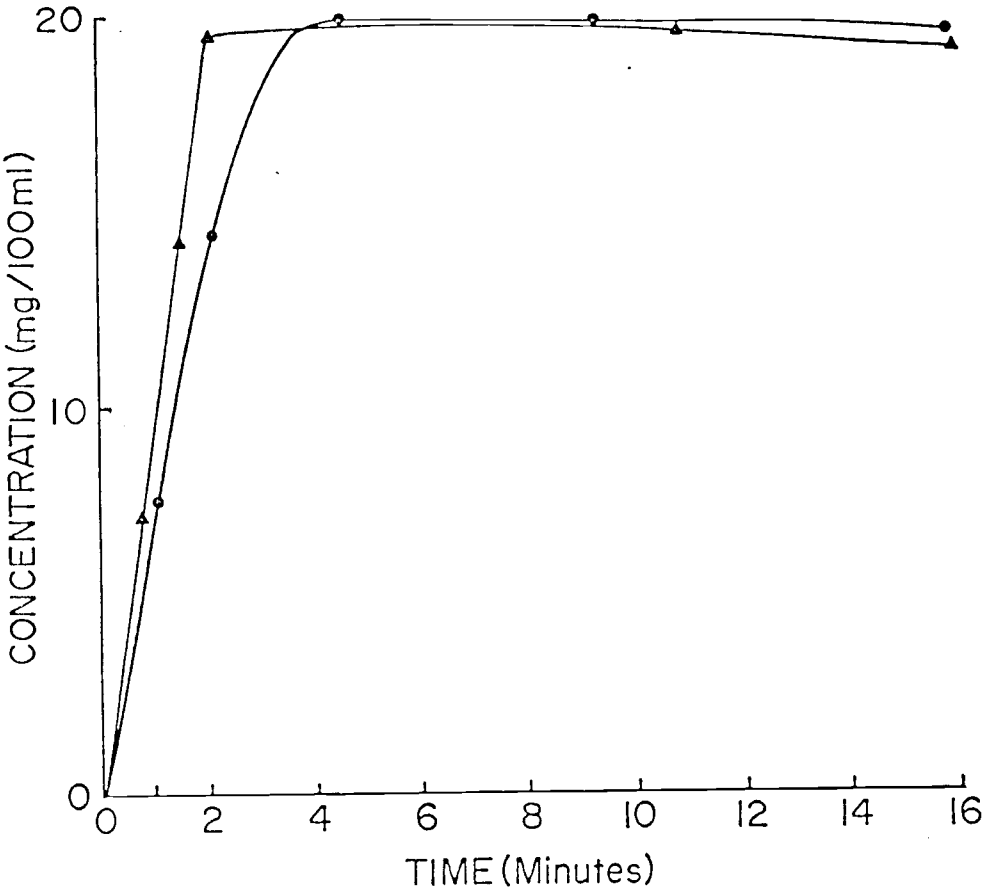

Figure 2. Dissolution of dyphylline tablets (200 mg).
Key: ▲, gastric fluid; ●, intestinal fluid.

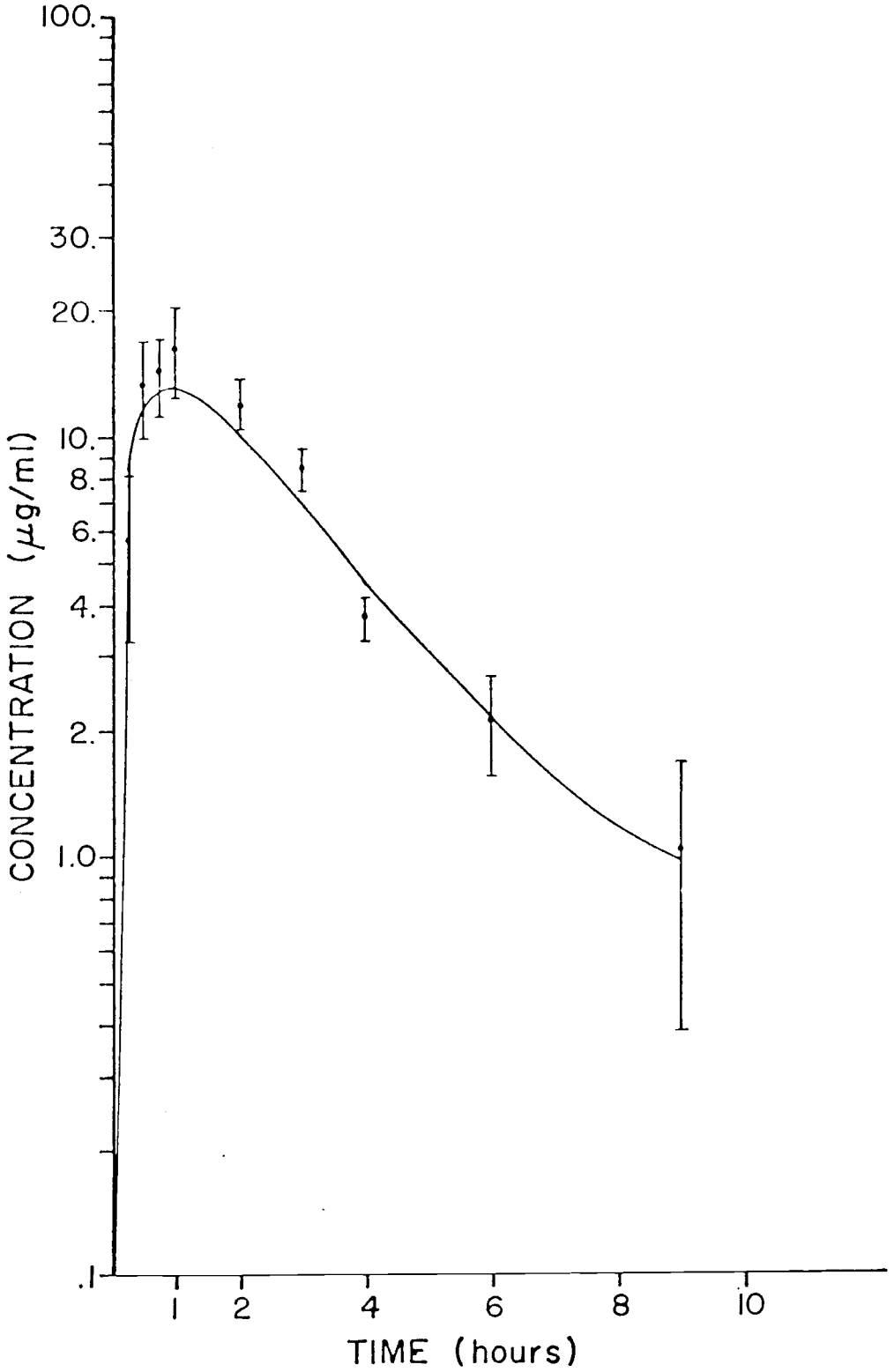

Figure 3 - Mean serum concentration of dyphylline vs. time following administration of a 200 mg tablet. ± SEM

with the AUC for intravenous administration of dyphylline. The mean percent absorption from the tablets was calculated to be 100 percent assuming the availability of the iv dose was 100 percent.

Pharmacokinetic parameter values (Table III) were determined by computer analysis of the plasma concentration (Cp) data obtained from tablet administration (Table II). The following general equation [6a] is appropriate for the assumptions being made:

$$Cp = A_1e^{-\alpha t} + A_2e^{-\beta t} + A_3e^{-kat} \qquad \text{(Eq. 2)}$$

Since computer generated values are dependent on the initial estimates provided, it can be advantageous to provide certain constraints for the computer analysis. This proved to be the case for this study. Two parameters were constrained (α and β). This proved to be necessary since interpretation of some of the computer generated values were physically inconsistent with some of the experimental data when no constraints were provided even though a good fit was obtained. The values determined for constraining α and β were 2.017 hr^{-1} and 0.178 hr^{-1}, respectively. These values were obtained for the computer generated results for the analysis of the intravenous administration of dyphylline (see Table III). Using this method of computer analysis provided just as good a computer

fit as with no constraints and the resultant values for the kinetic parameters were consistent with the experimental data. The resultant tri-exponential equation and pharmacokinetic parameters calculated from the data obtained following oral administration of dyphylline in rabbits, when assuming a two compartment open model, are presented in Table III.

Published serum levels of dyphylline [2a] in humans following oral administration of dyphylline tablets and elixir (different brands than the one used in this study) were also computer fitted[2a] to a two compartment open model with first order absorption in an attempt to correlate reported human parameters with those obtained in this study. The results are presented in Table III along with the values obtained in this study. Computer analysis of the human data was also accomplished by constraining two exponential terms. The α was constrained at 1.640 hr^{-1} and β was constrained to 0.178 hr^{-1}. The value for β was determined through application of linear regression techniques to published [2a] serum concentrations of dyphylline in humans who received oral dyphylline as tablets or elixir. Absorption was assumed to be complete within four hours and linear post absorption elimination characteristics for the logarithim of drug concentration vs. time appeared operative beyond this four hour mark. Appropriate linear regression

of the combined post four hour data for tablets and alcoholic solution administered to humans produced a β value of 0.178 hr^{-1} (r = 0.72). The α value was determined by computer analysis of the individual dosage forms in humans. This resulted in a mean α value of 1.640 hr^{-1}. The pharmacokinetic parameters presented for human data in Table III indicate relatively stable micro-constants with the different dosage forms which is as anticipated. It can also be noted that the micro-constants for rabbits are quite close to those for humans, especially when one considers the many differences between humans and rabbits.

It is interesting to note that the β value obtained (0.178 hr^{-1}) by linear regression of the human data is the same as that obtained by computer fitting of the rabbit iv data (see Table III). These β values correspond to a total body half-life of 3.89 hours which differs from the previously reported value of 2.11 hours [2a]. Since $t_{1/2}$ is dependent upon the value determined for β it is important to make certain that β does indeed reflect the post absorption and distribution linear portion of the serum concentration vs. time curve. Post absorption and distribution times were assumed to be 4 hours and longer in obtaining the $t_{1/2}$ of 3.89 hours in humans. If earlier times are considered a shorter $t_{1/2}$ would be obtained. As expected,

similar considerations hold for the data obtained in rabbits. The above considerations indicate that the $t_{1/2}$ of 3.89 hours is probably most correct for both humans and rabbits.

Crouthamel et al., [7a] have proposed that rabbits may be a poor choice for bioavailability studies due to the variations between the pH of human and rabbit duodenum and jejunum. Therefore, two rabbits from this study were sacrificed and the pH values along their entire gastrointestinal tracts were determined. The results indicate that the pH range was within the normal range for human pH values (Table IV). The pharmacokinetic results (Table III) and the gastrointestinal pH values shown in Table IV suggest that rabbits are a good choice for experimental work involving oral absorption following single dose administration of dyphylline.

Recommended dosage schedules for dyphylline tablets are [4a] 200 mg three or four times daily for prolonged therapy. Therefore, a multiple dosing study was completed by administering 200 mg tablets every six hours for four doses and appropriate blood samples were collected in an attempt to describe the plasma drug concentration vs. time curve for dyphylline following multiple dosing. This curve is shown in Figure 4. The parameters obtained from the single dose data were used with multiple dose kinetics [9a]

Table IV – Comparison of Rabbit and Human pH Values for Selected Segments of the Gastrointestinal Tract

Subject	Selected Segments			
	Stomach	Duodenum	Jejunum	Ileum
Rabbit	1.8	6.7	7.0	7.1
Human [6a,8a,9a]	1.0-3.5	5.8-7.6	6.2-7.3	6.1-7.3

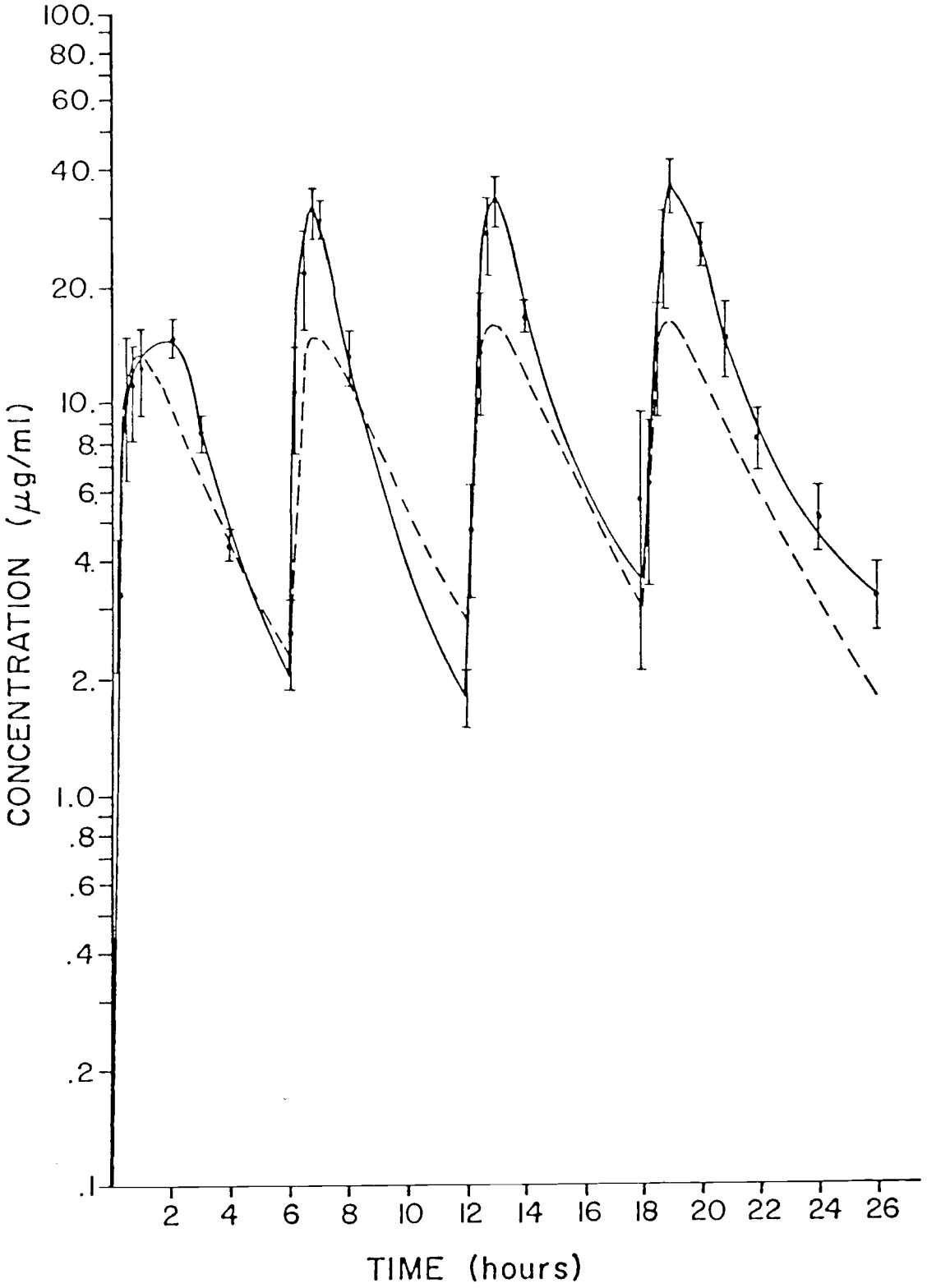

Figure 4 - Mean serum concentration of dyphylline vs. time following multiple dosing of 200 mg tablets every six hours (four doses) and computer predicted levels ± SEM. Key: --, computer prediction; —, experimental results.

to predict the dotted line in Figure 4. A poor correlation exists between the predicted line and the experimental points after the first 6.0 hours. Mitenko and Ogilvie have demonstrated that theophylline enters or binds to erythrocytes and is rapidly taken up by the cells [10a]. It would not be unexpected if dyphylline behaved in a similar manner since the molecular structures are so closely related. If this were true then an increased plasma concentration would be expected if the hematocrit were to decrease without a change in drug dose. Frequent blood sampling over an extended period of time would create a low hematocrit. During the multiple dosing studies dyphylline was administered every 6.0 hours and a total of 29 blood samples consisting of 6.0 milliliters were withdrawn from each rabbit during a 26 hour period. Approximate estimates of hematocrit were obtained for the latter time samples and a 50 percent or larger decrease was frequently detected. Therefore, the plasma concentration of dyphylline would be expected to be higher than those predicted from a single dose study where smaller changes in hematocrit occurred. The decreased hematocrit may be responsible for the differences between the observed and predicted plasma levels in Figure 4 although other changes in physiological functions associated with the study cannot yet be ruled out. It may also be noted that similar but

much smaller effects would have been operative at the end of the single dose study and the $t_{1/2}$ of 3.89 hour in rabbits may, therefore, not be exactly correct.

SUMMARY AND CONCLUSIONS

Dissolution results were obtained for a commercially available dyphylline product. Rabbits were administered dyphylline as iv injections or as the oral commercial tablet. Serum concentrations of dyphylline were determined for both single and multiple dosing schedules using high pressure liquid chromatography. Computer fitting of the experimental data as well as reported data for dyphylline in human subjects produced pharmacokinetic parameters for dyphylline in rabbits and humans.

It has been shown that for the product tested, dyphylline is completely dissolved within four minutes *in vitro*. Dyphylline is rapidly and completely absorbed following oral administration of the tablets to rabbits. The results of single dose studies in rabbits were compared to single dose studies in human subjects. Values obtained for the pharmacokinetic micro-constants and overall elimination half-life in rabbits correlate well with published values in humans. Also, it has been shown that the reported half-life for dyphylline in humans may be an underestimate of the actual value.

Single dose results do not correlate well with predicted multiple dose results in rabbits. A possible explanation may be attributed to the decreased erythrocyte binding of

dyphylline that occurs when the hematocrit is lowered as was seen in the multiple dose study. The current study does not purport to evaluate the efficacy of dyphylline in the treatment of asthma, but does support the conclusion that rabbits are useful in bioavailability studies involving single doses of dyphylline. Caution is advised in interpreting data which involves multiple blood sample collection in rabbits due to possible changes in hematocrit values.

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