AN ABSTRACT OF THE THESIS OF

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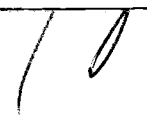
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Title: Pharmacokinetics of Dyphylline after Administration of

Prodrugs in Rabbits and Pigs

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James W. Ayres, Ph.D.

Dyphylline is an analogue of theophylline and the latter is the most popular member of the methylxanthine family in use for the treatment of asthma. Although dyphylline is devoid of some problems associated with theophylline, a reported short half-life for dyphylline (2.1 hours) has made its clinical usefulness limited. In order to avoid these problems a series of prodrugs of dyphylline previously synthesized to liberate dyphylline in a controlled manner after administration was evaluated as a method of drug delivery for dyphylline. Bioavailability comparisons were performed where both 'extent' and 'rate' of absorption were studied. Pharmacokinetic models for description of dyphylline plasma concentrations as delivered by prodrugs were evaluated and used as a basis for data analysis.

Numerical deconvolution and a statistical moments method were employed to analyze absorption kinetics. Parameters used to describe this derived absorption profile have better statistical precision than conventional estimates of rate of absorption and provide more information about drug input kinetics.

Preliminary experiments in New Zealand White rabbits showed that prolongation of duration of dyphylline blood concentration following oral administration of prodrug suspensions is probably due to slow and prolonged prodrug dissolution in the G-I tract. This characteristic of dyphylline prodrugs may provide a stratagem for design of an effective product. The pig, a carnivorous animal which is comparable with humans in size was chosen as another animal model because the results may give a projectable indication of dyphylline pharmacokinetics following prodrug administration in humans. Experimental data collected indicate that dipivaloyl dyphylline may be a useful approach to deliver dyphylline once the total dosage form has been optimized.

PHARMACOKINETICS OF DYPHYLLINE

AFTER ADMINISTRATION OF

PRODRUGS IN RABBITS AND PIGS

by

Hua-pin Huang

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CHAPTER I

INTRODUCTION

INTRODUCTION

Drugs available to improve pulmonary airflow include adrenergic drugs, xanthine bronchodilators and anti-inflamatory corticosteroids(1). Theophylline is the most widely used drug in the xanthine family. However, it is frequently reported to have side effects(2,3), a highly variable half-life(4-9), and a narrow therapeutic range(10-12).

Dyphylline, 7-(2,3-dihydroxypropyl) theophylline was synthesized in 1946(2) in an attempt to solve the above problems associated with theophylline. Although dyphylline is much less toxic(1,3,13-15) and has a relatively stable half-life of about two hours(16,17), the short half-life has made its clinical usefulness impractical. A drug is often administered every half-life in order to maintain plasma drug concentrations in the desirable range. With the short half-life of dyphylline, such a frequent dosing interval is not clinically acceptable. In order to improve the usefulness of dyphylline, a controlled release design through prodrug modification has been proposed(18).

A general description of possible disposition of a prodrug and its metabolite (or active drug) is illustrated in Figures 1 and 2. The mass balance equations are described in Appendix I. These schematic diagrams can be
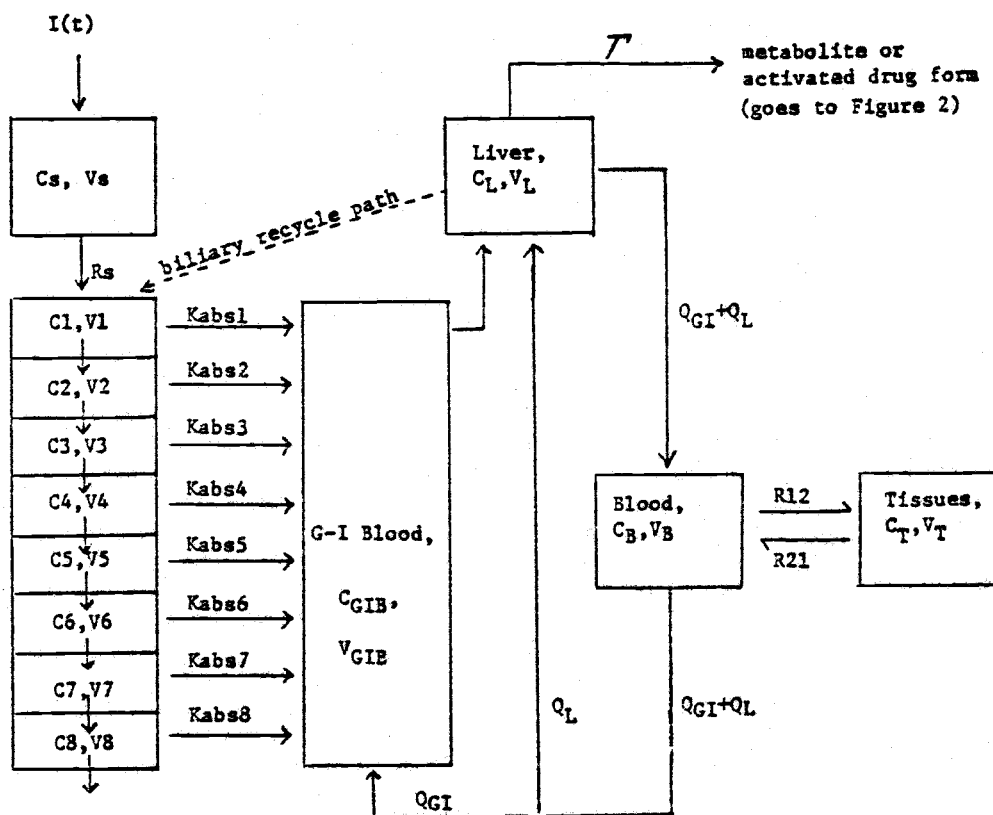
Chapter I

Figure 1. Schematic diagram of possible prodrug disposition following oral prodrug administration.

*For explanation of symbols see next page.

**For mass balance equations see Appendix I.

I(t):   Prodrug input function to the stomach

C_s: Prodrug concentration in the stomach

R_s: Prodrug mass transport rate constant in G-I tract

V_s: Volume of the stomach

C_i: Prodrug concentrations in different parts of the intestine, $i = 1,2,3,4,5,6,7,8.$

V_i: Volumes in different parts of the intestine, $i = 1,2,3,4,5,6,7,8.$

K_{absi}:   First order absorption rate constants, $i = 1,2,3,4,5,6,7,8.$

C_{GIB}:   Prodrug concentration in G-I blood

C_L: Prodrug concentration in the liver

C_B: Prodrug concentration in the general blood

C_T: Prodrug concentration in all other tissues

V_{GIB}:   Volume of the G-I blood

V_L: Volume of the liver

V_B: Volume of the general blood

V_T: Volume of all other tissues

Q_L: Blood flow rate from and into the liver

Q_{GI}:   Blood flow rate from and into the G-I tract

Γ: Liver metabolism rate constant of prodrug

[*] The above rate constants are assumed to be first order

[**] Units used for the above symbols may be defined as follow: I(t), mg/hr; Concentrations, mg/ml; R_s, mg/hr; Volumes, ml; Kabsi, hr^{-1}; Q(flow rates), ml/hr; Γ, mg/hr.
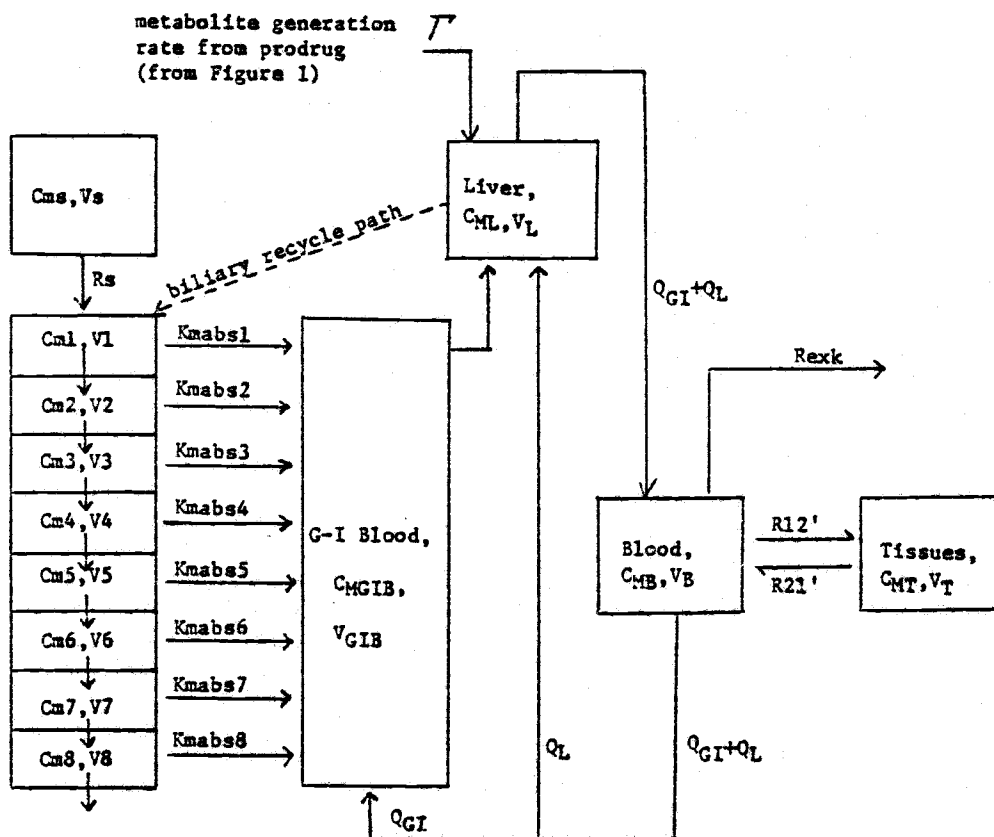
Chapter I
Figure 2. Schematic diagram of metabolite (active drug) disposition following oral prodrug administration.

*For explanation of symbols see next page.

** For mass balance equations see Appendix I.

C_{MS}: Drug concentration in the stomach

R_s: Drug mass transport rate constant from the stomach to the intestine

V_S: Volume of the stomach

C_{Mi}: Drug concentrations in different parts of the intestine, $i = 1,2,3,4,5,6,7,8$.

V_i: Volumes in different parts of the intestine, $i = 1,2,3,4,5,6,7,8$.

K_{mabsi}: First order absorption rate constants, $i = 1,2,3,4,5,6,7,8$.

C_{MGIB}: Drug concentration in G-I blood

C_{ML}: Drug concentration in the liver

C_{MB}: Drug concentration in the general blood

C_{MT}: Drug concentration in all other tissues

Q_L: Blood flow rate from and into the liver

Q_{GI}: Blood flow rate from and into the G-I tract

R_{exk}: Drug elimination rate constant

[*]The above rate constants are assumed to be first order.

[**]Concentrations, mg/ml; Volumes, ml; R_s, mg/hr; Kmabsi, hr^{-1}; Q(flow rates), ml/hr; Rexk, hr^{-1}.

used to describe a twenty-six compartment open pharmacokinetic model which includes 25 concentrations, 13 volumes and 25 microscopic rate constants. In order to measure these constants and volumes in each animal, the whole animal has to be anesthetized and fluid or blood samples have to be collected from at least 13 different areas at predetermined time intervals. Since anesthesia is highly likely to change the gastrointestinal tract movement, and change absorption and possibly the metabolic rate of the prodrug, this anatomio- physiologically more realistic model (Figure 1) is very difficult to validate experimentally.

In general, it is not possible to determine all of the characteristics of a process for which we desire a system model. The characteristics which we must describe are determined by the "goal" we set for the system, the allowable degree of complexity of the overall system, and its associated computational requirements(19).

The overall "goal" of the continuing dyphylline prodrug research is to control the input of dyphylline into the general blood circulation. This prolongs the duration of dyphylline concentration above the minimum effective concentration necessary to produce a bronchodilation effect. The overall input rate of dyphylline is of main interest. The overall "goal" of this thesis research is to administer dyphylline prodrugs

to animals in single doses, and to obtain sufficient pharmacokinetic parameters to be able to predict what will happen during multiple doses.

From reported data available, the overall input rate usually can be described by a simple equation, very often as a simple first order absorption process. That is, most of the "gut transport processes" in the above twenty-six compartment model (and even more complex models) can often be condensed to a simple input function I(t) for purposes of predicting approximate drug plasma concentrations. Therefore, in this thesis work, one object of the research will be to apply a deconvolution process to determine whether or not the obviously complex (and physiologically accurate) input function for dyphylline prodrugs can be described as a relatively simple first order process.

Assuming (i) the drug disposition of dyphylline follows a linear time invariant system and (ii) the disposition function of dyphylline following a bolus intravenous dose can be described as F(t), a drug input supply of I(t) will give a response function of R(t) = I(t) * F(t); where * is a symbol for convolution(20). Our goal in mathematical terms is this: We observe a drug disposition function, F(t), following a bolus intravenous dose and observe a drug disposition function, R(t), following oral prodrug administration. We then want to derive a suitable model to describe the unknown drug input

function, I(t). (See Appendix II for a detailed description of this method.)

In general, the order of the equation for input behavior is not known. However, for most of the input identification problems in pharmacokinetics, people usually assume first-order or consecutive first-order absorption processes, and use a non-linear least square regression procedure to estimate the absorption rate constant(s) as means of identifying the input function. For unknown input functions, procedure like the Wagner-Nelson method(21), Loo-Riegelman method(22) or a deconvolution method (See Appendix II) have been used.

In this thesis, a numerical deconvolution procedure (See Appendix III) is used for input function analysis. The input function of dyphylline through prodrug design gives us a continuous description of the dyphylline absorption profile which may be used for bioavailability studies of dyphylline for both extent and rate determinations.

Since the prodrugs in this study are new chemical entities which have not yet been approved by the Food and Drug Administration, pharmacokinetic testing in human subjects is prohibited. However, it is desirable to choose an animal model which is reasonably close anatomically, physiologically, and biochemically to humans, which can be used to study dyphylline prodrugs.

Experimental animals most widely used are small animals (e.g., mice, rats, rabbits). Larger mammalian species including dogs, rhesus monkeys and pigs are also often used.

Species variations in the fate of prodrug following oral prodrug administration may exit with respect to prodrug "metabolism" and "absorption". Comparative aspects of drug metabolism have been reviewed previously(23,24,25). Microsomal enzyme systems and blood flow rates to various organs including the liver may contribute significantly to drug metabolism for different species. For some drugs, pharmacokinetics in rats resemble pharmacokinetics in humans, for other drugs the dog is a better model. Of all animal species studied the pig is perhaps most comparable metabolically to humans(26).

Species variations in drug absorption are of concern with respect to the rate and extent of entry of the drug into the blood via the gastrointestinal tract. The choice of herbivorous ruminants such as sheep, cattle, or rabbits for studies involving gastrointestinal absorption may not be suitable for extrapolation of test data to carnivorous animals such as humans(27). In view of their anatomic, physiologic, biochemical similarities and the logistics of handling and holding of pigs compared to monkeys or primates, pigs represent a good animal model for pharmacokinetic studies of drug absorption.

Because economic factors were of primary consideration in a screening test, a less costly animal species was used to choose possible drug candidate(s) for further studies. In these dyphylline prodrug studies, New Zealand White rabbits were first used for preliminary tests. Promising drug candidate(s) were further extensively tested in Yorkshire pigs. The test data are expected to be projectable to humans.

In summary, the purposes of this study were to:

(1) Use a first-order input approximation for computer simulation of oral absorption of dyphylline to choose an optimal overall combination input rate which will give the desired duration of dyphylline concentration above the minimal effective concentration. (Chapter II)

(2) Use New Zealand White rabbits to study dyphylline pharmacokinetics and dyphylline prodrugs previously synthesized in our laboratory. (Chapter II)

(3) Use input function analysis to study the absorption characteristics of dyphylline after oral prodrug administrations. (Chapter II)

(4) Choose the most promising dyphylline prodrugs which give the optimal input rate of dyphylline after administration of oral prodrug in rabbits. (Chapter II)

(5) Determine whether or not the real-life complex pharmacokinetic model which describes dyphylline pharmacokinetics following prodrug administration

"collapses" to a relatively simple apparent first-order process when analyzed by deconvolution. (Chapters II and III)

(6) Use pigs as an animal model more closely approximating humans to study dyphylline pharmacokinetics and dyphylline prodrug absorption kinetics. (Chapter III)

(7) Choose the best dyphylline prodrug candidate that gives the most desirable dyphylline input rates and identify possible improvements for further dosage formulation optimization. (Chapter III)

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CHAPTER II

PHARMACOKINETICS OF DYPHYLLINE PRODRUGS IN RABBITS

INTRODUCTION

Theophylline has been widely used in the treatment of asthma since 1937. Since it frequently has adverse side effects(1,2), a highly variable half-life(3-8), and a narrow therapeutic range(9-11), several N-7-substituted theophylline derivatives have been introduced(8) in an attempt to eliminate the problems associated with theophylline. Dyphylline, dihydroxypropyl theophylline, was synthesized in 1946(1) and was designed to retain the therapeutic activity of theophylline, but have less of the associated side effects. Early pharmacokinetic studies of dyphylline reported a half-life from conventional drug dosage formulations of 2.11 ± 0.36 hours(12). To maintain plasma concentrations in the desired range, a drug is often administered every half-life, but with dyphylline this is unacceptable to patients because of the frequent dosing necessary to maintain plasma concentrations. An attempt to maintain plasma concentrations above the minimum effective level could be made by giving very large doses every six hours or so, but peak concentrations achieved shortly after administration might be associated with undesirable side effects and minimum effective concentrations might still not be maintained between doses. Therefore, prolonged release dyphylline preparations were previously synthesized in our

laboratory(13) and were predicted to provide a suitable amount of drug to the general circulation in a controlled manner which will be useful in the treatment of asthma.

Prolonging drug action can be accomplished by biological, physical or chemical means(14). The biological approach entails varying the route of administration. A greater degree of fexibility of drug modification is offered by the physical approach, commonly referred to as dosage form design. However, the highest degree of flexibility in altering drug efficacy is offered by the chemical approach(14). Controlled release of dyphylline through a prodrug approach was thus considered to be an appropriate way to overcome the problem of the short dyphylline half-life.

A prodrug has been defined as (a) a derivative of a drug which undergoes in vivo hydrolysis to the parent drug or (b) an analog which is metabolically transformed to a biologically active drug(15).

Dyphylline has two positions available for reversible modification (see Table I for structure) to produce a prodrug. A series of esters including four monoesters and four corresponding diesters has been previously synthesized to serve as prodrugs(16) which are expected to liberate dyphylline upon enzymatic attack in the body. These ester derivatives of dyphylline have been prepared and characterized by infrared(IR), proton nuclear magnetic resonance(PMR) and mass spectrometry(16).

Chapter II

Table 1 - STRUCTURE OF SOME DYPHYLLINE PRODRUGS
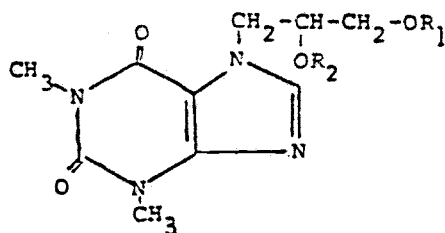
	Compound	R_1	R_2
I	Dyphylline	-H	-H
II	Monopropionoyldyphylline	$-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$	-H
III	Dipropionoyldyphylline	$-\overset{O}{\overset{\|}{C}}-CH_2-CH3$	$-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$
IV	Monoisobutyroyldyphylline	$-\overset{O}{\overset{\|}{C}}-CH-(CH_3)_2$	-H
V	Diisobutyroyldyphylline	$-\overset{O}{\overset{\|}{C}}-CH-(CH_3)_2$	$-\overset{O}{\overset{\|}{C}}-CH-(CH_3)_2$
VI	Monopivaloyldyphylline	$-\overset{O}{\overset{\|}{C}}-C(CH_3)_3$	-H
VII	Dipivaloyldyphylline	$-\overset{O}{\overset{\|}{C}}-C(CH_3)_3$	$-\overset{O}{\overset{\|}{C}}-C(CH_3)_3$
VIII	Mono-p-Cl-benzoyldyphylline	$-\overset{O}{\overset{\|}{C}}-\text{C}_6\text{H}_4-Cl$	-H
IX	Di-p-Cl-benzoyldyphylline	$-\overset{O}{\overset{\|}{C}}-\text{C}_6\text{H}_4-Cl$	$-\overset{O}{\overset{\|}{C}}-\text{C}_6\text{H}_4-Cl$

In the investigation of many other prodrugs(17,18,19), the evidence indicates that the rate of enzymatic hydrolysis by serum esterases was decreased with increasing steric bulk and branching of the acyl substituent. By varying bulkiness of substituents in the acyl moiety of dyphylline prodrugs (Table I), it was proposed that it would be possible to obtain different rates of conversion from prodrug to dyphylline(18).

For prolongation of drug action through prodrug design, there are at least three possible rate limiting steps: (1) slow absorption due to slow dissolution of the prodrug, (2) slow prodrug distribution into the central compartment from the peripheral compartment which acts as a prodrug depot position and (3) slow conversion rate from prodrug to drug (see Scheme I).

In previous dyphylline prodrug intravenous infusion studies in rabbits(20), it was shown that assayable concentrations of dyphylline prodrug no longer existed after 30 minutes postinfusion. The insignificant prodrug concentration and short presence of dyphylline prodrug in rabbit blood suggested that the prodrug must be quickly hydrolyzed to dyphylline in rabbits. The rapid appearance and high concentration of dyphylline in blood samples supported this evidence. The extremely rapid conversion rate _in vivo_ make k_{21}, k_{12} and process 3 in Scheme I insignificant; and process 1 becomes the controlling step
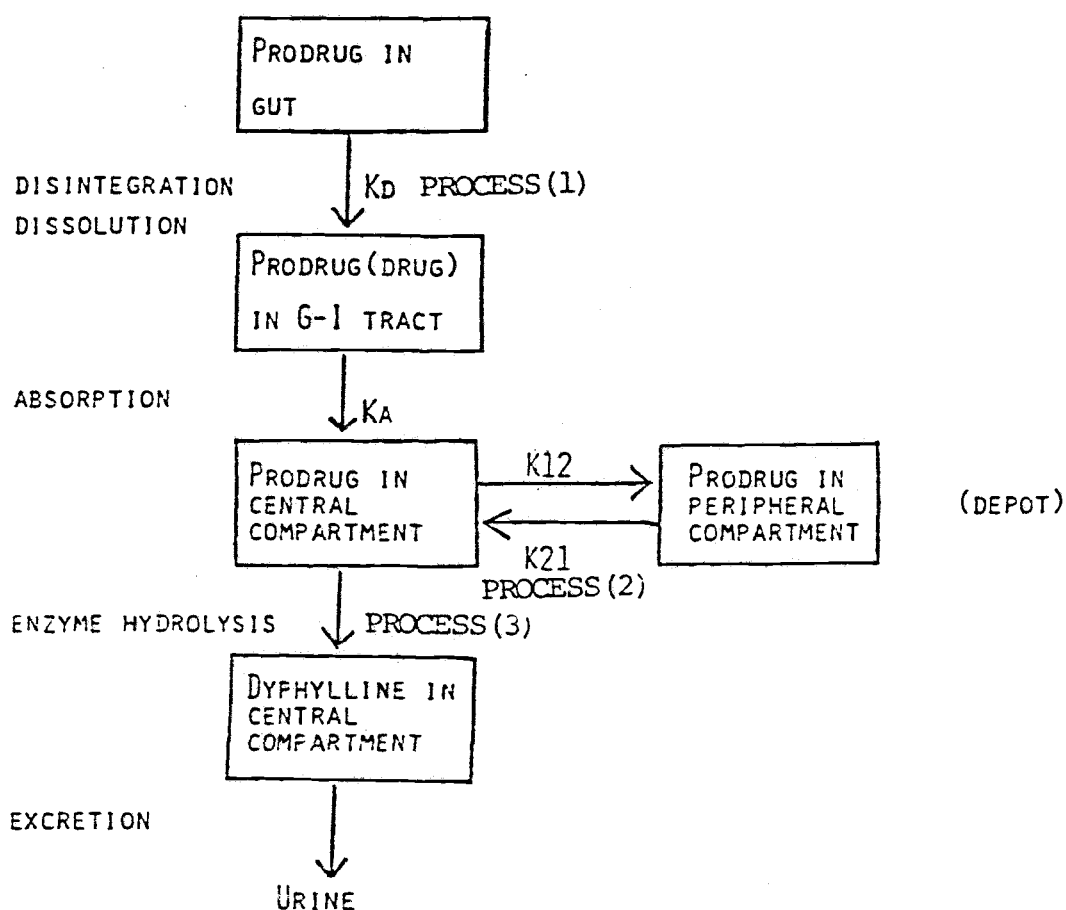
Scheme I. Dyphylline disposition following oral prodrug administration.

for dyphylline release. Since these prodrugs are converted to dyphylline at a fast rate in plasma, it is possible they will be converted to dyphylline by the first-pass effect due to high concentrations of enzymes in the liver, before the prodrug reaches the general circulation. These facts allow simplication of Scheme I to Scheme II where ka (or I(t)) is a hybrid mathematical description of absorpton kinetics of dyphylline in the gastrointestinal tract following oral administration of dyphylline prodrugs. Scheme II can now be used to approximate description of dyphylline concentration in plasma following oral administration of prodrugs of dyphylline.
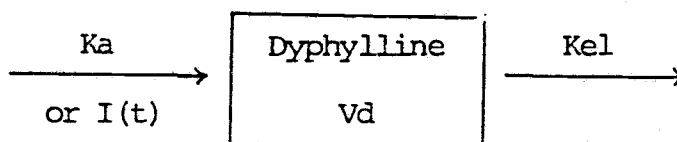
Scheme II- Dyphylline disposition following
oral prodrug administration (simplified).

In a previous study, Panomvana(21) suggested a complex model to show the dyphylline prodrug transformation and disposition where five compartments and eleven microconstants were used in a series of simulations. Though the model gives a clear view of disposition diagram and detailed simulations have been done, the microconstants in the model are not solvable. Wagner(22) indicated that there would be insufficient

information on plasma concentration - time data alone to solve the microscopic rate constants because some of the exponential terms derived from the full model will vanish on stripping or fitting of data. Disposition of many drugs follows multicompartment kinetics and dose calculations for sustained release products are based on one compartment kinetic considerations(23). It is thus considered appropriate to use the simplified model as depicted in Scheme II to study dyphylline prodrugs.

If a semilogarithmic plot of the amount of drug remaining to be absorbed vs time curve is a straight line, apparent first-order input kinetics exist and ka represents a first-order input rate constant which is a hybrid of dosage form disintegration, prodrug dissolution and enzymatic hydrolysis of dyphylline prodrug. If the semilogarithmic plot of the absorption data is non-uniform, then absorption kinetics can not be adequately described by the simple assumptions outlined above. In this case, the plots themselves may be helpful in identifying a kinetic scheme descriptive of drug transport into the general circulation.

Dosage forms or prodrugs that slowly release drug may alter the apparent half-life of the drug by controlling the input rate. This is known as the "flip-flop" phenomenon(24) and may be used to an advantage with prodrugs of dyphylline. If it is desired to prolong the

duration of action, then the prodrug with a suitably slower input rate will be more promising. However, the slower the input rate, the longer a portion of the dosage will remain in the gastrointestinal tract and the greater the possibility that smaller fractions of the dose administered will be absorbed. A suitable choice concerning these two factors must be optimized to obtain the most desirable prodrug design.

The purposes of this study were to: (1) use a first-order input approximation to simulate oral absorption of dyphylline and choose an optimal input rate as a criteria to design a slow release formulation; (2) inject dyphylline solution intravenously to determine a disposition function which can be used to resolve the input function from blood concentration data following oral administration of dyphylline prodrugs and (3) choose some promising dyphylline prodrugs for further studies based on the approximate absorption rate constant values which are described as derived from input function analysis of pharmacokinetic data generated by orally administering the prodrugs.

EXPERIMENTAL

Materials - Analytical grade chemicals were used without further purification. All water was distilled prior to use. Dyphylline was obtained from Lemmon Pharmacal Company. Eight dyphylline prodrugs (Table I) including four monoesters and four corresponding diesters were used; the details of synthesis, structure identification and preformulation stability study of these compounds have been reported previously(13).

Instrumentation - A high-pressure liquid chromatographic (HPLC) system consisted of a delivery pump[1], sample injection apparatus[2], 30-cm reverse phase C18 column[3], UV detector[4] with wavelength of 280 nm and a dual pen recorder[5] were used at ambient temperature. A guard column (14 cm long and packed with C18 packing powder[6]) was used to protect the column and was repacked when the back-pressure was beyond 3000 psig. The HPLC operating conditions included a mobile phase composed of acetonitrile (6% V/V) in distilled water and a flow rate of 2.0 ml/min. This system clearly separates dyphylline and theophylline.

Animals - All work was performed using female New Zealand White rabbits weighing 3.25-4.15 kg which were fed with standard commercial diets. They were fasted at least 12

hours before experiments involving oral dosing and allowed free access to water and food 4 hours after dosing. Rabbits were placed in animal restrainers during intravenous infusion experiments and when blood samples were collected from ear veins. Before each experiment, the rabbit ear was clipped and treated with hair remover lotion[7] for easy dosing and blood sample collection. The experiments were all single-dose studies. All oral prodrugs were administered once only to separate rabbits in this experiment. That is, there was no replication of oral prodrug administrations.

Drug administration –

1. Intravenous infusion of dyphylline –

Drug solutions containing 250 mg dyphylline in 10 ml distilled water were dissolved in a test tube with gentle flame heating to accelerate dissolution. The solution was prepared at the shortest possible time before administration. Sample was injected into an ear vein as a continuous infusion over a period of 15 minutes using an infusion pump[8].

2. Oral dyphylline prodrug administration –

For dyphylline prodrug oral administration, 1.967 mole of prodrug (equivalent to 500 mg of dyphylline) was suspended in 8 ml of distilled water after trituration with a small amount of glycerin as a wetting agent. Oral preparations were given by intubation using a rubber tubing which was about 1 cm in diameter and about 30 cm long. The tubing was flushed with an additional 8 ml water to carry all drug into the rabbit gut.

Plasma preparation - Blood samples were collected from an ear vein (opposite ear from the infusion site) at predetermined time intervals using a catheter[9] (20 G) with a heparin lock intermittent infusion plug . If the catheter became plugged with a clot samples were collected from ear veins using a needle (21 Ga.) and syringe.

Before collecting each sample through a needle, the rabbit ear was rubbed with xylene and procaine solution to dilate and anethetize ear veins. After a little more than 0.5 ml of blood was collected, the same volume of heparin solution (20 unit/ml) was injected. Immediately after each blood sample was drawn exactly 0.5 ml of blood was added to a centrifuge tube and 0.5 ml of acetonitrle (containing β-hydroxypropyl theophylline as internal standard) was added to precipitate the protein. After vortexing well the blood acetonitrile mixture was centrifuged (3000 xG, 10 minutes) and 10 microliters of

supernatant solution was injected into the HPLC. Rabbit blood is quite clear and use of whole blood in this manner rather than plasma or serum gave excellent analytical results and was very convenient.

Standard solutions -

Stock standard solutions were prepared to contain 0.5, 2, 4, 10, 30 and 50 mg of dyphylline in 100 ml of distilled water. Twenty five microliters of each was then added to 0.475 ml of rabbit blood to prepare working blood standard solutions. Linearity of peak height ratio versus dyphylline blood concentration curves was investigated. Although correlation coefficients were reasonably good, systemic deviations made use of parabolic regression more appropriate and correlation coefficients of 0.99 or more were obtained. The statistical information for standard curve fitting used in this experiment was reported previously(19).

Pharmacokinetic analysis -

Blood dyphylline concentrations during and after a constant intravenous infusion, of 250 mg dyphylline for T hours, were fitted to either a standard one- or two-compartment open model using the computer program SIMPLEX(20) according to Scheme III and IV.
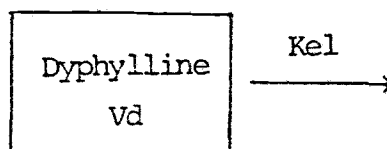
Scheme III- One compartment open model of drug distribution following intravenous injection of dyphylline.
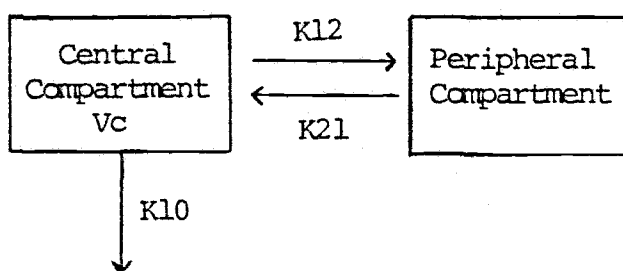
Scheme IV- Two compartment open model of drug distribution following intravenous injection of dyphylline.

For one-compartment open model fitting, postinfusion data were used and the best fit parameters found were corrected for infusion time(25). For a two-compartment open model, all the data were non-linearly least squares fitted to the following equation(26) directly:

$$C(t) = \frac{K_0(K_{21}-\alpha)}{V\alpha(\beta-\alpha)}\{(1-e^{-\alpha t})-U(t-T)(1-e^{-\alpha(t-T)}\}$$
$$+ \frac{K_0(K_{21}-\beta)}{V\beta(\alpha-\beta)}\{(1-e^{-\beta t})-U(t-T)(1-e^{-\beta(t-T)}\}$$
$$= A\{(1-e^{-\alpha t})-U(t-T)(1-e^{-\alpha(t-T)}\} + B\{(1-e^{-\beta t})-U(t-T)(1-e^{-\beta(t-T)}\}$$

where C(t) is the concentration of dyphylline in blood at time t. U(t) is the well known unit step function. The parameters were corrected to obtain the equivalent parameter values that would have been found if instantaneous bolus intravenous administration of the same dose were to be applied.

Input function analysis (see also Appendix II)-

A numerical deconvolution method(27) was used to resolve blood dyphylline concentration data obtained following oral dyphylline prodrug administration and to give the input function. Assuming linear pharmacokinetics for dyphylline , the function used to describe the blood dyphylline concentration data obtained following oral dyphylline prodrug administration, R(t), will be a combination of input function and disposition fuction(28):

$$R(t) = I(t) * F(t)$$

where I(t) is input function, F(t) is a disposition function (unit impulse) and * is a symbol for convolution. Knowing F(t) and R(t) from blood concentration data following intravenous injection and oral administration, I(t) can be derived by a numerical deconvolution process. Although the calculation for numerical deconvolution may seem to be quite complex, a computer program DECON, based on the general form in the original paper(26), readily allows the computation of percent drug unabsorbed in the gastrointestinal tract. The absorption profile may be assessed without specifying a model for a linear system of dyphylline disposition. If semilogarithmic plots of percent drug remaining to be absorbed versus time are linear, an apparent first-order absorption rate constant may be used to describe the input behavior of dyphylline from the prodrug according to the model presented in Scheme IV. In other cases, if rectilinear plots of percent drug remaining to be absorbed versus time are linear, an apparent zero-order absorption process may have occurred. The absorption rate constant calculated in this manner is an apparent absorption rate constant which will overestimate the true absorption rate constant if a simultaneous loss of drug from the absorption site occurs(29).

Human simulations -

Typical dyphylline oral administration data in human subjects was reported by Gisclon et al.(30). The published data were fitted to a two-compartment open model in the current study with first-order absorption using NONLIN(31). The fitted equation is

$$C_p = 10.46\ e^{-1.38t} + 17.37\ e^{-.318t} + 27.83\ e^{-4.32t}\ ,t>0$$

where the absorption rate constant, ka, is 4.32 hr and the dose used was 1.2 grams. Four simulations for humans in different dosing regimens were performed to demonstrate the effect of varying ka on the time of duration of dyphylline plasma concentrations greater than 10 mcg/ml. Time intervals used were 8, 8, 8 and 12 hours; doses used were 1.2, 3, 6 and 6 grams. In each simulation, 4 ka's, 4.32, 0.5, 0.3 and 0.1 hr^{-1} were employed. From the simulation data, an optimal "apparent" first-order absorption rate constant can be determined which will give maximum duration of dyphylline concentration in the assumed therapeutic range(10-20 mcg/ml).

NOTES

1. M-6000A Pump, Waters Associates, Milford, Ma.

2. Model U6K Injector, Waters Associates, Milford, Ma.

3. U Bondapak C18 Column, Waters Associates, Milford, Ma.

4. Model 440 Absorbance Detector, Milford, Ma.

5. Dual Pen Recorder, Soltec Company, Encino, Ca.

6. Bondapak C18/Corasil, Water Associates, Milford, Ma.

7. Nair, Carter-Wallace, Inc., New York, N.Y..

8. Model 941 infusion/withdrawal pump, Harvard Apparatus, Millis,Ma.

9. Quik-Cath (20 Ga), Travenol Laboratories, Inc., Deerfield, Il.

10. Sterile intermittent infusion plug, Argyle Co., St. Louis, Mo.

RESULTS AND DISCUSSION

Dyphylline intravenous infusion -

Log blood dyphylline concentration versus time plots following intravenous infusion of dyphylline are presented in Figure 1. The graph shows a linear decline after infusion stopped, suggesting that dyphylline distribution and elimination may be described by a standard one-compartment open model for the time period studied. Parameters obtained from linear least-squares fitting of the terminal data to a one-compartment model appear in Table II. The fitted line of the data can be seen in Figure 1. If the parameters used to describe the disposition of dyphylline in the blood are corrected for infusion time, the average equation will be as follow:

$$C = 104 \ e^{-1.05 \ t}$$

where t is the experimental time, 104 mcg/ml is the equivalent initial dyphylline blood concentration if bolus intravenous administration of the same dose had been administered and the drug had followed one compartment open pharmacokinetics from time zero (Dose administered was divided by volume of distribution calculated; see footnotes of Table II). Average parameter values are arithmetic means of three rabbits used in Figure 1.
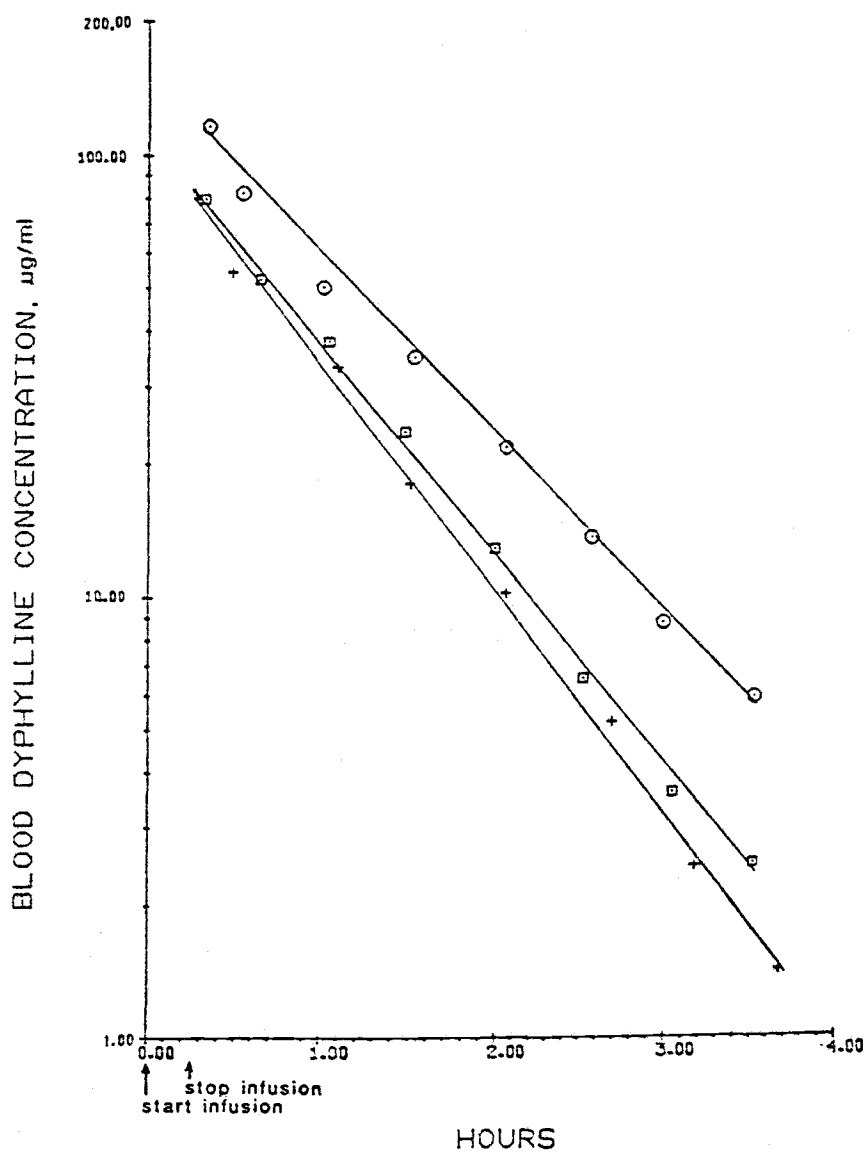
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Figure 1- Blood concentration versus time curves
of dyphylline after an intravenous infusion of
250 mg over 15 minutes in 3 rabbits. (Different
symbols represent plasma dyphylline concentrations
in each of three separate rabbits.)

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TABLE II - Pharmacokinetic Parameters[a] of Dyphylline in Rabbits After Intravenous Infusions[b] (one-compartment model).

	Rabbit-1	Rabbit-2	Rabbit-3	Mean	(S.D.)
C_{max}[c], mcg/ml	80.77	81.82	112.5	91.70	(18.02)
Kel, hr^{-1}	1.17	1.08	0.91	1.05	(0.13)
$t_{\frac{1}{2}}$[d], hr	0.59	0.64	0.76	0.66	(0.09)
Vd[e], liter	2.68	2.68	1.99	2.45	(0.40)

[a]Symbols of parameters are defined in "Pharmacokinetics" (1975) by Gibaldi and Perrier.

[b]The total dose was 250 mg/rabbit as a zero-order infusion over 15 minutes.

[c]Fitted blood dyphylline concentration at the time the infusion stopped

[d]$t_{\frac{1}{2}} = 0.693/Kel$

[e]$Vd = K_0 / C_{max} * Kel * (1-e^{-Kel*T})$; where K_0 is the infusion rate (250 mg 15 min) and T is the infusion time (15 minutes)

If a two compartment open model is preferred for description of the data, then the parameters obtained by fitting the data using the computer program SIMPLEX(20) and microscopic constants derived according to Scheme IV are presented in Table III. Since A and α values were fitted using 2 or 3 points only, their values may not be accurate enough to be used to describe the distribution phase precisely. Considering the rapid distribution, a one-compartment open model is an adequate approximation for dyphylline disposition in rabbits following intravenous injection.

Dyphylline prodrug oral administration -

Eight dyphylline prodrugs, 4 monoesters and their corresponding diesters, were administered orally in rabbits. The blood dyphylline concentration versus time curves are shown in Figures 2 and 3. From investigation of the terminal slopes in the figures and comparison to Figure 1, it is clear that the apparent biological half-lives of dyphylline following prodrug oral administration were significantly affected, probably due to slow dissolution of prodrugs in the gastrointestinal tract.

If a linear pharmacokinetic model were to be used to describe drug distribution following prodrug oral administration, Scheme I would be appropriate. However,

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TABLE III – Pharmacokinetic Parameter Estimates[a] of Dyphylline in Rabbits After Intravenous Infusions[b] (two-compartment model).

	Rabbit-1	Rabbit-2	Rabbit-3	Mean	(S.D.)
A, mcg/ml	58.0	56.8	63.7	59.5	(3.7)
B, mcg/ml	314	359	548	407	(124)
α, hr^{-1}	106.0	96.5	173.4	125.3	(41.9)
β, hr^{-1}	1.16	1.11	0.90	1.06	(0.14)
Vc[c], ml[c]	153.5	170.0	86.7	136.7	(44.1)
K_{21}[d], hr^{-1}	7.04	7.59	8.32	7.65	(0.64)
K_{12}[e], hr^{-1}	82.6	75.9	147.1	101.9	(39.3)
K_{el}[f], hr^{-1}	17.5	14.2	18.9	16.9	(2.4)
$T_{\frac{1}{2}}$[g], hr	.60	.62	.77	.66	(0.09)
V_B[h], ml[d]	2310	2170	1820	2100	(252)

[a] Symbols of parameters are defined in "Pharmacokinetics" (1975) by Gibaldi and Perrier.

[b] The total dose was 250 mg/rabbit as a zero-order infusion over 15 minutes.

[c] Apparent volume of the central compartment; Vc = Dose/(A+B)

[d] K_{21} = $(A\beta+B\alpha)/(A+B)$

[e] K_{12} = $\alpha+\beta-K_{21}-K_{el}$

[f] K_{el} = $\alpha\beta/K_{21}$

[g] $T_{\frac{1}{2}}$ = $0.693/\beta$

[h] Apparent volume of distribution of drug in the body; Vd = Vc * K_{el}/β
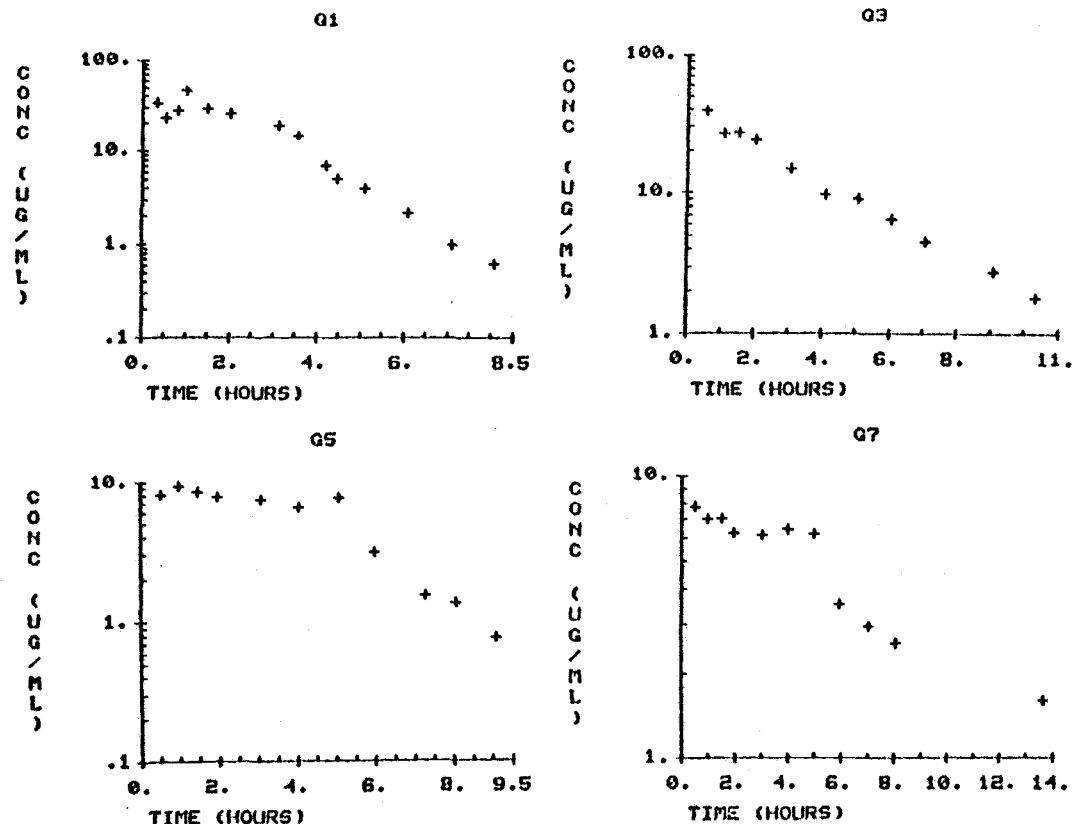
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Figure 2- Blood dyphylline concentration versus time curves following oral administration of four dyphylline monoesters. Key: Gl, monopropionoyl-dyphylline; G3, monoisobutyroyl dyphylline; G5, monopivaloyl dyphylline; G7, mono-p-Cl-benzoyl dyphylline.
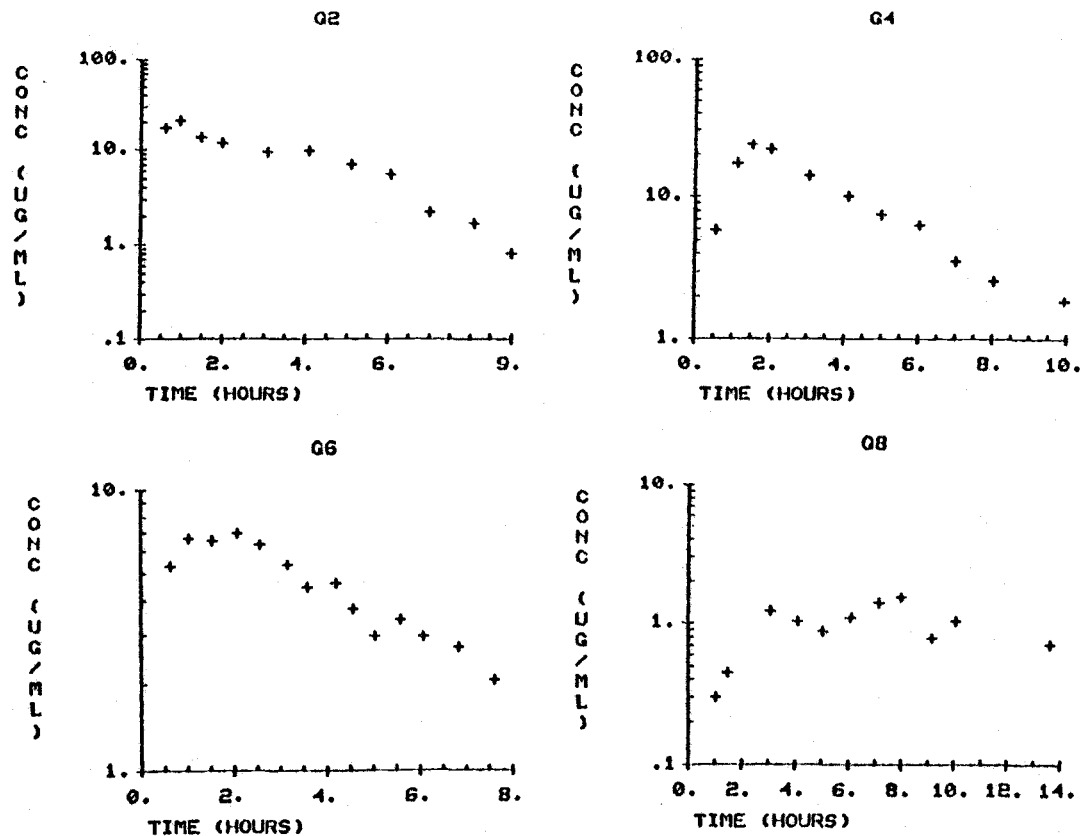
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Figure 3- Blood dyphylline concentration versus time curves following oral administration of four dyphylline diesters. Key: G2, dipropionoyl dyphylline; G4, diisobutyroyl dyphylline; G6, dipivaloyl dyphylline; G8, di-p-Cl-benzoyl dyphylline.

there is insufficient information to fit the data in Figures 2 and 3 to the equation which may be derived from this schematic model. In addition, since no assayable prodrug concentration was observed in the blood samples collected after 30 minutes following prodrug intravenous administrations(20) , it may be concluded that most prodrugs were converted to dyphylline in the first-pass through liver before getting into the general circulation. The fast conversion rate in vivo may be due to plasma enzymes or due to a first-pass effect. Due to this evidence and the inherent ambiguity of microconstants, the model in Scheme IV was applied for simplicity.

A model-independent input function analysis using a numerical deconvolution process(27) was performed to resolve the absorption profile; and the percent dyphylline remaining to be absorbed versus time curves are shown in Figures 4 and 5. Only the slope for percent dyphylline unabsorbed following oral administration of di-p-Cl-benzoyl dyphylline (Figure 5) showed a good linear relationship which suggests that zero-order absorption may have occurred. Semilogarithmic plots of the same data in Figures 6 and 7 showed that the other 7 prodrugs have apparent first-order absorption kinetics.

Based on the fact that all these prodrugs started from 100 % unabsorbed at time zero, least-square linear regression lines were forced through the (0,100) point and
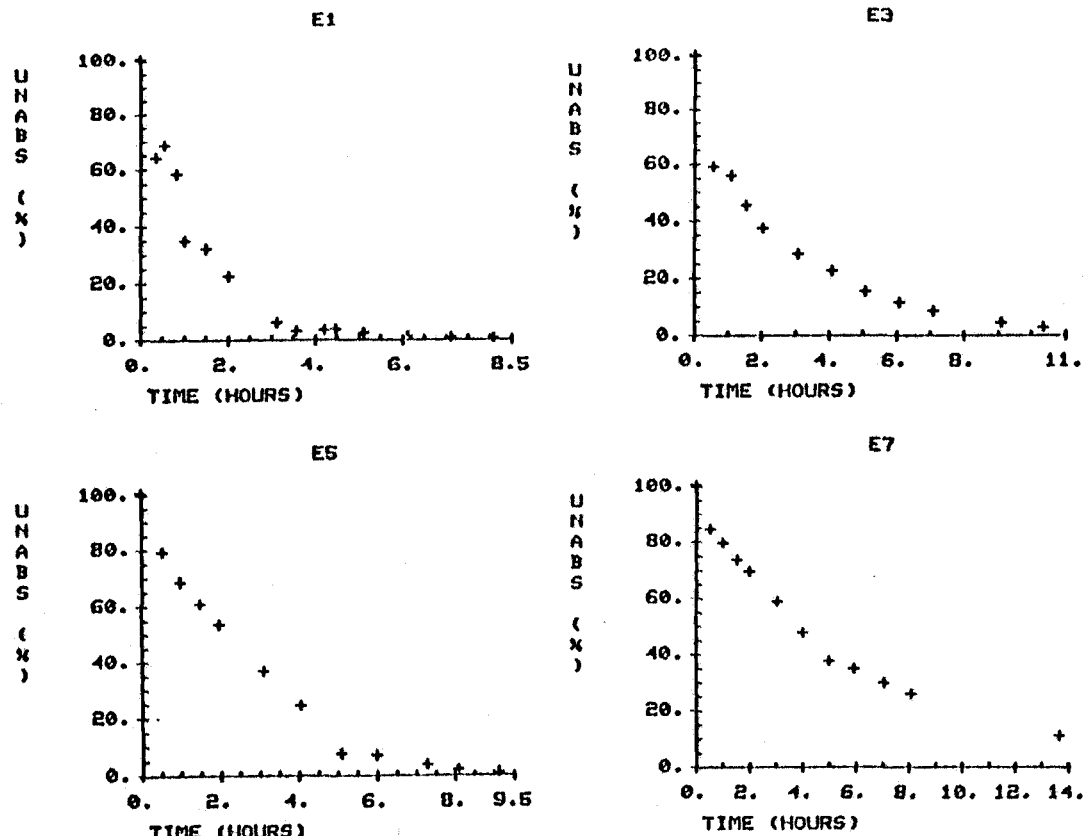
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Figure 4- Percent dyphylline unabsorbed versus time curves following oral administration of four dyphylline monoesters; data were derived by numerical deconvolution process. Key: E1, monopropionoyl dyphylline; E3, monoisobuty-royl dyphylline; E5, monopivaloyl dyphylline; E7, mono-p-Cl-benzoyl dyphylline.
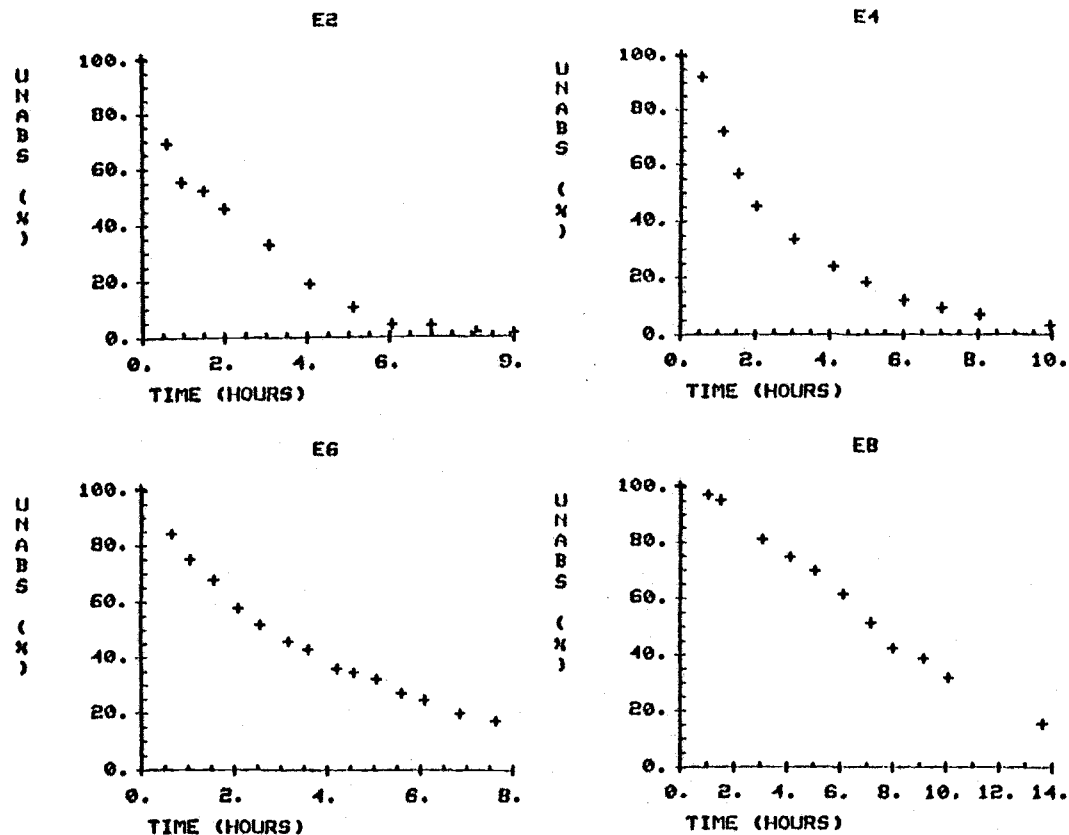
Chapter II

Figure 5- Percent dyphylline unabsorbed versus time curves following oral administration of four dyphylline diesters; data were derived by numerical deconvolution process. Key: E2, dipropionoyl dyphylline; E4, diisobutyroyl dyphylline; E6, dipivaloyl dyphylline; E8, di-p-Cl-benzoyl dyphylline.
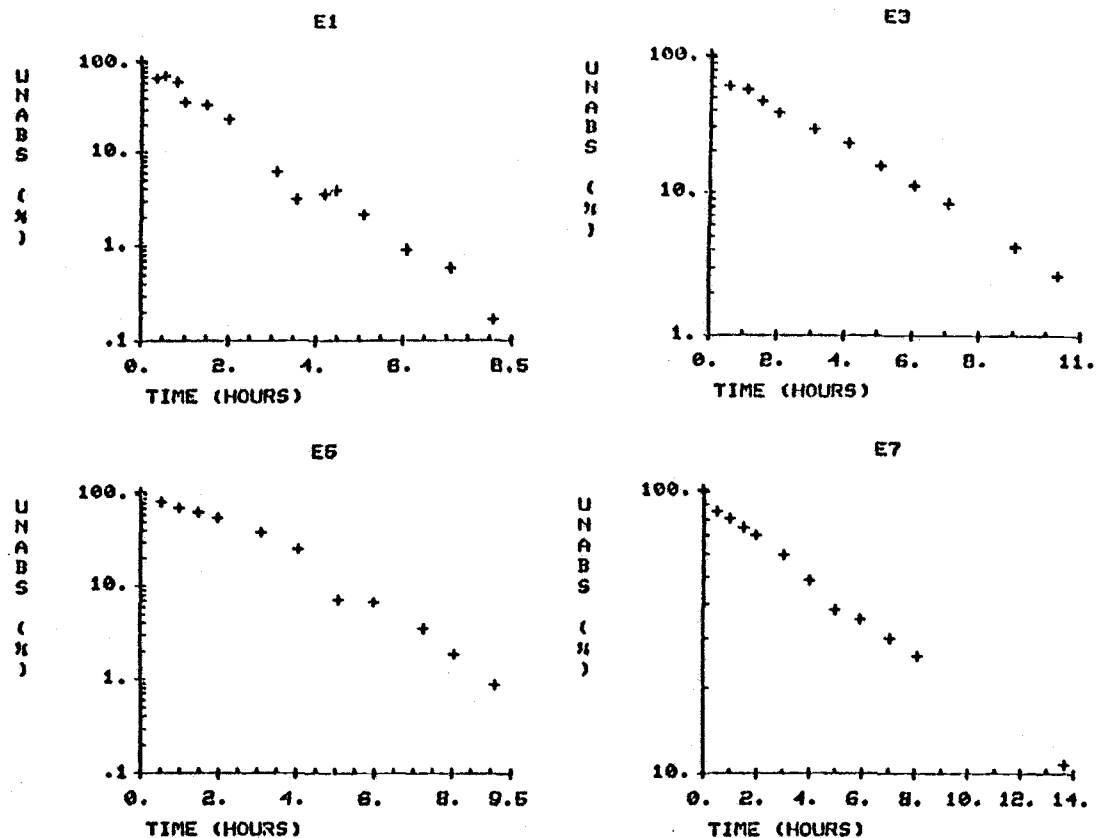
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Figure 6- Semilogrithmic plot of percent dyphylline unabsorbed versus time curves following oral administration of four dyphylline monoesters; data were derived by numerical deconvolution process. Key: E1, monopropionoyl dyphylline; E3, monoisobutyroyl dyphylline; E5, monopivaloyl dyphylline; E7, mono-p-Cl-benzoyl dyphylline.
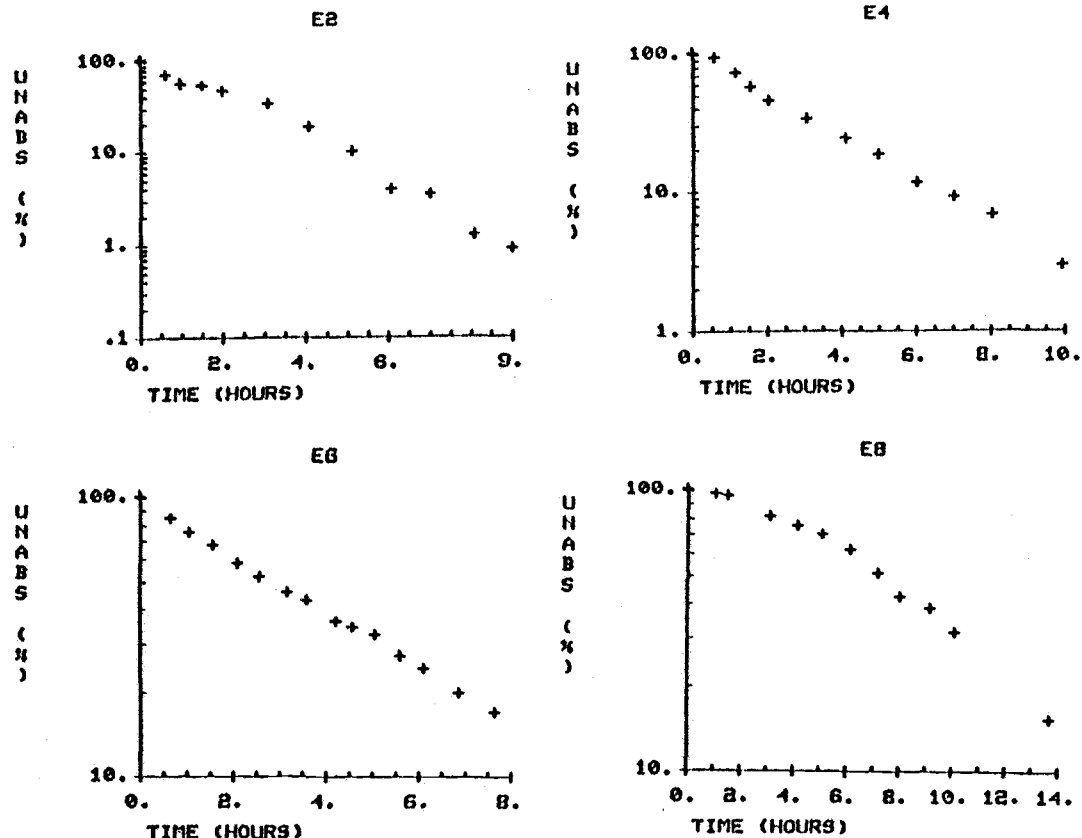
Figure 7- Semilogrithmic plot of percent dyphylline unabsorbed versus time curves following oral administration of four dyphylline diester; data were derived by numerical deconvolution process. Key: E2, dipropionoyl dyphylline; E4, diisobutyroyl dyphylline; E6, dipivaloyl dyphylline; E8, di-p-Cl-benzoyl dyphylline.

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TABLE IV – Absorption Rate Constants Derived From Deconvolution
Data Following Oral Dyphylline Prodrug Administration

Compounds	Kabs (hr^{-1})[a]
Monopropionoyldyphylline	0.79
Dipropionoyldyphylline	0.51
Monoisobutyroyldyphylline	0.36
Diisobutyroyldyphylline	0.35
Monopivaloyldyphylline	0.48
Dipivaloyldyphylline	0.24
Mono-p-Cl-benzoyldyphylline	0.17
Di-p-Cl-benzoyldyphylline	0.11

[a]First-order approximation of absorption rate constants.

used to fit the data in Figures 6 and 7; the absorption rate constants obtained from the slopes of these lines are prsented in Table IV.

Although complete pharmacokinetic analysis was not carried out for the dyphylline data following oral prodrug administration due to prolonged absorption preventing identification of beginning of the elimination phase, the absorption rate constants give us a measure of controlled drug delivery for the prodrugs.

Human dyphylline pharmacokinetic simulations-

In Figure 8, the assumed dosing interval is 8 hours and the dose is 1.2 grams. The first curve (Ka=4.32) is a theoretical disposition curve if Gisclon's data(30) is used for the simulation. A desired or effective range of 10-20 mcg/ml of dyphylline concentration was assumed and marked as horizontal lines. The duration of action predicted in Figure 8 is about one hour for ka=4.32 hr^{-1} and a highly fluctuating curve is observed. In order to reduce the fluctuation, a more frequent dosing interval with smaller doses will be necessary if ka stays at 4.32 hr^{-1}. However, this approach may not be clinically applicable. An alternative is to use a controlled release dosage formulation of dyphylline; the lower three curves in Figure 8 show that the fluctuation decreases with decreasing ka values. However, the decreased ka is
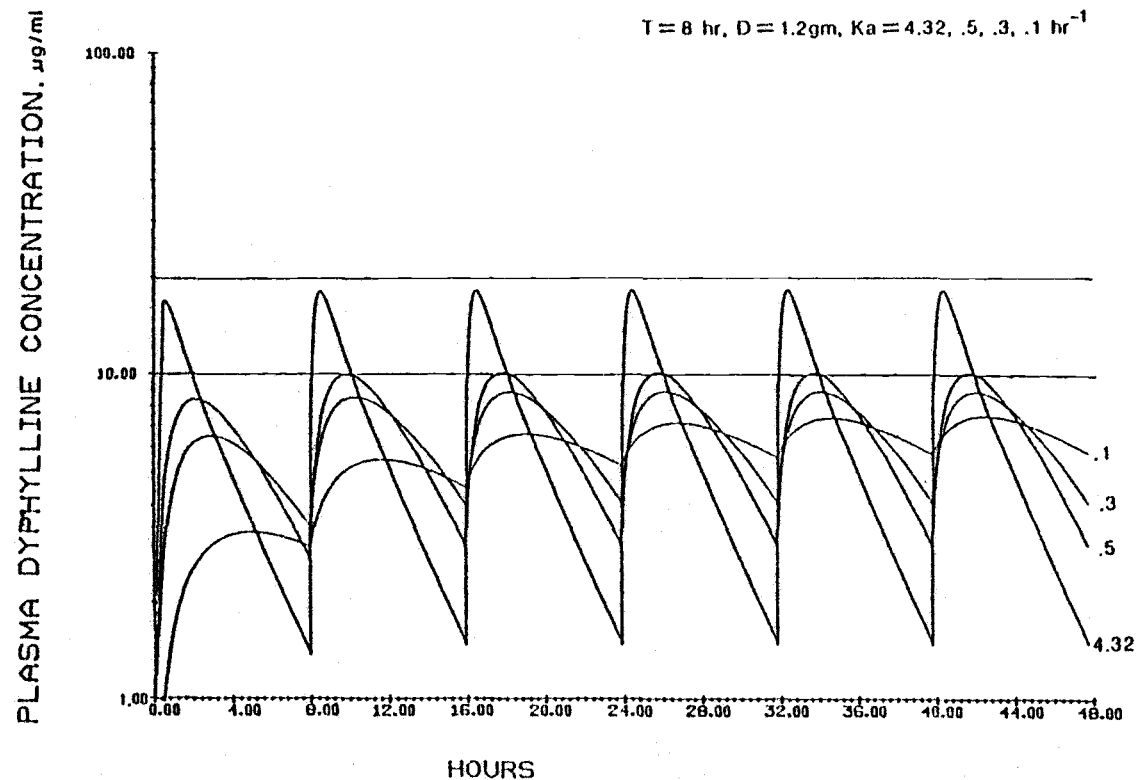
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Figure 8- Simulated dyphylline concentration versus time curves following oral administration of different absorption rates; D = 1.2 grams, T = 8 hours.

accompanied by a decrease in plasma dyphylline concentration and thus an increased dosage will be necessary to achieve the desired steady state plasma concentration.

In Figures 9 and 10, doses of 3 and 6 grams were employed for simulation. Figure 9 shows a desirable range of 10-20 mcg/ml can be achieved and Figure 10 shows a desirable range of 20-40 mcg/ml of dyphylline can be achieved depending on ka and dose. Figures 9 and 10 also show that duration of action increases with decreasing ka values. If it is desired to reach the therapeutic range by 12 hour dosing intervals, an input rate constant of 0.3 hr^{-1} may be most appropriate for maximizing the duration of action (See Figure 11). Although a smaller input rate gives a smaller fluctuation, it may not increase the duration of action as can be seen from the figures; and a decrease in extent of absorption is more likely with the smaller Ka value.

In Figure 11, the dose was assumed to be 6 grams and the dosing intervals were extended to be 12 hours. The simulation curves showed that an absorption rate constant of 0.2 to 0.3 hr^{-1} will be desirable.

Based on the above consideration, a dyphylline prodrug which has an absorption rate constant of about 0.25 hr^{-1} will be optimal. Table IV indicates that dipivaloyl dyphylline, with an absorption rate constant
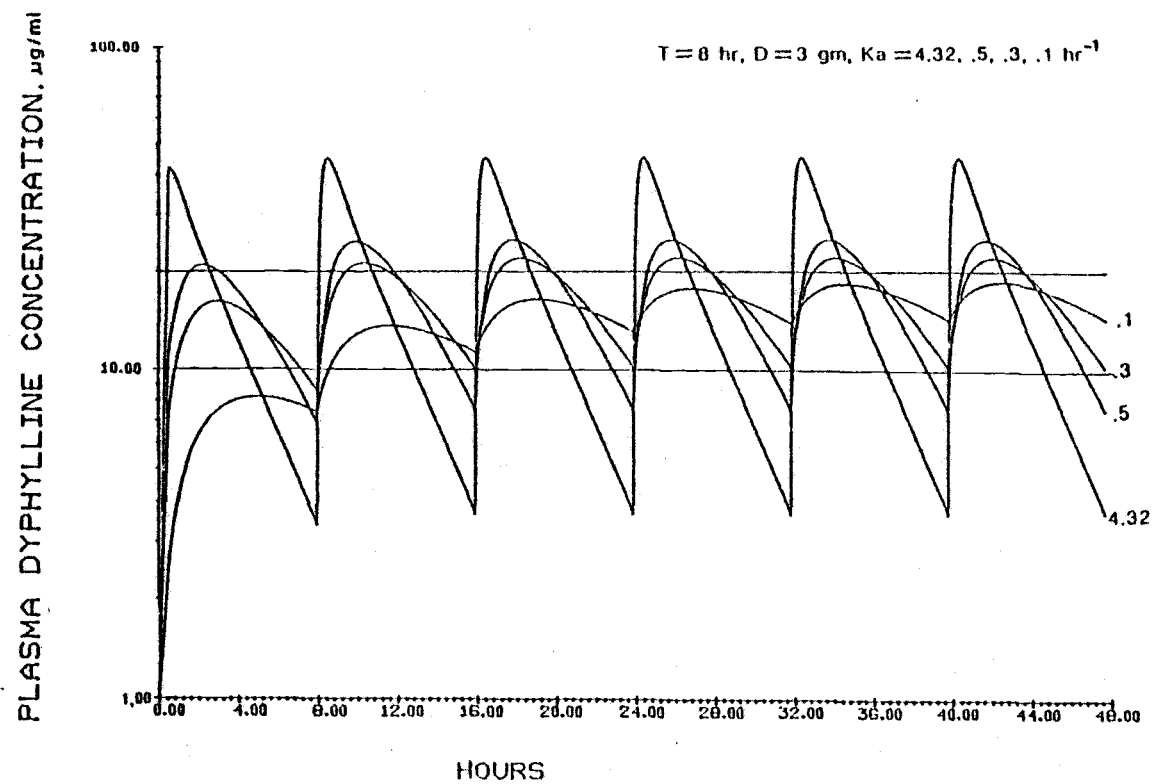
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Figure 9- Simulated dyphylline concentration versus time curves following oral administration of different absorption rates; D = 3.0 grams, T = 8 hours.
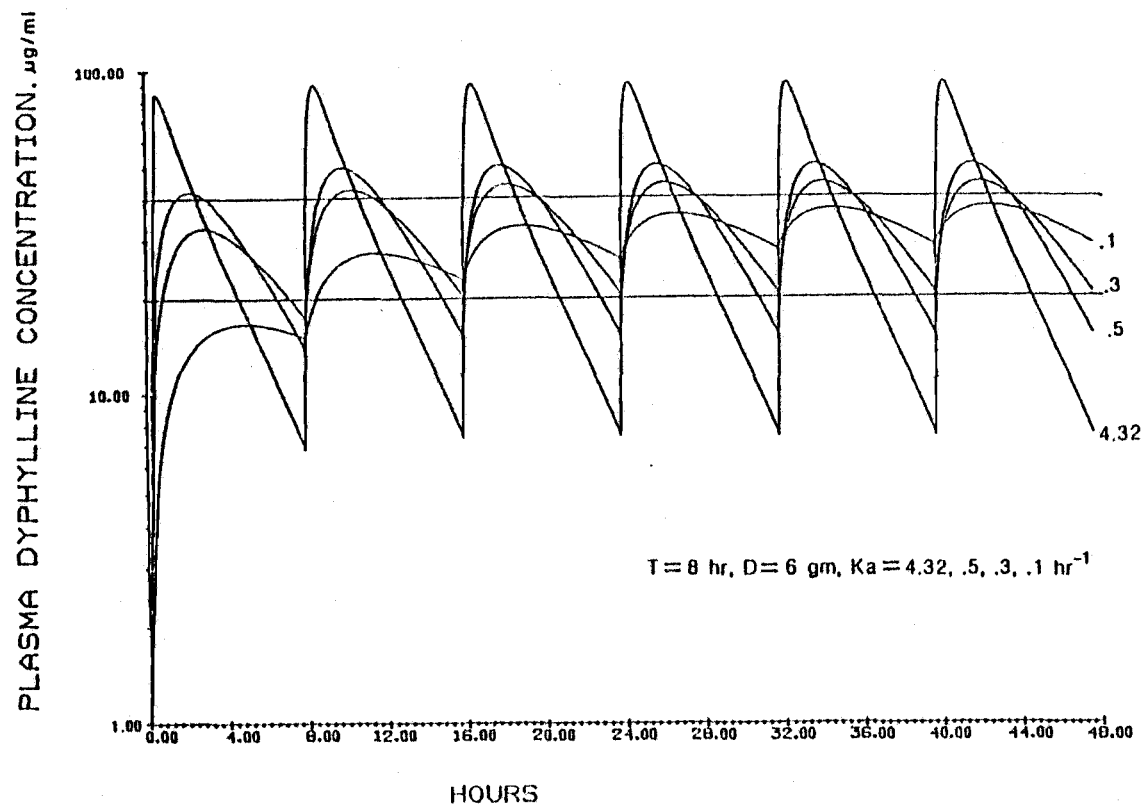
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Figure 10- Simulated dyphylline concentration versus time curves following oral administration of different absorption rates; D = 6.0 grams, T = 8 hours.
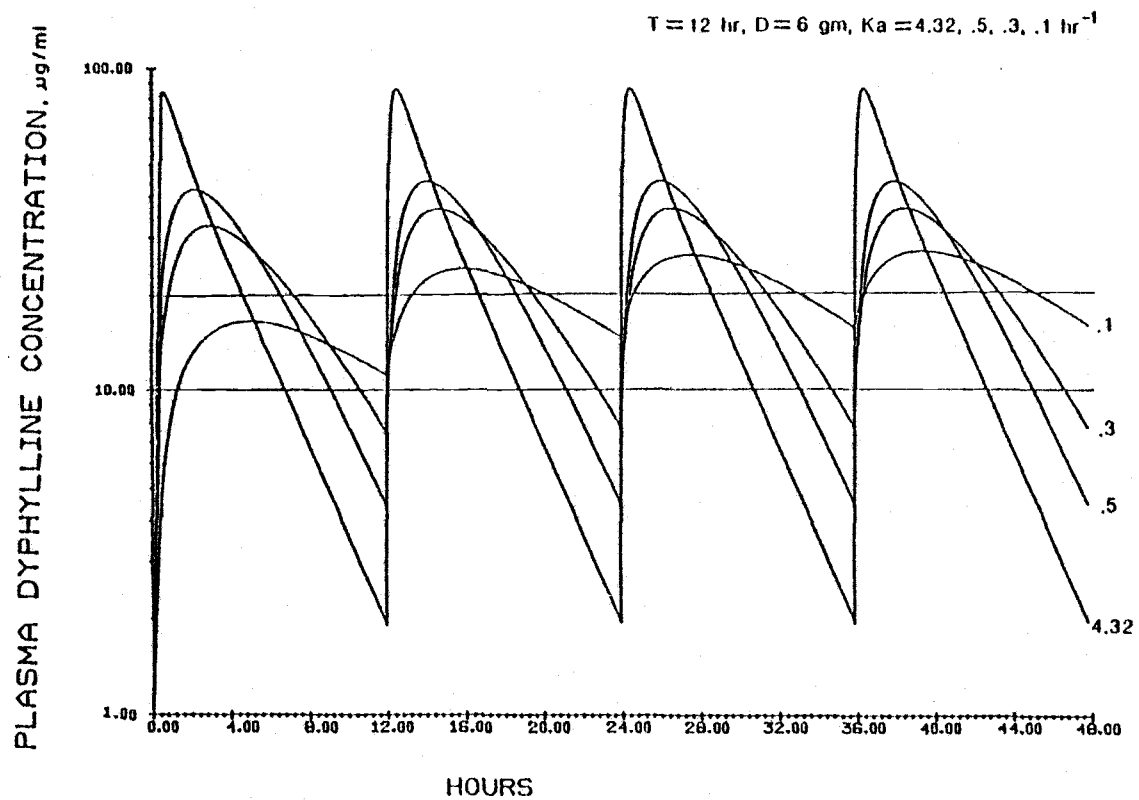
Figure 11- Simulated dyphylline concentration versus time curves following oral administration of different absorption rates; D = 6.0 grams, T = 12 hours.

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equal to 0.24 hr^{-1} in rabbits, is the best candidate for further testing based on the data available.

In these simulation studies which were used to determine the optimal Ka for maximizing duration of action, a therapeutic range of 10-20 mcg/ml or 20-40 mcg/ml were considered (See Figures 8-11). Although Simons et al. (12) suggested a minimum plasma dyphylline concentration of 12 mcg/ml is necessary and other authors(32,33) recommended much higher concentrations, no minimum effective concentration and optimal therapeutic range were extensively studied. With the fast elimination rate of dyphylline, a constant plasma concentration may be maintained using constant intravenous infusion and the optimal therapeutic range could thus be determined. Even though the optimal range may not be as suggested in these simulation studies, the optimal absorption rate will be similar, only the amount of dose will change. The simulation and preliminary pharmacokinetic studies presented in this research indicate that dipivaloyl dyphylline is worth further extensive studies.

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CHAPTER III

PHARMACOKINETICS OF DYPHYLLINE AND DYPHYLLINE

PRODRUGS IN PIGS: INPUT FUNCTION ANALYSIS

INTRODUCTION

Methylxanthines are widely used in the treatment of asthmatic patients. Theophylline is the most popular drug in this group. However, it is reported to have frequent adverse effects(1,2), highly variable half-life(3-8) and a narrow therapeutic range(9-11). Dyphylline (dihydroxypropyl theophylline) was introduced into clinical medicine in 1946 in an attempt to solve the above problems associated with theophylline. Although it has been marketed as a brochodilator, its rather short half-life has made its clinical usefulness limited. The half-life of dyphylline from conventional tablet formulations is reported to be 2.11 hrs(12) and 2.01 hrs(13). With this short half-life, optimal dosing intervals of every half-life is clinically impractical. Multiple dosing with longer dosing intervals and larger doses will cause large fluctuations of plasma dyphylline concentrations and may still have a short duration of action. A controlled release design of dyphylline may be developed to reduce fluctuations in blood concentrations and prolong the duration of action. A sustained release formulation of dyphylline has been reported wherein dyphylline was embedded in a water-insoluble plastic matrix and was slowly leached by gastrointestinal fluids(14). An apparent absorption half-life of 0.88 hour

and an apparent elimination half-life of 3.42 hours were reported. Previous simulation studies of dyphylline following oral administration(15) indicated that a first-order absorption rate constant of 0.2 to 0.3 hr^{-1} is desirable if the duration of action is to be maximized. Although an even lower absorption rate constant may further reduce fluctuations of plasma dyphylline concentration, a reduced extent of absorption may accompany and offset the advantages. The same simulation also shows, if a plasma dyphylline concentration of 10 to 20 mcg/ml is to be maintained by eight-hour dosing intervals of controlled release dyphylline preparation, at least 3 grams of such dyphylline preparation should be taken. Current physical approaches in preparing controlled release solid oral dosage forms generally results in products which must be swallowed whole and cannot be chewed or crushed since chewing or crushing prior to ingestion destroys the control of drug release. Since people cannot be expected to swallow 3 grams whole as a bulky dosage form, such a controlled release product is not feasible for dyphylline. However, chemical modification of dyphylline to produce a prodrug could result in a conventional chewable tablet. The prodrug would then release dyphylline in a controlled manner after chewing and swallowing so as to produce a continuous effective concentration of dyphylline in the body. A

series of ester derivatives of dyphylline was synthesized as dyphylline prodrugs(16). Previous studies of these derivatives in New Zealand White rabbits and simulation studies of dyphylline following oral administrations of different absorption rates(15) have shown that dipivaloyl dyphylline, with an apparent first-order absorption rate constant of 0.24 hr^{-1} in rabbits, may be the most promising prodrug of dyphylline for more extensive studies for prolonged release of dyphylline.

An animal larger than rabbits, Yorkshire pig, which is similar to humans both in size and diet was utilized in this study. Physiological conditions in the gastrointestinal tract of pigs are similar to humans, thus results from pigs may give a projectable indication of dyphylline pharmacokinetics and plasma concentrations following oral dyphylline derivative administration in humans. In these studies, absorption kinetics and bioavailability of dyphylline from dipivaloyl dyphylline suspension, monopivaloyl dyphylline suspension and dyphylline solution were compared in pigs.

As one may expect, drug absorption is generally less efficient when food is present in the gastrointestinal tract(17). This is especially important when controlled release dosage forms are involved. In order to test if bioavailability of dipivaloyl dyphylline -with respect to both 'rate' and 'extent'- is changed, drug absorption with

and without concomitant food intake were also investigated.

EXPERIMENTAL

Materials - Dyphylline injections[1] were donated by Lemmon Pharmacal Company. Two dyphylline ester derivatives, monopivaloyl dyphylline and dipivaloyl dyphylline, were synthesized in our laboratory utilizing pivaloyl chloride and dyphylline. The details of synthesis and identification were reported elsewhere(16). All reagents were analytical reagent grade quality. Deionized water was used for sample preparation.

Instrumentation - A high-pressure liquid chromatographic (HPLC) system consisted of a delivery pump[2], sample injection apparatus[3], 30-cm reverse phase C18 column[4], UV detector[5] with wavelength of 280 nm and a dual pen recorder[6] which were used at ambient temparature. A guard column (14 cm long and packed with C18 packing powder[7]) was used to protect the column and was repacked when the back pressure was over 3000 psig. HPLC operating conditions included a mobile phase composed of acetonitrile (6% V/V) in distilled water and a flow rate of 2.0 ml/min.

Animals - Yorkshire pigs weighing 68 ± 2 kilograms were used. A surgical operation was performed to place an indwelling catheter[8] in these pigs. Xylazine[9], 200 mg, was given intramuscularly as preanesthetic treatment and an initial dose of 150 mg of Ketamine[10] was administered via an ear vein as anesthetic medication; Ketamine

medication was continued to the end of surgery. The catheter was placed into the jugular vein; the tubing was run subcutaneously to the back of the neck. The catheter was filled with heparin solution (10 units/ml) and was sutured on the skin to avoid being ripped off. The animals were immediately put on 5 ml of antibiotics[11] intramuscularly and were dosed daily for the entire experiment. They were restrained in a farrowing cage and housed in a university pig barn. Food was restricted to keep the pigs at a nearly constant body weight through the experimental period. The pigs were fasted except for water from 24 hours before each treatment period began until 4 hours after dosing in oral administration experiments. The cage had two hinged doors on the front which close on both sides of the pig's neck; the animal was held with the head sticking outside the cage for dosing.

Drug administration -

a. Intravenous injection of dyphylline -

2 grams of dyphylline injection[1] were administered as a bolus injection through an indwelling catheter followed by 2 ml of haparin solution (10 unit/ml).

b. Oral dyphylline and dyphylline prodrug administration

Treatment A: Dyphylline powder (3 grams; 11.81 m moles) was dissolved in water (100 ml; 11.81 m moles) containing glycerin (about 15 ml) and Sodium carboxymethylcellulose (0.3 gram).

Treatment B: Monopivaloyl dyphylline powder (10 grams; 29.55 m moles) was wetted with glycerin (about 15 ml) and diluted to 100 ml with water containing Sodium carboxymethylcellullose (0.3 gram).

Treatment C: Dipivaloyl dyphylline powder (10 grams; 23.67 m moles) was prepared as described for treatment B.

Prodrug suspensions were dosed orally with a dosing gun and followed by about 100 ml of water.

c. Study design -

A typical Latin square cross over design was used to investigate the above three treatments. The Latin square design used is shown in Table I:

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TABLE I — Latin Square Design for Dyphylline
and Dyphylline Prodrug Oral
Administrations in 3 Pigs

	Periods		
Pigs	I	II	III
4	A	C	B
5	B	A	C
6	C	B	A

A = dyphylline solution
B = monopivaloyl dyphylline suspension
C = dipivaloyl dyphylline suspension

In the above design, time intervals between periods are 5 days which allowed complete elimination of residual dyphylline and dyphylline prodrugs in the body. (Note:

Pigs are numbered 4, 5 and 6 because preliminary work to establish procedures and techniques was conducted in pigs 1, 2 and 3.)

d. Food effects on oral dipivaloyl dyphylline administration -

For dipivaloyl dyphylline suspensions to be given orally with food, pigs were fed with solid pig chow prepared commercially at regular schedules. Ten grams of dipivaloyl dyphylline suspension was given by oral dosing gun between 2 halves of one feeding. For drug suspension to be given orally without food, pigs were fasted 24 hours before dosing and no food was allowed during the experiments. Free access of water was allowed.

Plasma preparation - Blood samples were collected from the indwelling catheter at predetermined time intervals. Before collecting each sample, 2 ml of heparin solution (10 unit/ml) was used to flush the catheter; 1.5 ml of blood was then drawn and discarded. Three milliliters of blood sample was collected and transferred to a plastic test tube which was pre-rinsed with anticoagulant sodium citrate solution (20% W/V). An additional 2 ml of heparin solution (10 unit/ml-for anticoagulation) was used to flush and fill the catheter after collection of each sample.

Blood samples were centrifuged and 0.5 ml of plasma was transferred to a small test tube. Half ml of acetonitrile containing internal standard(β -hydroxyethyl theophylline) was used to precipitate protein and 0.5 ml of 2 N sodium hydroxide solution was used to wash the plasma-acetonitrile mixture. After vortexing well, the plasma-acetonitrile mixture was centrifuged (2000 xG,10 minutes) and 1 ml of supernatant was transferred to a small bottle containing 4 ml of isopropanol/methylene chloride mixture (10/90 by volume). The mixture was shaken gently for 10 minutes and then transferred to a centrifuge tube.

After centrifuging for 10 minutes, the upper aqueous layer was removed by suction and the remaining solution was evaporated to dryness under nitrogen at $50°C$. The residual materials were reconstituted with 150 microliters of methanol and 15 microliters was injected into the HPLC. Standards - Stock standard solutions were prepared to contain 0.5, 1, 2, 10, 20, 30, 40 and 50 mg of dyphylline in 100 ml of distilled water. 50 microliters of each was then added to 0.95 ml of plasma to prepare working standard solutions. An investigation of linearity of peak height ratio versus dyphylline plasma concentration curves showed that the "log-log linear" fit is more suitable and precise(18,19). Correlation coefficients for the fit of standard curves to a line were between 0.99 to 1.00. A

typical linear regression function and its inverse estimates are listed in Table II.

Pharmacokinetic analysis - Plasma dyphylline concentration data for each pig following intravenous bolus injection of dyphylline were fitted to a standard two-compartment pharmacokinetic open model (Scheme I) (20) by a nonlinear least squares fitting procedure in PROPHET(21).
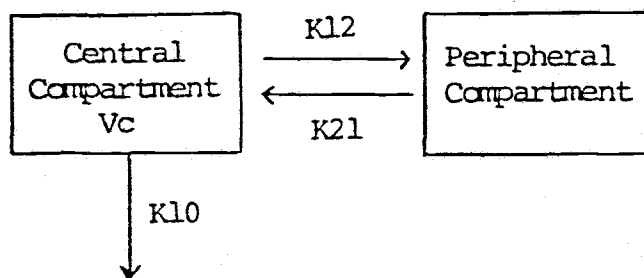
Scheme I- Two-compartment pharmacokinetic open model following bolus intravenous administration of dyphylline.

Initial parameter estimates for the nonlinear fitting were derived from curve stripping using the residual method(22) by hand calculator. Microconstant values defined in Scheme I were calculated by a standard procedure as described by Gibaldi and Perrier(20).

Cumulative amounts of dyphylline which reached systemic circulation following oral dyphylline prodrug administration can be calculated by a numerical deconvolution method(23) based on plasma concentration of dyphylline and a knowledge of pharmacokinetics of dyphylline following an intravenous bolus injection in the same subject. Percent dyphylline unabsorbed versus time

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TABLE II – Typical Standard Curve Data for Dyphylline
           Concentration Estimation Using log – log Linear
           Regression[a]

	CONC (mcg/ml)	PHR[b]	INV.EST.[c] (mcg/ml)	%THEORY[d]
ST1	0.5	0.044	0.51	102.0
ST2	1.0	0.087	1.04	104.0
ST3	2.0	0.158	1.90	95.0
ST4	10.0	0.764	9.47	94.7
ST5	20.0	1.54	19.35	96.8
ST6	30.0	2.54	32.19	107.3
ST7	40.0	3.17	40.39	101.0

[a]$R^2 = 0.999$

[b]Peak height ratio of dyphylline to internal standard.

[c]Inversely estimated concentration $= EXP[(PHR+2.4754)/.9813]$

[d]%THEORY =
        (inversely estimated concentration/known concentration)*100

plots can be generated to elucidate the input profile of dyphylline from the gastrointestinal tract after administration of the prodrug. This method may provide information for mechanistic model building and give a better understanding of the absorption kinetics. If the semilogarithmic plot of percent unabsorbed _versus_ time curves are linear, an apparent first-order absorption might be predominant. If the rectilinear plot of the same data are linear, the absorption kinetics might follow zero-order kinetics.

If the absorption kinetics are first order, the absorption rates of dyphylline following different prodrug preparations would be easier to be compared. However, if absorption is non-uniform, then the plots themselves are still useful in comparative bioavailability studies(24). In this case, statistical moments can be used to describe the time course of percent dyphylline unabsorbed following oral dyphylline prodrug administration. The zero and first moments for the curve are defined as follows(25,26):

$$AUC = \int_0^\infty C_p \, dt$$

$$MRT = \int_0^\infty t C_p \, dt \Big/ \int_0^\infty C_p \, dt$$

where t is time, AUC is the area under the plasma concentration-time curve, and MRT is the mean residence time of a drug in the body. Integration is performed using the log trapezoidal rule(25,27).

In order to estimate the fraction of drug absorbed at a given time and the mean residence time of dyphylline in the gastrointestinal tract, a simple relationship was derived by Yamaoka et al. as follows(26):

$$F = \frac{Mdyp}{Mtrt} \times \frac{AUCo}{AUCi.v.} \times 100\ \%$$

$$MRTg = MRTo - MRTi.v.$$

where F is the fraction of dose which is available from oral administration, Mdyp and Mtrt are the administered number of moles of dyphylline and tested drug, AUCo and AUCi.v. are the respective areas under the plasma dyphylline concentration-time curves from time zero to infinity after oral and intravenous administration. MRT's with subscript g, o or i.v. indicate mean residence time of the drug in the gastrointestinal tract and mean residence times of the drug in the blood stream following oral and intravenous administration. F and MRTg values are indicative of the extent and rate of bioavailabilities, respectively.

Statistical analysis - Two kinds of blocking variables, time and subject, are of interest in this study when treatments are to be compared. Since the full use of these two blocking variables in a complete block design may have the disadvantage of requiring too many experimental units, a Latin square design was utilized.

The Latin square arrangement shown in Table I allows elimination of both the time and inter-subject differences. Analysis of variance was used to determine differences between subjects, time periods, and treatments at the 95 percent significance level. Both extent and rate of absorption were compared.

NOTES

1. Neothylline injecton, 500 mg in 2 ml ampuls, Lemmon Pharmacal Company, Sellersville, PA.

2. M-6000A Pump, Water Associates, Milford, MA.

3. Model WISP 710 B Automatic injector, Water Associates, Milford, MA.

4. µ Bondapak C18 column, Water Associates, Milford, MA.

5. Model 440 Absorbance detector, Water Associates, Milford, MA.

6. Dual pen recorder, Soltec Co., Encio, CA.

7. Bondapak C18 powder, Water Associates, Milford, MA.

8. Cut-down catheter, 16 gauge, 36 inches, Deseret Pharmaceutical Co., Sandy, UH.

9. Rompun (Xylazine), 100 mg/ml, Cutter Laboratories, Inc., Shawnee, Ka.

10. Ketaset (Ketamine HCl), 100 mg/ml, Bristol Laboratories, Syracuse, NY.

11. Penstrep (each ml contains 200,000 units of Penicillin G and 250 mg of dihydrostreptomycin sulfate solution), Merck & Co., Inc., Rahway, NJ.

12. Stainless steel dosing gun which holds about 100 ml.

13. 2054 Tube, 12 x 75 mm, Falcon, Oxnard, CA.

RESULTS AND DISCUSSION

Intravenous administration-

Dyphylline injection (2 grams; 7.87 m moles) was administered intravenously in 6 different pigs (Numbers 1, 2 and 3 in a preliminary study and 4, 5 and 6 for the Latin square design). Table III shows the sampling times and assayed dyphylline concentrations in plasma. Semilogarithmic plots of plasma dyphylline concentration versus time data are presented in Figures 1 and 2. A typical two-compartment open pharmacokinetic model equation(20) was used to fit each set of data separately. Computer fitted regression lines for the data are also shown. The pharmacokinetic parameters obtained are tabulated in Table IV. Note that by visual inspection a terminal log-linear phase does not appear to have been achieved after 8 hours, especially for pigs 4, 5 and 6(Figure 2). This phenomenon of prolonged curvature in the semilogarithmic plot of dyphylline plasma concentration versus time has also been reported in humans(13). However, the curvature is very slight and the data can be quite well fit by computer (Figures 1 and 2) if it is assumed that a log-linear phase has been achieved, and that a two-compartment open pharmacokinetic model describes the data. Since such a model does a good job of describing the dyphylline plasma concentration versus time curve over 95% or more of the concentration

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TABLE III – Plasma Dyphylline Concentrations (mcg/ml) Following
Bolus Intravenous Administration of 2 grams of
Dyphylline in 6 Pigs

Time, Hour	Pig-1	Pig-2	Pig-3	Time Hour	Pig-4	Pig-5	Pig-6
0.4	22.27	24.06	28.50	0.5	23.32	25.35	28.54
0.5	19.88	22.26	27.71	0.67	21.39	22.62	26.33
0.75	14.63	16.47	22.61	0.83	19.54	18.27	22.41
1.0	11.20	12.05	17.60	1.0	17.85	16.43	22.06
1.25	9.46	9.51	15.82	1.5	11.76	9.61	15.94
1.5	9.18	8.80	13.23	2.0	9.72	7.55	11.14
2.0	6.40	6.20	8.99	2.5	7.75	5.14	8.56
2.5	4.44	4.54	7.70	3.0	6.06	4.32	6.02
3.0	3.53	3.84	6.10	3.5	4.86	3.44	4.88
3.5	2.71	2.74	4.80	4.0	3.54	2.57	4.00
4.0	2.14	2.41	4.09	4.5	2.81	1.98	3.47
5.0	1.40	1.44	2.74	5.5	1.78	1.34	1.96
6.0	0.93	1.14	2.23	6.5	1.22	0.96	1.51
7.0	0.66	0.76	1.47	7.5	0.84	0.70	0.99
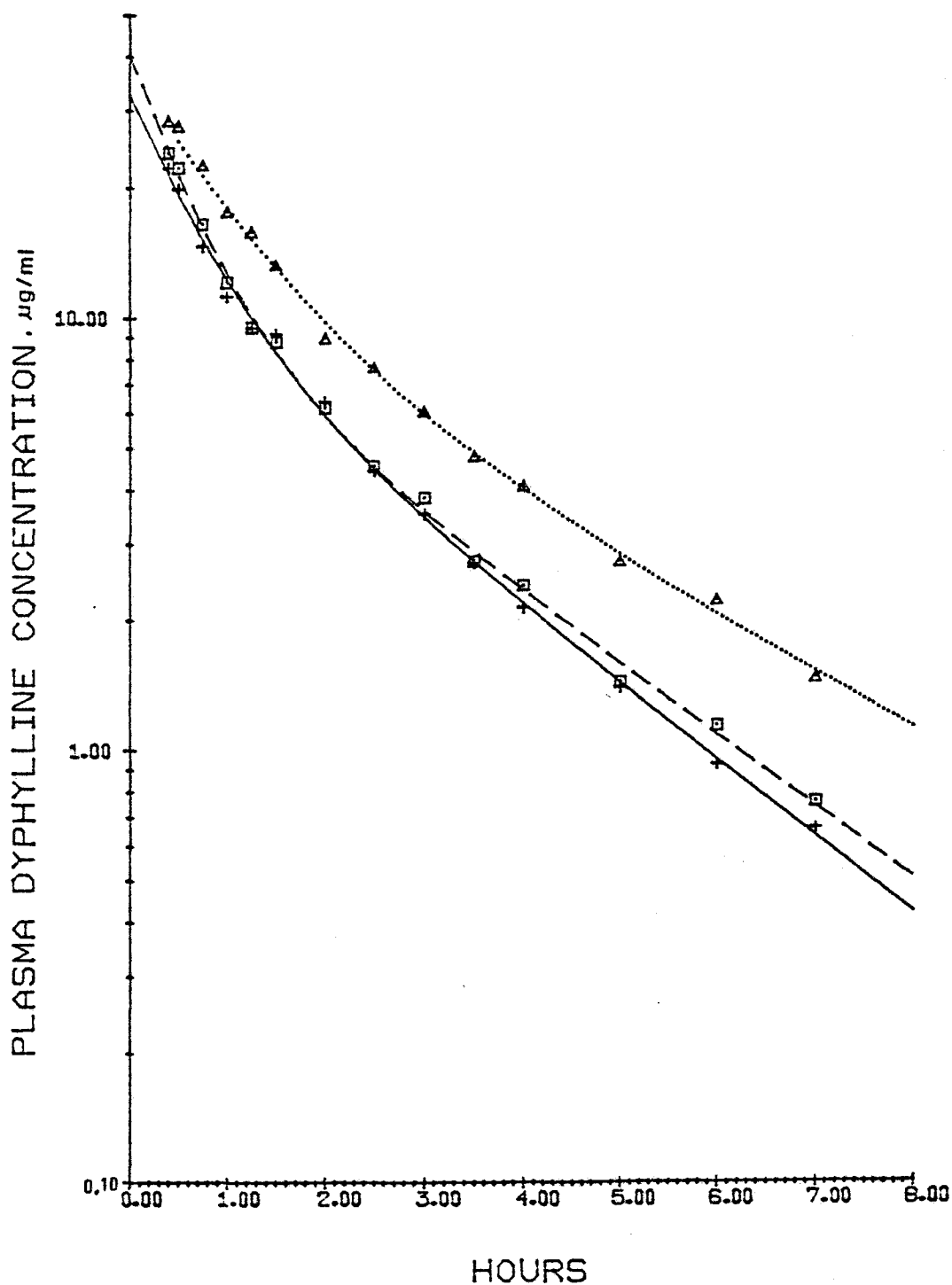
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Figure 1- Plasma dyphylline concentration versus time curves after a bolus intravenous administration of 2 grams of dyphylline in 3 pigs (pigs 1-3). Data points for each pig are represented by different symbols and the lines are computer fitted regression lines for the data.
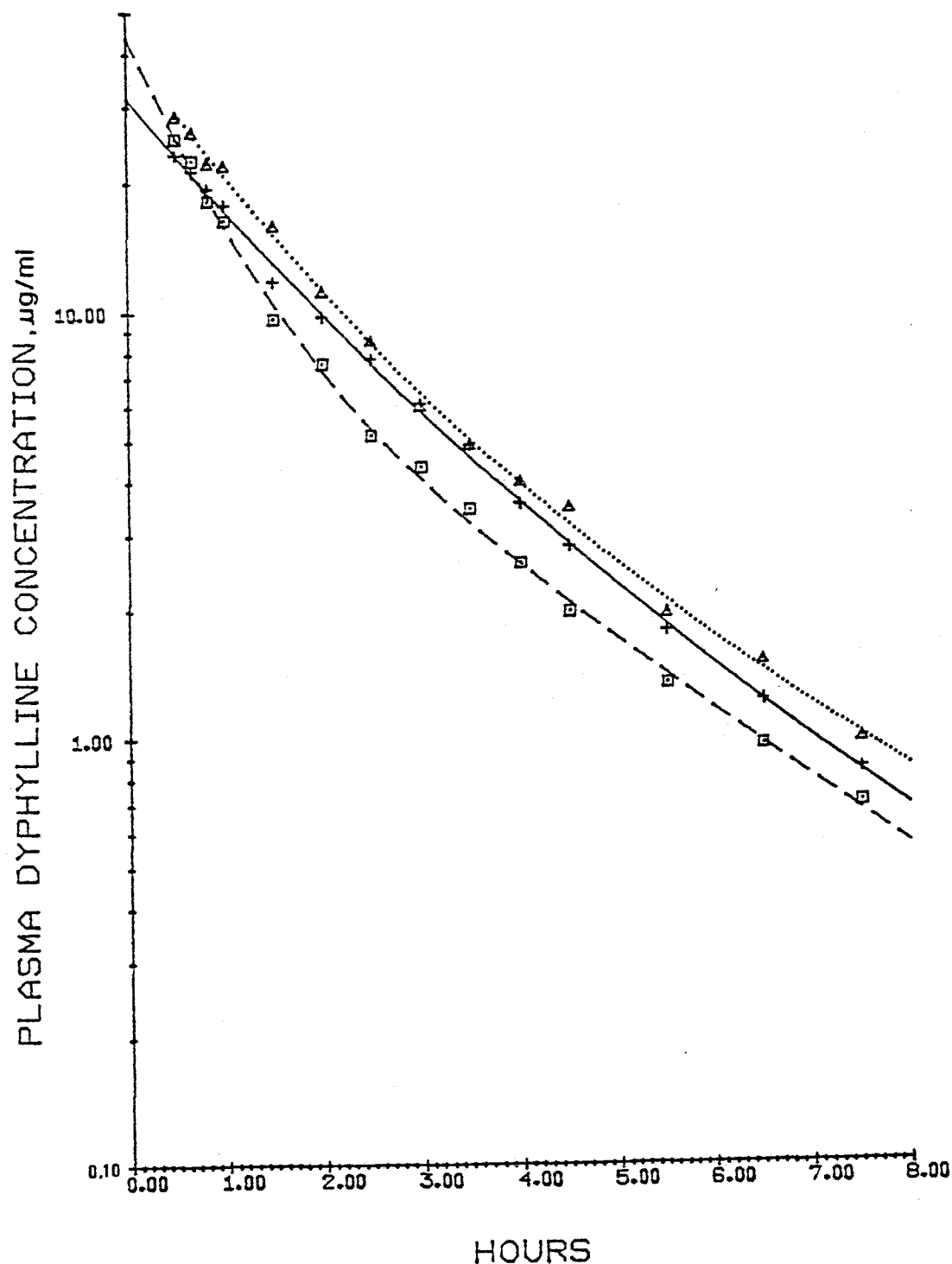
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Figure 2- Plasma dyphylline concentration versus time curves
after a bolus intravenous administration of 2 grams of dyphylline
in 3 pigs (pigs 4-6). Data points for each pig are represented by
different symbols and the lines are computer fitted regression
lines for the data.

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TABLE IV – Pharmacokinetic Parameter Estimates[a] Determined by Computer Analysis of Dyphylline Plasma Data in 6 Pigs

Parameter	Pig-1	Pig-2	Pig-3	Pig-4	Pig-5	Pig-6	Average(S.D.)
A, g/ml	21.81	29.69	26.04	23.11	33.28	31.45	27.56 (4.64)
B, g/ml	10.78	10.61	11.60	8.34	10.33	10.03	10.28 (1.09)
α, hr^{-1}	1.47	1.7	1.04	.71	1.34	.84	1.18 (.38)
β, hr^{-1}	.405	.380	.292	.327	.365	.314	.35 (.04)
K_{21}[b], hr^{-1}	.758	.728	.521	.429	.596	.441	.58 (.14)
K_{12}[c], hr^{-1}	.332	.465	.226	.068	.288	.115	.25 (.15)
K_{10}[d], hr^{-1}	.787	.888	.580	.543	.820	.598	.70 (.15)
Vc[e], liter	61.36	49.64	53.14	63.60	45.86	48.22	53.64 (7.28)
V_B[f], liter	119.11	116.02	105.62	105.74	103.08	91.75	106.89 (9.79)
$T_{\frac{1}{2}}$[g], hr	1.71	1.82	2.37	2.12	1.90	2.21	2.02 (.25)

TABLE IV (continued) – Standard Deviation Estimates for Parameters Used to Fit Dyphylline Plasma Data

	Pig – 1	Pig – 2	Pig – 3	Pig – 4	Pig – 5	Pig – 6
A, g/ml	5.55	2.31	10.46	28.17	4.22	15.78
B, g/ml	7.20	3.30	0.45	29.29	5.67	17.30
α, hr^{-1}	0.55	0.29	12.11	0.43	0.28	0.31
β, hr^{-1}	0.16	0.08	0.19	0.43	0.12	0.26

[a] Symbols and mathematical derivation of parameters are defined in "Pharmacokinetics" (1975) by Gibaldi and Perrier.

[b] $K_{21} = (A\beta+B\alpha)/(A+B)$

[c] $K_{12} = \alpha+\beta-K_{21}-K_{10}$

[d] $K_{10} = \alpha\beta/K_{21}$

[e] $Vc = Dose/(A+B)$

[f] $V_B = Vc * K_{10}/\beta$

[g] $T_{\frac{1}{2}} = 0.693/\beta$

range, it was used for estimation of model dependent
pharmacokinetic parameters of dyphylline in pigs.
Parameters for all six pigs were reasonably close over the
two different study days; average values and standard
deviations of each parameter for all six pigs were
calculated and are listed in the last column of Table IV.
The results show an average apparent half-life of 2.02 ±
0.25 hours which is comparable with the half-life of
dyphylline in humans as reported by Gisclon et. al. and
Simons et. al. (2.11 and 2.01 hours respectively)
(12,13). The similar dyphylline half-lives, shape of
plasma dyphylline concentration versus time curves, body
weights and diet indicate that pigs are a good animal
model for dyphylline prodrug studies.

Oral administration-

Tables V, VI and VII show sampling times and plasma
concentrations of dyphylline following oral administration
of dyphylline solution, monopivaloyl dyphylline suspension
and dipivaloyl dyphylline suspension, respectively. In
Figures 3, 4 and 5, the same data are presented in
semilogarithmic plots. Each response curve, R(t),
following oral administration is a hybrid of input
function, I(t), and disposition function, F(t). Response
curve characterestics are influenced by many factors
associated with both drug input and disposition. Since
factors which affect drug input behavior are of major

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TABLE V - Plasma Dyphylline concentrations and Percent Dyphylline
Unabsorbed Following Oral Administration of 3 Grams of
Dyphylline in Solution (treatment A) in 3 Pigs (pigs 4-6)

Time, hour	4A[a]		5A		6A	
	conc[b] mcg/ml	unabs,[c] %	conc, mcg/ml	unabs, %	conc, mcg/ml	unabs, %
0.	0.	100.	0.	100.	0.	100.
.25	13.66	67.15	21.06	59.87	13.40	71.89
.5	26.17	32.47	31.74	30.15	34.85	22.39
.75	33.04	6.21	32.43	15.02	35.04	10.33
1.	31.15	---	28.76	8.29	31.99	5.17
1.25	---	---	23.96	5.72	25.26	---
1.5	19.96	---	18.30	---	22.84	---
1.75	18.47	---	16.23	---	19.71	---
2.	16.94	---	13.82	---	16.81	---
3.	10.71	---	7.35	---	10.62	---
4.	6.80	---	4.26	---	6.60	---
5.	4.12	---	3.30	---	4.04	---
6.	2.71	---	1.96	---	2.90	---
7.	1.87	---	1.51	---	1.77	---
8.	1.34	---	1.21	---	1.55	---
9.	.94	---	.87	---	1.09	---
10.	.65	---	---	---	.88	---
11.	---	---	---	---	.59	---

[a] treatment A in pig 4.

[b] plasma dyphylline concentrations

[c] percent dyphylline unabsorbed in G-I tract; data derived from
deconvolution method. (see text for discussion)

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TABLE VI – Plasma Dyphylline Concentrations and Percent Dyphylline
Unabsorbed Following Oral Administration of 10 Grams of
Monopivaloyl Dyphylline in Suspensions (treatment B)
in 3 Pigs (pigs 4-6)

Time, hour	4B[a]		5B		6B	
	conc[b] mcg/ml	unabs,[c] %	conc, mcg/ml	unabs, %	conc, mcg/ml	unabs, %
0.	0.	100.	0.	100.	0.	100.
.25	37.77	66.03	1.46	98.02	1.66	98.22
.5	34.94	63.81	1.83	97.05	1.95	97.63
.75	34.61	59.76	6.17	90.6	7.47	91.39
1.	---	---	9.39	84.3	19.91	76.81
1.25	44.39	41.76	9.04	81.89	28.31	64.43
1.5	46.34	34.60	8.96	79.31	25.37	62.79
1.75	37.62	36.81	8.40	77.47	28.26	55.48
2.	39.05	31.03	9.96	72.99	26.86	52.32
3.	24.07	29.19	18.12	46.43	19.93	44.58
4.	18.98	23.97	20.73	21.68	18.26	34.74
5.	17.23	17.52	13.73	13.29	25.25	14.35
6.	13.76	13.76	9.65	8.07	20.97	4.78
7.	9.9	12.07	6.39	5.82	13.14	3.06
8.	9.28	8.57	4.74	3.83	8.61	2.01
9.	8.32	5.65	3.21	3.04	6.40	---
10.	6.15	4.47	2.27	2.47	3.99	---
11.	---	---	1.74	1.87	2.72	---
12.	4.44	1.63	1.30	1.44	---	---
13.	3.15	1.18	0.82	1.40	1.63	---
14.	2.28	---	0.73	1.03	1.07	---
15.	1.78	---	0.60	---	---	---
16.	1.26	---	---	---	---	---

[a]treatment B in pig 4.

[b]plasma dyphylline concentrations

[c]percent equivalent dyphylline unabsorbed in G-I tract; data
derived from deconvolution method. (see text for discussion)

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TABLE VII - Plasma Dyphylline Concentrations and Percent Dyphylline
Unabsorbed Following Oral Administration of 10 Grams of
Dipivaloyl Dyphylline in Suspensions (treatment C) in 3
Pigs (pigs 4-6)

Time, hour	4C[a] conc[b] mcg/ml	unabs,[c] %	5C conc, mcg/ml	unabs, %	6C conc, mcg/ml	unabs, %
0.	0.	100.	0.	100.	0.	100.
.25	.98	98.28	2.24	96.62	---	---
.5	4.45	91.97	3.62	93.75	---	---
.75	8.87	83.15	6.20	88.61	3.82	90.95
1.	10.37	78.36	9.99	80.77	7.02	83.31
1.25	10.30	75.95	8.89	79.03	8.19	78.73
1.5	9.87	74.26	8.42	76.83	9.20	74.12
1.75	10.81	70.27	8.12	74.61	8.36	72.95
2.	10.23	68.73	8.71	71.20	8.08	70.95
3.	7.66	64.78	7.53	63.29	9.22	58.40
4.	8.41	56.28	6.87	56.22	9.68	46.45
5.	8.60	48.31	7.14	48.16	10.38	33.53
6.	6.53	45.10	5.13	44.63	8.00	27.79
7.	5.92	40.73	6.33	36.86	5.38	25.74
8.	5.62	36.26	6.54	29.52	4.03	23.65
9.	6.23	30.06	5.87	23.81	2.97	22.35
10.	5.90	25.29	5.03	19.34	3.03	19.33
11.	5.00	22.06	5.11	13.94	2.98	16.38
12.	5.89	15.81	4.18	10.49	2.54	14.40
13.	5.76	10.89	3.35	7.98	2.01	13.12
14.	---	---	2.68	6.07	1.98	11.16
15.	3.90	5.89	1.89	5.15	1.68	9.87
16.	2.83	4.87	1.39	4.49	1.44	8.75
17.	2.14	4.01	1.23	3.60	1.19	7.91
18.	1.80	2.99	1.06	2.81	1.16	6.77
19.	1.46	2.26	.9	2.14	1.01	5.95
20.	1.21	1.61	.87	1.34	0.87	5.26
21.	---	---	.73	---	---	---
22.	.82	---	.61	---	---	---
23.	.62	---	.52	---	---	---
24.	.51	---	---	---	---	---

[a]treatment C in pig 4.

[b]plasma dyphylline concentrations

[c]percent equivalent dyphylline unabsorbed in G-I tract; data
derived from deconvolution method. (see text for discussion)
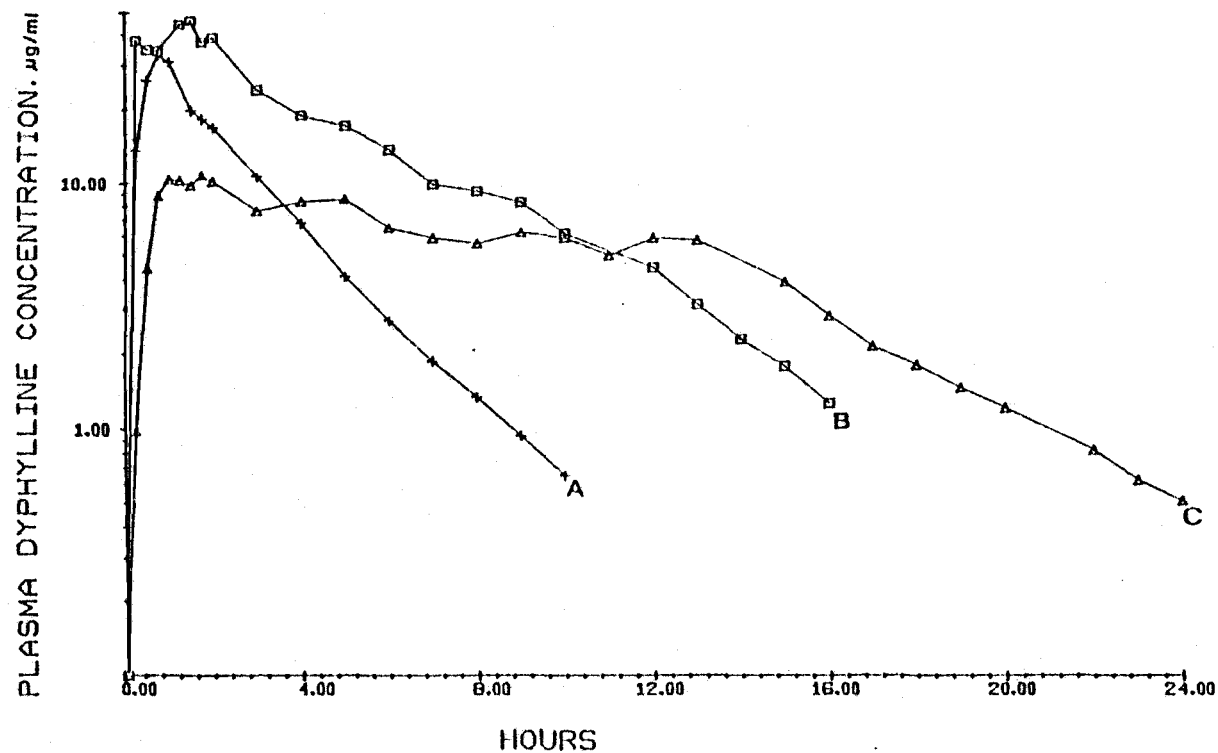
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Figure 3– Plasma dyphylline concentration versus time curves following 3 oral
dosage forms in pig 4. Key: A, 3 grams (11.81 m moles) of dyphylline; B, 10
grams (29.55 m moles) of monopivaloyl dyphylline; C, 10 grams (23.67 m moles)
of dipivaloyl dyphylline.
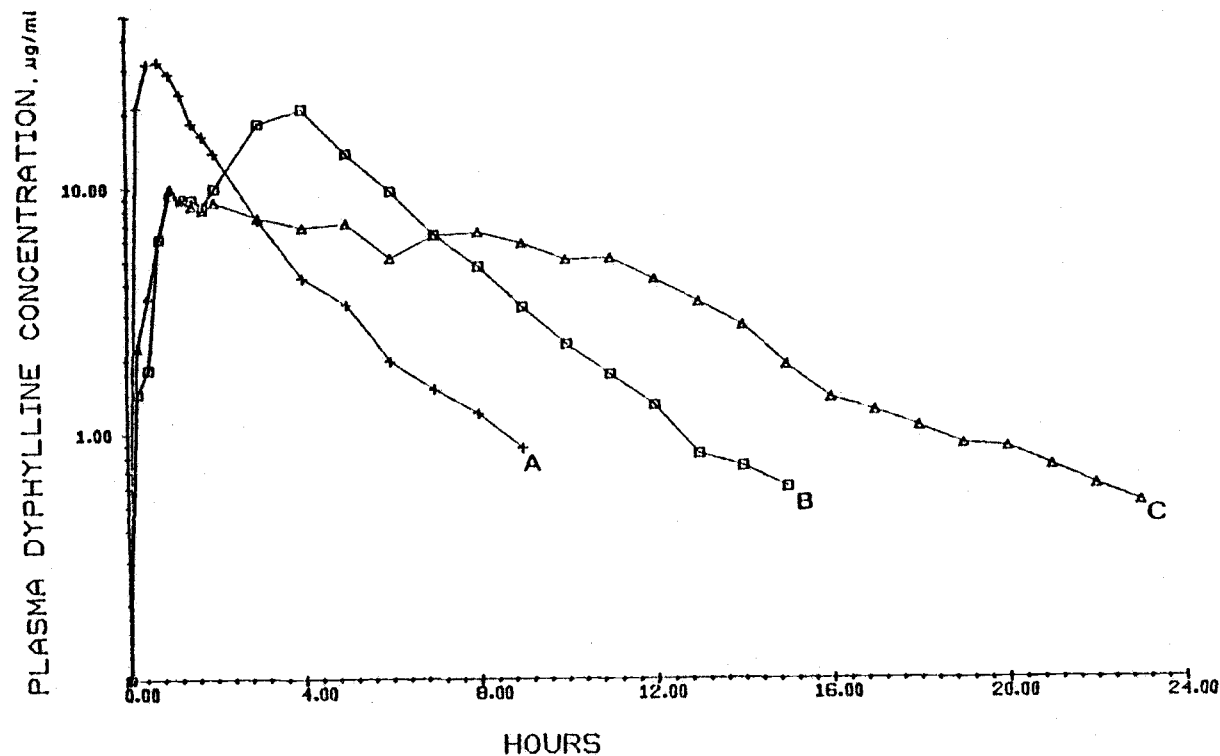
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Figure 4- Plasma dyphylline concentration versus time curves following 3 oral dosage forms in pig 5. Key: A, 3 grams (11.81 m moles) of dyphylline; B, 10 grams (29.55 m moles) of monopivaloyl dyphylline; C, 10 grams (23.67 m moles) of dipivaloyl dyphylline.
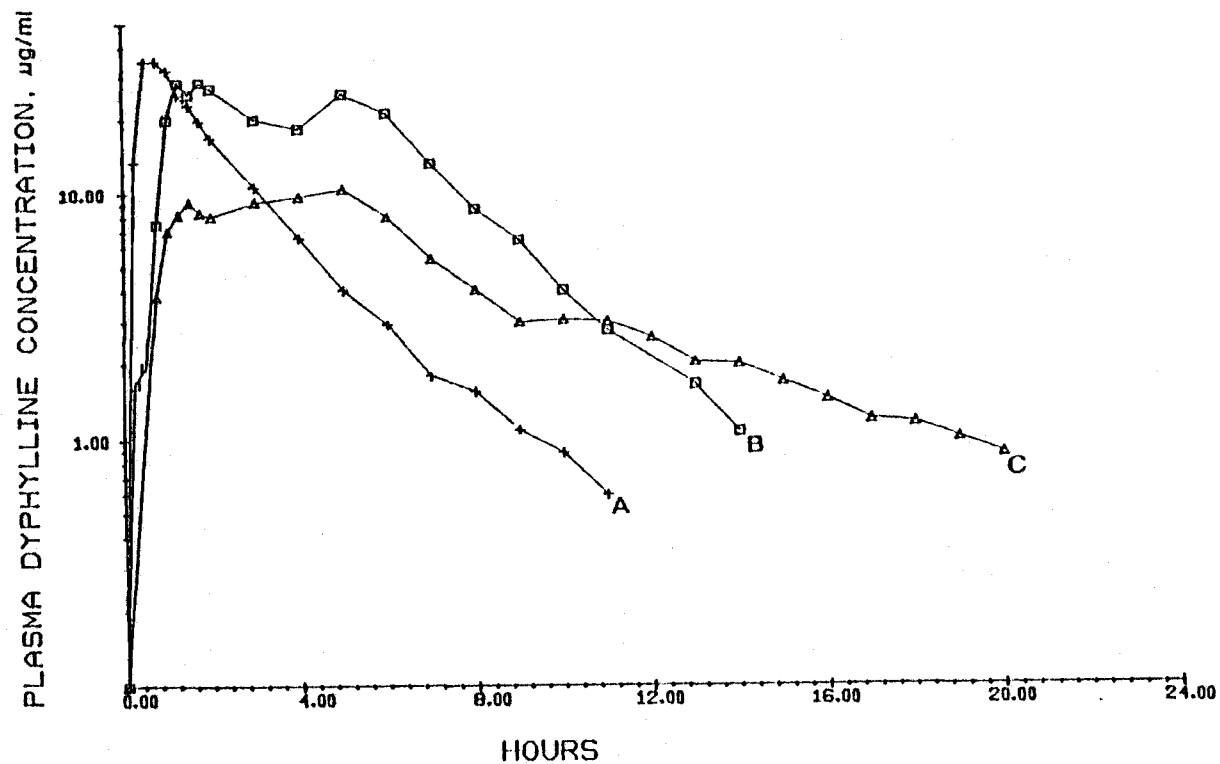
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Figure 5- Plasma dyphylline concentration versus time curves following 3 oral dosage forms in pig 6. Key: A, 3 grams (11.81 m moles) of dyphylline; B, 10 grams (29.55 m moles) of monopivaloyl dyphylline; C, 10 grams (23.67 m moles) of dipivaloyl dyphylline.

interest in oral dosage formulation design, it is desirable to resolve and define the input function. This was done by a numerical deconvolution method(23) which helps eliminate the effect of intersubject differences of drug disposition in systemic circulation on determining the input function. Assuming dyphylline follows linear pharmacokinetics, the following relationship holds:

$$R(t) = I(t) * F(t)$$

where * is a symbol of convolution. By knowing R(t) from plasma dyphylline concentration data derived from oral prodrug administration and F(t) from fitted regression function obtained from bolus intravenous administration of dyphylline in the same subject, the percent of available dyphylline remaining to be absorbed versus time curve can be derived by the numerical deconvolution process. The percent of dyphylline unabsorbed values are listed in Tables V, VI and VII for each dosage preparation separately. The percentage of dyphylline unabsorbed in the tables relates the amount of dyphylline remaining in absorption site to the amount of dyphylline eventually absorbed rather than to the administered dose. That is, sometimes for orally administered drugs, only a fraction of the dose is absorbed and the balance of the dose is excreted in the feces without being absorbed or degraded without being absorbed. Therefore, the calculation of

percent of drug unabsorbed involves determination of the amount of the dose which is eventually absorbed (Aa) and the amount of the dose which is unabsorbed or never absorbed (Au). The difference between the dose administered and the amount absorbed (Dose - Aa) is the amount unabsorbed (Au). Therefore, the percentage of dyphylline unabsorbed at time t is:

$$Aut = \frac{(D - Au) - At}{D - Au} = \frac{Aa - At}{Aa}$$

where Aut is percent of dyphylline unabsorbed at time t; At is percent of dyphylline has been absorbed at time t.

The absorption profiles after oral administration of dyphylline or dyphylline prodrugs are demonstrated in Figure 6 which show the time course of percent dyphylline unabsorbed calculated according to the above equation. A somewhat linear relationship was observed for the three treatments in all three pigs when plotted in semilogarithmic scale. The same data are presented by rectilinear plots in Figure 7. Since no linear relationship was found on rectilinear plots, zero-order input kinetics is not prevalent in these three dosage preparations. In these two figures, the non-uniform curvature of data for individual pigs make them hard to be described by any one specific absorption kinetic modeling process.

HOURS
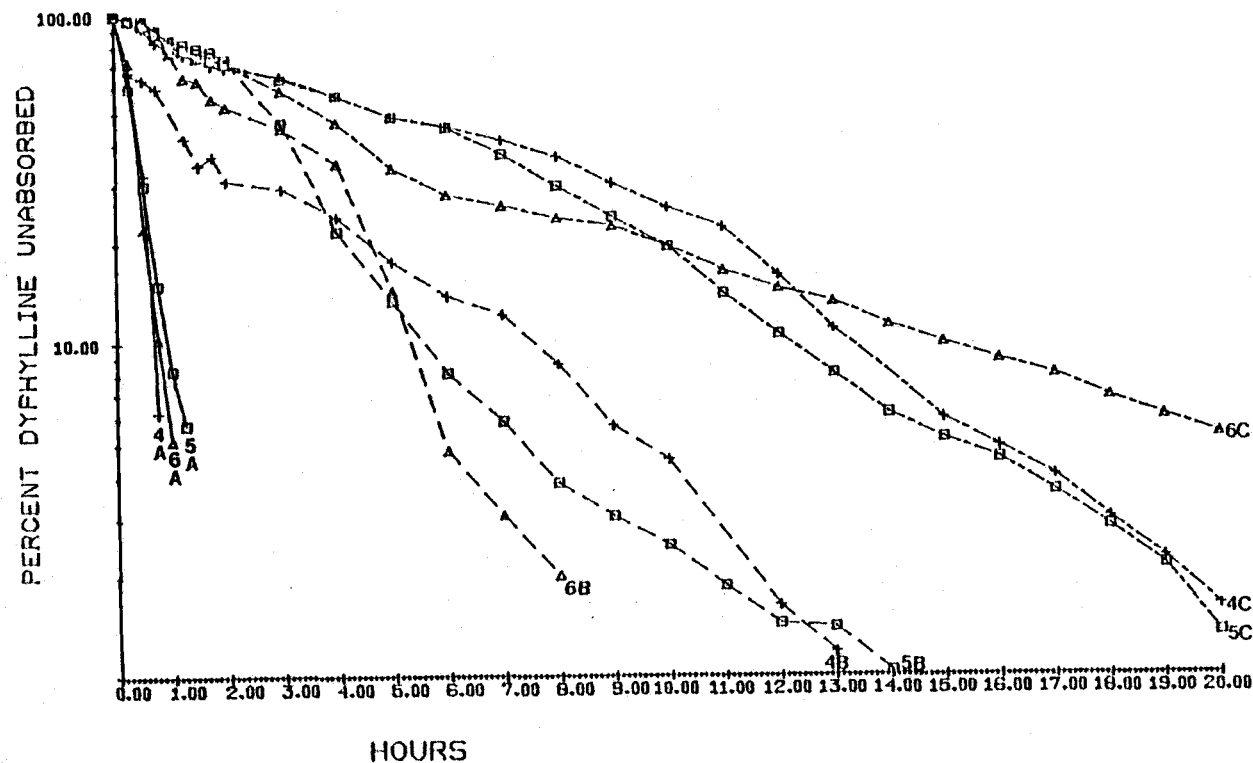
Chapter III

Figure 6- Semilogarithmic plot of percent dyphylline unabsorbed versus time curves in 3 pigs (pigs 4-6); data were derived by numerical deconvolution process. Key: A, 3 grams (11.81 m moles) of dyphylline; B, 10 grams (29.55 m moles) of mono-pivaloyl dyphylline; C, 10 grams (23.67 m moles) of dipivaloyl dyphylline.

HOURS
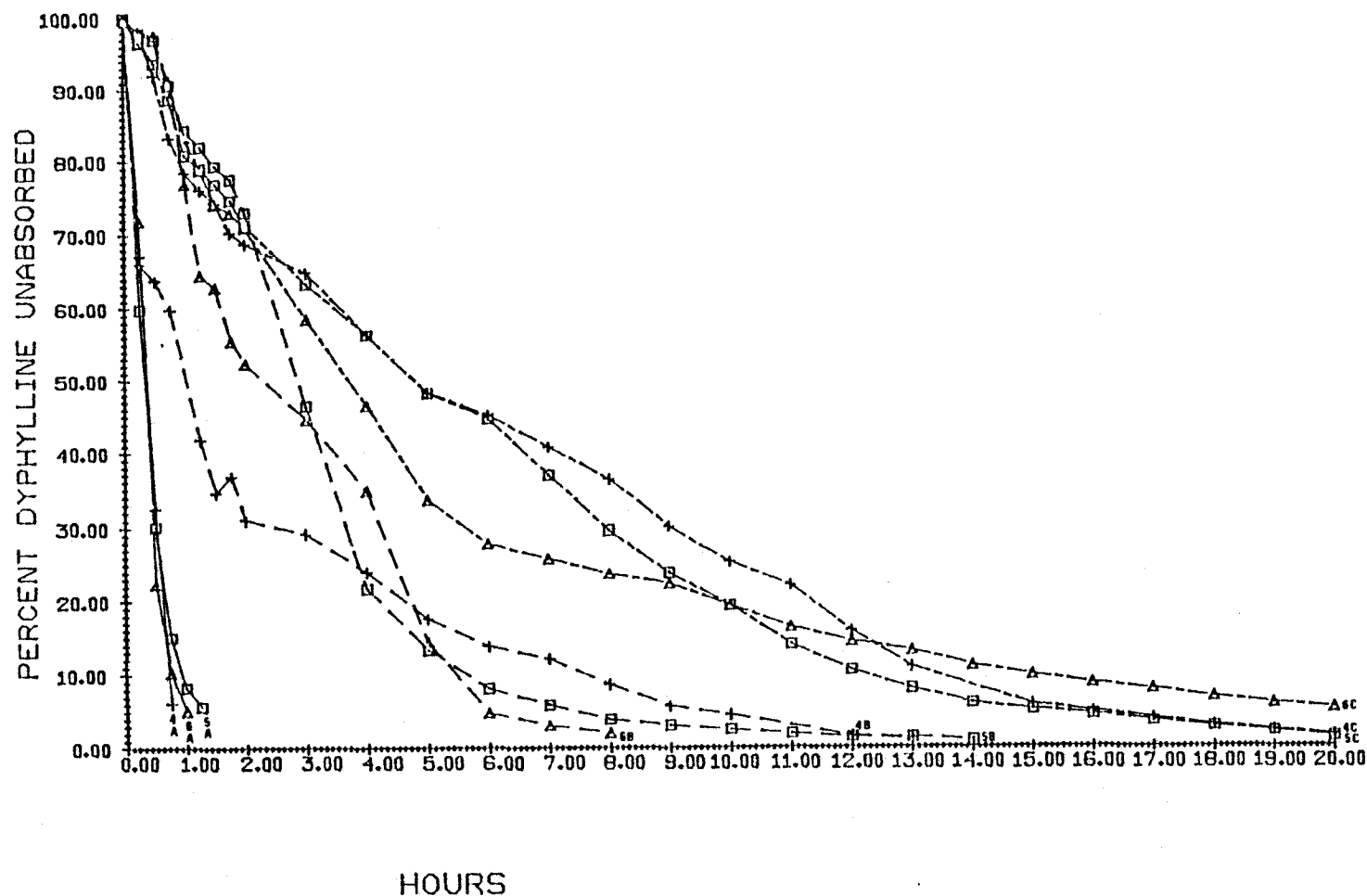
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Figure 7- Rectilinear plot of percent dyphylline unabsorbed versus time curves in 3 pigs (pigs 4-6); data were derived by numerical deconvolution process. Key: A, 3 grams (11.81 m moles) of dyphylline; B, 10 grams (29.55 m moles) of mono-pivaloyl dyphylline; C, 10 grams (23.67 m moles) of dipivaloyl dyphylline.

Cutler(28) and Yamaoka(26) et al. have proposed the application of statistical moments in pharmacokinetic analysis which is particularly useful in evaluating the time involved in _in vivo_ release and the absorption process. The zero-order statistical moments (AUC's) calculated for each pig following intravenous and oral administration are listed in Tables VIII and IX. Table X contains the calculated results of fraction of the drug absorbed. The first-order statistical moments (MRT's) calculated for each pig following intravenous and oral administration are listed in Tables XI and XII. Table XIII shows the calculated results of MRT's of dyphylline in the gastrointestinal tract. The formulae used in the above calculations are described in the experimental text. Table X and XIII list the results to show the calculation; the same data are rearranged corresponding to the study design arrangement in Tables XIV and XVI where the treatment in each cell is shown in parenthesis. The corresponding analysis of variance tables are given as Tables XV and XVII. The treatment and blocking effects are not significant at the 5 percent level in the analysis of variance test for fraction of the drug absorbed while the treatment effect is highly significant in the test of mean residence time in G-I tract. The blocking effects are very insignificant at the 5 percent level.

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TABLE VIII - Area Under the Curves Data Following Bolus Dyphylline
Intravenous Administration, $AUC_{i.v.}$[a]

	Pig-4	Pig-5	Pig-6
	58.16	53.09	69.61

[a]area under the curve from time zero to infinity calculated
from plasma dyphylline concentration data following bolus
intravenous administration.

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TABLE IX - Latin Square Data on AUC_o[a]

	Pig-4	Pig-5	Pig-6
Treatment - A	81.12	71.32	85.49
Treatment - B	217.22	101.57	170.2
Treatment - C	113.60	92.94	85.66

[a]area under the curve from time zero to infinity calculated from
plasma dyphylline concentration data following oral prodrug
administration.

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TABLE X - Latin Square Data on F[a]c%

	Pig-4	Pig-5	Pig-6
Treatment - A	93.0	89.6	81.9
Treatment - B	99.4	50.9	65.0
Treatment - C	64.9	58.1	40.9

[a]fraction of absorption;
$$F = \frac{Mdyp^{b}}{Mtrt} * \frac{Auc_o^{c}}{Auc_{i.v.}} * 100\%$$

[b]Mdyp and Mtrt are number of moles of dyphylline and treated
drugs.

[c]$Auc_{i.v.}$ and AUC_o are taken from TABLES VIII and IX.

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TABLE XI - Mean Residence Time Data Following Bolus Dyphylline
Intravenous Administration, $MRT_{i.v.}$

	Pig-4	Pig-5	Pig-6
	2.21	1.82	2.12

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TABLE XII - Latin Square Data on MRT_o[a]

	Pig-4	Pig-5	Pig-6
Treatment - A	2.72	2.47	2.75
Treatment - B	4.65	5.02	5.01
Treatment - C	8.46	7.79	7.16

[a]mean residence time calculated from data following dyphylline
and dyphylline prodrug oral administrations.

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TABLE XIII - Latin Square Data On MRT_g[a]

	Pig-4	Pig-5	Pig-6
Treatment - A	0.51	0.65	0.63
Treatment - B	2.44	3.20	2.89
Treatment - C	6.25	5.97	5.04

[a]mean residence time of treated drugs in gastrointestinal tracts,
$MRT_g = MRT_o - MRT_{i.v.}$

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TABLE XIV - Latin Square Data on Fractions of Absorption of
Dyphylline Following Dyphylline and Dyphylline Prodrugs
Oral Administration (Data Are Rearranged to Show
Calculations).

		PERIODS		
PIGS	I	II	III	$T_{i..}$[a]
4	93.0(A)	64.9(C)	99.4(B)	257.3
5	50.9(B)	89.6(A)	58.1(C)	198.6
6	40.9(C)	65.0(B)	81.9(A)	187.8
$T_{..k}$[b]	184.8	219.5	239.4	$T_{...}$[c] = 643.7

[a]Totals by pigs.
[b]Totals by periods.
[c]Grand total.
[d]Totals by treatment are: A-264.5; B-215.3; C-163.9.

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TABLE XV - Latin Square Analysis of Variance on Fraction of
Absorption of Dyphylline; F(%).

Source	SS	DF	MS	F[a]
Treatments	1687.00	2	843.5	9.70
Pigs	932.51	2	466.26	5.36
Periods	509.03	2	254.52	2.93
Error	173.98	2	86.99	
Total	3302.52	8		

[a]$F(.95; 2, 2) = 19$

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TABLE XVI - Latin Square Data on Mean Residence Time of Dyphylline
in G-I Tract Following Dyphylline and Dyphylline
Prodrugs Oral Administration (Data Are Rearranged To
Show Calculations).

PIGS	PERIODS			Ti..[a]
	I	II	III	
4	0.51(A)	6.25(C)	2.44(B)	9.20
5	3.20(B)	0.65(A)	5.97(C)	9.82
6	5.04(C)	2.89(B)	0.63(A)	8.56
T..k[b]	8.75	9.79	9.04	T...[c] = 27.58

[a]Totals by pigs.
[b]Totals by periods.
[c]Grand total.
[d]Totals by treatments are: A-1.79; B-8.53; C-17.26.

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TABLE XVII - Latin Square Analysis of Variance on Mean Residence
Time of Dyphylline in G-I Tract; MRTg.

Source	SS	DF	MS	F[a]
Treatments	40.107	2	20.054	61.8*
Pigs	0.265	2	0.133	0.41
Periods	0.192	2	0.096	0.3
Error	0.649	2	0.325	
Total	41.213	8		

[a]$F(.95; 2, 2) = 19$.
*Significant at the 95% level.

Determination of the effect of food on oral dyphylline prodrug administration was also investigated. Pigs to be dosed without food were fasted for 24 hours before beginning the experiment. Pigs used were the same ones used in the Latin square experimental design described earlier, but the treatment was separate and determination of food effects consisted of an independent experiment. Pigs to be dosed with food were given dipivaloyl dyphylline suspension between two halves of the food which was consumed at one feeding. Plasma dyphylline concentrations and percent dyphylline unabsorbed are listed in Table XVIII and XIX. Figure 8 shows the dyphylline concentration _versus_ time profiles, and Figure 9 shows the percent dyphylline unabsorbed _versus_ time profiles. The profiles indicate that food does not have much effect on the absorption of dipivaloyl dyphylline from suspension. Area under the curves for these experiments are tabulated in Table XX; and the calculated fractions of absorption are shown in Table XXI. There is no significant difference (p < 0.05) between these two treatments. Mean residence times of dyphylline following oral administrations in these experiments are listed in Table XXII; and the calculated mean residence times in G-I tract are shown in Table XXIII. There is no significant difference (p < 0.05) between these two treatments. The above results indicate that no statistical differences
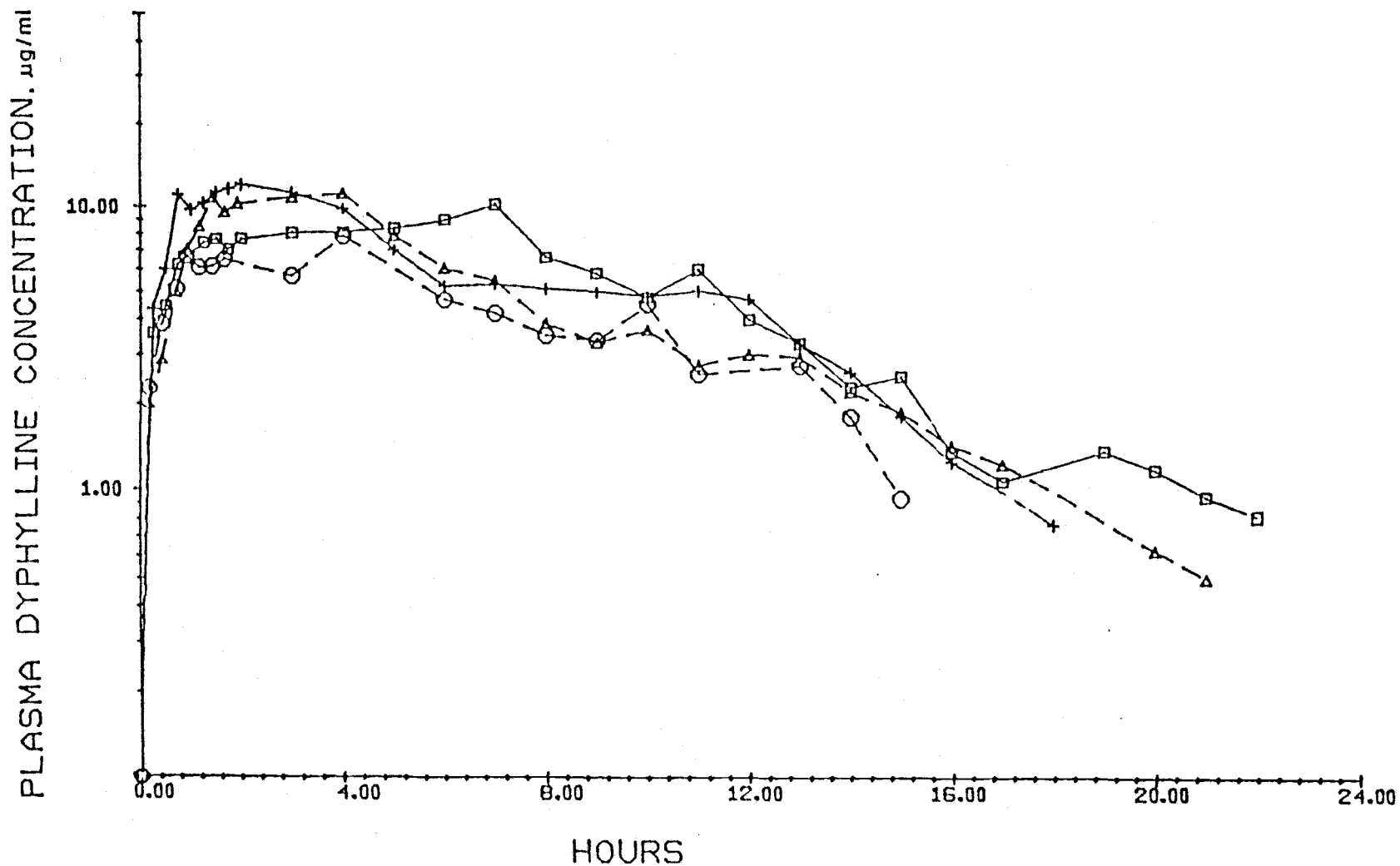
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Figure 8- Plasma dyphylline concentration versus time curves following 10 grams (23.67 m moles) of oral dipivaloyl dyphylline suspentions in pigs. Key: solid lines, with food; dashed lines, without food. (Note: Different symbols represent plasma dyphylline concentrations in different pigs. Data are independent from Figures 3,4,5)

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Figure 9- Percent dyphylline unabsorbed versus time curves in pigs; data were derived by numerical deconvolution process. Key: solid lines, with food; dashed lines, without food. (Note: Different symbols represent data from different pigs)

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TABLE XVIII - Plasma Dyphylline Concentrations and Percent
Dyphylline Unabsorbed Following 10 Grams of
Dipivaloyl Dyphylline Suspensions Administered
Orally With Food

Time, hour	Pig-4		Pig-6	
	conc[b], mcg/ml	unabs[c], %	conc, mcg/ml	unabs, %
0.	0.	100.	0.	100.
.25	4.35	91.30	3.58	93.84
.5	6.03	86.72	4.46	91.34
.75	11.00	75.11	6.25	87.05
1.	9.75	74.55	---	---
1.25	10.28	70.82	7.44	81.47
1.5	11.24	66.10	7.66	79.11
1.75	11.56	62.41	7.05	78.15
2.	12.03	58.35	7.70	75.21
3.	11.26	47.47	8.10	66.51
4.	9.88	39.17	8.17	58.31
5.	7.04	36.09	8.41	49.81
6.	5.28	33.49	9.03	40.33
7.	5.34	28.25	10.22	29.02
8.	5.13	23.54	6.64	26.95
9.	5.01	18.78	5.84	22.68
10.	4.85	14.21	4.80	19.69
11.	5.05	8.89	6.02	12.77
12.	4.72	4.65	4.00	11.55
13.	3.30	3.44	3.30	9.57
14.	2.62	1.94	2.31	8.86
15.	1.82	1.42	2.52	6.49
16.	1.26	1.13	1.37	6.78
17.	---	---	1.08	6.35
18.	.78	.34	---	---
19.	---	---	1.38	3.72
20.	---	---	1.19	2.88
21.	---	---	.96	2.32
22.	---	---	.83	1.76

[a]data for pig 5 was unavailable due to difficulty in dosing

[b]plasma dyphylline concentrations

[c]percent dyphylline unabsorbed

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TABLE XIX - Plasma Dyphylline Concentrations and Percent
Dyphylline Unabsorbed Following 10 Grams of
Dipavaloyl Dyphylline Suspensions Administered orally
without food.

Time, hour	Pig-4		Pig-5[a]	
	conc[b] mcg/ml	unabs[c] %	conc[b] mcg/ml	unabs %
0.	0.	100.	0.	100.
.17	2.02	95.70	2.29	95.41
.42	2.90	93.11	3.85	91.02
.72	5.00	87.69	5.13	86.07
.92	7.06	81.86	6.68	80.98
1.17	8.52	76.37	6.09	79.11
1.42	10.75	68.95	6.15	76.24
1.67	9.57	68.30	6.50	72.81
1.92	10.28	63.92	----	----
3.	10.85	49.43	5.70	61.13
4.	11.16	36.20	7.89	45.76
5.	7.94	32.27	----	----
6.	6.09	28.58	4.70	34.47
7.	5.51	23.65	4.21	29.20
8.	3.87	22.19	3.52	25.24
9.	3.33	19.65	3.38	20.74
10.	3.68	15.27	4.53	12.51
11.	2.77	13.79	2.57	11.33
12.	3.02	10.22	----	----
13.	2.96	7.14	2.75	3.45
14.	2.22	5.93	1.81	2.21
15.	1.88	4.51	.94	2.37
16.	1.44	3.72	----	----
17.	1.24	2.78	----	----
18.	----	----	----	----
19.	----	----	----	----
20.	0.64	1.41	----	----
21.	0.51	1.11	----	----

[a]data for pig 6 was incomplete due to blood clotting in catheter.

[b]plasma dyphylline concentrations.

[c]percent dyphylline unabsorbed.

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TABLE XX - Area Under the Curves of Plasma Dyphylline
          Concentration Versus Time Plot Following Dipivaloyl
          Dyphylline Oral Administrations, AUC_o; drugs were
          given with or without food.

	Pig-4	Pig-5	Pig-6
With food	98.62	---a	104.5
Without food	90.39	65.78	---b

[a]data unavailable due to dosing split.

[b]data incomplete due to blood clotting in catheter

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TABLE XXI - Fraction of Absorption of Dyphylline Following
           Dipivaloyl Dyphylline Oral Administrations, F(%)

	Pig-4	Pig-5	Pig-6
With food	56.3	---a	49.9
Without food	51.6	41.2	---b

[a]data unavailable due to dosing split.

[b]data incomplete due to blood clotting in catheter.

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TABLE XXII - Mean Residence Times of Dyphylline Following
Dipivaloyl Dyphylline Oral Administrations, MRT_o;
Drugs Were Given With or Without Food

	Pig-4	Pig-5	Pig-6
With food	6.56	---a	7.96
Without food	6.79	6.55	---b

[a] data unavailable due to dosing split.

[b] data incomplete due to blood clotting in catheter

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TABLE XXIII - Mean Residence Time of Dyphylline in G-I Tract
Following Dipivaloyl Dyphylline Oral
Administrations, MRT_g; Drugs Were Given With or
Without Food

	Pig-4	Pig-5	Pig-6
With food	4.35	---a	5.84
Without food	4.58	4.73	---b

[a] data unavailable due to dosing split.

[b] data incomplete due to blood clotting in catheter.

were observed for both 'extent' and 'rate' of absorption
of dyphylline when dipivaloyl dyphylline was taken with or
without concomitant food intake.

In a previous study(15), eight dyphylline prodrugs
including four monoesters and four corresponding diesters
were investigated in New Zealand White rabbits. Since
rabbits have some different pharmacokinetic parameters for
dyphylline compared to humans, especially a shorter
half-life of 40 minutes compared to about 2 hours in
humans, it may not be a good animal model for studies of
dyphylline prodrugs. In addition, rabbits have a shorter
gastrointestinal tract; a shorter residence time for a
dosage form may cause incomplete bioavailability. A
physiologically similar gastrointestinal tract condition
exists between pigs and humans which may make pigs more
suitable for bioavailability studies of dyphylline
prodrugs. The results in this study showed that pigs have
an apparent elimination half-life of 2.01 hours which is
very close to human data. The apparent volume of
distribution is 1.6 liters/Kg which is twice as much as
human's. However, the volume of distribution may be
corrected easily in human simulation studies. Based on
these similarities, data derived from pig studies are
considered to be projectable to humans for simulation of
dyphylline plasma concentrations through prodrug
administration.

In bioavailability comparison studies for three treatments in three pigs, the Latin square cross over design was considered suitable. In the Latin square design, restrictions placed on randomnization reduce experimental error at the expense of the degree of freedom. This means less precision in estimating the error variance(29). In our study, the degree of freedom was reduce from 6 to 2. After discovering that blocking variables had no significant effect, the sum of squares for times and pigs may be pooled with the error sums of squares to obtain a more precise estimation of experiment error variance. However, the pooled estimate of experiment error variance is still relatively large for the fraction of absorption comparisons; and the F-value for treatment effects is still insignificant at the 5 percent level. In this study, although we may conclude that the absorption rates are statistically different (p < 0.05) between these three treatments while the extent of absorptions are not statistically different, the equivalency in extent of absorption may be a result of large experimental errors. A larger sample size will be necessary for more powerful test of the extent of absorption in pigs.

After the Latin square analysis of variance study and the treatment effects had been found to be significant, Tukey's multiple comparison procedure(30) was performed.

The results showed that the absorption rate of dipivaloyl dyphylline was slower than both dyphylline and monopivaloyl dyphylline while monopivaloyl dyphylline did not show a significantly slower absorption rate when compared with dyphylline.

As shown in Figure 6, the absorption profile for dyphylline solution is a straight line in semilogarithmic plot, the absorption profile for dipivaloyl dyphylline is approximately straight. However, the absorption for monopivaloyl dyphylline is not as uniform; a linear approximation may not be adequate. For bioavailability comparison, a model independent parameter, mean absorption time, was used to indicate the rate of absorption. The two prodrug treatments (B and C) showed a slower release of dyphylline which may be due to slower in vivo dissolution of prodrug in the gastrointestinal tract. Dipivaloyl dyphylline showed the slowest absorption rate in these three treatments and gave a relatively straight absorption profile (See Figure 6). A relatively straight line was also observed after oral administration of dipivaloyl dyphylline suspensions with or without food as shown in Figure 9. Linear regressions for these curves produces first-order absorption rate constants which may be used to approximate the absorption kinetics. The absorption rate constant was calculated to be 0.20 ± 0.04 hr which is ideal from former simulation studies and

very close to what was found in rabbits(31). The absorption rate constant calculated using the numerical deconvolution procedure gives an apparent absorption rate constant which may be higher than the true absorption rate constant. Like Wagner-Nelson or Loo-Riegelman methods, nonlinear least-squares regression analysis, or the method of residuals, the absorption rate constant obtained by the numerical deconvolution procedure is the sum of the true absorption rate constant and any rate constants for parallel first-order loss of drug from the absorption site(32). However, the apparent absorption rate constant gives accurate estimations of plasma dyphylline concentrations and is a better parameter for studying dyphylline controlled release design than the true absorption rate constant.

The average fraction of absorption of dyphylline after oral administration of dipivaloyl dyphylline (with and without concomitant food intake) is $52.7 \pm 7.8\%$. In spite of the optimal rate of absorption, the small fraction of dose absorbed needs further improvement. Some of the dosage form was not absorbed and was, therefore, eventually eliminated from the gastrointestinal tract. A refined dosage form preparation is necessary for long term application of this prodrug. Various formulation factors, such as particle size reduction and addition of surfactants, may be utilized to enhance the 'extent' of

absorption and thus reduce the dosage necessary to maintain effective plasma dyphylline concentration level. Because of HPLC sensitivity limitations, dyphylline plasma concentrations were not detectable 24 hours after oral administration of dipivaloyl dyphylline suspensions. The accumulation effect of this dosage form after multiple dosing remains undetermined. An additional multiple dosing study will be necessary to determine dosage regimens that will optimize and maintain plasma dyphylline concentrations in the therapeutic range. Experimental data collected in this study indicated that dipivaloyl dyphylline may be a useful drug delivery system once the total dosage form has been optimized.

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CHAPTER IV

CONCLUSION

CONCLUSION

A pharmacokinetic study of dyphylline following oral dyphylline tablet administration in humans has been reported by Gisclon (See Chapter II). The data were fitted to a two compartment open model with first order absorption using a non-linear least squares procedure. The results show a large first order absorption rate constant (4.32 hr^{-1}) which means plasma dyphylline concentration in the bloodstream will be controlled by the dyphylline elimination rate following a conventional tablet dosage form. However, the dyphylline elimination rate is also fast and a highly rapid increasing-peaking-decreasing pharmacokinetic curve was observed in a simulation study. This phenomenon was described in Chapter II of this thesis. If conventional dyphylline tablets are taken every eight hours, the plasma dyphylline concentration will not stay above the minimum effective concentration for more than one hour. The effective duration of action of dyphylline can thus be maintained for only one eighth of the dosing interval.

Several absorption rate constants were used to simulate plasma dyphylline concentrations following a proposed controlled release design of dyphylline. The results show that an optimum duration of concentration of dyphylline will be obtained if a controlled release

delivery system can be designed with an apparent absorption rate constant of about 0.25 hr^{-1}. Generally, if Ka is less than about 0.1 hr^{-1}, then total absorption may be compromised due to drug passing through G-I tract without absorption. If Ka is greater than about 0.5 hr^{-1}, then a flip-flop phenomenon will not exist and the plasma concentration of dyphylline will not stay in the 10-20 mcg/ml range over the dosing interval. Computer simulations also teach that knowledge of the mechanism for the input function is not necessary since an overall input rate of about 0.25 hr^{-1} will give the desired results.

Eight dyphylline prodrugs were tested in New Zealand White rabbits (Chapter II) to determine which prodrugs, if any, were absorbed with a Ka which was close to the optimum predicted by computer simulation. Dipivaloyl dyphylline gives an apparent absorption rate constant which is close to the optimal one. The percent dyphylline remaining to be absorbed versus time curve shows a linear decline when plotted in semilogarithmic scale. Therefore, an apparent first order absorption is adquate to describe the complex input mechanism as described in Chapter I.

Since New Zealand White rabbits are ruminant animals, the results may not be adquately projectable to humans. Therefore, the pig (a carnivorous animal) was chosen as another experimental animal to study dipivaloyl dyphylline. Its corresponding monoester, monopivaloyl

dyphylline, and parent drug, dyphylline, were also tested for comparison.

In order to eliminate experimental errors from the effect of period and inter-subject differences, a Latin square cross-over design was used. The results show that pigs have an dyphylline elimination half-life of about two hours which is in good agreement with human's elimination half-life. Results also show that dipivaloyl dyphylline has a rate of absorption which is close to the optimal expectation. Input function analysis also shows the complex input collapses to an apparent first order input. However, the fraction of drug absorbed data shows incomplete absorption of dyphylline in the prodrug form. In order to improve the extent of absorption of dyphylline through oral dipivaloyl dyphylline administration, the physical properties of dipivaloyl dyphylline should be extensively investigated. After the dosage formulation has been optimized, dipivaloyl dyphylline may be a good prodrug for controlled release of dyphylline.

Studies in this thesis demonstrate the pharmacokinetics of dyphylline in two animal models. Absorption kinetics of dyphylline prodrugs have been studied by using the mathematical techniques of input function analysis (numerical deconvolution). It is believed that using the pig as an animal model and numerical deconvolution analysis are especially useful

tools in studying drug delivery systems and controlled release drug design.

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APPENDIX

APPENDIX I   MASS BALANCE EQUATIONS

The following mass balance equations are used to describe the compartmental model illustrated in Figures 1 and 2 of Chapter 1. (See Chapter 1 for schematic diagram and explanations of symbols).

Prodrug Model

$$\frac{d(C_S V_S)}{dt} = I(t) - R_S C_S$$

$$\frac{d(C_1 V_1)}{dt} = C_S R_S - C_1 R_S - C_1 K_{hyd1} - C_1 K_{abs1}$$

$$\frac{d(C_i V_i)}{dt} = C_{i-1} R_S - C_i R_S - C_i K_{hydi} - C_i K_{absi}; \ i=2,3,4,5,6,7,8$$

$$\frac{d(C_{GIB} V_{GIB})}{dt} = \sum_{i=1}^{8} C_i K_{absi} + Q_{Gi} C_b - Q_{GI} C_{GIB}$$

$$\frac{d(C_L V_L)}{dt} = Q_{GI} C_{GIB} + Q_L C_B - (Q_{GI} + Q_L) (C_B - C_L) - T\ C_L$$

$$\frac{d(C_B V_B)}{dt} = (Q_{GI} + Q_L) C_L - (Q_{GI} + Q_L) C_B - R_{12} C_B + R_{21} C_T$$

$$\frac{d(C_T V_T)}{dt} = R_{12} C_B - R_{21} C_T$$

Metabolite Model

$$\frac{d(C_{Ms} V_S)}{dt} = C_S K_{hyd} - C_{Ms} R_S$$

$$\frac{d(C_{Mi V1})}{dt} = C_{Ms} R_S - C_{M1} R_S + C_1 K_{hyd1} - C_{M1} K_{abs1}$$

$$\frac{d(C_{Mi}V_i)}{dt} = C_{Mi-1}R_S - C_{Mi}R_S + C_iK_{hydi} - C_{Mi}K_{mabsi};$$

$$i = 2,3,4,5,6,7,8.$$

$$\frac{d(C_{MGIB}V_{GIB})}{dt} = \sum_{i=1}^{8} C_{Mi}K_{Mabsi} + Q_{GI}C_{MB} - Q_{GI}C_{MGIB}$$

$$\frac{d(C_{ML}V_L)}{dt} = Q_{GI}C_{MGI} + Q_L C_{MB} - (Q_{GI} + Q_L)C_{ML} + \Gamma C_L$$

$$\frac{d(C_{MB}V_B)}{dt} = (Q_{GI} + Q_L)C_{ML} - (Q_{GI} + Q_L)C_{MB} - R_{exk}C_{MB} - R_{12}' C_{MB} + R_{21}' C_{MT}$$

$$\frac{d(C_{MT}V_T)}{dt} = R_{12}' C_{MB} - R_{21}' C_{MT}$$

APPENDIX 2   METHOD OF NUMERICAL DECONVOLUTION

In pharmacokinetics it is frequently desirable to determine
the rate and extent of absorption of a drug to the general cir-
culation.   The extent of absorption is generally determined by
comparing the area under the plasma concentration-time curve
following oral and intravenous administration.   The rate of
absorption may be assessed by comparing time to peak con-
centration, magnitude of peak concentration, or absorption rate
constant(s) estimated by computer fitting.   The first two
approaches are model-independent.   However, their estimates may
be subject to large experimental errors because the peak may not
be determined accurately if the sampling time is not frequent
enough.   For estimation of absorption rate constant(s), a model
has to be specified before using a computer-fitting procedure.
Though the above methods give point estimator rate of absorption
comparisons, they don't supply a continuous absorption profile.
It is often desirable to observe the drug input profile before
assigning absorption kinetics, especially if the kinetic behavior
is not known.   In this appendix, a point-area numerical decon-
volution procedure described in the literature is reviewed in
order to show how application of deconvolution provides infor-
mation relative to underlying drug absorption kinetics.   The
extent of absorption can also be determined in this procedure.

The nature of our problem is represented in the following
scheme:

I(t)	Linear time-invariant system;F(t)	R(t)
→ input		→ output
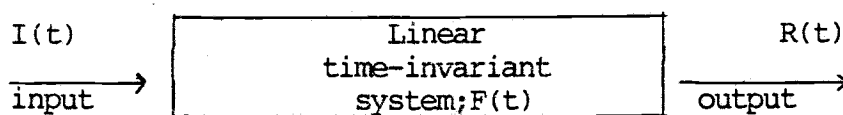
Where I(t) is the drug input rate into the system.

(1) Let a unit amount of drug of interest be introduced into this system at time zero t=0, and let F(t) be the resulting response.

   R(t) = F(t); when I(0) = 1

   F(t) is called the "unit impulse response."

(2) If D units amount of drug is introduced into this system at time zero, and the system is assumed to be linear, then the resulting response is DF(t).

   R(t) = D F(t); when I(0) = D

In the above two cases, a bolus of drug is introduced into a system at time zero; and I(t) is a constant.

(3) If the amount of drug introduced into the system follows a continuous process, then I(t) is not a constant. Over the time interval $(T, T + \Delta T)$, where ΔT is a small time increment, the amount of drug introduced is approximately I(T) dT. If the unit impulse response is independent of the time at which the unit impulse is administered, the resulting contribution to the response, measured at time t, is $F(t-T) \cdot I(t) \Delta T$, for $0 \leq T \leq t$. Note that t-T is the time elapsed after the amount I(T) dT is introduced, and the contribution of this amount to the response depends on the time elapsed and not on the time at which this amount is introduced.

Systems with this property are called "time invariant

systems." The overall response R(t) is obtained by adding

these contributions.

$$R(t) = \sum_{o \leq T \leq t} F(t-T) \, I(T) \, \Delta T$$

The summation becomes the convolution integral when dT

approaches zero.

$$R(t) = \int_o^t F(t-T) \, I(T) \, dT$$

For input function analysis in pharmacokinetic studies, the

unit impulse response F(t) may be obtained by administering a

unit dose of drug into the general circulation. An equation may

be used to fit the plasma concentration - time curve, and an

equation may be derived. If an unknown input function I(t) is

introduced into this system (i.e.) by oral drug administra-

tion in the same subject), and assuming this system is linear,

the response will follow from the above convolution integral.

The problem now is this: By knowing the plasma concentration -

time data (the response) and F(t), the unit impulse response, or

the underlying pharmacokinetic disposition model for this par-

ticular compartment, we want to determine the functional form of

I(t). We will only observe the response at N periodically

sampled time values in the time interval [o,t]. On the basis of

this data, we will approximately determine the input function at

data points over the time interval [o,t].

A staircase input is defined as a finite set of rectangular

pulses of duration $P_j - P_j - 1$ and intensity I_j, each commencing at

$t = P_j-1$ and ending at $t = P_j$ $(j = 1,2,3,---)$. We can approximate the input function I(t) by assuming that it is a constant equal to I_j between two adjacent sample points P_j and P_j-1.

$$R(P_j) = \sum_{i=1}^{j} I_i \left[\int_0^{P_j-P_{i-1}} F(T)\, dT - \int_0^{P_j-P_i} F(T)\, dT \right]$$

Where $j = 1,2,3,---$, $P_0 = 0$, and $R(0) = 0$.

The above working equation was taken from Vaughan's published article (see references), and the following example has been created as a teaching tool.

Example 1

Suppose a unit impulse response function $F(t) = 21e^{-.43t} + 54e^{-5.5t}$ is known; and $I(t) = 0.4e^{-0.25t}$ is introduced into this system. The overall response function R(t) can be derived as shown below. Following Laplace transformation of F(t) and I(t) we have:

$$i(s) = \frac{0.4}{s+0.25} \quad \text{and} \quad f(s) = \frac{21}{s+0.43} + \frac{54}{S+5.5}$$

Now as $r(s) = i(s) \cdot f(s)$; we have

$$r(s) = \frac{0.4}{s+0.25} \left(\frac{21}{s+0.43} + \frac{54}{s+5.5} \right)$$

or $$r(s) = \frac{30S + 55.49}{(s+0.25)(s+0.43)(s+5.5)}$$

Which upon application of the well known inversion theorem for Laplace transforms,

$$R(t) = 50.781e^{-0.25t} - 46.667e^{-0.43t} - 4.114e^{-5.5t}$$

Using the above response function, a series of concentration vs time data can be generated as follows.

t	1	2	3	4	5	6	7	8
R(t)	9.174	11.053	11.141	10.325	9.113	7.795	6.524	5.376

t	9	10	12	14	16	18	21	24	27	30
R(t)	4.379	3.535	2.260	1.420	0.882	0.544	0.261	0.124	0.059	0.028

Now our problem is knowing the above point data and unit impulse response function $F(t) = 21e^{-043t} + 54e^{-5.5t}$, we want to derive $I(t)$.

Solution

Since $R(P_j) = \sum_{i=1}^{j} I_i [\int_0^{P_j - P_j - 1} F(T)dT - \int_0^{P_j - P_j} F(T)dT]$

when $j = 1$; $R(P_1) = I_1 [\int_0^{P_1 - 0} F(T)dT]$

$$I_1 = \frac{R(P_1)}{\int_0^1 F(T)dT} = \frac{9.174}{26.846} = 0.3417$$

when $j = 2$; $R(P_2) = I_1 [\int_0^{P_2 - P_0} F(T)dT - \int_0^{P_2 - P_1} F(t)dT]$

$$+ I_2 [\int_0^{P_2 - P_1} F(T)dT - \int_0^{P_2 - P_2} F(T)dT]$$

$$I_2 = \frac{R(P_2) - I_1 [\int_0^2 F(T)dT - \int_0^1 F(T)dT]}{\int_0^1 F(T)dT}$$

$$= \frac{11.053 - (0.342)(37.989 - 26.846)}{26.846} = 0.2699$$

following the same manner for further calculations, the estimated cumulative input may be calculated. The comparison of the cumulative drug input and that determined by the deconvolution procedure are listed in the following table.

		Exact Value		Estimated Value	
Hours	Conc	Cumulative Input	% unabs	Cumulative Input,(% Error) [**]	% unabs(% Error)
1	9.174	0.354	77.88	0.342(-3.4)	78.09(0.3)
2	11.053	0.630	60.65	0.612(-2.9)	60.79(0.2)
3	11.141	0.844	47.24	0.823(-2.5)	47.26(0.0)
4	10.325	1.011	36.79	0.987(-2.4)	36.70(-0.2)
5	9.113	1.142	28.65	1.116(-2.3)	28.48(-0.6)
6	7.795	1.243	22.31	1.215(-2.3)	22.08(-1.0)
7	6.524	1.322	17.38	1.293(-2.2)	17.09(-1.7)
8	5.376	1.384	13.53	1.354(-2.2)	13.21(-2.4)
9	4.379	1.431	10.54	1.401(-2.1)	10.18(-3.4)
10	3.535	1.469	8.21	1.438(-2.1)	7.83(-4.6)
12	2.260	1.520	4.98	1.486(-2.2)	4.73(-5.0)
14	1.420	1.552	3.02	1.516(-2.3)	2.83(-6.3)
16	0.882	1.571	1.83	1.534(-2.4)	1.67(-8.7)
18	0.544	1.582	1.11	1.545(-2.3)	0.97(-12.6)
21	0.261	1.592	0.52	1.553(-2.4)	0.43(-17.3)
24	0.124	1.596	0.25	1.557(-2.4)	0.18(-28.0)
27	0.059	1.598	0.12	1.559(-2.4)	0.06(-50.0)
30	0.028	1.599[*]	0.06	1.560(-2.4)	0.00(-----)

[*] Cumulative input = 0.4/0.25 = 1.6 (units)
  if time is extended to infinity.

[**] Error analysis has been studied by Cutler and Vaughan (See references).

From the data listed in the last column of the above table, a drug unabsorbed _vs_ time curve can be generated. The above data, when plotted in semilogarithmic graph paper, shows a linear decline which means a first order absorption proces may be used to describe the input kinetics. The slope of the line is calculated to be 0.256 hr^{-1}. Since percentages below 1% are subject to a relative large error, they are not used in the linear regression (See last column in page 126). Percentage error listed in page 126 (the fifth column) shows that the estimated cumulative input is underestimated for about 2.4%. However, if slope is used to estimate apparent first order absorption, a consistent underestimation of each point will not affect this estimation too much. The cumulative input up to 30 hours shows that 1.56 units have been absorbed which is about 98% of the theroretical data. It is thus possible to use the deconvolution method to calculate the extent of absorption in addition to a rate of absorption.

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Example 2

The following table shows time, concentrations and percent unabsorbed dyphylline data obtained after oral administration of dipivaloyl dyphylline in pig 5 (see page 80). The unit impulse response function used is

$F(t) = 33.28e^{-1.34r}+10.33e^{-0.35}$ which is derived by fitting a two exponential equation to plasma dyphylline concentration time data obtained after administering 2 grams of dyphylline solution intravenously. (See page 76)

TIME	CONC	CUM. INPUT	% UNABSORBED
0	0.	0.	100.
0.25	2.24	0.0076	96.62
0.5	3.62	0.0663	93.75
0.75	6.20	0.1162	88.61
1.0	9.99	0.2055	80.77
1.25	8.89	0.3416	79.03
1.5	8.42	0.3718	76.83
1.75	8.12	0.4100	74.61
2.	8.71	0.4487	71.20
3.	7.53	0.5078	63.29
4.	6.87	0.6452	56.22
5.	7.14	0.7680	48.16
6.	5.13	0.9081	44.63
7.	6.33	0.9693	36.86
8.	6.54	1.1042	29.52
9.	5.87	1.2318	23.81
10.	5.03	1.3311	19.34
11.	5.11	1.4087	13.94
12.	4.18	1.5025	10.49
13.	3.35	1.5623	7.98
14.	2.68	1.6060	6.07
15.	1.89	1.6391	5.15
16.	1.39	1.6551	4.49
17.	1.23	1.6666	3.60
18.	1.06	1.6820	2.81
19.	0.9	1.6957	2.14
20.	0.87	1.7073	1.34
21.	0.73	1.7213	---
22.	0.61	1.7314	---
23.	0.52	1.7395	---

*   The percent dyphylline unabsorbed <u>vs</u> time curve gives

the absorption profile of dyphylline after oral dipivaloyl

dyphylline administration; a continuous rate of absorption

is observed.

**   The cumulative input of dyphylline up to 23 hours is

calculated to be 1.74 units which is 58% of the

total unit administered***.   The extent of

absorption is in good agreement with that calcu-

lated from area under the curve comparisons.

*** The total unit administered is calculated as follows:

10 gm of oral dipivaloyl dyphylline contains

$10 \times 254.24/422.22 = 6.02$ gm of dyphylline which is equal to

3.01 units compared with unit dose 2 gm injected intrave-

nously.