



UNIVERSITI PUTRA MALAYSIA

**PHARMACOKINETICS OF OXYTETRACYCLINE IN THE
SWAMP BUFFALO (BUBALUS BUBALIS)**

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Pharmacokinetics of oxytetracycline in the
swamp buffalo (Bubalus bubalis)

by
Kalthum bt Hashim, DVM

A thesis
submitted in partial fulfilment
of the requirements for the degree of
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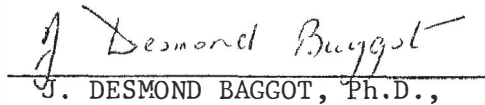
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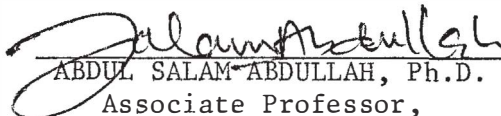
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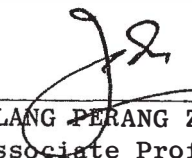
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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vii
ABSTRACT	xi
 CHAPTER	
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	4
PHARMACOKINETICS	4
TETRACYCLINES	18
OXYTETRACYCLINE	21
MINIMUM INHIBITION CONCENTRATION . .	23
III. MATERIALS AND METHODS	26
ANTIBIOTIC ASSAY	29
PHARMACOKINETIC ANALYSIS	36
MINIMUM INHIBITION CONCENTRATION . .	37
IV. RESULTS	40
ANTIBIOTIC ASSAY	40
PHARMACOKINETIC ANALYSIS	43
MINIMUM INHIBITION CONCENTRATION . .	65
V. DISCUSSION	67
ANTIBIOTIC ASSAY	68
PHARMACOKINETIC ANALYSIS	69
MINIMUM INHIBITION CONCENTRATION . .	77



VI. SUMMARY	84
VII. CONCLUSIONS	86
BIBLIOGRAPHY	87



LIST OF TABLES

Table		Page
I	Experimental design	27
II	Sequence of application of samples into their respective wells	35
III	Zones of inhibition for six standard concentrations of oxytetracycline	41
IV	Pharmacokinetic parameters describing disposition of oxytetracycline after intravenous injection of a single dose in swamp buffalo cows	46
V	Pharmacokinetic parameters describing disposition of oxytetracycline after intravenous injection of a single dose in buffalo cows and buffalo heifers	47
VI	Pharmacokinetic parameters describing disposition of oxytetracycline after intravenous injection of a single dose in swamp buffalo heifers	51
VII	Pharmacokinetic parameters describing disposition of oxytetracycline after intramuscular injection of a single dose in swamp buffalo cows	58
VIII	Pharmacokinetic parameters describing disposition of oxytetracycline after intramuscular injection of a single dose in swamp buffalo heifers	61
IX	Pharmacokinetic parameters describing disposition of oxytetracycline after intramuscular injection of a single dose in buffalo cows and buffalo heifers	64



X	Minimum inhibition concentrations for 11 bacterial isolates from diseased buffaloes	66
XI	Calculated intramuscular maintenance dose of OTC in mg/kg for different dosage intervals and micro-organism of different susceptibility	82



LIST OF FIGURES

Figure		Page
1	Diagrammatic presentation of the compartment open models of the body.	5
2	Plasma concentration-time profile of a hypothetical drug after intravenous administration of a single bolus dose	9
3	Graphical method of estimating a half life of a drug	13
4	Molecular structure of oxytetracycline	22
5	Diagrammatic presentation showing the zone of inhibition of the test organism to an antibiotic	30
6	The standard curve of oxytetracycline	42
7	Semilogarithmic graph depicting the time course of oxytetracycline hydrochloride in serum of a buffalo cow after intravenous administration of a single dose	44
8	Relationship of experimental oxytetracycline concentrations in serum as a function of time to computer-generated curves for the fraction of administered dose in the central and peripheral compartments and eliminated during the same period after intravenous administration of a single dose	49
9	Semilogarithmic graph depicting the time course of oxytetracycline hydrochloride in serum of a buffalo heifer after an intravenous administration of a single dose	50



10	Relationship of experimental oxytetracycline concentration in serum as a function of time to computer-generated curves for the fraction of administered dose in the central and peripheral compartments and eliminated during the same period after intravenous administration of a single dose given to buffalo heifers	53
11	Concentrations of oxytetracycline in serum of buffalo cows and heifers at fixed times after intravenous administration of a single dose	55
12	Concentration-time profile of oxytetracycline in serum of buffalo cows and heifers after intramuscular administration	57
13	Apparent first-order absorption of oxytetracycline following intramuscular administration of a single dose to buffalo cows	60
14	Apparent first-order absorption of oxytetracycline following intramuscular administration of a single dose to buffalo heifers	63
15	Plot of maintenance doses vs intervals after intramuscular administration of oxytetracycline for different organisms and their respective desired plasma concentrations	81



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By

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ABSTRACT

Pharmacokinetics of oxytetracycline (OTC) was studied in swamp buffalo heifers and cows after a single injection of 20 mg/kg OTC hydrochloride intravenously and OTC long acting (LA) formulation (Terramycin/LA, Pfizer) intramuscularly using a cross-over study. The concentration of OTC in serum samples was measured by a bioassay technique using Bacillus cereus (ATCC 11778) as the test organism.

The disposition curve for oxytetracycline activities after intravenous administration was best described by a two-compartment open model. The serum concentrations at time zero, C_0 , for buffalo cows and buffalo heifers were 61.37 ± 19.62 mcg/ml and



33.09±9.93 mcg/ml respectively. The elimination half-lives for buffalo cows (10.13±2.72 h) and heifers (8.72±2.14 h) after intravenous injection of the drug were not significantly different ($P>0.05$). The apparent volume of distribution was significantly higher in buffalo cows (2.84±0.46 L/kg) than in heifers (2.0±0.42 L/kg) but the total body clearance did not differ significantly ($P>0.05$).

Following a single intramuscular injection of OTC-LA formulation only one rate constant of absorption was noted in buffalo cows ($k_a-0.05 \text{ h}^{-1}$) but two rate constants of absorption were noted for heifers - a fast rate ($k_{a_1}-3.62 \text{ h}^{-1}$) and a slow rate ($k_{a_2}-0.04 \text{ h}^{-1}$). The peak serum concentration of OTC for buffalo cows was 4.56±1.48 mcg/ml at 4.67±1.91 hours and for buffalo heifers was 4.40±0.57 mcg/ml at 1.67±0.26 hours. The bioavailability of OTC was 64 and 24 per cent for buffalo cows and heifers respectively. Plasma concentrations above 0.5 mcg/ml were maintained for approximately 50-60 hours. The minimum inhibition concentration (MIC) for eleven isolates from diseased buffaloes ranged from 0.25 to 4.0 mcg/ml.

For the swamp buffalo OTC-LA is recommended at a dosing rate of 20 mg/kg injected intramuscularly at 48-hour dosage intervals for highly susceptible organisms ($\text{MIC}<1 \text{ mcg/ml}$). However it is preferable to use the conventional OTC formulation intramuscularly for less susceptible organisms ($\text{MIC}>2 \text{ mcg/ml}$).



CHAPTER I

INTRODUCTION

Water buffaloes (Bubalus bubalis) may be classified into river and swamp types according to their wallowing habits. Water buffaloes are among the most important domestic ruminants providing draft power, milk and meat in over 40 countries. The world buffalo population is approximately 150 million or one-eighth of the population of cattle. Over 95 per cent of the buffaloes in Malaysia are of the swamp type. They are primarily used for draft power and are concentrated in the rice growing areas where they are utilized for ploughing, harrowing and puddling of rice fields before planting. At the end of their working life, swamp buffaloes provide about half the present meat supply in Malaysia.

The important bacterial diseases of buffaloes include haemorrhagic septicaemia (Pasteurella multocida), infectious keratitis (Haemophilus bovis), suppurative pneumonia (Strep. pyogenes and Pasteurella multocida) and arthritis (E. coli and Corynebacterium pyogenes). These diseases particularly haemorrhagic septicaemia cause severe economic losses to the country.

There is a lack of accurate information on the use of antibiotic therapy in the water buffalo. Much of our knowledge on antibiotic therapy in the water buffalo is based on information from cattle. Pharmacokinetic studies are essential to establish



dosage regimens adequate for producing effective antimicrobial drug concentrations in vivo. This information is of importance especially to veterinarians who have been treating diseased buffaloes with dosages recommended for cattle.

Oxytetracycline (OTC) is an important antimicrobial drug and is commonly used in veterinary medicine. Until recently, parenteral OTC therapy has involved the daily administration over several days. A depot formulation of oxytetracycline containing 200 mg/ml of OTC (Terramycin-LA, Pfizer) allows a reduction in the frequency of administration. The main advantage of this product is that a single dose (20 mg/kg) sustains effective levels for over three to five days in cattle and sheep.

OTC-LA would be a very valuable antibiotic in the water buffalo as it would overcome the problem of daily administration of OTC to diseased buffaloes under range conditions where restraining facilities are inadequate. However, very few studies have been conducted in the buffalo to determine effective blood levels of OTC-LA. There is also no information on the absorption, distribution and elimination of oxytetracycline in swamp buffaloes after parenteral administration.

This study was therefore conducted in healthy female swamp buffaloes:

- a. To determine the disposition kinetics of oxytetracycline hydrochloride following intravenous injection of a single dose.
- b. To ascertain the rate of absorption and systemic availability of OTC following administration of OTC-LA by the intramuscular route.

- c. To establish minimum inhibitory concentrations for different bacterial isolates from diseased buffaloes.
- d. To recommend dosing rate for OTC-LA that would maintain "effective" serum oxytetracycline concentrations in the swamp buffalo.

CHAPTER II

REVIEW OF LITERATURE

PHARMACOKINETICS

Pharmacokinetics deals in part with the mathematical description of the time course of drug absorption, distribution and elimination by means of a suitable model (Levy and Gibaldi, 1972).

To facilitate the study of the pharmacokinetic behaviour of drugs, the body is depicted as a system made up of one, two or three distribution compartment open models (Figure 1). The principle adopted in analysing the serum concentration-time profiles is to employ the pharmacokinetic model with the least number of compartments that can adequately describe the data.

If a drug distributes very rapidly relative to the rate of elimination, the disposition kinetics of the drug behaves as a single homogeneous distribution and the one-compartment open model is applied (Figure 1a). This model assumes that a change in the drug concentration in one tissue is accompanied by a corresponding change in drug concentration in all other tissues (including plasma) at the same time. The following expression satisfies the one-compartment open model (Baggot, 1977):

$$C_p = B e^{-kt} \quad (1)$$

where C_p is the concentration of the drug in plasma at any time



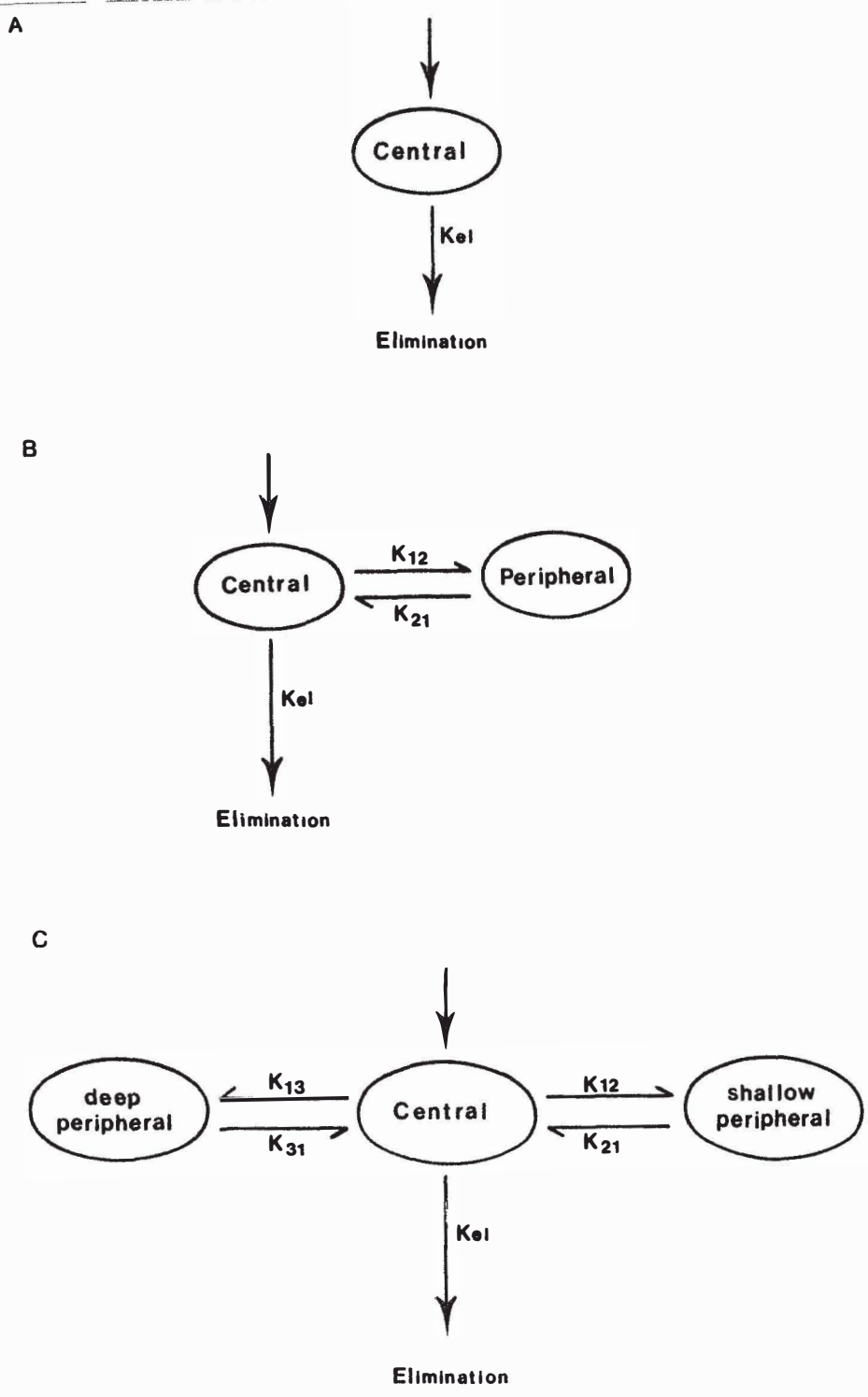


FIGURE 1: DIAGRAMMATIC PRESENTATION OF COMPARTMENTAL PHARMACOKINETIC MODELS

A: One-compartment model; B: Two-compartment model; C: Three-compartment model



t, B (mcg/ml) is zero-time intercept extrapolated from the overall elimination rate constant " β " and "e" the base of the natural logarithm. Drugs that can be adequately described by a one-compartment open model include amphetamine (Baggot and Davis, 1973), chloramphenicol (Davis et al., 1972) and quinidine (Neff et al., 1972).

Disposition kinetics of many drugs including erythromycin and tylosin (Baggot and Gingerich, 1976), ketamine (Baggot and Blake, 1976), trimethoprim (Alexander and Collett, 1974), can be described by a two-compartment open model (Figure 1b).

It is assumed that a drug introduced into the central compartment, which consists of blood plasma and the extracellular fluid of highly perfused organs such as lungs, liver, heart and kidneys, distributes instantaneously and homogeneously, and that it is eliminated exclusively from the central compartment. Distribution into the second or peripheral (tissue) compartment, which consists of less perfused tissues such as muscle, skin and body fat, occurs more slowly. A two-compartment open model can be described by the following expression:

$$C_p = A e^{-\alpha t} + B e^{-\beta t} \quad (2)$$

where C_p is the concentration of the drug in the plasma at any time t , A and B (mcg/ml) are extrapolated intercepts " α " and " β " are the rate constants for the distribution and elimination phases respectively, expressed in unit reciprocal time (h^{-1}), and "e" represents the base of the natural logarithm (Baggot, 1978). The sum of A and B (mcg/ml) gives the plasma concentration at time zero.

Disposition kinetics of a few drugs such as sulphadimethoxine (Boxenbaum and Kaplan, 1975) and oxytetracycline in dogs (Baggot et al., 1977) can be described by a three-compartment open model (Figure 1c) as follows:

$$C_p = P e^{-\pi t} + A e^{-\alpha t} + B e^{-\beta t} \quad (3)$$

where " π " and " α " (in unit reciprocal time) are the rate constants of distribution phases and " β " (in unit reciprocal time) is the rate constant of elimination phase, P, A, and B (mcg/ml) are zero-time extrapolated intercepts respectively, and "e" is the base of the natural logarithm. The individual rate constants associated with three-compartment open model (k_{12} , k_{21} , k_{13} , k_{31} and k_{el}) can be calculated (Gibaldi and Perrier, 1975).

The rate constants of the absorption and the elimination of a drug are assumed to follow first-order kinetics, i.e., constant fraction of the drug present is eliminated per unit time (Goodman and Gilman, 1975). The elimination of most drugs is exponential since drug concentrations usually do not approach those required for saturation of the elimination process. Suitable dosage regimens for drugs exhibiting dose-dependent kinetics in the therapeutic dose range defy easy calculation and are established by careful titration of drug level in the patient (Levy, 1968), e.g., salicylate in cats (Yeary and Swanson, 1973).

In certain exceptional cases, the drug elimination processes may become saturated and the result is zero-order kinetics i.e., a constant amount of the drug present is eliminated per unit time. An increase in dosage in such cases results in a more prolonged half-life and a disproportionately greater accumula-

tion of the drug in the body (Goodman and Gilman, 1975). The elimination of phenylbutazone in dog (Dayton et al., 1967) and in horses (Piperno et al., 1968) obeys zero-order kinetics. The rate of elimination of a drug may be influenced by extensive binding to plasma protein, the rate of various metabolic pathways and the efficiency of excretion processes particularly glomerular filtration (Baggot, 1977).

Principle of Pharmacokinetic Analysis

Intravenous injection is the most reliable method of administration of a drug for the purpose of pharmacokinetic analysis. The biexponential expression (Gibaldi et al., 1969; Baggot, 1977)

$$C_p = A e^{-\alpha t} + B e^{-\beta t} \quad (4)$$

describes the decline of a drug concentration-time curve after intravenous administration of a single bolus dose (Figure 2). The biexponential expression of the two-compartment open model assumes that when injected intravenously, the drug distributes instantaneously throughout the central compartment. The initial steep decline in plasma concentration of a drug is due to the distribution of the drug (by passive diffusion) from the central to the peripheral (tissue) compartment. Once apparent (pseudo) distribution equilibrium is attained, the rate of decline in plasma drug concentration is decreased and the linear terminal portion is referred to as the " β " or the elimination phase. Iterative least square linear regression analysis is used to find the best fit curve. The slope of the linear terminal portion of the curve may be defined as $-\beta/2.303$ and an extrapolation of

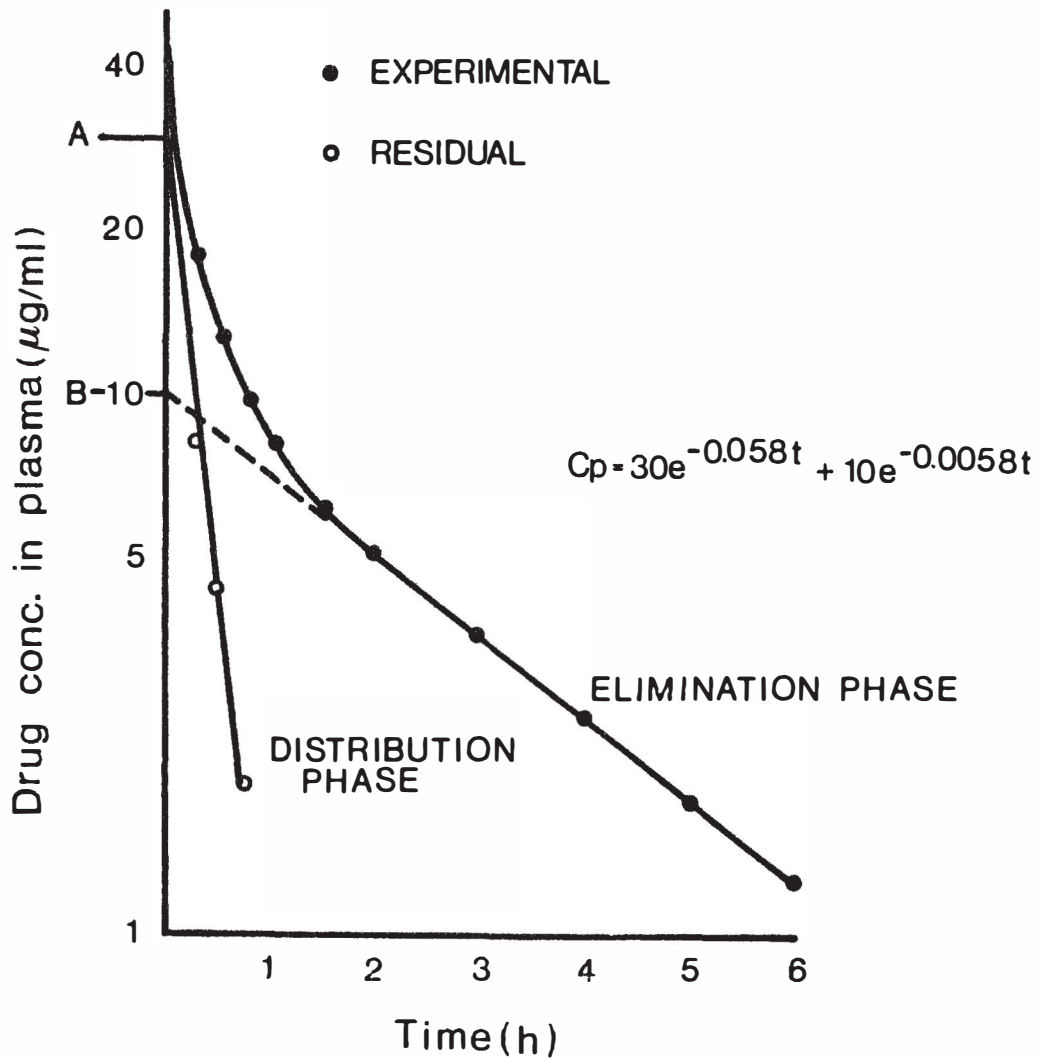


FIGURE 2: PLASMA CONCENTRATION-TIME PROFILE OF A HYPOTHETICAL DRUG AFTER INTRAVENOUS ADMINISTRATION OF A SINGLE BOLUS DOSE (From Baggot, J. D. (1977): Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. W. B. Saunders Co. Philadelphia. USA.)

this line gives the zero-time intercept, B (mcg/ml) which is an estimate of the drug concentration that would have been attained if the distribution were instantaneous.

The residual data points, representing " α " (the distribution phase) are obtained by subtracting the extrapolated portion of " β " (the elimination phase) from the experimental data of the initial steep portion of the concentration-time curve. Least square regression analysis for the residual data is used to obtain the best fit curve of the distribution phase " α " and extrapolation of this line yields zero-time intercept, A .

For the three-compartment open model, the second set of residual data points are obtained by subtracting the extrapolated portion of the first distribution phase from its steep portion. Least squares regression analysis for the second set of residual data is used to obtain the best fit curve of the second distribution phase " π ", the extrapolation yields the zero-time intercept, P (mcg/ml).

Actual Pharmacokinetic Rate Constants

The plasma drug concentration-time curve provides the only accurate information for drug measurement in the body. The experimental constants (A , B , α and β) are "hybrid" pharmacokinetic parameters and are used to calculate the actual pharmacokinetic rate constants (k_{12} , k_{21} and k_{el}) associated with the two-compartment open model (Baggot, 1978).

$$k_{12} = \frac{A\beta + B\alpha}{A + B} \quad (5)$$

$$k_{21} = \alpha + \beta - k_{12} - k_{el} \quad (6)$$

are the rate constants for distribution between central and peripheral compartments, and

$$k_{el} = \frac{\alpha\beta}{k_{21}} \quad (7)$$

is the rate constant for elimination. All these expressions are given in units of reciprocal time (h^{-1}).

Determination of the microconstants permits an assesment of the relative contribution of distribution and elimination processes (which may be altered in disease state) to the concentration-time profile of a drug (Baggot, 1977). A computer programme, using values of the individual rate constants of the model, can be used to predict curves which describe the levels of a drug (expressed as fraction of a single dose) in the central and peripheral compartments as a function of time, as well as elimination curve. Based on the hybrid and actual pharmacokinetic parameters, other parameters associated with the two-compartment open model can be calculated (Baggot, 1977).

Half-life ($t_{1/2}$)

The half-life for the elimination phase or the (biological) half-life of a drug is defined as the time required for the body to eliminate one-half of a particular drug, i.e., the time taken for the serum concentration of a drug to decline by fifty per cent during the elimination phase of the disposition curve. It is assumed that once pseudodistribution equilibrium has been established, the ratio of the drug in peripheral to central compartments remains constant. The half-life value is obtained from the expression,

$$t_{1/2} = \frac{\ln 2}{\beta} = \frac{0.693}{\beta} \quad (8)$$

β is the overall elimination rate constant, $t_{1/2}$ is expressed in unit of time (h). An estimate of the half-life may also be obtained graphically (Figure 3). A large value of β corresponds to a short half-life and indicates rapid elimination (Baggot, 1977).

For drugs that obey zero-order kinetics, their half-lives become progressively longer as the doses increase (Baggot, 1978), e.g., elimination of phenylbutazone in dogs (Dayton et al., 1967) horses (Piperno et al., 1968) and salicylate in cats (Yeary and Swanson, 1973). Knowledge of the half-life is very useful particularly for design of rational dosage regimens.

The half-lives of most drugs are independent of the dose and route of administration. Intravenous injection of a single dose of the drug is the only satisfactory procedure to determine the half-life value since the apparent overall rate of elimination following other routes may be influenced by the rate of absorption (Byron and Notari, 1976).

Apparent Volume of Distribution (Vd)

It is defined as the volume of fluid which would be required to contain the amount of drug in the body if it was uniformly distributed at a concentration equal to that in the plasma. The body is assumed to behave as a single homogeneous compartment with respect to the drug. The value of Vd serves as a proportionality constant relating the plasma concentration of a drug to the amount in the body at any time after distribution equilibrium has been attained. Vd can be expressed as,