PHARMACOKINETIC STUDY OF DOXYCYCLINE AND DEMECLOCYCLINE IN GOAT

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

BIHAR

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

PHARMACOLOGY

By

Vijay Kumar Jha

DEPARTMENT OF PHARMACOLOGY

BIHAR VETERINARY COLLEGE

PATNA

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By
Vijay Kumar Jha

Department of Pharmacology
Bihar Veterinary College,
Patna

1985

DEDICATED TO

MY

BELOVED PARENT

Department of Veterinary Pharmacology,
Bihar Veterinary College, Patna.
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR.

C E R T I F I C A T E - I

This is to certify that the thesis entitled "Pharmacokinetic study of Doxycycline and Demeclocycline in goat" submitted in partial fulfilment of the requirement for the Degree of Master of Veterinary Science (Veterinary Pharmacology) of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by Dr. Vijay Kumar Jha under my supervision and guidance. No part of the thesis has been submitted for any other Degree and Diploma.

It is further certified that such help or informations received during the course of this investigation and preparation of the thesis have been duly acknowledged.

28/3/85

(M. K. Singh)
Associate Professor & Head,
Department of Veterinary Pharmacology.

C E R T I F I C A T E - II

We, the undersigned, member of the Advisory Committee of Dr. Vijay Kumar Jha, a candidate for the Degree of Master of Veterinary Science with major in Veterinary Pharmacology have gone through the manuscript of the thesis and agree that the thesis entitled "Pharmacokinetic study of Doxycycline and Demeclocycline in goat" may be submitted by Dr. Vijay Kumar Jha in partial fulfilment of the requirements for the Degree.

28/3/85

(M. K. Singh)
Chairman, Advisory Committee.

Members of the Advisory Committee:

1. Dr. S. D. Singh :- 28/3/85

2. Dr. H. N. Jha :- 28/3/85

3. Dr. M. Murtuza :- 28/3/85

C E R T I F I C A T E - III

This is to certify that the thesis entitled "Pharmacokinetic study of Doxycycline and Demeclocycline in goat" submitted in partial fulfilment of the requirements for the Degree of Master of Veterinary Science (Veterinary Pharmacology) of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Bihar, was examined and approved on 1.7.....1985.

1/7/85

(M. K. Singh)
Chairman, Advisory Committee.

B.D. Garg 1/7/85

(B. D. Garg)
External Examiner.

Members of Advisory Committee:

1. Dr. S. D. Singh :-

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3. Dr. M. Murtuza :-

Dean, Post-Graduate studies.

A C K N O W L E D G E M E N T

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(V. K. Jha)

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C O N T E N T S

INTRODUCTION

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The current century has witnessed the replacement of emperical therapy with more potent and specific drugs in the armamentarium of modern physicians particularly with regard to cure for infectious diseases. The development of tetracyclines as chemotheraputic agent was a landmark for the prevention and cure of various infections caused by gram-positive and gram-negative bacteria, rickettsiae, mycoplasma, spirochetes, actinomyces and certain protozoa (Jones et al., 1977). Among the tetracyclines, Oxytetracycline and Tetracycline are most widely and routinely used to treat a variety of bacterial infections in veterinary practice. In recent years, newer tetracyclines like Demeclocycline and Doxycycline have attained an important place in human therapy due to their high bioavailability, prolonged maintenance of therapeutic concentration and greater efficacy against many infective organisms. However, only a few reports of systematic pharmacokinetic study of these drugs are available in animals particularly in goat and hence, the drugs are yet to find a suitable place in veterinary proctice.

The goat which is considered as a "poor man's cow" contributes significantly to the meat and leather industries in India and deserves proper attention for its adequate health coverage.

The level and persistance of an antimicrobial agent in plasma alone had been relied upon till last decade in determining the therapeutic efficacy. It is now proved and well appreciated that the concentration of an antibiotic in serum does not necessarily reflect its antimicrobial activity at the site of infection (Tan, 1978). The distribution patterns of a drug vary considerably in various body fluids such as plasma, milk, urine, interstitial fluid etc. because of the specialised nature of the membranes at each site (Varvey et al., 1965). Physico-chemical properties of an antimicrobial agent such as molecular weight, lipid solubility, degree of ionization, protein binding etc. are found to influence the pattern and extent of distribution as well as rate of elimination. In addition to individual variations in organ function and enzyme activity, there are distinct anatomical and physiological differences among the species of animals (Baggot, 1977). In any clinical situation, dosage of the appropriate drug product should be based on relevant pharmacokinetic data. Pharmacokinetic studies give proper perspective for efficent use of an antibiotic in man and animals. Levels of routinely used antibiotics in body fluids such as plasma, milk, urine, interstitial fluid etc. are required to be known while preparing dosage regimen.

To be effective in a particular affection, the drug should reach the target organ in desired concentration. The estimation of antibiotic in milk, urine and interstitial fluid

following oral/parenteral administration will be more convincing proof of anticipated therapeutic success by systemic route(s) for treating local affections such as mastitis, urinary tract infections as well as systemic infections. Besides, contamination of antibiotic in milk is a public health problem and requires withdrawal of milk from human consumption for sufficient period of time after cessation of therapy. A detailed knowledge of the residual persistance of the drug in various biological fluids will enable us to suggest the time limit for withdrawal of milk and meat from human consumption.

Keeping in view of the aforesaid aims and objectives, detailed pharmacokinetics of doxycycline and demeclocycline with reference to their distribution in plasma, milk, interstitial fluid and urine, as well as bioavailability, elimination half life, apparent volume of distribution etc. have been studied in the present investigation since such informations are lacking in goat. Based on the kinetic parameters, appropriate dosage regimen have been determined. Such informations would definitely lead to the judicious use of these drugs in veterinary practice particularly in goat.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The modern era of the chemotherapy of bacterial infection started with the clinical use of sulfonamide in 1936. The "golden age" of antimicrobial therapy began with the accidental discovery of penicillin by Alexander Fleming in 1928 and establishment of its clinical success against streptococcal and staphylococcal infections which were refractory to all other drugs then used in therapy. The advent of streptomycin in 1944 by prof. Waksman and his collegues from the soil organism streptomyces griseus led to the isolation of many potent antibiotics by a thorough systematic search of microorganisms. One such group of antibiotics is tetracyclines, with a broad spectrum of activity against many organisms. The first of these compounds, chlortetracycline was obtained from Streptomyces aureofaciens and clinically used in 1948. Oxytetracycline which was isolated from streptomyces rimosus, became available two years later. Tetracycline, was produced semisynthetically from chlortetracycline in 1952. Demethylchlortetracycline (Demeclocycline), obtained from a mutant of the strain of Streptomyces aureofaciens in 1957, became available for general use in 1959. Methacycline, Doxycycline and Minocycline which were introduced in 1961, 1966 and 1972, respectively, are all semisynthetic derivatives of tetracycline molecule.

CHEMISTRY :

The tetracyclines are close congeneric derivatives of the polycyclic naphthacenecarboxamide. Their structural formula are shown in Fig. 1.

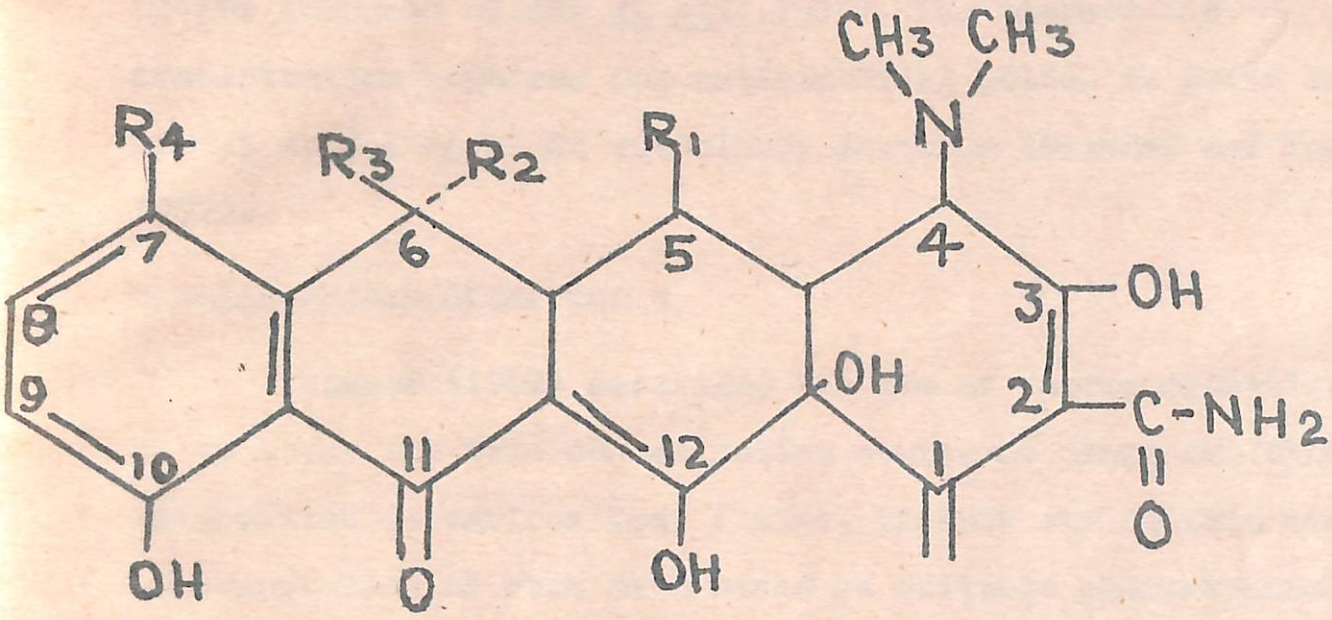
All tetracyclines are crystalline bases and faintly yellow, odourless and slightly bitter compounds. They are only slightly soluble in water at pH 7 (0.25 to 0.5 mg/ml), but they form soluble sodium salts and hydrochlorides, The bases and the hydrochlorides are quite stable as dry powders, but these agents loose their activity relatively rapidly when present in solution.

ANTIMICROBIAL EFFICACY :

The tetracyclines possess a wide range of activity against gram-positive and gram-negative bacteria. Besides, they are also effective against some microrganisms innately insensitive to many chemotherapeutic agents, such as rickettsiae, Mycoplasma, Chlamydia, some atypical mycobacteria, and amoebae. Individually, they have little activity against true fungi although they may exert an antifungal action when combined with amphotericin (Lew et al., 1977).

These drugs are primarily bacteriostatic and in higher concentrations, they are frequently bactericidal. The sensitivity or resistance of a particular microorganism to each of the congeners is similar. However, minocycline is usually the most active, followed by doxycycline and

FIG. 1



Name	R_1	R_2	R_3	R_4
Tetracycline	H	OH	CH_3	H
Chlortetracycline	H	OH	CH_3	Cl
Demeclocycline	H	OH	H	Cl
Oxytetracycline	OH	OH	CH_3	H
Methacycline	OH	$=CH_2$	-	H
Minocycline	H	H	H	$-N(CH_3)_2$
Doxycycline	OH	H	CH_3	H

demeclocycline. Tetracycline and oxytetracycline are the least active (Goodman Gilman et al., 1980). The therapeutic concentration required for antibacterial action is found to be 0.5 to 1.0 µg/ml in veterinary practice (Brander and Pugh, 1977).

GENERAL PHARMACOKINETICS :

Wagner (1968) described the aim of pharmacokinetics as to study the time concentration course of drugs and their metabolites in various body fluids, tissues and excreta and interpretation of such data based on suitable pharmacokinetic models (compartment models).

The compartment model is a hypothetical structure which can be used to characterise with reproducibility of behaviour and fate of drugs in a biological system, when given by a certain route of administration, in a particular dosage form. Compartment is an entity which has a definite volume and concentration of a drug in that volume at any time. An open compartment model indicates free movement of drugs from one compartment to another compartment (i.e. blood to tissue & vice-versa).

The one compartment open model fits well when the drug distribution is instantaneous between the blood and tissues. Any change in drug concentration in the blood reflects directly the quantitative change in its tissue levels. The rate of drug elimination from the body is proportional to the concentration

in the blood reflects directly the quantitative change in its tissue levels. The rate of drug elimination from the body is proportional to the concentration of the drug in blood (Baggot, 1974). The drug concentration in plasma, in this model, is expressed by the following mathematical formula as a function of time.

$$Cp = Be^{-\beta t} \quad \text{..... Eq. 1.}$$

Where Cp is the concentration of drug in plasma, B is the extrapolated zero time intercept of monoexponential curve, β is the overall elimination rate constant, t is the time elapsed after drug administration and e represents the base of natural logarithm. The one compartment open model is particularly useful in describing the time course of most drugs in plasma after oral or i.m. administration (Baggot, 1977).

A two compartment open model accurately describes the pharmacokinetics of most drugs after i.v. administration. In this model drug distribution is instantaneous and homogeneous into the central compartment (blood and other readily accessible tissues like liver and kidney) and more slowly into the peripheral compartment, comprising of less perfused organs and tissues, such as muscle and fats (Baggot, 1974). The two compartment open model specifices that distribution and elimination processes follow the first order kinetics and elimination takes place exclusively from central compartment after an i.v. injection. A biexponential expression frequently

describes the drug concentration in plasma, which is given by the following equation :

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \quad \ldots\ldots\ldots\ldots \text{Eq. 2.}$$

Where Cp represents plasma concentration of the drug, A and B are the zero time intercept of distribution and elimination phases, α and β are the distribution and elimination rate constants, respectively, e is the base of natural logarithm and t is the time elapsed after drug administration.

To calculate the other pharmacokinetic rate constants (K_{12}, K_{21} and K_{el}) associated with two compartmentopen model, the value of A, B$\alpha$and β are essential. The value of these rate constants give an idea of the relative contribution of distribution and elimination processes to the drug concentration time data (Baggot, 1977).

The disposition kinetics of some drugs may also follow three or multiple compartment model, where plasma drug concentrations after single i.v. administration are described by a triexponential expression :

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} + Ge^{-\gamma t} \quad \ldots\ldots\ldots \text{Eq. 3.}$$

The residual methods are employed to estimate the additional constant G and γ. Gibaldi and Perrier (1975) reported that above constants can be used to calculate K_{13} and K_{31}.

RELEVANCE OF KINETIC PARAMETERS TO CLINICAL PRACTICE :

The clinical application of pharmacokinetic studies comprises of determination of drug bioavailability following different routes of administration, calculation of dosages regimen of a drug in a particular species of animal and estimation of the drug withdrawal period for drug residues in milk and tissues of food producing animals.

Baggot (1977) reported that the overall elimination rate constant (β) is the most important pharmacokinetic parameter as it is used to calculate the half life ($t_{1/2\beta}$), volume of distribution by area method (Vd_{area}) and body clearance (Cl_B). It is also used to predict the drug withdrawal period for drug residues in milk and tissues of food producing animals (Mercer et al., 1977).

Gibaldi and Weintraub (1971) described the elimination half life as time required to reduce the drug concentration in plasma or serum to its half during the elimination phase of the drug concentration time profile. The half life is inversely proprtional to the overall elimination rate constant. Half life is of prime importance in determining the duration of drug action in the body. The half life of a first order process is independent of the route of administration and the dose. This means that doubling the dose, does not double the duration of action of drug but increases it by one half life. Knowledge of the half life of a drug is extremely helpful in predicting the design of rational dosage regimen.

The apparent volume of distribution is an important parameter in the pharmacokinetic characterization of drugs. The apparent volume of distribution (Vd) is a hypothetical volume of body fluid that would be required to dissolve the total amount of the drug to attain the same concentration as that found in the blood. The calculated value of volume of distribution is not dependent upon the method used for its calculation, if the drug distributes truely according to one compartment model (Riegelman et al., 1968). Baggot (1977) stated that apparent volume of drug distribution gives an idea of the extent or magnitude of distribution without providing any clue whether the drug is uniformly distributed or restricted to certain tissues. A large volume of distribution indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. The small volume of distribution means, that the drug is restricted to certain fluid compartments, namely plasma water, extracellular fluid etc. This is due to the high protein binding or low lipid solubility of a drug.

After i.v. administration, whole of the drug is available for distribution, metabolism and excretion processes and its peak level in blood is attained immediately. The peak plasma concentration following extravascular administration is delayed and its magnitude decreases. The bioavailability refers to both, the rate of drug absorption and the extent of

absorption of a drug in pharmacologically active form. The extent of absorption (F), commonly called bioavailability, is determined experimentally from the ratio of the area under the plasma concentration time curve following extravascular and i.v. administration (Baggot, 1977; Sams, 1978). The extent of bioavailability is the main factor that determines the relation between drug dosage and intensity of action.

Some drugs have affinity to plasma protein, mainly albumin and gets bound with them. Binding of drugs with plasma protein affect drug distribution (high molecular weight of plasma protein prevents bound drug from diffusing out of capillaries into tissues), drug effects (free drug fraction is alone pharmacologically active, since it can penetrate to the region of target organ) and drug elimination (free drug is alone filtered at the glomerulous and also excreted into saliva, milk etc.). Besides that the protein bound drug can act as a reservoir.

Another parameter, total body clearance (Cl_B) indicates the sum of the clearance of each eliminating organ, mainly liver and kidney. For most of the drugs, the half life is a complex function which depends upon the process of drug distribution, biotransformation and renal excretion. The parameter, body clearance, on the other hand is independent of these processes and gives a proper expression of the rate of drug removal from the body. Unlike β and $t_{1/2}\,\beta$ which are hybrid constants and depend upon K_{12}, K_{21} and Kel, the body

clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland et al., 1973).

Jusko and Gibaldi (1972) indicated that the various constant α, β, A, B, $t_{1/2\alpha}$, $t_{1/2\beta}$ and Vd_{area} etc. change disproportionally with the magnitude of the elimination rate constant (Kel) and therefore, should not be used individually as a direct or safe measure of a change in drug elimination or distribution.

Dose is a quantitative term estimating the amount of drug which must be administred to produce a particular biological response, i.e. to establish a certain effective concentration of drug in the body fluids. Maintenance of therapeutic concentration of a drug in the body requires the administration of maintenance dose at a particular dose interval after administering the priming or loading dose, so that concentration must be above a minimum effective level and below a level producing excessive side effects and toxicity. Thus, the objective of a multiple dosage regimen is to maintain the plasma concentration of the drug within the limits of the maximum safe concentration and the minimum effective levels.

DOXYCYCLINE :

Doxycycline, a semisynthetic derivatives of tetracycline was introduced in 1966 (Goodman Gilman et al., 1980). Pharmacokinetic study of doxycycline is mainly reported in human beings. However, a few literature are available in different species of animals.

Blood and tissue level :

Steigbigel et al., (1968) reported the serum concentration of doxycycline in normal human beings after a single oral dose of 300 mg. They found the mean peak serum concentration of 3.64 µg/ml at 8 hr. and at 48 hr the drug concentration was found to be 0.64 µg/ml. Nielsen et al. (1971) conducted a multiple dose trial in normal human subjects with an initial dose of 400 mg doxycycline and subsequent doses of 200 mg every 24 hr orally and found an average minimum serum concentration of between 1 and 2 µg doxycycline per ml of serum. Leibowitz et al. (1972) found the peak plasma concentration of 3 µg/ml at 2 hr and the drug concentration was maintained above 1 µg/ml for 8 to 12 hr after an oral dose of 200 mg of doxycycline in man. Klastersky et al. (1972) observed the mean peak level of 1.3 µg/ml and 5 to 6 µg/ml of doxycycline in serum, when given in the same dose (200 mg) by oral and i.v. routes, respectively, in human patients. Mardh et al. (1975) studied the concentration of doxycycline in serum and in thoracic duct lymph after oral and i.v. administration of 200 mg (doxycycline) in human patients. He found the mean peak serum concentration of 2.4 µg/ml at 3 hr after oral and 4.5 µg/ml just after i.v. administration. The mean peak concentration of drug in thoracic duct lymph were 1.6 µg/ml at 3-6 hr and 3.26 µg/ml at 1 hr after oral and i.v. administration, respectively. Heaney and Eknoyan (1978) reported a peak concentration of 9.68 µg/ml of doxycycline in

serum of noramlhuman volunteers just after the i.v. infusion in dose of 200 mg. Thadepalli et al. (1980) measured the concentration of doxycycline in the human lung and pleural tissues following i.v. administration. The average serum levels were 9.3, 7.0 and 3.2 µg/ml at 1, 2 and 3 hr. The lung tissue levels were 6.8, 3.5 and 2.3 µg/g while the pleural tissue levels were 2.5, 1.5 and 1.5 µg/g at the corresponding time intervals.

Ranade et al. (1981) estimated the doxycycline levels in serum, milk, saliva and interstitial fluid of lactating goats after single i.v. injection in the dose of 200 mg/animal. The peak level in serum, milk, saliva and interstitial fluid was found to be 8.2 µg/ml, 0.63 µg/ml, 0.43 µg/ml and 0.8 µg/ml at ½ hr, 2 hr, ½ hr and 1 hr, respectively.

Bioavailability :

The percentage of an oral dose that is absorbed (oral bioavailability) is reported to be 93% (Fabre et al., 1971), 95% (Barza and Scheife, 1977) and 93% (Neu, 1978) for doxycycline in human subjects.

Protein binding :

The plasma protein binding in human beings was noted to be 82% (Schach Von Wittenau and Yeary, 1963), 93% (Rosenblatt et al., 1966), 80 to 95% (Kunin, 1967; Neu, 1978) and 90.8 ± 3.52% (Raghuram and Krishnaswamy, 1982).

Schach Von Wittenau and Yeary (1963) reported canine serum protein binding of doxycycline to be 80% after i.v. administration of the drug to dogs whereas Schach Von Wittenau and Delahunt (1966) observed the same to be 82%. Ziv and Sulman (1974) found the protein binding of doxycycline in serum of cows and ewes upto 90.2 ± 2.4%.

Elimination half life :

The mean elimination half life of doxycycline in human beings was reported to be 15 hr (Migliardi and Wittenau, 1967), 15.1 hr (Rosenblatt et al., 1967), 8.3 hr (Doluisio and Dittert, 1969), 20 hr (Merier et al., 1969/70), 11.6 hr (Nielsen et al., 1971), 16.4 hr (Lee et al., 1972), 17 to 20 hr (Leibowitz et al., 1972), 15.5 hr (Curtis et al., 1973), 13.8 hr (Heaney and Eknoyan, 1978), 15 to 22 hr (Neu, 1978) and 16.3 hr (Raghuram and Krishnaswamy, 1982).

Ziv and Sulman (1974) reported the mean elimination half life of doxycycline to be 24.75 hr in cows and ewes after a single i.v. injection in the dose of 20 mg/kg body weight. Michael et al. (1979) observed the half life of doxycycline phosphate as 11.5 hr in dogs after oral administration of the drug at the dose rate of 10 mg/kg body weight.

Apparent volume of distribution :

The volume of distribution of the tetracyclines are generally found to be higher, denoting that the drugs are well distributed in different tissues and body fluids

(Goodman Gilman et al., 1980). Ceccarelli et al. (1971) reported the volume distribution of doxycycline between 0.9 to 1.8 L/kg in children. Raghuram and Krishnaswamy (1982) noted the volume distribution of doxycycline in adult human beings to be 0.75 ± 0.089 L/kg.

Ziv and Sulman (1974) found the volume of distribution of doxycycline as 2.285 ± 0.31 L/kg in cows and ewes. Michael et al. (1979) observed the volume distribution of doxycycline to be 3.25 L/kg in dogs. The higher value for volume of distribution noted by the above workers, denotes a good distribution of the drug which is supported by the ovservation of Neu (1978), who showed the highest distribution of doxycycline in liver, kidney, brain and sputum.

Concentration in urine and urinary excretion rate :

Steigbigel et al. (1968), after a single oral dose of 300 mg of doxycycline in a normal young man, reported that the concentration of drug in urine varies between 5.7 to 93.7 µg/ml with a peak value of 93.7 µg/ml at 0-8 hr and minimum value of 5.7 µg/ml between 72 to 96 hr. Merier et al. (1969/70) found the urinary excretion of doxycycline to be 40 ± 4% after oral ingestion of 200 mg of the drug followed by 100 mg every 24 hr in normal subjects. Nielsen et al. (1971) reported that 42% of the drug administered was excreted in the urine in an active form after a multiple dose trial, with an initial dose of 400 mg and subsequent doses of 200 mg every 24 hr of doxycycline in normal human subjects. They further reported that the average

urine concentration of doxycycline varied between 100 and 200 µg/ml of urine. Neu (1978) observed the urinary excretion of doxycycline in human beings as 35% after parenteral administration. Heaney et al. (1978) noted that 57% of the drug was excreted in urine after a single i.v. infusion of 200 mg doxycycline in human volunteers. Raghuram and Krishnaswamy (1982) observed the percentage of dose excreted upto 48 hr after i.v. administration of doxycycline at the dose rate of 3 mg/kg body weight in normal subjects, to be 40.6 ± 5.28.

OTHER KINETIC PARAMETERS :

Raghuram and Krishnaswamy (1982) conducted the detailed pharmacokinetic study of doxycycline in human by giving a dose of 3 mg/kg body weight in 200 ml of 5% dextrose solution in water through i.v. infusion over a period of 0.5 ± 0.09 hr. The authors reported the AUC as 87.7 ± 9.10 µg/ml.hr, distribution half life ($t_{\gamma 2}\alpha$) as 0.87 ± 0.293 hr, extrapolated zero time concentration in plasma (Co=A+B) as 6.9 ± 0.70 µg/ml, the rate constant for drug transfer from central to peripheral (K_{12}) as 0.50 ± 0.140 hr^{-1}, peripheral to central compartment (K_{21}) as 0.74 ± 0.163 hr^{-1}, elimination from central compartment (Kel) as 0.074 ± 0.0083 hr^{-1}, total body clearance (Cl_B) as 28.9 ± 2.65 ml/kg/min, renal clearance as 14.7 ± 1.44 ml/kg/min, and non-renal clearance as 13.9 ± 3.30 ml/kg/min.

Ziv and Sulman (1974) reported similar kinetic pattern of doxycycline after a single i.v. injection of 20 mg/kg body weight in lactating cows and ewes and pharmacokinetic parameters of the drug were found to be A = 38.0 ± 6.9 µg/ml, B = 21.4 ± 3.7 µg/ml, α = 1.248 ± 0.11 hr^{-1}, β = 0.028 ± 0.006 hr^{-1}, K_{12} = 0.734 ± 0.08 hr^{-1}, K_{21} = 0.468 ± 0.07 hr^{-1}, Kel = 0.075 ± 0.008 hr^{-1}, and Fc = 0.37 ± 0.09. Michael et al. (1979) studied the serum kinetics of doxycycline phosphate (DPP) in dogs after oral administration at the dose rate of 10 mg/kg body weight and the kinetic parameters were reported as, A = 6.87 µg/ml, B = 3.19 µg/ml, Ka = 1.645 hr^{-1}, Ke = 0.061 hr^{-1}, t_{y2a} = 0.4 hr, t_{max} = 2.6 hr and Cp max = 2.6 µg/ml.

DEMECLOCYCLINE :

Demeclocycline, which was obtained from a mutant of the strain of Streptomyces aureofeciens from which chlortetracycline was originally produced, was initially described by McCormick et al. (1957). The pharmacokinetic parameters of this drug are mainly reported in human beings whereas very scanty literature is available in animals.

Serum concentration :

Finland and Garrod (1960) reported the peak serum concentration around 2.5 µg/ml at 6 hr after oral administration of 500 mg of demeclocycline in normal subjects. They noted that the drug concentration was greater than 1 µg/ml upto 24 hr. The drug was even detectable beyond 72 hr. The mean peak serum

concentration around 22 µg/ml was obtained immediately after i.v. infusion of same dose in normal subjects (Kunin et al., 1959). Steigbigel et al. (1968) observed peak serum concentrations of 1.74 µg/ml and 1.20 µg/ml at 4 hr while the concentrations were 0.17 µg/ml and 0.10 µg/ml at 48 hr after oral administration of 300 mg and 150 mg of demeclocycline, respectively, in normal young man. Miller et al. (1980) found a mean plasma level of 2.7 ± 0.25 µg/ml of demeclocycline after oral administration of the drug in the dose of 900-1200 mg daily for a period of 7 days in normal human volunteers.

Bioavailability :

Barza and Scheife (1977) noted that the percentage of an oral dose that is absorbed (oral bioavailability) was similar for three tetracyclines viz. oxytetracycline, demeclocycline and tetracycline, ranging between 60 to 80% in human beings. Neu (1978) reported the oral bioavailability of demeclocycline as 66% in normal man.

Protein binding :

Kunin et al. (1959) and Finland and Garrod (1960) observed the human serum protein binding of demeclocycline by equilibrium dialysis method to be 41% whereas Kunin (1967) reported a higher value (90.8%) by ultracentrifugation method. Schach Von Wittenau and Yeary (1963) noted the human serum protein binding as 75% by UV-determination. Neu (1978) found the plasma protein binding of demeclocycline to be 80 to 90% in human beings.

Schach Von Wittenau and Yeary (1963) reported the canine serum protein binding of demeclocycline as 77%, while it was reported to be 75% by Schach Von Wittenau and Delahunt (1966). Ziv and Sulman (1974) observed the protein binding of demeclocycline to 72.6 ± 8.5% in the serum of lactating cows and ewes.

Elimination half life :

Kunin and Finland (1958) reported the mean elimination half life of demeclocycline as 11.8 hr in human beings, while it was observed to be 11.8 hr and 12.7 hr after a single oral dose of 250 mg and 500 mg, respectively, in human beings (Kunin et al., 1959). Sweeny et al. (1959) reported the elimination half life as 10 hr in normal subjects. Rosenblatt et al. (1967) noted the elimination half life to be 12.7 hr and more or less a similar value of 12.3 hr was also reported by Kunin (1967), after a single oral dose in normal young man. Doluisio and Dittert (1969) found the serum elimination half life of the drug to be 9 hr after repetitive oral dosing in man. Neu (1978) observed the serum half life of demeclocycline as 15 hr in normal human subjects. Goodman Gilman et al. (1980) reported the half life of demeclocycline about 16 hr in man.

Ziv and Sulman (1974) reported the mean serum elimination half life of 18.24 hr after a single i.v. injection of demeclocycline at the dose rate of 20 mg/kg body weight in lactating cows and ewes.

Apparent volume of distribution :

The apparent volume of distribution of demeclocycline was reported to be 1.79 L/kg and 1.48 L/kg in human beings after an oral dose of 500 mg and 250 mg, respectively (Kunin et al., 1959; Finland and Garrod, 1960). Kunin and Finland (1959) showed the concentration of demeclocycline in bile to be 20 to 32 times higher than in the serum 7 to 24 hr after an intravenous dose. Lichter and Gobel (1960) recorded the concentration of drug in synovial fluid equal to that of serum in patients under going treatment with demeclocycline. It is observed that the penetration of all the tetracyclines into most tissues and fluids is exellent (Goodman Gilman et al., 1980). However, the drug was detected in low concentration (1/20th to 1/50th of the serum) in cerebrospinal fluid of uninflammed meninges (Boger and Gavin, 1959/60). Similar is the observation in cases of meningitis (Fujii et al., 1959/60; Lichter and Gobel, 1960).

Ziv and Sulman (1974) reported the volume of distribution as 1.996 $\pm$ 0.22 L/kg in cows and ewes after i.v. injection of demeclocycline at the dose rate of 20 mg/kg body weight.

Concentration in urine and urinary excretion :

Various workers had shown that the excretion of demeclocycline is of same order and found to be 39 to 43% in 96 hr (Finland and Garrod, 1960), 42% (Kunin, 1967) and 40% (Neu, 1978) in human beings.

The mean concentration of demeclocycline in urine of normal subjects at various time intervals after oral administration of 300 mg was found to be 105, 59.7, 27.2, 9.7 and 3.8 µg/ml at 0-8, 8-24, 24-48, 48-72 and 72-96 hr, respectively (Steigbigel et al., 1968).

OTHER KINETIC PARAMETERS :

Ziv and Sulman (1974) reported a detailed pharmacokinetic study of demeclocycline in dairy cows and ewes after a single i.v. infusion of the drug at the dose rate of 20 mg/kg body weight. The value of the kinetic parameters obtained by them was as follows: A = 41.0 ± 3.8 µg/ml, B = 23.6 ± 2.0 µg/ml, α = 1.196 ± 0.21 hr^{-1}, β = 0.038 ± 0.01 hr^{-1}, K_{12} = 0.674 ± 0.06 hr^{-1}, K_{21} = 0.461 ± 0.05 hr^{-1}, Kel = 0.098 ± 0.01 hr^{-1} and Fc = 0.39 ± 0.05.

OTHER TETRACYCLINES :

The newer tetracyclines like Demeclocycline, Methacycline, Doxycycline and Minocycline possess certain advantages over older tetracyclines like Chlortetracycline, Oxytetracycline and Tetracycline (Goodman Gilman et al., 1980). They are :

(1) High bioavailability.

(2) Higher biological half life and hence exert longer duration of action.

(3) The sensitivity of a particular microorganism to each of the tetracyclines is similar. However, the antibacterial potency is of the order of Minocycline > Doxycycline > Methacycline and Demeclocycline > Tetracycline and Oxytetracycline.

The purpose of the present study is restricted to the pharmacokinetic study of doxycycline and demeclocycline, but for the convenience of the readers the important kinetic data of all the common tetracyclines obtained from the literature are given in Table 1.

TABLE - 1

Some of the important kinetic data of tetracyclines

Kinetic data	Chlortetra-cycline	Oxytetra-cycline	Tetra-cycline	Demeclo-cycline	Metha-cycline	Doxy-cycline	Mino-cycline
Bioavailability (%)							
Man	- -	58[a]	77[a]	60-80[b];66[a]	-	93[a];95[b]	98[a];100[b]
Protein binding (%)							
Man	47[c]	20[c];35[d]	24[c];55-65[a]	75[d];90.8[e]	78[d]	82[d];93[f]	80-95[a]
Dog	-	27[g];29[d]	80[d]	75[g];77[d]	94[g];95[d]	80[d];82[g]	92[g]
Cattle	37.5[h]	23[h]	36[h]	72.6[h]	88.8[h]	90.2[h]	80[h]
Elimination half life (hr)							
Man	5.6[c]	9[a];9.6[e]	8.2[i]	9[j];11.8[i];16[k]	7[j];14.3[e]	8.3[j];15[l];20[m]	14.6[n];15[a]
Cattle	11.2[h]	9.2[h]	12.8[h]	18.2[h]	23.1[h]	24.8[h]	21.7[h]
Volume distribution (L/kg)							
Man	1.48[c]	1.89[c]	1.59[c]	1.48[c];1.79[o]	-	0.75[p];0.9-1.8[q]	-
Dog	-	-	-	-	-	3.25[r]	-
Cattle	1.91[h]	5.36[h]	3.32[h]	2.00[h]	2.45[h]	2.29[h]	2.34[h]

a - Neu (1978) b - Barza and Scheife (1977) c - Kunin et al. (1959) d - Schach Von Wittenau and Yeary (1963) e - Kunin (1967) f - Rosenblatt et al. (1966) g - Schach Von Wittenau and Delahunt (1966) h - Ziv and Sulman (1974) i - Kunin and Finland (1958) j - Doluisio and Dittert (1969) k - Goodman Gilman et al. (1980) l - Migliardi and Wittenau (1967) m - Merier et al. (1969/70) n - Heaney and Eknoyan (1978) o - Finland and Garrod (1960) p - Raghuram and Krishnaswamy (1982) q - Ceccarelli et al. (1971) r - Michael et al. (1979).

MATERIALS AND METHODS

MATERIALS AND METHODS

In the present study, twelve clinically healthy female lactating goats of non-descript breed, between 1.5 to 2 years of age and 25 to 30 kg weight, were used. The animals were housed in animal shed with concrete floor. They were maintained on Korai, Chunni and greens. Water was given ad lib.

EXPERIMENTAL SCHEDULE :

Each drug (Doxycycline/Demeclocycline) was studied on a group of six animals. An interval of three weeks was allowed to elapse before administration of the next dose. The drugs were administered by intravenous (i.v.) and intramuscular (i.m.) routes in each animal to study their bioavailability.

DRUGS USED :

(a) Doxycycline:

Duracycline capsule, a commercial preparation of Unichem Laboratories Ltd., containing Doxycycline Hydrochloride equivalent to 100 mg of Doxycycline base was used.

(b) Demeclocycline:

Ledermycin capsule, a commercial preparation of Cyanamid India Ltd., containing 150 mg of Demeclocycline

Hydrochloride was used.

COLLECTION OF BIOLOGICAL SAMPLES AND THEIR TIMINGS :

The samples of various biological fluids mentioned below were collected post i.v. administration whereas the sample of blood alone was collected after i.m. administration of the drugs.

(a) Blood :

Hairs around the jugular vein on either side of neck of the animals were shaved and cleaned with ether. The site was sterilized prior to each collection with rectified spirit. Blood samples were collected in sterile centrifuge tubes containing appropriate amount of sodium oxalate by venipuncture of jugular vein prior to and at 5, 15, 30 & 45 min and 1, 2, 4, 6, 8, 12, 24, 30, 36, 48 hr post drug administration. The blood samples were centrifuged at 5000 r.p.m. for 10 min for the separation of the plasma. The plasma thus obtained was kept in a refrigerator. For preparation of plasma standards of the drug, plasma collected prior to drug administration was used.

(b) Milk :

In order to collect milk samples, the udder of the goat were washed with soap water and dried with clean soft towel. The milk was collected in sterile test tubes by hand milking. Samples of milk were taken before drug administration

for the preparation of standards. After administration of the drug, the milk samples were collected at the intervals of 5, 15, 30 & 45 min and 1, 2, 4, 6, 8, 12, 24, 30, 36 & 48 hr. The samples thus collected were kept in a refrigerator and the drug concentrations were measured in following days.

(c) Urine :

On the day of experiment, a Foley's balloon catheter (No. 14), made sterile by dipping into dettol solution and lubricated with glycerine, was introduced through urethra into the bladder of the experimental goat with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 20 ml of air through a syringe to keep the catheter in position. The catheter was fixed with a pressure clip to check dripping of urine. Normal urine sample was collected in a sterile test tube prior to the drug administration for the preparation of standards. After administration of the drug, the urine samples were collected in sterile test tubes at 5, 15, 30 & 45 min and 1, 2, 4, 6, 8, 12, 24, 30, 36 & 48 hr. The samples were kept in a refrigerator and were analysed on successive days.

(d) Interstitial fluid :

For collection of interstitial fluid, two multiperforated (each perforation of 6 mm diameter) table tennis balls were aseptically implanted subcutaneously in the

neck and flank region using the technique of Kozak et al. (1977). A period of 4 to 5 weeks after implantation was allowed for the development of fibrous membrane around the ball. Part of the skin over the balls were sterilized with ethanol and dried prior to collection of samples. Samples of clear interstitial fluid were aspirated from the ball cavity using a sterile syringe and hypodermic needle by directly piercing through the skin and ball hole and collected in sterile vials. Interstitial fluid was collected from both balls before the administration of the drug for preparation of the drug standards. The samples of interstitial fluid were collected alternately from both the balls at 5, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, 24, 30, 36 & 48 hr post drug administration. The samples were kept in a refrigerator until assay was carried out within a few days.

ADMINISTRATION OF DRUGS :

(a) Doxycycline :

A capsule containing 100 mg of doxycycline was opened and the drug was dissolved in 20 ml of sterile distilled water. The drug was injected in the dose of 5 mg/kg body weight by i.v. as well as i.m. routes.

(b) Demeclocycline :

A capsule having 150 mg of demeclocycline was opened and made soluble in 30 ml of sterile distilled water. The drug was injected i.v. as well as i.m. in the dose of 5 mg/kg.

PROCEDURE ADOPTED FOR THE MICROBIOLOGICAL ASSAY :

(a) Sterilization of glasswares and needles :

All glasswares and needles were washed with detergent solution in running tape water. These were again rinsed with glass distilled water and air dried. Test tubes, centrifuge tubes, vials and measuring cyclinders were plugged with cotton wool and assay plates, pipettes, syringe etc. were wrapped by paper. The materials were sterilized in hot air oven at 160°C for an hour.

(b) Preparation of media :

(i) Assay agar:

Dehydrated Tetracycline Assay Agar (commercial preparation of Hindustan Dehydrated Media) was used in this study. The composition of the media was as follows:

	Ingredients	Per liter
1.	Beef Extract	1.5 gm
2.	Yeast Extract	3.0 gm
3.	Peptone	6.0 gm
4.	Agar	15.0 gm

pH 5.9 ± 0.2 at 25°C (approx.)

To rehydrate, 25.5 gm of this medium was suspended in 1000 ml of cold glass distilled water, as per direction given. The media was heated to dissolve and the solution was

transferred into conical flask which was later plugged with cotton wool. Wet sterilization of media was done by autoclaving at 15 pounds pressure (121°C) for 20 min.

(ii) Nutrient broth :

Nutrient broth of the following composition was prepared.

	Ingredients	Per liter
1.	Sodium chloride	5 gm
2.	Peptone	10 gm
3.	Beef Extract	10 gm

These ingredients were dissolved in 1000 ml of glass distilled water and pH was adjusted to 7.4 (approx.). Sterilization of the broth was done as mentioned above.

(c) Preparation of assay agar plates :

Melted tetracycline assay agar (20 ml) was poured gently into each of the sterile special assay plate (Borosil), kept on a horizontally plane surface with the aid of a sterile measuring cyclinder to get uniform thickness of agar. The plates were left at room temperature for about 2 hr for solidification of agar. The plates were kept inside the incubator at 37°C for 24 hr to ascertain any growth indicating microbial contamination. The growth free plates were then stored in a refrigerator until assay was carried out.

(d) Preparation of test organism :

The test organism was grown on the slant of tetracycline assay agar at 37°C for 24 hr and then stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity.

(e) Preparation of standards in blological samples :

The drug (Doxycycline or Demeclocycline) was dissolved in sterile glass distilled water and diluted to have different strengths, viz. 80 µg/ml, 40 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml and 0.625 µg/ml. From each standard solution, 0.1 ml was added to a sterile vial containing 0.9 ml of plasma, milk, urine or interstitial fluid collected prior to drug administration. This yielded drugs standards of 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.25 µg/ml, 0.125 µg/ml and 0.0625 µg/ml in the above noted biological samples. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentration in the test samples.

(f) Assay procedure :

The quantitative estimation of doxycycline and demeclocycline in the biological samples of plasma, milk, urine and interstital fluid were done by microbiological assay (cylinder plate diffusion method) using Bacillus cereus

(ATCC-111778) as the test organism (British pharmacopoeia, 1980).

The test organism was grown in nutrient broth for 2 to 3 hr until the growth was seen (turbid by naked eye). Tetracycline assay agar plates were flooded with the broth containing the organism and the excess broth was drained out. The plates were dried in the incubator at 37°C for a period of about half an hour. Sterile polythene cylinders (prepared from polythene tubes of 6 mm diameter obtained from commercial sources) were placed at appropriate distance along the circumference in the innoculated agar plates. Ninety microliter of standard solution of various strength as well as test samples of the drug was poured in separate cylinders in an assay plate. Such plates were left on the table for about 2 hr and then kept in the incubator at 37°C overnight for the growth of organism. The diameter of the bacterial zone of inhibition produced by standards as well as test samples of the drug were measured. The concentration of the drug in different test samples of a biological fluid was estimated from the standard curve plotted from the zone of inhibition versus concentration of the drug on a semilog scale.

Standard solutions of doxycycline and demeclocycline in plasma, milk, urine and interstitial fluid were kept under refrigeration (0°C to 4°C). The drug concentration in each sample was assayed using freshly prepared drug standards daily

for a period of seven days to test the drug stability in biological fluids. The results showed that the concentration of demeclocycline started declining from 3rd day whereas doxycycline was stable upto 5 days.

CALCULATION OF THE PHARMACOKINETIC PARAMETERS :

The following pharmacokinetic parameters of doxycycline and demeclocycline after a single i.v. administration were calculated from the semilog plot of plasma drug concentration versus time curve. The experimental data was analysed using one compartment or two compartment open model (Gibaldi and Perrier, 1975).

The concentration of the drug in plasma at any time is obtained by the formula:

(i) $C_p = Be^{-\beta t}$ .....(1-Compartment model)

(ii) $C_p = Ae^{-\alpha t} + Be^{-\beta t}$.....(2-Compartment model)

Where C_p is the drug concentration in plasma at time 't'. The description and calculation of the kinetic parameters (A, B, α & β) used in the above formulae are noted below.

(a) A, the zerotime concentration of the drug in plasma and α, the regression coefficient, for distribution phase, were calculated by the method of residual yields (Appendix I).

(b) B, the zero time concentration of the drug in plasma and β, the regression coefficient, for elimination phase, were calculated by the method of least squares (Appendix I).

(c) A, the zero time concentration of the drug in plasma and Ka, the regression coefficient, for absorption phase after i.m. administration of the drug, were calculated by the method of residual yields (Appendix I).

(d) C_o^p, the theoretical concentration in plasma at zero time.

$$C_o^p = A + B$$

(e) $t_{\gamma 2}Ka$, $t_{\gamma 2}\alpha$ and $t_{\gamma 2}\beta$, the half life of the drug in absorption, distribution and elimination phase, respectively.

$$t_{\gamma 2}Ka = \frac{0.693}{Ka}, \quad t_{\gamma 2}\alpha = \frac{0.693}{\alpha}, \quad t_{\gamma 2}\beta = \frac{0.693}{\beta}$$

(f) AUC, the total area under the curve.

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad \text{(2-Compartment model)}$$

$$AUC = \frac{B}{\beta} \quad \text{(1-Compartment model)}$$

(g) F, the bioavailability.

$$F = \frac{AUC\ i.m.}{AUC\ i.v.} \times 100 \quad \text{(for i.m. study)}$$

(h) K_{21}, rate of transfer of the drug from the peripheral (tissue) compartment to the central (blood) compartment.

$$K_{21} = \frac{A \cdot \beta + B \cdot \alpha}{A + B}$$

(i) Kel, the elimination rate constant of the drug from central compartment.

$$Kel = \frac{\alpha \cdot \beta}{K_{21}}$$

(j) K_{12}, the rate of transfer of the drug from central to the peripheral compartment.

$$K_{12} = \alpha + \beta - K_{21} - Kel.$$

(k) Fc, the fraction of drug available for elimination from central compartment.

$$Fc = \frac{\beta}{Kel}$$

(l) Vd, the volume of distribution, based on distribution and elimination.

$$Vd = \frac{D}{A + B}$$

D = Dose rate (mg/kg)

(m) Vd_B, the volume of distribution based on elimination.

$$Vd_B = \frac{D}{B}$$

(n) Vd_{area}, the volume of distribution, based on total area.

$$Vd_{area} = \frac{D}{(A/\alpha + B/\beta) \cdot \beta} \quad \text{(2-Compartment model)}$$

$$Vd_{area} = \frac{D}{B} \quad \text{(1-Compartment model)}$$

(o) $Vd_{s.s.}$, the total volume of distribution at steady state.

$$Vd_{s.s.} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd.$$

(p) Cl_B, the total body clearance.

$$Cl_B = Vd_{area} \times \beta$$

CALCULATION OF DOSAGES REGIMEN :

Dosage regimen has been calculated to maintanin minimum inhibitory concentration (MIC) in plasma at desired dosage interval using the formulae described by Notari (1980).

Maintenance dose (Do):

The maintenance dose required to provide any desired minimum plasma concentration ($C_p^{\infty}min$ = MIC) can be arrived from the formula:

$$C_p^{\infty}min = \frac{fB}{(1 - f)}$$

B = zero time concentration during elimination phase.

f = fraction of dose remains at a particular dosage interval (τ). f can be derived from the formula:

$$f = \frac{Inf}{-\beta}$$

Since, B is directly proportional to dose, the maintenance dose can be arrived at for a particular dosage interval (τ) and for a specific MIC.

Loading dose (D^*):

$$D^* = \frac{D_o}{(1 - f)}$$

Prediction of steady state maximum plasma concentration (Cpmax):

During repetitive administration the theoretical steady state maximum plasma concentration is calculated by the derivation :

$$Cp^{\infty}max = \frac{B}{(1 - f)}$$

The method of calculation of dosage regimen is shown in Appendix II.

RESULTS

R E S U L T

DOXYCYCLINE:

Pharmacokinetic study of doxycycline after i.v. administration:

1. Plasma levels:

The plasma drug concentration profile at various time intervals after a single i.v. dose (5 mg/kg) of doxycycline in goat has been shown in Table-2 and Fig.-2. The plasma concentration of the drug at 5 min was found to be 6.04 ± 0.81 µg/ml and the value ranged from 2.50 to 8.20 µg/ml. The mean therapeutic concentration (≥ 0.5 µg/ml) of the drug in plasma was maintained from 5 min to 2 hr. The drug was detectable in plasma samples upto 48 hr. The mean plasam concentration at 48 hr was 0.05 ± 0.01 µg/ml.

2. Kinetic parameters:

The plasma drug concentration versus time profile has confirmed the two compartment open model as depicted in Fig-3. Table-3 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A) and elimination phase (B) was observed to be 2.55 ± 0.41 and 0.41 ± 0.03 µg/ml respectively. The mean value for theoretical zero time concentration of drug in plasma (C_o^p = A + B) was found to be

TABLE-2

Plasma concentrations (µg/ml) of Doxycycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	8.20	5.80	5.65	2.50	6.70	7.40	6.04 ± 0.81
15 min	2.80	2.25	2.80	1.00	2.40	3.00	2.38 ± 0.30
30 min	1.70	1.43	1.20	0.90	1.90	1.80	1.49 ± 0.16
45 min	1.20	1.15	1.00	0.78	1.60	1.50	1.21 ± 0.13
1 hr	1.00	0.96	0.90	0.70	1.25	1.00	0.97 ± 0.07
2 hr	0.70	0.80	0.60	0.50	0.98	0.62	0.70 ± 0.07
4 hr	0.50	0.48	0.42	0.35	0.70	0.36	0.47 ± 0.05
6 hr	0.30	0.34	0.35	0.32	0.41	0.26	0.33 ± 0.02
8 hr	0.25	0.27	0.30	0.30	0.37	0.22	0.29 ± 0.02
12 hr	0.18	0.23	0.25	0.26	0.24	0.19	0.23 ± 0.01
24 hr	0.10	0.15	0.18	0.14	0.16	0.14	0.15 ± 0.01
30 hr	0.08	0.12	0.17	0.12	0.10	0.09	0.11 ± 0.01
36 hr	0.05	0.09	0.15	0.08	0.07	0.04	0.08 ± 0.02
48 hr	0.04	0.06	0.09	0.05	0.03	0.000	0.05 ± 0.01

FIG. 2

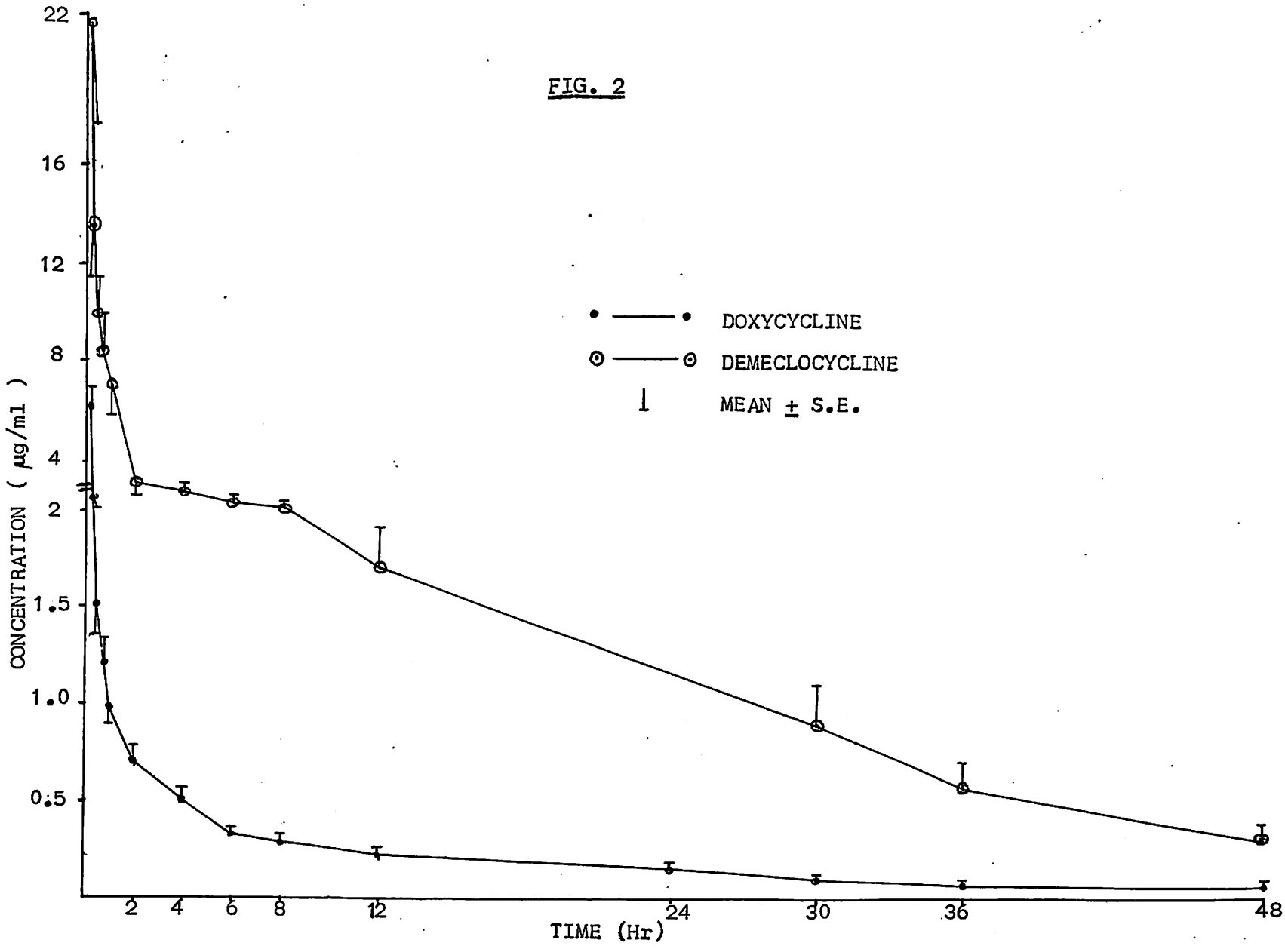

22
16
12
8
4
2
1.5
1.0
0.5
CONCENTRATION (µg/ml)
DOXYCYCLINE
DEMECLOCYCLINE
MEAN ± S.E.
2
4
6
8
12
24
30
36
48
TIME (Hr)

FIG. 3

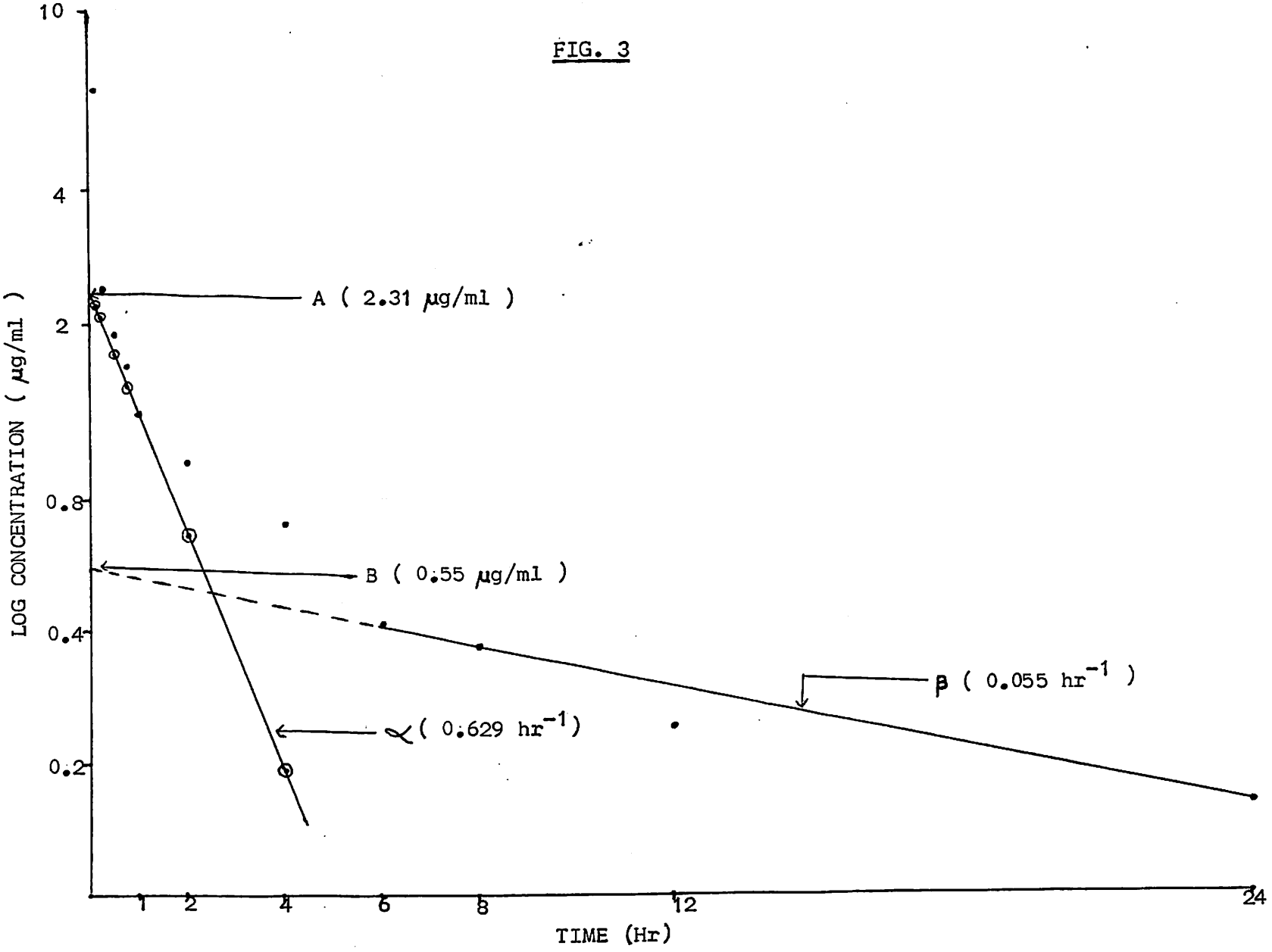

10
4
2
0.8
0.4
0.2
LOG CONCENTRATION (µg/ml)
A (2.31 µg/ml)
B (0.55 µg/ml)
β (0.055 hr^{-1})
α (0.629 hr^{-1})
1
2
4
6
8
12
24
TIME (Hr)

TABLE - 3

Kinetic parameters of Doxycycline in goat following single intravenous dose of 5 mg/kg.

Parameter	Animal No. 1	2	3	4	5	6	Mean ± S.E.
A(µg/ml)	2.49	2.14	3.01	1.16	2.31	4.20	2.55 ± 0.41
B(µg/ml)	0.38	0.39	0.41	0.42	0.55	0.32	0.41 ± 0.03
C_o^p(µg/ml)	2.87	2.53	3.42	1.58	2.86	4.52	2.96 ± 0.40
$\alpha(hr^{-1})$	0.758	0.790	1.534	1.253	0.629	1.432	1.066± 0.158
$t_{\gamma 2}\alpha$(hr)	0.91	0.88	0.45	0.55	1.10	0.48	0.73 ± 0.11
$\beta(hr^{-1})$	0.054	0.040	0.030	0.043	0.055	0.039	0.044± 0.004
$t_{\gamma 2}\beta$(hr)	12.83	17.33	23.10	16.12	12.60	17.77	16.63 ± 1.58
$K_{12}(hr^{-1})$	0.387	0.471	1.135	0.783	0.309	0.928	0.669± 0.135
$K_{21}(hr^{-1})$	0.147	0.156	0.210	0.365	0.165	0.138	0.197± 0.035
$Kel(hr^{-1})$	0.278	0.203	0.219	0.148	0.210	0.405	0.244± 0.036
Fc	0.194	0.197	0.137	0.291	0.262	0.096	0.196± 0.030
AUC(mg/L.hr)	10.32	12.46	15.63	10.69	13.67	11.14	12.32 ± 0.83
Vd(L/kg)	1.74	1.98	1.46	3.16	1.75	1.11	1.87 ± 0.29
Vd_B(L/kg)	13.16	12.82	12.20	11.90	9.09	15.63	12.47 ± 0.86
Vd_{area}(L/kg)	8.97	10.03	10.66	10.88	6.65	11.51	9.78 ± 0.72
$Vd_{s.s.}$(L/kg)	6.32	7.96	9.35	9.94	5.03	8.57	7.86 ± 0.76
Cl_B(ml/kg/min)	8.07	6.69	5.33	7.80	6.10	7.48	6.91 ± 0.43

2.96 ± 0.40 µg/ml. The distribution rate constant of the drug (α) ranged from 0.629 to 1.534 hr^{-1} with a mean value of 1.066 ± 0.158 hr^{-1} while its elimination rate constant (β) ranged from 0.030 to 0.055 hr^{-1} with a mean value of 0.044 ± 0.004 hr^{-1} in plasma. The mean distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) half life of the drug were observed to be 0.73 ± 0.11 and 16.63 ± 1.58 hr. The average rate of transfer of the drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartments were calculated to be 0.669 ± 0.135, 0.197 ± 0.035 & 0.244 ± 0.036 hr^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) was noted to be 0.196 ± 0.030. The value of area under the curve in plasma (AUC) was found to be 12.32 ± 0.83 mg/L.hr. The various values for volume of distribution of the drug in goat, calculated by different methods are shown in Table-3. The mean values of Vd_{area} and $Vd_{s.s.}$ were observed to be 9.78 ± 0.72 & 7.86 ± 0.76 L/kg. The total body clearance (Cl_B) ranged from 5.33 to 8.07 ml/kg/min with a mean of 6.91 ± 0.43 ml/kg/min.

3. Concentration of doxycycline in various biological fluids:

(a) Interstitial fluid:

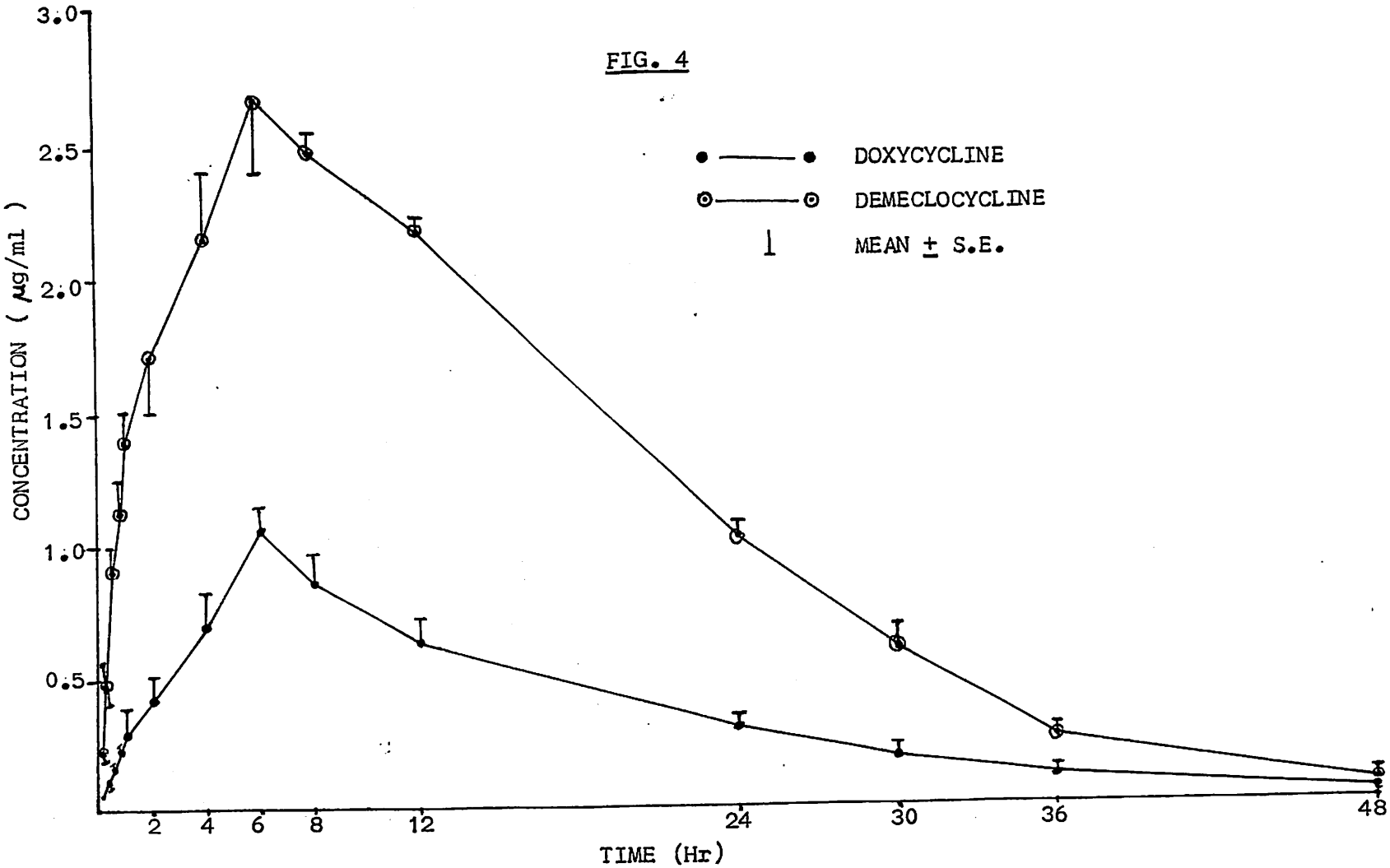
The distribution of doxycycline in interstitial fluid after a single i.v. dose (5 mg/kg) has been presented in Table-4 and depicted in Fig-4. The drug was detectable in the

TABLE - 4

Interstitial fluid concentrations (µg/ml) of Doxycycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	0.08	0.10	0.066	0.00	0.00	0.05	0.05 ± 0.02
15 min	0.12	0.14	0.08	0.07	0.11	0.09	0.10 ± 0.01
30 min	0.16	0.26	0.09	0.13	0.14	0.12	0.15 ± 0.02
45 min	0.19	0.58	0.09	0.18	0.17	0.15	0.23 ± 0.07
1 hr	0.25	0.72	0.18	0.20	0.21	0.19	0.29 ± 0.09
2 hr	0.36	0.80	0.25	0.35	0.43	0.32	0.42 ± 0.08
4 hr	0.78	1.20	0.35	0.56	0.67	0.56	0.69 ± 0.12
6 hr	1.26	1.00	0.90	1.00	1.20	0.98	1.06 ± 0.06
8 hr	0.96	0.77	0.82	0.54	1.28	0.76	0.86 ± 0.10
12 hr	0.65	0.62	0.64	0.40	0.93	0.54	0.63 ± 0.07
24 hr	0.36	0.28	0.37	0.22	0.34	0.24	0.30 ± 0.03
30 hr	0.29	0.13	0.12	0.10	0.23	0.20	0.18 ± 0.03
36 hr	0.16	0.05	0.09	0.04	0.15	0.14	0.11 ± 0.02
48 hr	0.07	0.00	0.05	0.00	0.08	0.06	0.04 ± 0.01

FIG. 4


3.0
2.5
2.0
1.5
1.0
0.5
CONCENTRATION (µg/ml)
2
4
6
8
12
24
30
36
48
TIME (Hr)
DOXYCYCLINE
DEMECLOCYCLINE
MEAN ± S.E.

samples of interstitial fluid collected even at 5 min in four out of six animals. The mean peak concentration of 1.06 ± 0.06 µg/ml was obtained at 6 hr. Thereafter, the drug concentration declined steadily and was detectable in all the samples collected upto 36 hr. The drug was detectable in most of the animals even at 48 hr. The mean drug concentration at 36 hr was found to be 0.11 ± 0.02 µg/ml. The mean therapeutic concentration (≥0.05 µg/ml) was maintained between 4 to 12 hr.

(b) Milk :

The concentration of doxycycline in milk following i.v. administration (5 mg/kg) is presented in Table-5 and Fig-5. In contrast to interstitial fluid, the drug appeared in milk samples of all the animals at 1 hr and was detectable upto 48 hr in most of the animals. Mean peak concentration of 1.64 ± 0.25 µg/ml in milk was attained at 6 hr. The mean therapeutic concentration (≥0.5 µg/ml) was achieved at 2 hr and maintained even beyond 12 hr.

(c) Urine :

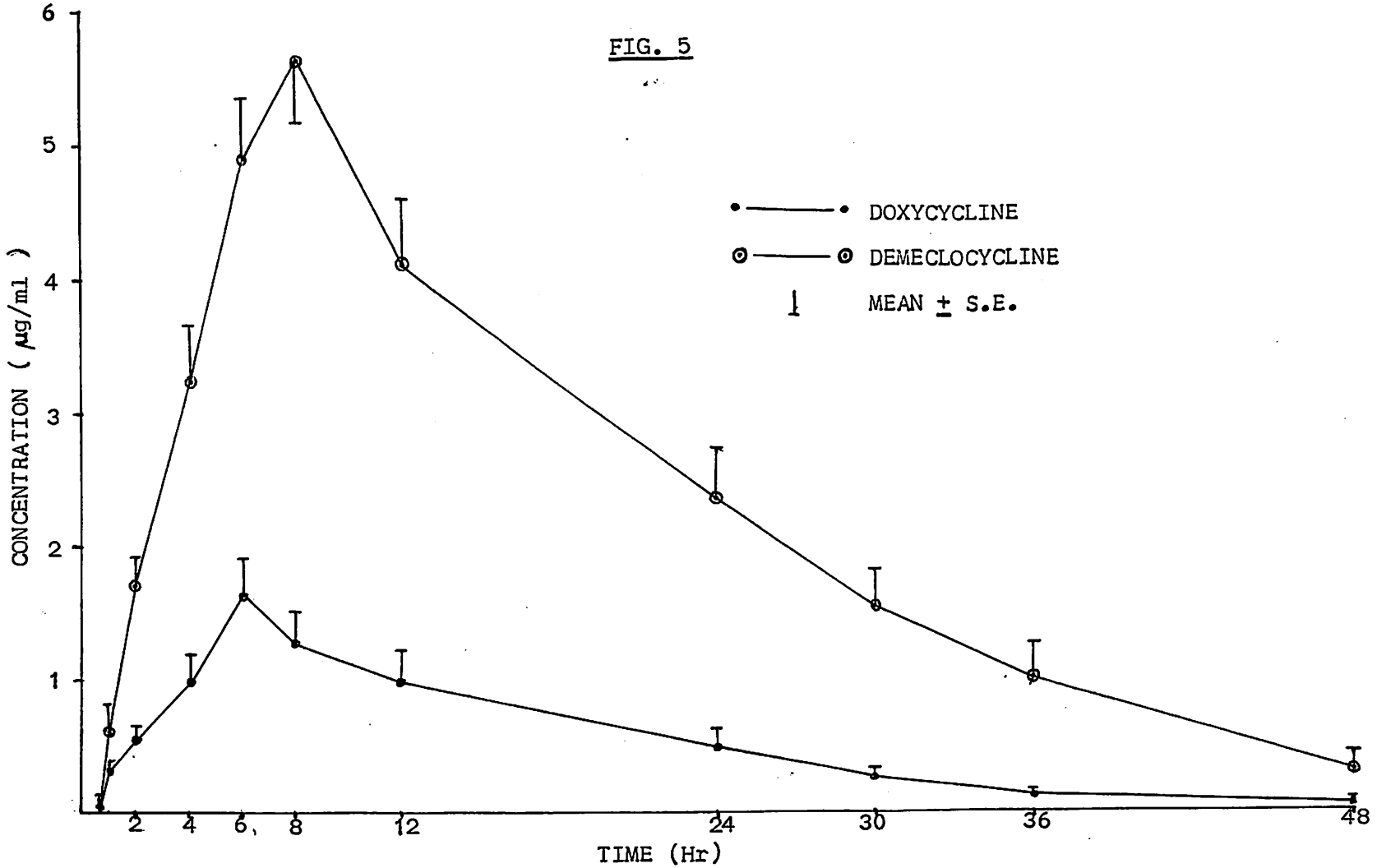
The concentration of drug (5 mg/kg) in urine post i.v. administration has been presented in Table-6 and Fig-6. The drug appeared in effective concentration (≥0.5 µg/ml) within 5 min and was also detectable in the last urine sample (48 hr). The mean drug concentration of 3.12 ± 0.70 &

T A B L E - 5

Milk concentrations (µg/ml) of Doxycycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
15 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
30 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
45 min	0.00	0.25	0.00	0.00	0.00	0.00	0.04 ± 0.04
1 hr	0.35	0.38	0.22	0.29	0.20	0.42	0.31 ± 0.04
2 hr	0.48	0.75	0.34	0.36	0.30	0.87	0.52 ± 0.10
4 hr	0.86	1.36	0.68	0.50	0.68	1.74	0.97 ± 0.20
6 hr	2.82	1.14	1.70	1.45	1.52	1.22	1.64 ± 0.25
8 hr	2.45	0.84	1.15	0.92	1.25	0.96	1.26 ± 0.25
12 hr	2.15	0.72	0.83	0.63	0.85	0.72	0.98 ± 0.24
24 hr	1.05	0.32	0.52	0.14	0.45	0.38	0.48 ± 0.13
30 hr	0.53	0.26	0.15	0.08	0.24	0.27	0.26 ± 0.06
36 hr	0.13	0.10	0.10	0.06	0.12	0.14	0.11 ± 0.01
48 hr	0.09	0.00	0.07	0.00	0.08	0.10	0.06 ± 0.02

FIG. 5

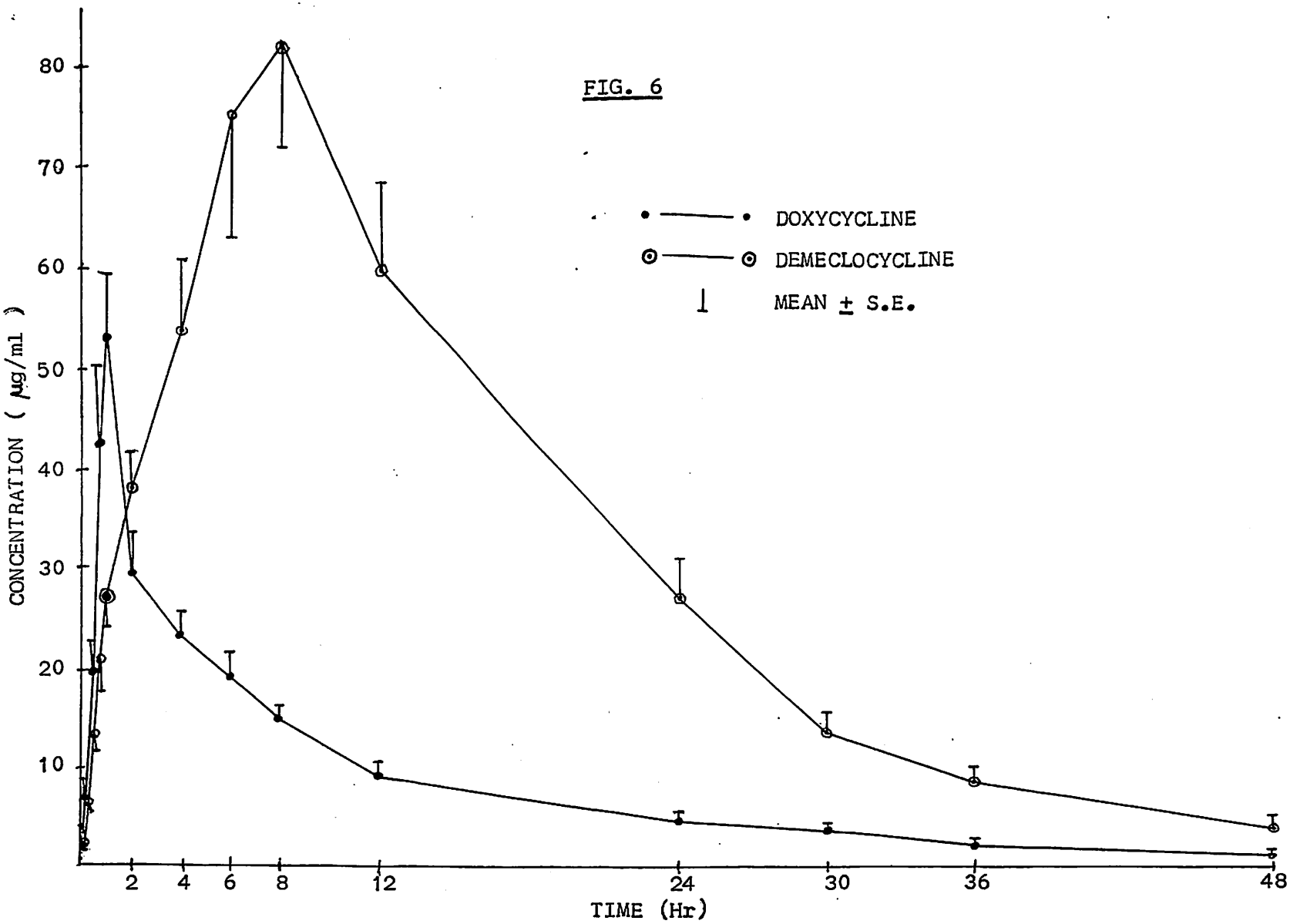

6
5
4
3
2
1
CONCENTRATION (µg/ml)
DOXYCYCLINE
DEMECLOCYCLINE
MEAN ± S.E.
2
4
6
8
12
24
30
36
48
TIME (Hr)

T A B L E - 6

Urine concentrations (µg/ml) of Doxycycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	2.82	1.85	4.84	1.25	5.58	2.40	3.12 ± 0.70
15 min	5.84	4.41	12.15	2.36	12.17	6.23	7.19 ± 1.67
30 min	16.30	15.25	25.25	11.45	31.78	15.75	19.30 ± 3.11
45 min	66.12	21.35	48.10	28.92	60.23	31.21	42.66 ± 7.44
1 hr	32.40	58.05	72.21	62.15	38.36	54.22	52.90 ± 6.11
2 hr	18.15	24.25	46.31	30.75	29.68	27.95	29.52 ± 3.84
4 hr	13.30	19.14	33.00	25.80	23.90	24.20	23.22 ± 2.70
6 hr	12.32	14.23	26.86	22.85	20.25	17.78	19.05 ± 2.21
8 hr	10.33	11.42	21.79	15.46	15.86	13.65	14.75 ± 1.16
12 hr	7.52	6.82	16.50	8.57	7.23	6.82	8.91 ± 1.54
24 hr	4.80	4.23	8.24	3.53	3.45	3.50	4.63 ± 0.75
30 hr	3.54	2.72	6.80	2.18	2.32	2.12	3.28 ± 0.74
36 hr	2.13	1.91	4.25	1.40	1.50	1.25	2.07 ± 0.46
48 hr	1.20	1.00	2.45	0.93	0.82	0.63	1.17 ± 0.27

FIG. 6


80
70
60
50
40
30
20
10
CONCENTRATION (µg/ml)
2
4
6
8
12
24
30
36
48
TIME (Hr)
DOXYCYCLINE
DEMECLOCYCLINE
MEAN ± S.E.

1.17 ± 0.27 µg/ml was observed at 5 min and 48 hr, respectively. The mean peak urine concentration of 52.90 ± 6.11 µg/ml was observed at 1 hr. The therapeutic concentration was maintained in the urine samples of all animals collected from 5 min to 48 hr post drug administration.

4. Dosage regimen :

The dosage regimen required to maintain the minimum therapeutic concentration (0.5 to 1.5 µg/ml) in plasma for i.v. administration is presented in Table-7. The suitable dosage regimen i.e. loading dose (D^*) and maintenance dose (D_0) were tabulated at convenient dosage interval (τ) of 12 & 24 hr. The table also shows the maximum concentration ($Cp^{ss}max$) in plasma expected to be achieve following administration of required doses of the drug as designed in the dosage regimen.

Pharmacokinetic study of doxycycline after i.m. administration:

1. Plasma levels:

The plasma concentration of doxycycline at different time intervals after i.m. administration has been presented in Table-8 and Fig-7. The drug appeared in the samples of all the animals at 5 min and persisted upto 36 hr except in animal no. 4. The drug was detectable at 48 hr only in the samples of animal no. 3. The mean peak plasma

T A B L E - 7

Dosage regimen of Doxycycline for intravenous route in goat.

	Animal No. 1	2	3	4	5	6	Mean ± S.E.
$Cp^{\infty}min$=0.5 µg/ml							
D^*	12.68	10.42	8.87	10.05	8.84	12.54	10.57 ± 0.70
γ=12 hr D_o	6.05	3.97	2.68	4.05	4.27	4.69	4.29 ± 0.45
$Cp^{\infty}max$	0.96	0.81	0.73	0.84	0.97	0.80	0.85 ± 0.04
D^*	23.93	16.82	12.59	16.63	16.98	20.05	17.83 ± 1.56
γ=24 hr D_o	17.37	10.38	6.46	10.71	12.45	12.19	11.59 ± 1.45
$Cp^{\infty}max$	1.80	1.31	1.03	1.40	1.87	1.28	1.45 ± 0.13
$Cp^{\infty}min$=1.0 µg/ml							
D^*	25.37	20.84	17.75	20.10	17.68	25.08	21.14 ± 1.39
γ=12 hr D_o	12.11	7.95	5.37	8.10	8.55	9.38	8.58 ± 0.90
$Cp^{\infty}max$	1.93	1.63	1.46	1.69	1.95	1.60	1.71 ± 0.08
D^*	47.85	33.65	25.19	33.26	33.97	40.10	35.67 ± 3.11
γ=24 hr D_o	34.74	20.77	12.93	21.43	24.91	24.38	23.19 ± 2.90
$Cp^{\infty}max$	3.60	2.63	2.07	2.80	3.74	2.57	2.90 ± 0.26
$Cp^{\infty}min$=1.5 µg/ml							
D^*	38.05	31.26	26.62	30.15	26.52	37.62	31.70 ± 2.09
γ=12 hr D_o	18.16	11.92	8.05	12.14	12.82	14.06	12.86 ± 1.34
$Cp^{\infty}max$	2.89	2.44	2.19	2.53	2.92	2.41	2.56 ± 0.12
D^*	71.78	50.47	37.78	49.89	50.95	60.15	53.50 ± 4.67
γ=24 hr D_o	52.11	31.15	19.39	32.14	37.36	36.56	34.79 ± 4.35
$Cp^{\infty}max$	5.45	3.94	3.10	4.19	5.61	3.85	4.36 ± 0.40

$Cp^{\infty}min$ = Minimum plasma level $Cp^{\infty}max$ = Maximum plasma level

D^* = Loading dose D_o = Maintenance dose γ = Dosage interval

concentration of 1.47 ± 0.17 µg/ml was attained at 1 hr. The mean therapeutic concentration appeared at 15 min and was maintained upto 2 hr.

2. Kinetic parameters:

The plasma drug concentration versus time profile has confirmed the two compartment open model after i.m. administration of doxycycline (5 mg/kg) as depicted in Fig-8. The values of different kinetic parameters calculated on the basis of two compartment open model is presented in Table-9.

The mean extrapolated zero time concentration during distribution phase (A) and elimination phase (B) was observed to be 3.67 ± 1.02 & 0.37 ± 0.05 µg/ml, respectively. The mean value of theoretical zero time concentration of the drug in plasma (C_o^p = A + B) was found to be 4.04 ± 0.98 µg/ml. The absorption rate constant (Ka) ranged from 2.206 to 2.697 hr^{-1} with a mean value of 2.468 ± 0.074 hr^{-1}. The distribution rate constant (α) varied from 0.477 to 1.803 hr^{-1} with a mean of 1.035 ± 0.209 hr^{-1}. The range of elimination rate constant (β) was 0.033 to 0.056 hr^{-1} with an average of 0.044 ± 0.004 hr^{-1}. The mean absorption half life ($t_{1/2}Ka$), distribution half life ($t_{1/2}\alpha$) and elimination half life ($t_{1/2}\beta$) were observed to be 0.28 ± 0.01 hr, 0.85 ± 0.19 hr and 16.25 ± 1.34 hr, respectively. The average rate of drug transfer from central to peripheral (K_{12}), peripheral to

T A B L E - 8

Plasma concentrations (µg/ml) of Doxycycline in goat following single intramuscular dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	0.54	0.36	0.20	0.30	0.42	0.32	0.36 ± 0.05
15 min	0.70	0.56	0.34	0.35	0.63	0.45	0.51 ± 0.06
30 min	0.90	0.80	0.54	0.60	0.90	0.60	0.72 ± 0.07
45 min	1.20	1.90	1.75	1.40	1.20	0.88	1.39 ± 0.16
1 hr	1.40	1.00	1.00	2.00	1.80	1.60	1.47 ± 0.17
2 hr	1.00	0.70	0.58	0.80	0.88	0.50	0.74 ± 0.08
4 hr	0.60	0.32	0.35	0.20	0.68	0.35	0.42 ± 0.07
6 hr	0.36	0.30	0.32	0.15	0.35	0.24	0.29 ± 0.03
8 hr	0.30	0.28	0.29	0.13	0.32	0.22	0.26 ± 0.03
12 hr	0.24	0.23	0.26	0.12	0.26	0.18	0.22 ± 0.02
24 hr	0.12	0.15	0.18	0.07	0.14	0.12	0.13 ± 0.02
30 hr	0.10	0.11	0.14	0.06	0.09	0.08	0.10 ± 0.01
36 hr	0.05	0.06	0.12	0.00	0.07	0.05	0.06 ± 0.02
48 hr	0.00	0.00	0.06	0.00	0.00	0.00	0.01 ± 0.01

FIG. 7

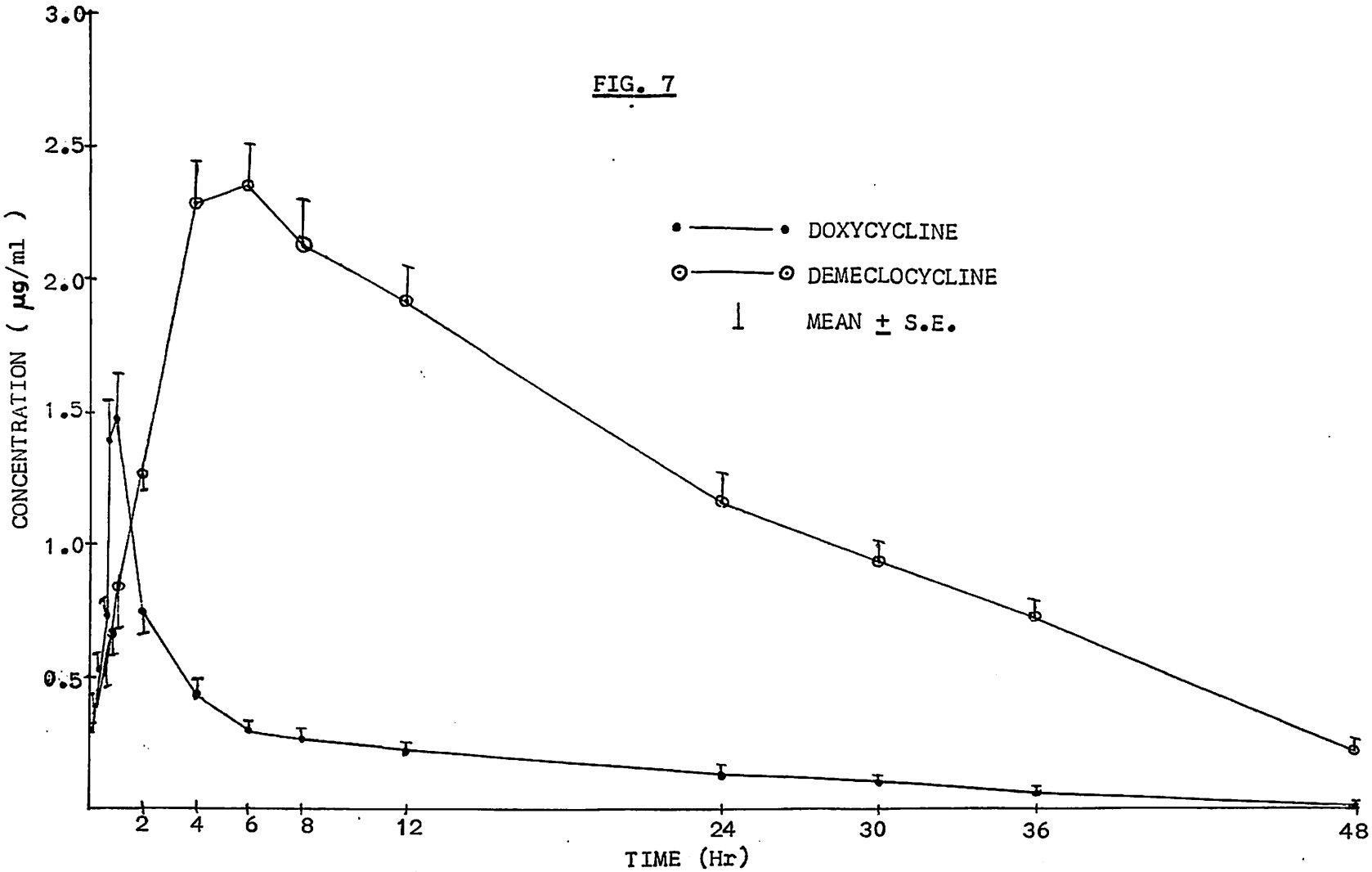
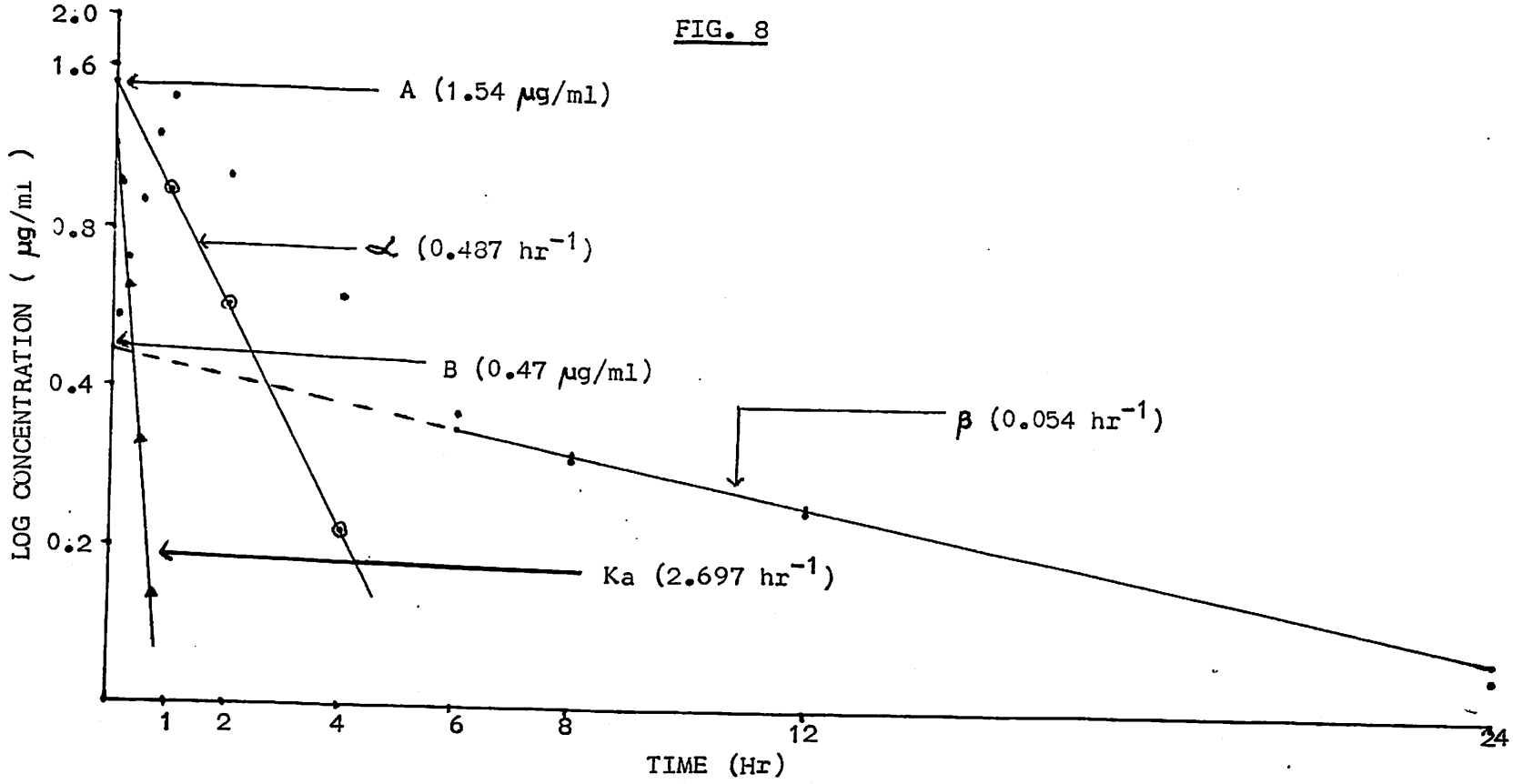

3.0
2.5
2.0
1.5
1.0
0.5
CONCENTRATION (µg/ml)
DOXYCYCLINE
DEMECLOCYCLINE
MEAN ± S.E.
2
4
6
8
12
24
30
36
48
TIME (Hr)

FIG. 8


2.0
1.6
0.8
0.4
0.2
LOG CONCENTRATION (µg/ml)
A (1.54 µg/ml)
α (0.487 hr⁻¹)
B (0.47 µg/ml)
β (0.054 hr⁻¹)
Ka (2.697 hr⁻¹)
1
2
4
6
8
12
24
TIME (Hr)

T A B L E - 9

Kinetic parameters of Doxycycline in goat following single intramuscular dose of 5 mg/kg.

Parameter	Animal No. 1	2	3	4	5	6	Mean ± S.E.
A(µg/ml)	1.54	2.49	3.13	5.28	1.65	7.93	3.67 ± 1.02
B(µg/ml)	0.47	0.38	0.39	0.18	0.50	0.31	0.37 ± 0.05
C_o^p(µg/ml)	2.01	2.87	3.52	5.46	2.15	8.24	4.04 ± 0.98
Ka(hr^{-1})	2.697	2.453	2.206	2.319	2.584	2.549	2.468± 0.074
t_{y_2}Ka(hr)	0.26	0.28	0.31	0.30	0.27	0.27	0.28 ± 0.01
α(hr^{-1})	0.487	1.018	1.363	1.064	0.477	1.803	1.035± 0.209
$t_{y_2}\alpha$(hr)	1.42	0.68	0.51	0.65	1.45	0.38	0.85 ± 0.19
β(hr^{-1})	0.054	0.041	0.033	0.038	0.056	0.043	0.044± 0.004
$t_{y_2}\beta$(hr)	12.83	16.90	21.00	18.24	12.38	16.12	16.25 ± 1.34
K_{12}(hr^{-1})	0.216	0.643	0.966	0.468	0.206	1.026	0.588± 0.146
K_{21}(hr^{-1})	0.155	0.170	0.180	0.072	0.154	0.109	0.140± 0.017
Kel(hr^{-1})	0.170	0.246	0.250	0.562	0.173	0.711	0.352± 0.093
Fc	0.250	0.167	0.132	0.068	0.324	0.060	0.167± 0.042
AUC(mg/L.hr)	11.87	11.71	14.11	9.70	12.39	11.61	11.90 ± 0.58
Vd(L/kg)	2.49	1.74	1.42	0.92	2.33	0.61	1.59 ± 0.31
Vd_B(L/kg)	10.64	13.16	12.82	27.78	10.00	16.13	15.09 ± 2.69
Vd_{area}(L/kg)	7.80	10.41	10.74	13.56	7.21	10.02	9.96 ± 0.93
$Vd_{s.s.}$(L/kg)	5.96	8.32	9.04	6.90	5.45	6.35	7.00 ± 0.57
Cl_B(ml/kg/min)	7.02	7.11	5.91	8.59	6.73	7.18	7.09 ± 0.36
F(%)	115.02	93.98	90.28	90.74	90.64	104.22	97.48 ± 4.12

central (K_{21}) and elimination from central (Kel) compartments were calculated to be 0.588 ± 0.146, 0.140 ± 0.017 & 0.352 ± 0.093 hr^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) was noted to be 0.167 ± 0.042. The value of area under the curve in plasma (AUC) is found to be 11.90 ± 0.58 mg/L.hr. The various values for volume of distribution calculated by different methods are shown in Table-9. The mean value for Vd_{area} and $Vd_{s.s.}$ was observed to be 9.96 ± 0.93 L/kg and 7.00 ± 0.57 L/kg, respectively. The total body clearance (Cl_B) varied between 5.91 to 8.59 ml/kg/min with an average of 7.09 ± 0.36 ml/kg/min. The bioavailability (F) ranged from 90.28 to 115.02% with a mean of 97.48 ± 4.12%.

3. Dosage regimen:

The dosage regimen required to maintain the minimum therapeutic concentration along with expected maximum concentration (Cp max) in plasma at suitable dosage interval of 12 & 24 hr for i.m. administration is presented in Table-10.

T A B L E - 10

Dosage regimen of Doxycycline for intramuscular route in goat.

	Animal No. 1	2	3	4	5	6	Mean ± S.E.
$Cp^{\infty}min$=0.5 µg/ml							
D^*	10.25	10.82	9.42	22.02	9.82	13.60	12.66 ± 1.97
γ=12 hr D_o	4.89	4.21	3.08	8.06	4.80	5.48	5.09 ± 0.68
$Cp^{\infty}max$	0.96	0.82	0.73	0.79	0.98	0.84	0.85 ± 0.04
D^*	19.34	17.65	14.06	34.38	19.22	22.55	21.20 ± 2.87
γ=24 hr D_o	14.04	11.05	7.69	20.56	14.20	14.52	13.68 ± 1.74
$Cp^{\infty}max$	1.82	1.34	1.10	1.24	1.92	1.40	1.47 ± 0.13
$Cp^{\infty}min$=1.0 µg/ml							
D^*	20.50	21.65	18.84	44.04	19.63	27.20	25.31 ± 3.94
γ=12 hr D_o	9.79	8.42	6.15	16.11	9.60	10.97	10.17 ± 1.36
$Cp^{\infty}max$	1.93	1.65	1.47	1.58	1.96	1.69	1.71 ± 0.08
D^*	38.68	35.30	28.12	68.76	38.43	45.09	42.40 ± 5.73
γ=24 hr D_o	28.09	22.11	15.38	41.11	28.40	29.03	27.35 ± 3.49
$Cp^{\infty}max$	3.64	2.68	2.19	2.47	3.84	2.80	2.94 ± 0.27
$Cp^{\infty}min$=1.5 µg/ml							
D^*	30.75	32.47	28.26	66.07	29.45	40.79	37.97 ± 5.91
γ=12 hr D_o	14.68	12.63	9.23	24.17	14.40	16.45	15.26 ± 2.04
$Cp^{\infty}max$	2.89	2.47	2.20	2.38	2.94	2.53	2.57 ± 0.12
D^*	58.02	52.96	42.18	103.14	57.65	67.64	63.60 ± 8.60
γ=24 hr D_o	42.13	33.16	23.08	61.67	42.60	43.55	41.03 ± 5.23
$Cp^{\infty}max$	5.45	4.03	3.29	3.71	5.76	4.19	4.41 ± 0.40

$Cp^{\infty}min$ = Minimum plasma level $Cp^{\infty}max$ = Maximum plasma level

D^* = Loading dose D_o = Maintenance dose γ = Dosage interval

DEMECLOCYCLINE :

Pharmacokinetic study of demeclocycline after i.v. administration:

1. Plasma levels:

The plasma concentration of demeclocycline in goat at various time intervals following single i.v. dose (5 mg/kg) has been shown in Table-11 and Fig-2. The concentration of the drug at 5 min varied between 7.40 to 31.50 µg/ml (Mean ± S.E. = 21.70 ± 4.06). The mean therapeutic concentration ($\geq$ 0.5 µg/ml) was maintained in all the animals upto 36 hr. The mean plasma concentration at 36 hr was found to be 0.57 ± 0.12 µg/ml. The drug was present even at 48 hr samples (0.31 ± 0.07 µg/ml) of all the animals.

2. Kinetic parameters:

The values of various kinetic parameters calculated from the log plasma concentration of the drug versus time profile using two compartment open model are presented in Table-12 and Fig-9.

The extrapolated zero time concentration obtained during distribution phase (A) varied from 6.81 to 27.95 µg/ml with a mean of 17.81 ± 3.67 µg/ml. The extrapolated zero time concentration during elimination phase (B) ranged from 2.04 to 3.55 µg/ml with a mean of 2.88 ± 0.27 µg/ml. The mean value of theoretical zero time concentration of drug in plasma (C_o^p = A+B)

TABLE - 11

Plasma concentrations (µg/ml) of Demeclocycline in goat following single intravenous dose of 5 mg/kg.

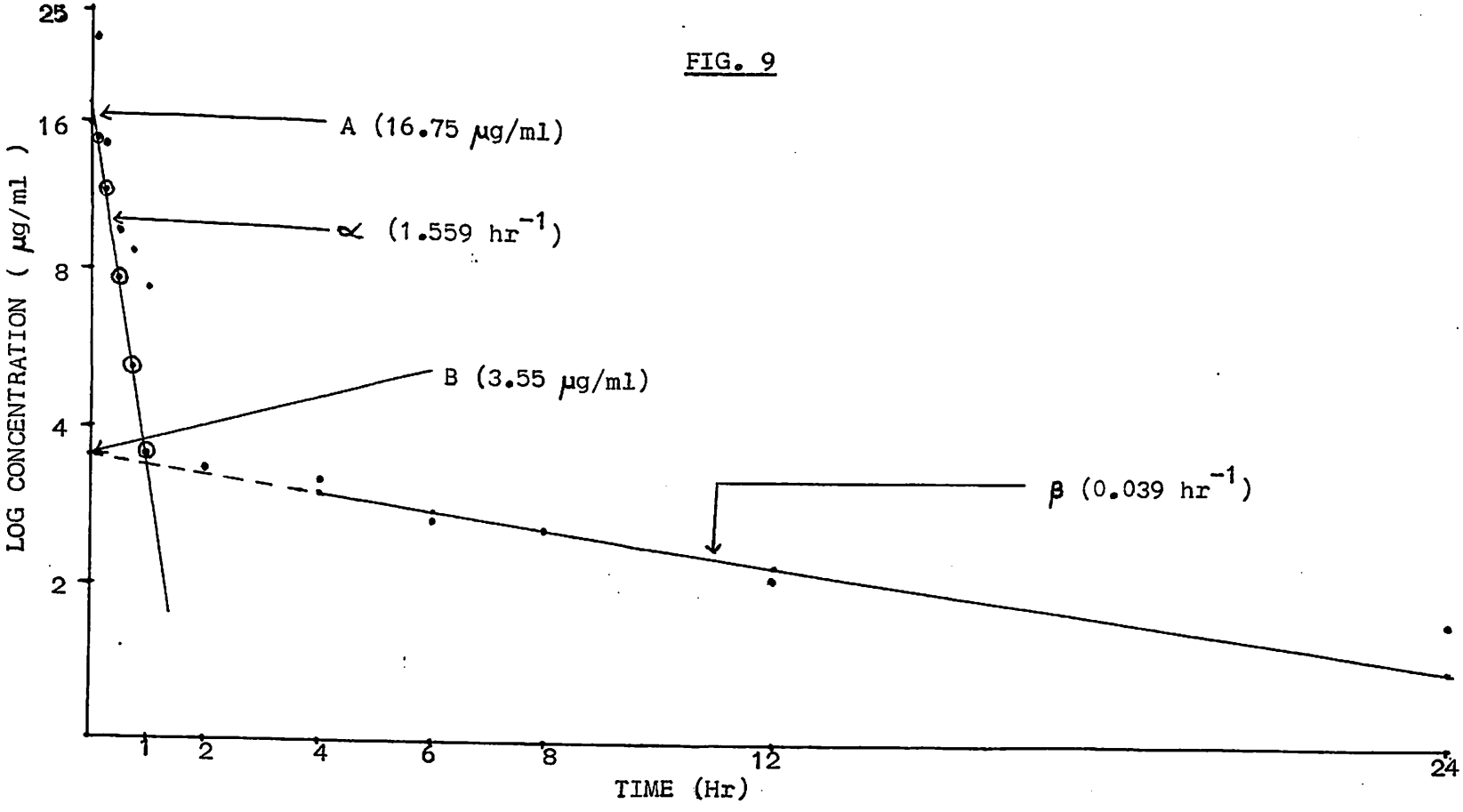
Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	31.50	21.80	7.40	29.50	12.00	28.00	21.70 ± 4.06
15 min	18.00	13.70	6.20	19.50	9.20	14.50	13.52 ± 2.07
30 min	14.50	9.50	3.80	14.00	6.40	12.00	9.87 ± 1.67
45 min	10.50	8.70	2.80	12.60	5.25	10.50	8.39 ± 1.50
1 hr	8.00	7.40	2.60	10.00	4.65	9.20	6.98 ± 1.15
2 hr	2.00	3.30	2.00	4.40	3.30	3.60	3.10 ± 0.39
4 hr	2.00	3.20	1.90	3.20	2.45	3.50	2.71 ± 0.28
6 hr	1.65	2.68	1.80	2.82	2.30	2.40	2.28 ± 0.19
8 hr	1.55	2.60	1.60	2.78	2.10	2.30	2.16 ± 0.21
12 hr	0.90	2.05	1.20	1.78	1.85	2.20	1.66 ± 0.21
24 hr	0.65	1.75	0.88	1.50	1.20	1.84	1.30 ± 0.20
30 hr	0.56	0.96	0.76	0.60	1.05	1.20	0.86 ± 0.11
36 hr	0.21	0.65	0.70	0.18	0.82	0.85	0.57 ± 0.12
48 hr	0.10	0.32	0.46	0.13	0.45	0.40	0.31 ± 0.07

T A B L E - 12

Kinetic parameters of Demeclocycline in goat following single intravenous dose of 5 mg/kg.

Parameter	Animal No. 1	2	3	4	5	6	Mean ± S.E.
A(ug/ml)	27.95	16.75	6.81	26.74	8.18	20.42	17.81 ± 3.67
B(ug/ml)	2.13	3.55	2.04	3.50	2.77	3.26	2.88 ± 0.27
C_o^p(ug/ml)	30.08	20.30	8.85	30.24	10.95	23.68	20.68 ± 3.76
$\alpha(hr^{-1})$	1.605	1.559	2.534	1.541	1.301	1.350	1.648± 0.184
$t_{\gamma 2}\alpha$(hr)	0.43	0.44	0.27	0.45	0.53	0.51	0.44 ± 0.04
$\beta(hr^{-1})$	0.048	0.039	0.032	0.038	0.033	0.031	0.037± 0.003
$t_{\gamma 2}\beta$(hr)	14.44	17.77	21.66	18.24	21.00	22.35	19.24 ± 1.22
$K_{12}(hr^{-1})$	1.007	1.094	1.824	1.091	0.859	0.972	1.141± 0.141
$K_{21}(hr^{-1})$	0.158	0.305	0.609	0.212	0.354	0.213	0.309± 0.067
$Kel(hr^{-1})$	0.488	0.199	0.133	0.276	0.121	0.196	0.236± 0.055
Fc	0.098	0.196	0.241	0.138	0.273	0.158	0.184± 0.027
AUC(mg/L.hr)	61.79	101.77	66.44	109.46	90.23	120.29	91.66 ± 9.61
Vd(L/kg)	0.17	0.25	0.56	0.17	0.46	0.21	0.30 ± 0.07
Vd_B(L/kg)	2.35	1.41	2.45	1.43	1.81	1.53	1.83 ± 0.19
Vd_{area}(L/kg)	1.69	1.26	2.35	1.20	1.68	1.34	1.59 ± 0.18
$Vd_{s.s.}$(L/kg)	1.25	1.15	2.24	1.04	1.58	1.17	1.41 ± 0.18
Cl_B(ml/kg/min)	1.35	0.82	1.25	0.76	0.92	0.69	0.97 ± 0.11

FIG. 9


25
16
8
4
2
LOG CONCENTRATION (µg/ml)
A (16.75 µg/ml)
∝ (1.559 hr^{-1})
B (3.55 µg/ml)
β (0.039 hr^{-1})
1
2
4
6
8
12
24
TIME (Hr)

was found to be 20.68 ± 3.76 µg/ml. The distribution rate constant (α) varied between 1.301 to 2.534 hr^{-1} with a mean of 1.648 ± 0.184 hr^{-1}. The elimination rate constant (β) ranged from 0.031 to 0.048 hr^{-1} with a mean of 0.037 ± 0.003 hr^{-1}. The mean distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) half life were found to be 0.44 ± 0.04 hr and 19.24 ± 1.22 hr, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) compartment and elimination rate constant (Kel) from central conpartment were calculated to be 1.141 ± 0.141 hr^{-1}, 0.309 ± 0.067 hr^{-1} and 0.236 ± 0.055 hr^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) was observed to be 0.184 ± 0.027. The value of area under the curve (AUC) in plasma was found to be 91.66 ± 9.61 mg/L .hr. Various values calculated for valume distribution are shown in Table-12. The Vd_{area} and $Vd_{s.s.}$ are found to be 1.59 ± 0.18 L/kg and 1.41 ± 0.18 L/kg, respectively. The total body clearance (Cl_B) ranged from 0.69 to 1.35 ml/kg/min with a mean of 0.97 ± 0.11 ml/kg/min.

3. Concentration of demeclocycline in various biological fluids:

(a) Interstitial fluid:

The concentration of drug in interstitial fluid at various time intervals has been shown in Table-13 and Fig-4. The drug appeared even at 5 min in all the animals. The mean peak concentration of 2.67 ± 0.23 µg/ml was reached at 6 hr.

TABLE - 13

Interstitial fluid concentrations (µg/ml) of Demeclocycline in goat following signle intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	0.25	0.18	0.15	0.22	0.32	0.19	0.22 ± 0.03
15 min	0.63	0.46	0.35	0.38	0.65	0.28	0.46 ± 0.06
30 min	1.00	0.92	0.58	0.85	1.30	0.75	0.90 ± 0.10
45 min	1.25	1.15	0.76	1.14	1.65	0.85	1.13 ± 0.13
1 hr	1.42	1.18	0.92	1.56	1.85	1.35	1.38 ± 0.13
2 hr	1.75	1.25	1.10	2.00	2.50	1.68	1.71 ± 0.21
4 hr	2.00	1.68	1.45	2.80	2.75	2.20	2.15 ± 0.23
6 hr	2.80	2.32	1.84	2.50	3.25	3.30	2.67 ± 0.23
8 hr	2.40	2.58	2.22	2.25	2.57	2.82	2.47 ± 0.09
12 hr	1.96	2.26	2.10	2.12	2.26	2.30	2.17 ± 0.05
24 hr	0.78	0.98	1.00	0.96	1.15	1.25	1.02 ± 0.07
30 hr	0.45	0.53	0.48	0.42	0.75	0.95	0.60 ± 0.09
36 hr	0.21	0.22	0.31	0.12	0.25	0.45	0.26 ± 0.05
48 hr	0.00	0.08	0.09	0.00	0.10	0.16	0.07 ± 0.03

The drug was present in the samples of all the animals upto 36 hr. The mean therapeutic concentration appeared at 30 min and was maintained upto 30 hr.

(b) Milk :

Table-14 and Fig-5 show the concentration of drug in milk at various time intervals. The drug did not appear in any of the samples collected upto 45 min. The drug was detectable in milk samples of 4 animals at 1 hr and in other animals at 2 hr. The mean peak concentration of 5.65 ± 0.45 µg/ml was attained at 8 hr. The mean therapeutic concentration ($\geq$0.5 µg/ml) was maintained from 1 to 36 hr. The drug was present in milk samples of all the animals upto 30 hr.

(c) Urine :

The concentration of demeclocycline in urine at different time intervals has been shown in Table-15 and Fig-6. The drug appeared in urine within 5 min after drug administration and persisted beyond 48 hr. The mean peak concentration of 82.23 ± 10.06 µg/ml in urine was attained at 8 hr. The therapeutic concentration ($\geq$0.5 µg/ml) was maintained in all the samples collected from 5 min to 48 hr.

4. Dosage regimen:

The suitable dosage regimen of demeclocycline to maintain the desired minimum therapeutic concentration (Cp^{∞}_{min}) alongwith expected maximum concentration (Cp^{∞}_{max}) at

T A B L E - 14

Milk concentrations (µg/ml) of Demeclocycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
15 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
30 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
45 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00
1 hr	1.15	0.84	0.00	0.98	0.00	0.65	0.60 ± 0.20
2 hr	2.25	1.98	1.00	2.10	1.45	1.52	1.72 ± 0.19
4 hr	5.12	3.10	2.00	3.80	2.80	2.75	3.26 ± 0.44
6 hr	6.20	4.40	2.80	5.80	5.40	4.78	4.90 ± 0.50
8 hr	4.80	6.30	4.80	4.62	7.40	6.00	5.65 ± 0.45
12 hr	3.14	5.20	3.65	2.65	5.80	4.20	4.11 ± 0.50
24 hr	1.60	2.80	2.25	1.10	3.75	2.72	2.37 ± 0.38
30 hr	1.00	1.30	1.80	0.65	2.40	2.15	1.55 ± 0.28
36 hr	0.74	0.80	1.20	0.00	1.90	1.45	1.02 ± 0.27
48 hr	0.00	0.00	0.60	0.00	0.50	0.68	0.30 ± 0.14

T A B L E - 15

Urine concentrations (µg/ml) of Demeclocycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	2.00	1.80	2.42	0.72	2.90	1.75	1.93 ± 0.30
15 min	4.80	8.20	6.45	4.80	6.83	5.90	6.16 ± 0.53
30 min	18.00	12.35	15.30	8.56	12.24	11.48	12.99 ± 1.33
45 min	32.50	15.25	18.13	10.75	24.15	22.65	20.57 ± 3.11
1 hr	38.00	19.82	24.18	19.62	28.88	32.54	27.17 ± 3.00
2 hr	45.00	29.00	47.26	27.18	36.98	41.36	37.80 ± 3.39
4 hr	72.30	34.38	67.76	32.37	53.25	60.45	53.42 ± 6.87
6 hr	128.00	52.85	85.00	45.95	67.06	71.07	74.99 ±12.00
8 hr	92.20	90.00	104.87	34.00	84.35	87.95	82.23 ±10.06
12 hr	82.00	62.12	70.15	17.89	58.02	68.30	59.75 ± 9.01
24 hr	28.00	23.67	33.30	10.22	31.48	35.68	27.06 ± 3.78
30 hr	13.20	11.80	12.80	7.78	14.24	22.13	13.66 ± 1.92
36 hr	11.00	6.43	7.21	4.48	8.65	12.70	8.41 ± 1.24
48 hr	8.00	1.22	2.50	1.00	4.28	5.20	3.70 ± 1.10

TABLE - 15

Urine concentrations (ug/ml) of Demeclocycline in goat following single intravenous dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	2.00	1.80	2.42	0.72	2.90	1.75	1.93 ± 0.30
15 min	4.80	8.20	6.45	4.80	6.83	5.90	6.16 ± 0.53
30 min	18.00	12.35	15.30	8.56	12.24	11.48	12.99 ± 1.33
45 min	32.50	15.25	18.13	10.75	24.15	22.65	20.57 ± 3.11
1 hr	38.00	19.82	24.18	19.62	28.88	32.54	27.17 ± 3.00
2 hr	45.00	29.00	47.26	27.18	36.98	41.36	37.80 ± 3.39
4 hr	72.30	34.38	67.76	32.37	53.25	60.45	53.42 ± 6.87
6 hr	128.00	52.85	85.00	45.95	67.06	71.07	74.99 ±12.00
8 hr	92.20	90.00	104.87	34.00	84.35	87.95	82.23 ±10.06
12 hr	82.00	62.12	70.15	17.89	58.02	68.30	59.75 ± 9.01
24 hr	28.00	23.67	33.30	10.22	31.48	35.68	27.06 ± 3.78
30 hr	13.20	11.80	12.80	7.78	14.24	22.13	13.66 ± 1.92
36 hr	11.00	6.43	7.21	4.48	8.65	12.70	8.41 ± 1.24
48 hr	8.00	1.22	2.50	1.00	4.28	5.20	3.70 ± 1.10

T A B L E - 16

Dosage regimen of Demeclocycline for intravenous route in goat.

	Animal No. 1	2	3	4	5	6	Mean ± S.E.
$Cp^{\infty}min$=0.5 µg/ml							
D^*	2.10	1.12	1.76	1.12	1.31	1.13	1.42 ± 0.17
γ=12 hr D_o	0.92	0.42	0.56	0.41	0.43	0.35	0.52 ± 0.09
$Cp^{\infty}max$	0.89	0.80	0.72	0.79	0.73	0.74	0.78 ± 0.03
D^*	3.71	1.81	2.65	1.77	1.97	1.60	2.25 ± 0.33
γ=24 hr D_o	2.54	1.10	1.42	1.06	1.08	0.84	1.34 ± 0.25
$Cp^{\infty}max$	1.58	1.28	1.08	1.24	1.10	1.05	1.22 ± 0.08
$Cp^{\infty}min$=1.0 µg/ml							
D^*	4.20	2.25	3.51	2.24	2.63	2.25	2.85 ± 0.34
γ=12 hr D_o	1.83	0.85	1.13	0.83	0.87	0.71	1.04 ± 0.17
$Cp^{\infty}max$	1.78	1.60	1.44	1.58	1.47	1.48	1.56 ± 0.05
D^*	7.43	3.62	5.30	3.55	3.95	3.20	4.51 ± 0.66
γ=24 hr D_o	5.07	2.20	2.84	2.11	2.17	1.69	2.68 ± 0.50
$Cp^{\infty}max$	3.16	2.57	2.16	2.47	2.19	2.10	2.44 ± 0.16
$Cp^{\infty}min$=1.5 µg/ml							
D^*	6.30	3.37	5.27	3.36	3.94	3.38	4.27 ± 0.51
γ=12 hr D_o	2.75	1.27	1.69	1.24	1.30	1.06	1.55 ± 0.25
$Cp^{\infty}max$	2.67	2.41	2.16	2.38	2.20	2.22	2.34 ± 0.08
D^*	11.14	5.43	7.95	5.32	5.92	4.80	6.76 ± 0.98
γ=24 hr D_o	7.61	3.30	4.26	3.17	3.25	2.53	4.02 ± 0.75
$Cp^{\infty}max$	4.74	3.85	3.25	3.71	3.29	3.14	3.66 ± 0.24

$Cp^{\infty}min$ = Minimum plasma level $Cp^{\infty}max$ = Maximum plasma level

D^* = Loading dose D_o = Maintenance dose γ = Dosage interval

convenient dosage interval (γ) for i.v. administration in goat has been presented in Table-16.

Pharmacokinetic study of demeclocycline (5 mg/kg) after i.m. administration :

1. Plasma levels:

The plasma drug concentration at various time intervals after i.m. administration has been shown in Table-17 and Fig-7. The drug appeared in plasma samples of all the animals at 5 min and persisted till 48 hr. The mean peak plasma concentration of 2.35 ± 0.17 was attained at 6 hr. The mean therapeutic concentration ($\geq$0.5 µg/ml) was reached at 30 min and was maintained upto 36 hr.

2. Kinetic parameters:

The plasma drug concentration versus time profile has confirmed one compartment open model after i.m. administration of demeclocycline (5 mg/kg) as depicted in Fig-10. Table-18 shows the values of different kinetic parameters calculated by one compartment open model.

The extrapolated zero time concentration of elimination phase (B) varied between 2.19 to 3.56 µg/ml with a mean of 2.97 ± 0.21 µg/ml. The absorption rate constant (Ka) ranged from 0.260 to 0.516 hr^{-1} with a mean of 0.357 ± 0.035 hr^{-1}. The elimination rate constant (β) ranged from 0.029 to 0.046 hr^{-1} with a mean of 0.039 ± 0.002 hr^{-1}. The mean

T A B L E - 17

Plasma concentrations (µg/ml) of Demeclocycline in goat following single intramuscular dose of 5 mg/kg.

Time	Animal No. 1	2	3	4	5	6	Mean ± S.E.
5 min	0.25	0.28	0.20	0.30	0.24	0.40	0.28 ± 0.03
15 min	0.38	0.42	0.25	0.36	0.28	0.52	0.37 ± 0.04
30 min	0.60	0.58	0.32	0.54	0.34	0.70	0.51 ± 0.06
45 min	0.84	0.80	0.40	0.60	0.43	0.86	0.66 ± 0.09
1 hr	1.00	1.10	0.45	0.80	0.50	1.10	0.83 ± 0.12
2 hr	1.50	1.35	0.86	1.30	1.20	1.60	1.30 ± 0.11
4 hr	2.20	1.95	2.00	2.20	2.35	3.00	2.28 ± 0.16
6 hr	2.00	2.60	1.80	2.80	2.20	2.70	2.35 ± 0.17
8 hr	1.70	2.30	1.70	2.60	2.00	2.45	2.13 ± 0.16
12 hr	1.60	2.10	1.55	2.20	1.75	2.30	1.92 ± 0.13
24 hr	0.86	1.20	1.10	1.40	0.98	1.50	1.17 ± 0.10
30 hr	0.68	1.00	0.92	1.10	0.84	1.15	0.95 ± 0.07
36 hr	0.50	0.80	0.74	0.84	0.62	0.92	0.73 ± 0.06
48 hr	0.28	0.44	0.40	0.50	0.38	0.52	0.42 ± 0.04

FIG. 10

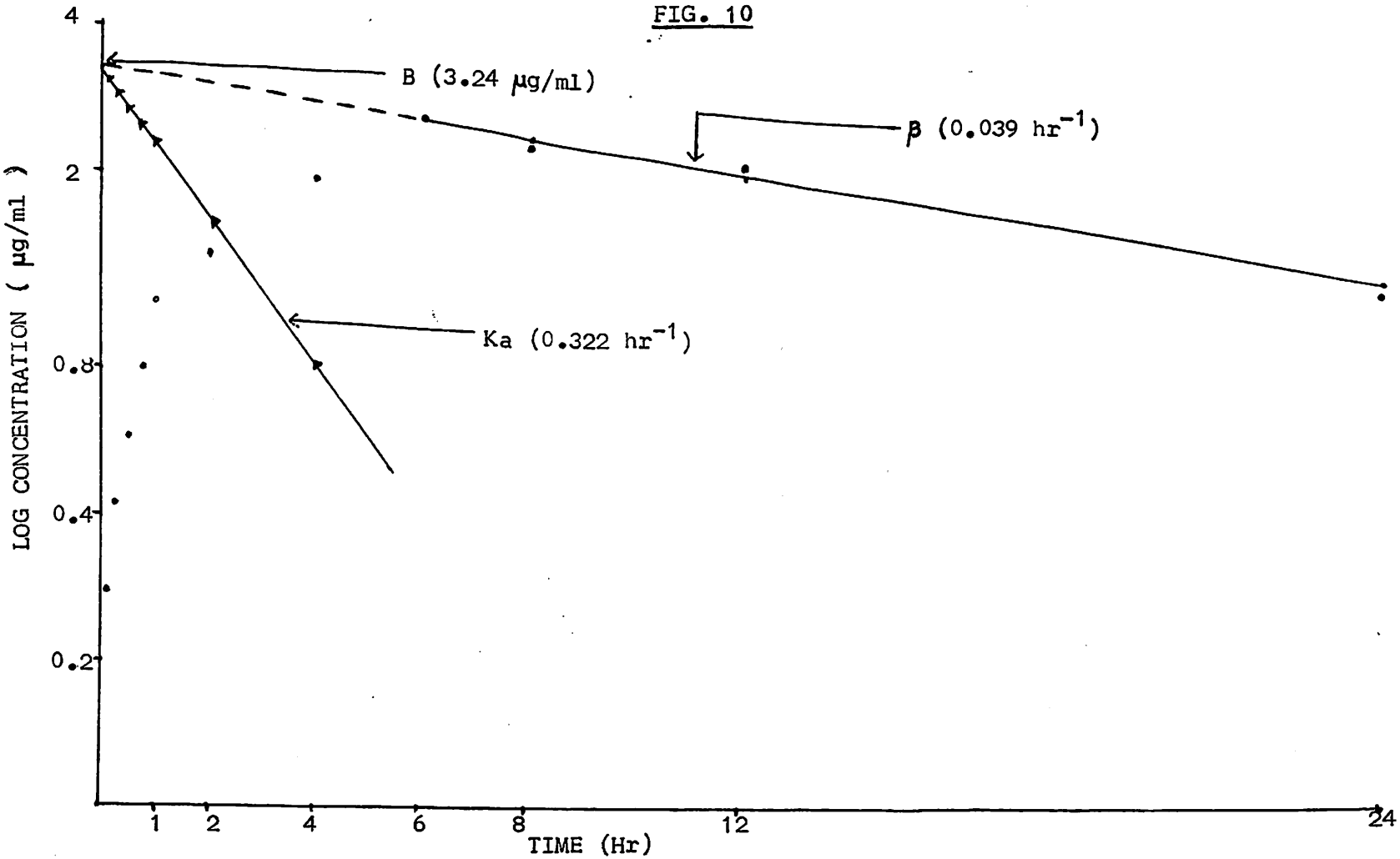

B (3.24 µg/ml)
β (0.039 hr⁻¹)
Ka (0.322 hr⁻¹)
LOG CONCENTRATION (µg/ml)
4
2
0.8
0.4
0.2
1
2
4
6
8
12
24
TIME (Hr)

TABLE - 18

Kinetic parameters of Demeclocycline in goat following single intramuscular dose of 5 mg/kg.

Parameter	Animal No. 1	2	3	4	5	6	Mean ± S.E.
B(ug/ml)	2.61	3.24	2.19	3.56	2.81	3.41	2.97 ± 0.21
$\beta(hr^{-1})$	0.046	0.039	0.029	0.040	0.042	0.036	0.039± 0.002
$t_{1/2}\beta$(hr)	15.07	17.77	23.90	17.33	16.50	19.25	18.30 ± 1.25
$Ka(hr^{-1})$	0.516	0.322	0.260	0.345	0.355	0.341	0.357± 0.035
$t_{1/2}$Ka(hr)	1.34	2.15	2.67	2.01	1.95	2.03	2.03 ± 0.17
AUC(mg/L.hr)	56.74	83.08	75.52	89.00	66.90	94.72	77.66 ± 5.79
Vd_{area}(L/kg)	1.92	1.54	2.28	1.40	1.78	1.47	1.73 ± 0.14
Cl_B(ml/kg/min)	1.47	1.00	1.10	0.93	1.25	0.88	1.11 ± 0.09
F(%)	91.83	81.64	113.67	81.31	74.14	78.74	86.89 ± 5.86

TABLE - 19

Dosage regimen of Demeclocycline for intramuscular route in goat.

	Animal No.						
	1	2	3	4	5	6	Mean ± S.E.
Cp^{∞}min=0.5 µg/ml							
D^*	1.67	1.23	1.63	1.15	1.49	1.14	1.39 ± 0.10
γ=12 hr D_o	0.71	0.46	0.48	0.44	0.59	0.40	0.51 ± 0.05
Cp^{∞}max	0.87	0.80	0.71	0.81	0.83	0.77	0.80 ± 0.02
D^*	2.89	1.97	2.28	1.85	2.44	1.74	2.20 ± 0.18
γ=24 hr D_o	1.93	1.20	1.14	1.14	1.55	1.01	1.33 ± 0.14
Cp^{∞}max	1.51	1.28	1.00	1.31	1.37	1.19	1.28 ± 0.07
Cp^{∞}min=1.0 µg/ml							
D^*	3.35	2.46	3.27	2.31	2.98	2.28	2.78 ± 0.20
γ=12 hr D_o	1.42	0.93	0.96	0.87	1.17	0.79	1.02 ± 0.09
Cp^{∞}max	1.75	1.60	1.43	1.63	1.67	1.54	1.60 ± 0.04
D^*	5.78	3.95	4.55	3.70	4.88	3.49	4.39 ± 0.35
γ=24 hr D_o	3.87	2.41	2.28	2.28	3.10	2.02	2.66 ± 0.28
Cp^{∞}max	3.02	2.57	2.00	2.63	2.74	2.38	2.56 ± 0.14
Cp^{∞}min=1.5 µg/ml							
D^*	5.02	3.69	4.90	3.46	4.47	3.42	4.16 ± 0.30
γ=12 hr D_o	2.13	1.39	1.44	1.31	1.76	1.19	1.54 ± 0.14
Cp^{∞}max	2.62	2.41	2.14	2.44	2.50	2.31	2.40 ± 0.07
D^*	8.67	5.92	6.83	8.96	7.32	5.23	7.16 ± 0.60
γ=24 hr D_o	5.80	3.61	3.42	3.41	4.64	3.04	3.99 ± 0.42
Cp^{∞}max	4.54	3.85	2.99	3.94	4.11	3.58	3.84 ± 0.21

Cp^{∞}min = Minimum plasma level Cp^{∞}max = Maximum plasma level

D^* = Loading dose D_o = Maintenance dose γ = Dosage interval

absorption half life ($t_{1/2}Ka$) and elimination half life ($t_{1/2}\beta$) were observed to be 2.03 ± 0.17 hr and 18.30 ± 1.25 hr, respectively. The total area under the curve (AUC) in plasma was found to be 77.66 ± 5.79 mg/L.hr. The total body clearance was observed to be 1.11 ± 0.09 ml/kg/min. The bioavailability (F) ranged from 74.14% to 113.67% with a mean value of 86.89 ± 5.86%.

3. Dosage regimen :

The appropriate dosage regimen required to maintain the minimum therapeutic concentration at different levels in plasma ($Cp^{ss}min$) for i.m. route has been presented in Table-19. The table also shows the expected maximum concentration of drug in plasma ($Cp^{ss}max$) at concerned dose rate. The dosage regimen has been designed at suitable dosage interval (τ).

COMPARISION BETWEEN DOXYCYCLINE AND DEMECLOCYCLINE:

Doxycycline and demeclocycline are compared here, since these drugs belong to a same group (tetracyclines) and administered in equal dose rate (5 mg/kg) following i.v. and i.m. administration in goat.

1. Concentrations in biological fluids:

(a) Plasma :

The plasma drug concentration after i.v. administration of these drugs are analysed statistically and shown in Table-20. Doxycycline attained significantly low blood level throughout from 5 min to 48 hr as compared to demeclocycline.

Table-21 shows the mean plasma levels of doxycycline and demeclocycline after i.m. injection. The concentration of doxycycline at 5 & 15 min was not significantly different than that of demeclocycline. Doxycycline attained a higher concentration at 30 & 45 min and 1 hr while it attained significantly low level starting from 2 to 48 hr. Doxycycline attained its peak level earlier (1 hr) than demeclocycline (6 hr).

(b) Interstitial fluid:

The concentration of doxycycline at different times was foun to be consistently lower except at 48 hr where there is no significant difference between these two drugs (Table-20). The mean peak concentration was attained at 6 hr for both drugs.

TABLE - 20

Comparision between concentrations (ug/ml) of Doxycycline and Demeclocycline in biological fluids after a single intravenous dose of 5 mg/kg in goat.

TIME	DOXYCYCLINE (MEAN ± S.E.)				DEMECLOCYCLINE (MEAN ± S.E.)			
	Plasma	I.S.F.	Milk	Urine	Plasma	I.S.F.	Milk	Urine
5 min	6.04±0.81	0.05±0.02	0.00±0.00	3.12±0.70	21.70±4.06**	0.22±0.03***	0.00±0.00	1.93±0.30+
15 min	2.38±0.30	0.10±0.01	0.00±0.00	7.19±1.67	13.52±2.07**	0.46±0.06***	0.00±0.00	6.16±0.53+
30 min	1.49±0.16	0.15±0.02	0.00±0.00	19.30±3.11	9.87±1.67**	0.90±0.10***	0.00±0.00	12.99±1.33+
45 min	1.21±0.13	0.23±0.07	0.04±0.04	42.66±7.44	8.39±1.50**	1.13±0.13***	0.00±0.00	20.57±3.11*
1 hr	0.97±0.07	0.29±0.09	0.31±0.04	52.90±6.11	6.98±1.15**	1.38±0.13***	0.60±0.20+	27.17±3.00**
2 hr	0.70±0.07	0.42±0.08	0.52±0.10	29.52±3.84	3.10±0.39**	1.71±0.21***	1.72±0.19***+	37.80±3.39+
4 hr	0.47±0.05	0.69±0.12	0.97±0.20	23.22±2.70	2.71±0.28**	2.15±0.23***	3.26±0.44***+	53.42±6.87**
6 hr	0.33±0.02	1.06±0.06	1.64±0.25	19.05±2.21	2.28±0.19**	2.67±0.23***	4.90±0.50***+	74.99±12.00**
8 hr	0.29±0.02	0.86±0.10	1.26±0.25	14.75±1.16	2.16±0.21**	2.47±0.09***	5.65±0.45***+	82.23±10.06***
12 hr	0.23±0.01	0.63±0.07	0.98±0.24	8.91±1.54	1.66±0.21**	2.17±0.05***	4.11±0.50***+	59.75±9.01**
24 hr	0.15±0.01	0.30±0.03	0.48±0.13	4.63±0.75	1.30±0.20**	1.02±0.07***	2.37±0.38***+	27.06±3.78**
30 hr	0.11±0.01	0.18±0.03	0.26±0.06	3.28±0.74	0.86±0.11**	0.60±0.09**	1.55±0.28**+	13.66±1.92***
36 hr	0.08±0.02	0.11±0.02	0.11±0.01	2.07±0.46	0.57±0.12**	0.26±0.05*	1.02±0.27**+	8.41±1.24***
48 hr	0.05±0.01	0.04±0.01	0.06±0.02	1.17±0.27	0.31±0.07**	0.07±0.03+	0.30±0.14+	3.70±1.10*

+ Non-significant * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

I.S.F. = Interstitial fluid

T A B L E - 21

Comparision between plasma concentration (µg/ml) of Doxycycline and Demeclocycline after a single intramuscular dose of 5 mg/kg in goat.

Time	VALUE (MEAN ± S.E.)	
	Doxycycline	Demeclocycline
5 min	0.36 ± 0.05	0.28 ± 0.03+
15 min	0.51 ± 0.06	0.37 ± 0.04+
30 min	0.72 ± 0.07	0.51 ± 0.06*
45 min	1.39 ± 0.16	0.66 ± 0.09**
1 hr	1.47 ± 0.17	0.83 ± 0.12*
2 hr	0.74 ± 0.08	1.30 ± 0.11***
4 hr	0.42 ± 0.07	2.28 ± 0.16***
6 hr	0.29 ± 0.03	2.35 ± 0.17***
8 hr	0.26 ± 0.03	2.13 ± 0.16***
12 hr	0.22 ± 0.02	1.92 ± 0.13***
24 hr	0.13 ± 0.02	1.17 ± 0.10***
30 hr	0.10 ± 0.01	0.95 ± 0.07***
36 hr	0.06 ± 0.02	0.73 ± 0.06***
48 hr	0.01 ± 0.01	0.42 ± 0.04***

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

+ Non-significant

(c) Milk:

Milk drug concentration of both drugs are presented in Table-20. The table reveals that doxycycline attained lower concentration between 2 to 36 hr than demeclocycline. The peak concentration was attained at 6 hr and 8 hr for doxycycline and demeclocycline, respectively.

(d) Urine :

The concentration of doxycycline in urine was non-significant at 5, 15 & 30 min, significantly higher at 45 min and 1 hr, while it was significantly lower throughout from 4 to 48 hr (Table-20). Doxycycline attained its mean peak level at 1 hr while demeclocycline reached its peak level at 8 hr.

2. Kinetic parameters:

The mean values of different kinetic parameters of doxycycline and demeclocycline obtained after i.v. administration (5 mg/kg) are presented in Table-22. The value of A, B and C_o^p of doxycycline was found to be significantly lower in comparision to demeclocycline. A statistically significant lower value of α and higher $t_{y2}\alpha$ was obtained for doxycycline whereas the value of β & $t_{y2}\beta$ was non-significant. The rate of transfer of drug from central to peripheral compartment (K_{12}) was significantly lower while

T A B L E - 22

Comparision between kinetic parameters of Doxycycline and Demeclocycline after a single intravenous dose of 5 mg/kg in goat.

Kinetic parameter	VALUE (MEAN ± S.E.)	
	Doxycycline	Demeclocycline
A(ug/ml)	2.55 ± 0.41	17.81 ± 3.67***
B(ug/ml)	0.41 ± 0.03	2.88 ± 0.27***
C_o^p(ug/ml)	2.96 ± 0.40	20.68 ± 3.76***
α(hr^{-1})	1.066±0.158	1.648±0.184*
$t_{1/2}\alpha$(hr)	0.73 ± 0.11	0.44 ± 0.04*
β(hr^{-1})	0.044±0.004	0.037±0.003+
$t_{1/2}\beta$(hr)	16.63 ± 1.58	19.24 ± 1.22+
K_{12}(hr^{-1})	0.669±0.135	1.141±0.141*
K_{21}(hr^{-1})	0.197±0.035	0.309±0.067+
Kel(hr^{-1})	0.244±0.036	0.236±0.055+
Fc	0.196±0.030	0.184±0.027+
AUC(mg/L.hr)	12.32 ± 0.83	91.66 ± 9.61***
Vd(L/kg)	1.87 ± 0.29	0.30 ± 0.07***
Vd_B(L/kg)	12.47 ± 0.86	1.83 ± 0.19***
Vd_{area}(L/kg)	9.78 ± 0.72	1.59 ± 0.18***
$Vd_{s.s.}$(L/kg)	7.86 ± 0.76	1.41 ± 0.18***
Cl_B(ml/kg/min)	6.91 ± 0.43	0.97 ± 0.11***

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

+ Non-significant

T A B L E - 23

Comparision between kinetic parameters of Doxycycline and Demeclocycline after a single intramuscular dose of 5 mg/kg in goat.

Kinetic parameter	VALUE (MEAN ± S.E.) Doxycycline (2-Compt.)	Demeclocycline (1-Compt.)
A(ug/ml)	3.67 ± 1.02	-
B(ug/ml)	0.37 ± 0.05	2.97 ± 0.21***
C_o^p(ug/ml)	4.04 ± 0.98	-
Ka(hr^{-1})	2.468± 0.074	0.357± 0.035***
t_{y_2}Ka(hr)	0.28 ± 0.01	2.03 ± 0.17***
α(hr^{-1})	1.035± 0.209	-
$t_{y_2}\alpha$(hr)	0.85 ± 0.19	-
β(hr^{-1})	0.044± 0.004	0.039± 0.002+
$t_{y_2}\beta$(hr)	16.25 ± 1.34	18.30 ± 1.25+
K_{12}(hr^{-1})	0.588± 0.146	-
K_{21}(hr^{-1})	0.140± 0.017	-
Kel(hr^{-1})	0.352± 0.093	-
Fc	0.167± 0.042	-
AUC(mg/L.hr)	11.90 ± 0.58	77.66 ± 5.79***
Vd(L/kg)	1.59 ± 0.31	-
Vd_B(L/kg)	15.09 ± 2.69	-
Vd_{area}(L/kg)	9.96 ± 0.93	1.73 ± 0.14***
$Vd_{s.s.}$(L/kg)	7.00 ± 0.57	-
Cl_B(ml/kg/min)	7.09 ± 0.36	1.11 ± 0.09***
F(%)	97.48 ± 4.12	86.89 ± 5.86+

*** $p < 0.001$ + Non-significant

T A B L E - 24

Comparison of dosage regimen of Doxycycline and Demeclocycline for intravenous route in goat.

		VALUE (MEAN ± S.E.)	
		Doxycycline	Demeclocycline
$Cp^{\infty}min$=0.5 µg/ml			
γ=12 hr	D*	10.57 ± 0.70	1.42 ± 0.17***
	D_o	4.29 ± 0.45	0.52 ± 0.09***
	$Cp^{\infty}max$	0.85 ± 0.04	0.78 ± 0.03+
γ=24 hr	D*	17.83 ± 1.56	2.25 ± 0.33***
	D_o	11.59 ± 1.45	1.34 ± 0.25***
	$Cp^{\infty}max$	1.45 ± 0.13	1.22 ± 0.08+
$Cp^{\infty}min$=1.0 µg/ml			
γ=12 hr	D*	21.14 ± 1.39	2.85 ± 0.34***
	D_o	8.58 ± 0.90	1.04 ± 0.17***
	$Cp^{\infty}max$	1.71 ± 0.08	1.56 ± 0.05+
γ=24 hr	D*	35.67 ± 3.11	4.51 ± 0.66***
	D_o	23.19 ± 2.90	2.68 ± 0.50***
	$Cp^{\infty}max$	2.90 ± 0.26	2.44 ± 0.16+
$Cp^{\infty}min$=1.5 µg/ml			
γ=12 hr	D*	31.70 ± 2.09	4.27 ± 0.51***
	D_o	12.86 ± 1.34	1.55 ± 0.25***
	$Cp^{\infty}max$	2.56 ± 0.12	2.34 ± 0.08+
γ=24 hr	D*	53.50 ± 4.67	6.76 ± 0.98***
	D_o	34.79 ± 4.35	4.02 ± 0.75***
	$Cp^{\infty}max$	4.36 ± 0.40	3.66 ± 0.24+

*** $p < 0.001$ + Non-significant

T A B L E - 25

Comparision of dosage regimen of Doxycycline and Demeclocycline for intramuscular route in goat.

		VALUE (MEAN ± S.E.)	
		Doxycycline	Demeclocycline
$Cp^{\infty}min$=0.5 µg/ml			
γ=12 hr	D*	12.66 ± 1.97	1.39 ± 0.10***
	D_o	5.09 ± 0.68	0.51 ± 0.05***
	$Cp^{\infty}max$	0.85 ± 0.04	0.80 ± 0.02+
γ=24 hr	D*	21.20 ± 2.87	2.20 ± 0.18***
	D_o	13.68 ± 1.74	1.33 ± 0.14***
	$Cp^{\infty}max$	1.47 ± 0.13	1.28 ± 0.07+
$Cp^{\infty}min$=1.0 µg/ml			
γ=12 hr	D*	25.31 ± 3.94	2.78 ± 0.20***
	D_o	10.17 ± 1.36	1.02 ± 0.09***
	$Cp^{\infty}max$	1.71 ± 0.08	1.60 ± 0.04+
γ=24 hr	D*	42.40 ± 5.73	4.39 ± 0.35***
	D_o	27.35 ± 3.49	2.66 ± 0.28***
	$Cp^{\infty}max$	2.94 ± 0.27	2.56 ± 0.14+
$Cp^{\infty}min$=1.5 µg/ml			
γ=12 hr	D*	37.97 ± 5.91	4.16 ± 0.30***
	D_o	15.26 ± 2.04	1.54 ± 0.14***
	$Cp^{\infty}max$	2.57 ± 0.12	2.40 ± 0.07+
γ=24 hr	D*	63.60 ± 8.60	7.16 ± 0.60***
	D_o	41.03 ± 5.23	3.99 ± 0.42***
	$Cp^{\infty}max$	4.41 ± 0.40	3.84 ± 0.21+

*** $p < 0.001$ + Non-significant

K_{21}, Kel and Fc did not differ significantly. The various values of volume distribution obtained by different methods and total body clearance (Cl_B) was significantly higher ($P < 0.001$) for doxycycline.

The comparision between kinetic parameters of doxycycline and demeclocycline after a single i.m. dose (5 mg/kg) is depicted in Table-23. The data of doxycycline fitted well in two compartment open model whereas demeclocycline confirmed one compartment open model. The value of B for doxycycline was significantly lower than that of demeclocycline. A significantly higher value of absorption rate constant (Ka) and lower absorption half life ($t_{1/2}Ka$) was obtained for doxycycline whereas there is no difference between bioavailability (F) of both drugs. The difference between the value of β and $t_{1/2}\beta$ of both drugs were non-significant. A very high value of Vd_{area} and Cl_B were obtained for doxycycline.

3. Dosage regimen:

The dosage regimen for i.v. and i.m. route in goat are presented in Table-24 & 25. Doxycycline required a very high loading (D^*) as well as maintenance (D_o) doses at each level of therapeutic concentration ($Cp^{ss}min$) of the dosage interval (τ) of 12 and 24 hr.

DISCUSSION

a mean of 6.04 ± 0.81 µg/ml. The drug was detectable even at 48 hr in 5 out of 6 animals (Table-2). Following i.m. administration, the drug appeared in plasma within 5 min, attained its mean peak concentration of 1.47 ± 0.17 µg/ml at 1 hr and was detectable upto 36 hr in 5 out of 6 animals (Table-8). Ranade et al. (1981) observed a similar result except that the drug was detectable only upto 8 hr following a single i.v. injection of 200 mg/goat. Various factors such as sensitivity of the analytical methods, variation in breed & strain etc. may be attributed to the longer persistance of the drug observed in the present study. Steigbigel et al. (1968) reported a peak serum level of 3.64 µg/ml at 8 hr and the drug was detectable beyond 48 hr after an oral dose of 300 mg in normal human volunteers.

The plasma log concentration versus time curve following i.v. administration of doxycycline in goat suggests a two compartment open model for calculation of kinetic parameters (Fig-3). It is generally observed for most drugs, the kinetic data is best fitted in one compartment open model for i.m. and oral routes (Baggot, 1977). However, in the present study, the plasma log concentration versus time profile following i.m. administration of doxycycline produced a biphasic curve (Fig-8) after achieving its peak level and thus suggests two compartment open model for calculation of kinetic parameters.

The absorption rate constant (Ka) of 2.468 ± 0.074 hr^{-1} and absorption half life ($t_{1/2}Ka$) of 0.28 ± 0.01 hr of doxycycline obtained in the present study after i.m. administration indicates a rapid absorption of the drug from the site of injection. Michael et al. (1979) also observed almost similar result for doxycycline phosphate with Ka and $t_{1/2}Ka$ of 1.645 hr^{-1} and 0.4 hr, respectively after oral administration in dog. In the present study, the bioavailability of doxycycline by i.m. route has been observed to be 97.48 ± 4.12% in goat. The oral bioavailability of doxycycline in human was reported to be 93% (Fabre et al., 1971; Neu, 1978).

In the present investigation the distribution rate constant (α) and distribution half life ($t_{1/2}\alpha$) obtained after i.v. (Table-3) and i.m. (Table-9) are nearly similar, denoting the rapid distribution of doxycycline. The value of α and $t_{1/2}\alpha$ of doxycycline was found to be 1.248 ± 0.11 hr^{-1} and 0.56 hr in lactating cows and ewes after a single i.v. dose of 20 mg/kg (Ziv and Sulman, 1974). Raghuram and Krishnaswamy (1982) reported $t_{1/2}\alpha$ of 0.87 ± 0.293 hr after an i.v. infusion of 3 mg/kg in human beings. The studies by several workers amply suggest a similar pattern of distribution in cow, ewe, goat and man.

The mean elimination rate constant (β) has been observed to be 0.044 ± 0.004 hr^{-1} after i.v. and i.m. administration. The mean elimination half life ($t_{1/2}\beta$) is found

to be 16.63 ± 1.58 hr after i.v. and 16.25 ± 1.34 hr after i.M. administration during present investigation (Table-3 & 9). Michael et al. (1979) reported β as 0.06 hr^{-1} and $t_{1/2}\beta$ as 11.5 hr after oral administration of doxycycline phosphate (10 mg/kg) in dog. Ziv and Sulman (1974) found the β and $t_{1/2}\beta$ as 0.028 ± 0.006 hr^{-1} and 24.75 hr, respectively, in cows and ewes after single i.v. injection (20 mg/kg). In human beings a wide variation in elimination half life ranging from 8.3 hr to 22 hr was reported by various workers (Doluision and Dittert, 1969; Neu, 1978; Raghuram and Krishnaswamy, 1982). From these findings it could be concluded that the persistance of doxycycline in blood is fairly longer in various species of animals including man. This provides an option for spacing the dosing schedule at 12 & 24 hr intervals.

The high value of α and low value of β obtained in this study, indicate a rapid distribution but slow elimination of doxycycline suggesting the drug to be a long acting antibiotic in goat.

The value of rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartments is noted to be 0.669 ± 0.135 hr^{-1}, 0.197 ± 0.035 hr^{-1}, 0.244 ± 0.036 hr^{-1} after i.v. and 0.588 ± 0.146 hr^{-1}, 0.140 ± 0.017 hr^{-1}, 0.352 ± 0.093 hr^{-1} after i.m. administration, respectively (Table-3 & 9). The ratio between K_{12}:K_{21}-β during elimination

phase reflects the expected tissue distribution of any drug after a single i.v. dose (Notari, 1980). The mean value for tissue : plasma ratio in this study is calculated to be 4.37, which indicates the good distribution of the drug in poripheral compartments. This fact gets support by a higher drug concentration obtained in interstitial fluid and milk as compare to plasma during elimination phase. The low values of Kel ($0.244 \pm 0.036\ hr^{-1}$ for i.v. and $0.352 \pm 0.093\ hr^{-1}$ for i.m.) and β (Table-3 & 9) indicate that the drug is slowly eliminated from the body of goat. The fraction of drug in the central compartment which at any time is available for elimination (Fc) is presented in Table-3 & 9. The low value of Fc (0.196 ± 0.030 for i.v. and 0.167 ± 0.042 for i.m.) shows that only 19.6% and 16.7% of total amount of drug present in central compartment was available for elimination at any time. All these parameters contribute to a longer persistance of drug in plasma after both i.v. and i.m. injection. Ziv and Sulman (1974) reported the value of K_{12}, K_{21}, Kel and Fc to be $0.734 \pm 0.08\ hr^{-1}$, $0.468 \pm 0.07\ hr^{-1}$, $0.075 \pm 0.008\ hr^{-1}$ and 0.37 ± 0.09, respectively in cows. Raghuram and Krishnaswamy (1982) found the value of K_{12}, K_{21} and Kel as $0.50 \pm 0.140\ hr^{-1}$, $0.74 \pm 0.163\ hr^{-1}$ and $0.074 \pm 0.0083\ hr^{-1}$, respectively in man. These findings demonstrate higher expected tissue : plasma ratio in goat than in cow and man. This may be due to higher musele fat content in goat

than in cattle (Baruah, 1984) and thus permitting greater distribution to this lipophilic drug in goat tissues.

Values of volume distribution obtained by various methods are presented in Table-3 for two compartment open model. Notari (1980) observed that for a two compartment open model the value of $Vd_B > Vd_{area} > Vd_{s.s.} > Vd$. He further stated that among these values of volume distribution, only Vd_{area} correctly predicts the amount of drug in the body during elimination phase whereas Vd_B overestimates and $Vd_{s.s.}$ underestimates the amount of drug in the body. The value of Vd_{area} for i.v. and i.m. routes are 9.78 $\pm$ 0.72 L/kg and 9.96 $\pm$ 0.93 L/kg, respectively in goat. In children apparent valume of distribution was reported to be 0.9 to 1.8 L/kg (Ceccarelli et al., 1971) and 0.75 $\pm$ 0.089 L/kg in adults (Raghuram and Krishnaswamy, 1982). In animals, Vd_{area} of 2.285 $\pm$ 0.31 L/kg was reported in cow and ewes by Ziv and Sulman (1974) while Michael et al. (1979) observed a Vd value of 3.25 L/kg in dog for doxycycline. Thus, a highly variable value for Vd was observed in different species of animals including man. A very high value of Vd_{area} obtained during the present study may be attributed to the wide distribution of the drug coupled with its storage in tissue depots, more likely in fat, since doxycycline is highly lipophilic in nature (Schach Von wittenau and Yeary, 1963; Schach Von Wittenau and Delahunt, 1966).

In the present study the total body clearance (Cl_B) is observed to be 6.91 ± 0.43 ml/kg/min and 7.09 ± 0.36 ml/kg/min after i.v. and i.m. administration. Cl_B in human beings was reported to be 28.9 ± 2.65 ml/kg/min after i.v. administration (Raghuram and Krishnaswamy, 1982). The lower value of Cl_B in goat than in man indicates that the drug is removed at a lower rate in goat as compare to man.

(b) Concentrations in various biological fluids:

Concentrations of doxycycline in interstitial fluid, milk and urine are presented in Table-4, 5 & 6.

The drug appeared within 5 min and the mean peak concentration of 1.06 ± 0.06 µg/ml reached at 6 hr in interstitial fluid. In milk the drug appeared at 1 hr, showing a longer lag phase probably, it may be due to specialized nature of membrane at the site (Varvey et al., 1965). The mean peak concentration of 1.64 ± 0.25 µg/ml in milk was obtained at 6 hr. Ranade et al. (1981) found a mean peak concentration of 0.8 µg/ml at 1 hr and 0.63 µg/ml at 2 hr in interstitial fluid and milk, respectively after a single i.v. injection of 200 mg/goat. Delayed appearance of peak concentration in interstitial fluid and milk during present investigation may be due to the rise in the level of the drug in these fluids only after relative saturation of fat depots with the drug.

The drug appeared within 5 min in urine at a higher concentration in comparision to interstitial fluid and milk. The mean peak concentration of 52.90 ± 6.11 µg/ml at 1 hr was obtained in goat in this study (Table-6). Steigbigel et al. (1968) reported the urinary excretion of doxycycline varying between 5.7 to 93.7 µg/ml with a peak value of 93.7 µg/ml at 0-8 hr after a single oral dose of 300 mg in a normal young man. This shows a more or less similar pattern of excretion of doxycycline via urine in goat as in man.

DEMECLOCYCLINE :

(a) Pharmacokinetics:

In the present investigation, various pharmacokinetic parameters have been calculated from plasma drug concentration after i.v. and i.m. administration of demeclocycline. Following i.v. administration, the mean peak plasma concentration of 21.70 ± 4.06 µg/ml appeared at 5 min and the drug was detectable upto 48 hr (Table-11). The peak level was achieved at 6 hr and the drug persisted upto 48 hr after i.m. administration (Table-17). Kunin et al. (1959) obtained the mean peak serum concentration around 22 µg/ml immediately after single i.v. infusion of 500 mg demeclocycline in normal human subjects. Finland and Garrod (1960) reported the peak serum concentration around 2.5 µg/ml

at 6 hr and the drug was even detectable beyond 72 hr after oral administration of 500 mg of demeclocycline in man. Steigbigel et al. (1968) observed peak serum concentration of 1.74 µg/ml at 4 hr after oral administration of 300 mg in normal young men. The results of this investigation in goat show a similar pattern with the reports of various workers in human.

The graphic representation of log plasma concentration of demeclocycline versus time following i.v. route suggests a two compartment open model (Fig-9) and after i.m. administration fits well in one compartment open model (Fig-10) for calculation of kenetic parameters. The values of different kinetic parameters obtained after i.v. and i.m. administration are depicted in Table-12 & 18.

The absorption rate constant (Ka) of 0.357 ± 0.035 hr^{-1} and absorption half life ($t_{1/2}Ka$) of 2.03 ± 0.17 hr of demeclocycline obtained in the present study after i.m. administration indicates that the drug is absorbed slowly from the site of injection. This result shows resembalance with the findings of Finland and Garrod (1960). They reported that demeclocycline reached its peak concentration slowly, and its level also declined gradually.

A similar order of bioavailability by i.m. route has been observed to be 86.89 ± 5.86% in goat during present investigation. The oral bioavailability of demeclocycline in human was reported to be 60 to 80% (Barza and Scheife, 1977; Neu, 1978).

The distribution rate constant (α) of 1.648 ± 0.184 hr^{-1} and distribution half life ($t_{1/2}\alpha$) of 0.44 ± 0.04 hr confirms the rapid distribution of demeclocycline in tissue. Ziv and Sulman (1974) also observed a more or less similar value of α (1.196 ± 0.21 hr^{-1}) in cows & ewes.

The elimination rate constant (β) has been observed to be 0.037 ± 0.003 hr^{-1} after i.v. and 0.039 ± 0.002 hr^{-1} following i.m. administration. The elimination half life ($t_{1/2}\beta$) is found to be 19.24 ± 1.22 hr and 18.30 ± 1.25 hr after i.v. and i.m. administration, respectively. Ziv and Sulman (1974) also reported a similar value of β (0.038 ± 0.01 hr^{-1}) and $t_{1/2}\beta$ (18.24 hr) after a single i.v. injection of demeclocycline (20 mg/kg) in cows and ewes. A wide variation in elimination half life ranging from 9 to 16 hr in human beings was reported by various workers (Rosenblatt et al., 1967; Doluisio and Dittert, 1969; Goodman Gilman et al., 1980). The findings indicate that demeclocycline is eliminated from the body at a lower rate and thus persists in blood for a longer period.

The rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartments is noted to be 1.141 ± 0.141 hr^{-1}, 0.309 ± 0.067 hr^{-1} and 0.236 ± 0.055 hr^{-1}, respectively in the present study (Table-12). The higher value of K_{12} indicates that the drug is well distributed in peripheral

compartment. The low value of Kel and β denote that the drug is slowly eliminated from the body. The mean value for tissue: plasma ($K_{12} : K_{21}-\beta$) ratio in this study has been calculated to be 4.19. This indicates that demeclocycline is well dostributed in tissues and body fluids. This fact is further supported by a comparative higher concentration obtained in interstitial fluid and milk than in plasma during elimination phase in the present study. A low value of Fc (0.184 ± 0.027) depicts that only 18.4% of the total amount of drug, present in the central compartment is available for elimination. This also explains towards longer persistance of drug in plasma. The value of K_{12}, K_{21}, Kel and Fc was reported to be 0.674 ± 0.06 hr^{-1}, 0.461 ± 0.05 hr^{-1}, 0.098 ± 0.01 hr^{-1} and 0.39 ± 0.05, respectively in cows & ewes (Ziv and Sulman, 1974). The above finding shows that the expected tissue: plasma ratio is low for cow and ewe than goat.

Values of volume distribution obtained by different methods for two compartment open model are presented in Table-12. Among all the values of volume distribution only Vd_{area} correctly predicts the amount of drug in the body during elimination phase. In one compartment open model all values of volume distribution yield same numerical value (Notari, 1980). Vd_{area} of 1.59 ± 0.18 L/kg and 1.73 ± 0.14 L/kg after i.v. and i.m. administration,

The mean peak concentration of 5.65 ± 0.45 µg/ml at 8 hr is observed during present investigation.

The drug appeared immediately at 5 min in urine in higher concentration as compared to plasma (Table-15). The mean peak concentration of 82.23 ± 10.06 µg/ml at 8 hr is noted in urine. Steigbigel et al. (1968), reported the mean peak urine concentration of 105 µg/ml between 0-8 hr after a single oral dose of 300 mg in normal young man.

COMPARISION BETWEEN DOXYCYCLINE AND DEMECLOCYCLINE:

(a) Pharmacokinetics:

The concentrations of doxycycline and demeclocycline in plasma at different time intervals after administration of 5 mg/kg i.v. and i.m. are presented in Table-20 & 21, respectively. The plasma level of doxycycline has been observed to be significantly low than that of demeclocycline fron 5 min to 48 hr post i.v. administration. Following i.m. administration, plasma level of doxycycline was significantly higher at 30 min ($P < 0.05$), 45 min ($P < 0.01$) and 1 hr ($P < 0.05$). The peak concentration of doxycycline noted at 1 hr while demeclocycline achieved its peak 6 hr (Table-21). The finding suggests a higher rate of absorption for doxycycline as compare to demeclocycline. Beyond 1 hr, the concentration of doxycycline was significantly low than demeclocycline. Schach Von Wittenau and Yeary (1963) stated

respectively has been obtained in the present study. Ziv and Sulman (1974) reported the volume of distribution as 1.996 ± 0.22 L/kg after i.v. injection in cows & ewes. In human beings the volume of distribution was found to be 1.79 L/kg after an oral dose of 500 mg (Kunin et al., 1959; Finland and Garrod, 1960). The studies on volume distribution indicate that demeclocycline is fairly distributed throughout the body in all species.

The total body clearance (Cl_B) has been observed to be 0.97 ± 0.11 ml/kg/min and 1.11 ± 0.09 ml/kg/min after i.v. and i.m. administration in the present investigation. The low value of Cl_B indicates slow elimination of demeclocycline from the body.

(b) Concentrations in various biological fluids:

The concentrations of demeclocycline in interstitial fluid, milk and urine are presented in Table-13, 14 & 15.

In interstitial fluid, the drug appeared within 5 min and persisted upto 36 hr in all animals. The immediate appearance of drug may be due to its higher distribution rate constant. The mean peak concentration of 2.67 ± 0.23 µg/ml was obtained at 6 hr in this study.

The drug appeared in milk at 1 hr in most of the animals showing a sufficiant lag phase, which may be due to specialized nature of membrane at the site (Varvey et al. 1965).

that a highly lipid soluble tetracycline will be more concentrated in tissue depots than a less lipid soluble analogue and hence, will show lower drug concentration in serum. Doxycycline having very high lipid solubility than demeclocycline (Schach Von Wittenau and Delahunt, 1966) is bound to get concentrated in lipid fraction of the tissue leading to low plasma drug concentration.

The comparative kinetic parameters of doxycycline and demeclocycline for i.v. and i.m. routes in goat are presented in Table-22 and 23, respectively.

The results reveal that doxycycline is absorbed at a much faster rate than demeclocycline which is evident by a highly significant Ka value of doxycycline (2.468 $\pm$ 0.074 hr^{-1}) in comparision to demeclocycline (0.357 $\pm$ 0.035 hr^{-1}). This fact is also supported by a low t_{y2}Ka value of doxycycline than demeclocycline. The bioavailability of doxycycline (97.48 $\pm$ 4.12%) by i.m. route has been found slightly higher than that of demeclocycline (86.89 $\pm$ 5.86%), but the value is statistically non-significant. It reveals that the both drugs are absorbed from i.m. site in almost equal amount.

The demeclocycline is quickly distributed than doxycycline as revealed by its significant high α and low $t_{y2}\alpha$ values after i.v. administration (Table-22). Lower protein binding of a drug has been attributed to be an

important factor for quick distribution. Demeclocycline has low protein binding capacity than doxycycline (Goodman Gilman et al., 1980). This may be the possible explanation for quick distribution of demeclocycline.

The K_{12} value of the drugs differ significantly, but the ratio between K_{12}: K_{21}-β comes to 4.37 for doxycycline and 4.19 for demeclocycline. This reveals that both the drugs get distributed in a higher concentration in tissues and fluids. A comparatively higher concentration of the drugs observed during elimination phase in interstitial fluid and milk than in plasma of goat supports well distribution of both drugs.

The values of Vd_{area} is found to be 9.78 $\pm$ 0.72 L/kg and 9.96 $\pm$ 0.93 L/kg for doxycycline and 1.59 $\pm$ 0.18 L/kg and 1.73 $\pm$ 0.14 L/kg for demeclocycline following i.v. and i.m. administration, respectively (Table-22 & 23). Apparent volume of drug distribution gives an idea of the extent or magnitude of distribution without providing any clue whether the drug is uniformly distributed or restricted to certian tissues. A large volume of distribution indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above (Baggot, 1977). The higher values ($Vd > 1$ L/kg) of volume distribution of these drugs obtained in the present study suggest that apart from their wide distribution in body

tissues and fluids, the drugs having high lipid solubility, get bound/stored in tissues mainly in fat. The partition coefficient between chloroform and water was reported to be 475×10^3 and 72×10^3 for doxycycline and demeclocycline, respectively by Schach Von Wittenau and Delahunt (1966). The basic tetracycline molecule has three pKa values of 3.3, 7.7 & 9.5 (Leeson et al., 1963), and hence, degree of ionization for both the drugs is expected to be approximately similar. Further a drug having effective partition coefficient (EPC), product of lipid solubility coefficient and unionized/ionized fraction of the drug in plasma is greater/equal to unity, readily diffuses across a biological membrane (Singh et al., 1975). In the present case, the value of EPC for both drugs are greater than unity and hence readily distributed in various tissues and fluids. The values of Vd_{area} is noted to be 9.78 & 9.96 L/kg for doxycycline and 1.59 & 1.73 L/kg for demeclocycline for i.m. & i.v. administration. This shows that the value of Vd_{area} for doxycycline is nearly 6.2 & 5.8 times higher than demeclocycline in i.v. & i.m. route, respectively. Both drugs are having nearly equal degree of ionisation but doxycycline has 6.6 times higher lipid solubility than demeclocycline and, hence, expected to yield 6.6 times higher Vd_{area}. This may be the possible explanation of the very high Vd value of doxycycline as compared to demeclocycline obtained in the present study.

The elimination rate constant (β) and elimination half life ($t_{1/2}\beta$) of doxycycline and demeclocycline in i.v. and i.m. routes are statistically non-significant and explain about the persistance of the drugs in plasma for longer period. The values of the fraction of the drugs available at any time for elimination from central compartment (Fc) are also statistically non-significant. The data shows that only a small fraction of each drug is available for elimination and this subscribes to a prolnged persistance of the drugs in different body fluids. The non-significant Kel values of both the drugs suggest a similar rate of elimination. The total body clearance (Cl_B) for doxycycline is significantly higher than demeclocycline for both i.v. and i.m. routes.

Concentrations in various biological fluids:

The comparision between concentrations of doxycycline and demeclocycline after i.v. administration in interstitial fluid, milk and urine is presented in Table-20.

It has been observed that the concentrations of doxycycline is significantly low in interstitial fluid and milk than demeclocycline. The significant lower level of doxycycline obtained in these fluids is best explained by the observation of Schach Von Wittenau and Yeary (1963).

They stated that a highly lipid soluble tetracyclines will be more concentrated in tissues and less in interstitial fluid than a less lipid soluble analogue and therefore, will show lower free drug concentration in serum. Doxycycline being more lipid soluble than demeclocycline is expected to be stored in greater amount in tissues and appear in low concentration in interstitial fluid and milk. The concentration of both drugs is higher in interstitial fluid and milk as compared to that of plasma during elimination phase (Table-20). This finding well corrlates with the result of rate of drug transfer discussed earlier.

It has been observed in this study that the doxycycline is excreted in urine in comparatively higher concentration than demeclocycline in early phase whereas in lower concentration at later phase (Table-20).

(c) Dosage regimen:

The loading dose (D^*) and maintenance dose (D_o) for i.v. and i.m. routes required to maintain the minimum therapeutic concentration ($Cp^{\infty}min$) at convenient dose interval (γ) is presented in Table-24 & 25.

The study of the tables reflect that doxycycline requires a significantly higher ($P < 0.001$) dose than demeclocycline at all the therapeutic concentrations (0.5 to 1.5 µg/ml) at same dosage interval for both routes. A very

high dose of doxycycline in goat may not be popular with the clinicians and owners as well, due to the cost of treatment, though the drug fulfills the desirable criteria as a therapeutic agent. Demeclocycline may be prefered on this account in goat. Demeclocycline has been reported to have hepatotoxic and phototoxic properties in human (Goodman Gilman et al., 1980; Neu, 1978). No such studies have been conducted in the field of veterinary science. A detailed study on this aspect of this drug seems imperative before recommending it for field use.

SUMMARY

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A detailed pharmacokinetic study of doxycycline and demeclocycline had been undertaken in healthy lactating goats between 1.5 to 2 years of age and weighing 25-30 kg. Attempts have been made to calculate the rational dosage regimen of these drugs based on the kinetic data obtained in the present study.

(1) With single i.v. dose of doxycycline (5 mg/kg), the mean therapeutic concentration ($\geq$0.5 µg/ml) was maintained upto 2 hr only whereas it was upto 36 hr with demeclocycline. Doxycycline maintained significantly low plasma concentration as compared to demeclocycline, at all the time intervals.

(2) Various kinetic parameters were obtained from plasma concentration versus time profile after i.v. dose. The distribution half life ($t_{\frac{1}{2}\alpha}$) was observed to be statistically higher for doxycycline (0.73 $\pm$ 0.11 hr) than demeclocycline (0.44 $\pm$ 0.04 hr). There was nonsignificant difference in the elimination half life ($t_{\frac{1}{2}\beta}$) of doxycycline (16.63 $\pm$ 1.58 hr) and demeclocycline (19.24 $\pm$ 1.22 hr). The longer elimination half life of these drugs reveal that both these drugs are long acting in goat. The rate of transfer of drug from central to peripheral compartment (K_{12}) was significantly low for

doxycycline ($0.669 \pm 0.135\ hr^{-1}$) in comparision to demeclocycline ($1.141 \pm 0.141\ hr^{-1}$). The rate of transfer of drug from peripheral to central (K_{21}) and elimination from central (Kel) compartment did not show any significant difference between these drugs. All the values of volume of distribution calculated by different methods revealed a highly significant ($P < 0.001$) value for doxycycline than demeclocycline. A higher Vd_{area} of 9.78 ± 0.72 L/kg was noted for doxycycline while it was only 1.59 ± 0.18 L/kg for demeclocycline. The total body clearnce (Cl_B) of doxycycline (6.91 ± 0.43 ml/kg/min) was noted to be highly significant as compared to demeclocycline (0.97 ± 0.11 ml/kg/min).

(3) A consistantly significant low concentration of doxycycline at different time intervals (except 48 hr) was observed as compared to demeclocycline post i.v. administration (5 mg/kg). However, the mean peak concentration in interstitial fluid reached at 6 hr for both drugs. The present study revealed that doxycycline attained significantly low concentration in milk between 2 to 36 hr than demeclocycline. The peak milk concentration was achieved at 6 hr for doxycycline while it was 8 hr for demeclocycline. A higher drug concentration was

observed in intarstitial fluid and milk as compared to plasma during elimination phase which may be due to a high theoretical tissue plasma ratio (K_{21}: K_{21}-β). The value of K_{12}: K_{21}-β was observed to be 4.37 and 4.19 for doxycycline and demeclocycline, respectively. Concentration of doxycycline in urine was non significant at 5,15 & 30 min, significantly higher at 45 min & 1 hr and significantly lower throughout from 4 to 48 hr. Doxycycline attained its mean peak level at 1 hr while demeclocycline reached at 8 hr.

(4) Doxycycline requires a highly significant ($P < 0.001$) higher loading dose (D^*) as well as maintenance dose (D_o) at MIC (C_p^{∞}min) of 0.5, 1 & 1.5 µg/ml at the fixed dosage interval (γ) of 12 & 24 hr as compared to demeclocycline by i.v. route.

(5) Following i.m. administration (5 mg/kg) there was no significant difference in the plasma levels of doxycycline and demeclocycline at 5 & 15 min while doxycycline attained significantly higher concentration at 30 min, 45 min and 1 hr. Beyond 1 hr, the concentration of doxycycline remained significantly low throughout the various time intervals as compared to demeclocycline.

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(7) Doxycycline requires a highly significant ($P < 0.001$) higher loading (D^*) and maintenance (D_o) dose than demeclocycline at MIC (C_p^{∞}min) of 0.5, 1.0 & 1.5 µg/ml at the dosage interval (γ) of 12 & 24 hr by i.m. route as well.

The observations made in the present study revealed that both doxycycline and demeclocycline possess many desirable properties including high bioavailability, longer duration of action (greater $t_{\gamma 2}\beta$), better distribution in body fluids and tissues (high tissue : plasma ratio). Though doxycycline possesses many desirable kinetic properties, the concentration of the drug in plasma, milk and interstital fluid was maintained below therapeutic level in the dose of 5 mg/kg and requires a higher loading and maintenance dose for maintaining the therapeutic concentration. Hence, doxycycline may not be popular with the clinicians and owners dueto its high cost of treatment. Demeclocycline on the other hand may be prefered on this account. However, the drug is known to have phototoxic and hepatotoxic properties in human which need detailed study on this aspect in animals.

(6) By i.m. route, the plasma drug concentration versus time profile showed a biphasic curve after attaining its peak level and thus suggested two compartment open model for calculation of kinetic parameters of doxycycline whereas demeclocycline produced a monophasic curve depicting one compartment open model. The absorption half life ($t_{1/2}Ka$) was found to be highly significant for doxycycline (0.28 ± 0.01 hr) as compared to demeclocycline (2.03 ± 0.17 hr) thus indicating a rapid absorption of doxycycline. The bioavailability of doxycycline (97.48 ± 4.12%) by i.m. route was slightly higher than that of demeclocycline (86.89 ± 5.86%), but the value is statistically non significant. It reveals that both the drugs are absorbed well and in almost equal amount from i.m. site. The elimination half life was found to be more or less similar for both doxycycline (16.25 ± 1.34 hr) and demeclocycline (18.30 ± 1.25 hr). The volume of distribution was significantly ($P < 0.001$) higher for doxycycline (Vd_{area} = 9.96 ± 0.93 L/kg) as compared to demeclocycline (Vd_{area}=1.73). The total body clearance (Cl_B) was observed to be significantly ($P < 0.001$) high for doxycycline (7.09 ± 0.36 ml/kg/min) than demeclocycline (1.11 ± 0.09 ml/kg/min).

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APPENDIX-I

CALCULATION OF KINETIC PARAMETERS:

Kinetic parameters were calculated from the plasma drug concentration versus time profile. An example is shown here from the data of animal no.3 obtained after i.v. injection of doxycycline (5 mg/kg). The data showed a biphasic curve and hence fits well in a two compartment open model.

Calculation of β and B (values were taken which fits into straight line):

Plasma conc. (µg/ml)	X (Time in hr)	X^2	Log(Y x 10) drug conc.	XY
0.42	4	16	0.623	2.493
0.35	6	36	0.544	3.264
0.30	8	64	0.477	3.817
0.25	12	144	0.398	4.775
0.18	24	576	0.255	6.127
0.17	30	900	0.230	6.913
0.15	36	1296	0.176	6.339

$\xi X = 120$ $\xi X^2 = 3032$ $\xi Y = 2.704$ $\xi XY = 33.729$

$(\xi X)^2 = 14400$ $\bar{Y} = 0.386$

$\bar{X} = 17.143$

b, slope of line $= \frac{n.\Sigma XY - \Sigma X.\Sigma Y}{n.\Sigma X^2 - (\Sigma X)^2}$

where, X = Time
Y = drug conc.
n = number of samples.

$= \frac{7 \times 33.729 - 120 \times 2.704}{7 \times 3032 - 14400}$

$= - 0.0130 \text{ hr}^{-1}$

β, elimination rate constant = b x - 2.303
(slope of line in natural log)

$= - 0.0130 \times - 2.303$
$= 0.030 \text{ hr}^{-1}$

Y, intercept (Zero time conc. during elimination phase) can be obtained from the formula:

$\bar{Y} = a + b\bar{X}$ where, $\bar{Y}$ = mean log conc.
$\bar{X}$ = mean time
b = slope of line
a = zero time conc.

Therefore, $a = \bar{Y} - b\bar{X}$
$= \log 0.386 - (-0.0130 \times 17.143)$
$= \log 0.6089$

Zero time conc. = antilog of 0.6089 = 4.06 µg/ml.

Since plasma concentration is multiplied earlier by 10 in the above calculation, the value of 4.06 µg/ml should be divided by 10 to get the actual zero time concentration. Hence, zero time concentration (B) = 0.406 µg/ml or 0.41 µg/ml.

Similarly the theoretical plasma concentration (Y) can be calculated by putting the values of time (X) in the above equation during the time intervals of distribution phase. ($Y = a + bX$).

Substracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution constant, α) and the zero time intercept (zero time concentration during distribution phase, A) can be calculated as per the method adopted for calculation of β and B. The calculated values are

$$\alpha = 1.534 \text{ hr}^{-1}$$

$$A = 3.01 \text{ } \mu g/ml$$

C_o^p, theoretical plasma concentration at time zero

$$C_o^p = A + B$$

$$= 3.01 + 0.41 = 3.42 \text{ } \mu g/ml$$

$t_{y_2}\alpha$, distribution half life

$$t_{y_2} = \frac{0.693}{\alpha}$$

$$= \frac{0.693}{1.534} = 0.45 \text{ hr}$$

$t_{y_2}\beta$, elimination half life

$$t_{y_2}{}^B = \frac{0.693}{\beta}$$

$$= \frac{0.693}{0.030} = 23.10 \text{ hr}$$

AUC, Area under curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

$$= \frac{3.01}{1.533} + \frac{0.41}{0.030} = 15.63 \text{ mg/L.hr}$$

K_{21}, rate constant for drug transfer from peripheral to central compartment.

$$K_{21} = \frac{A\beta + B\alpha}{C_o^p}$$

$$= \frac{3.01 \times 0.030 + 0.41 \times 1.534}{3.42} = 0.210 \text{ hr}^{-1}$$

Kel, the elimination rate constant of the drug from central compartment.

$$Kel = \frac{\alpha \cdot \beta}{K_{21}}$$

$$= \frac{1.534 \times 0.030}{0.210} = 0.219 \text{ hr}^{-1}$$

K_{12}, rate constant of drug tranfer from central to peripheral compartment.

$$K_{12} = \alpha + \beta - K_{21} - Kel$$

$$= 1.534 + 0.030 - 0.210 - 0.219$$

$$= 1.135 \text{ hr}^{-1}$$

Fc, the fraction of drug available for elimination from central compartment.

$$Fc = \frac{\beta}{Kel}$$

$$= \frac{0.030}{0.219} = 0.137$$

Vd, the volume of distribution based on distribution and elimination.

$$Vd = \frac{D}{A + B} \quad \text{where } D = \text{Dose rate (mg/kg)}$$

$$= \frac{5}{3.42} = 1.46 \text{ L/kg}$$

Vd_B, the volume of distribution based nn elimination

$$Vd_B = \frac{D}{B} = \frac{5}{0.41} = 12.20 \text{ L/kg}$$

Vd_{area}, the volume of distribution based on total area.

$$Vd_{area} = \frac{D}{\left(\frac{A}{\alpha} + \frac{B}{\beta}\right) . \beta}$$

$$= \frac{5}{\left(\frac{3.01}{1.534} + \frac{0.41}{0.030}\right) .030} = 10.66 \text{ L/kg}$$

$Vd_{s.s.}$, the volume of distribution at steady state.

$$Vd_{s.s.} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd$$

$$= \frac{1.135 + 0.210}{0.210} \times 1.46 = 9.35 \text{ L/kg}$$

Cl_B, the total body clearance.

$$Cl_B = Vd_{area} \times \beta$$

$$= 15.63 \times 0.030 = 0.468 \text{ L/kg/hr}$$

$$= 7.80 \text{ ml/kg/min.}$$

APPENDIX - II

Dosage regimen were calculated to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage interval using the formulae described by Notari (1980). The data of animal no.3 obtained after i.v. injection of doxycycline has been used as an example for calculation of dosage regimen for MIC ($C_p^{\infty}min$) of 0.5 µg/ml at the dosage interval (γ) of 12 hr. The calculation is as follows.

Calculation of Maintenance dose (D_o) :

For calculation of D_o, fraction of dose (f) remains in circulation at a particular dosage interval (γ) should be obtained, which is derived from the formula:

$$\gamma = \frac{\ln f}{-\beta} \; ; \; \beta = \text{elimination rate constant.}$$

$$\ln f = -\beta \times \gamma = -0.030 \times 12 = 0.36$$

$$f = 0.698$$

For calculation of D_o, zero time concentration of drug during elimination phase should be arrived for a particular MIC ($C_p^{\infty}min$) and fraction of dose (f) remained at particular dosage interval (γ). This is derived from the formula:

$$C_p^{\infty}min = \frac{f \times B}{(1 - f)}$$

$$B = \frac{(C_p^{\infty}min)(1-f)}{f} = \frac{0.5\ (1-0.698)}{0.698} = 0.22\ \mu g/ml$$

B is directly proportional to dose.

The value of B for a given dose of 5 mg/kg i.v. in the present experiment was found to be 0.41 µg/ml (Table-3). Hence, the dose required to obtain the value of B as 0.22 µg/ml is derived as below.

$$D_o = \frac{5 \times 0.22}{0.41} = 2.68 \text{ mg/kg}$$

Loading dose (D^*)

Loading dose is arrived from the formula:

$$D^* = \frac{D_o}{(1-f)} = \frac{2.68}{(1-0.698)} = 8.87 \text{ mg/kg}$$

Prediction of steady state maximum plasma concentration ($C_p$max)

During repetitive administration, the theoretical steady state maximum plasma concentration (C_p^{∞}max) is calculated from the derivation:

$$C_p^{\infty}\text{max} = \frac{B}{(1-f)} = \frac{0.22}{(1-0.698)} = 0.73 \text{ µg/ml.}$$