EFFECT OF PROCAINE AND ANTIPYRINE ON THE PHARMACOKINETICS OF DIMINAZENE IN BUFFALO CALVES

THESIS

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of

MASTER OF VETERINARY SCIENCE

VETERINARY PHARMACOLOGY AND TOXICOLOGY

(Minor Subject : Veterinary Physiology)

By

Jaspreet Kaur
(L-98-V-310-M)

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College of Veterinary Science
PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA - 141 004.

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CERTIFICATE I

This is to certify that the thesis entitled **"Effect of procaine and antipyrine on the pharmacokinetics of diminazene in buffalo calves"** submitted for the degree of **M.V.Sc.** in the subject of **Veterinary Pharmacology and Toxicology** (Minor subject : **Veterinary Physiology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Jaspreet Kaur** (L-98-V-310-M) under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Srivastava
25/5/2000

(A.K. Srivastava)
Major Advisor

CERTIFICATE II

This is to certify that the thesis **"Effect of procaine and antipyrine on the pharmacokinetics of diminazene in buffalo calves"** submitted by **Jaspreet Kaur** (L-98-V-310-M) to the Punjab Agricultural University, Ludhiana, in partial fulfilment of the requirements for the degree of **M.V.Sc.** in the subject of Veterinary Pharmacology and Toxicology (Minor subject : **Veterinary Physiology**) has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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Jaspreet

(Jaspreet Kaur)

Ludhiana
Dated

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ABSTRACT

The present study was planned to investigate the effect of procaine and antipyrine on the pharmacokinetics, urinary excretion and dosage regimen of diminazene in buffalo calves. In addition, *in vitro* erythrocytic partitioning and plasma protein binding of diminazene were also studied.

Diminazene was administered at the dose rate of 3.5 $mg.kg^{-1}$ along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) by single intramuscular route in healthy buffalo calves in two different dosage forms i.e. granules and suspension. The heparinized blood samples were collected at various predetermined time intervals and diminazene was estimated by diazotization method. After i.m. administration of granular form of diminazene, appreciable concentration (1.53 ± 0.15 $\mu g.ml^{-1}$) appeared at 1 min and the drug was detected upto 7 days (1.05 ± 0.11 $\mu g.ml^{-1}$). Similarly following administration of suspension form, plasma level at 1 min was (1.50 ± 0.07 $\mu g.ml^{-1}$) and at the end of 7 days it was (1.25 ± 0.10 $\mu g.ml^{-1}$). On comparing the results of various pharmacokinetic parameters of both forms of diminazene, it was revealed that there was no significant difference in

most of the pharmacokinetic parameters. The values of $t_{0.5}Ka$, $t_{0.5}\alpha$, $t_{0.5}\beta$, $V_{d(ss)}$ and Cl_T ranged between 0.057-0.446 h, 0.314-0.400 h, 538.3-667.6 h, 2.11-2.70 $L.kg^{-1}$ and 3.49-3.66 $ml.kg^{-1}.h^{-1}$, respectively. For evaluating the effect of procaine and antipyrine on pharmacokinetics of diminazene, the data of present investigation was compared with already established data following alone administration of diminazene. The results revealed that procaine and antipyrine causes slow elimination of diminazene from body (as the $t_{0.5}\beta$ was increased to 8-10 folds) and prolongs the therapeutic effect of diminazene in body for more than 20 days. Diminazene bound to plasma proteins to the extent of 34.0 ± 1.40 per cent. At various blood concentrations ranging from 1.25 to 20 $\mu g.ml^{-1}$, the erythrocytic partitioning was 23.6 ± 3.25 per cent. The appropriate dosage regimen of diminazene in buffalo calves, when administered along with procaine and antipyrine would be 4.3 $mg.kg^{-1}$ followed by 1.9 $mg.kg^{-1}$ at 15 days interval.

Srivastava 25/5/2002

Signature of Major Advisor

Jaspreet

Signature of Student

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CHAPTER I

INTRODUCTION

The treatment of trypanosomiasis has drawn the attention of many scientists and veterinarians for over a century. Diminazene is considered as an ideal chemotherapeutic drug (Raisinghani and Lodha 1980, Gilbert and Newton 1982, Mdachi *et al* 1995) and is effectively used in treatment of trypanosomiasis caused by *Trypanosoma vivax, T. congolense, T. evansi, T. brucei, T. gambiense* and *T. rhodesiense*. The human sleeping sickness, especially at early stages of infection is also treated with diminazene (Bailey 1968, Ogada *et al* 1973).

Trypanocidal activity of diminazene was first reported by Bauer (1955a,b). The detailed pharmacology, indications, usefulness, tolerance and toxicity of diminazene have also been extensively investigated (Singh *et al* 1980, Heerden 1981, Hemeida 1981, Farewell *et al* 1982, Hunter *et al* 1992, Anene *et al* 1997). Four features of this drug distinguish it from other trypanocidal drugs : its low toxicity both local and systemic, high therapeutic index than other trypanocides (Fairclough 1963, Williamson 1970), low incidence of resistance (Williamson 1976) and it is effective against trypanosomes which are resistant to most other trypanocides (Williamson 1970).

For effective and judicious use of antitrypanocidal drugs, their detailed pharmacokinetic studies are prerequisite. The disposition pattern and pharmacokinetics of diminazene have been extensively investigated in goats (Mamman and Peregrine 1994), dogs (Ravi Prakash *et al* 1994), cross bred cow calves (Kaur 1998), sheep (Aliu and Odegaard 1985), pregnant and lactating cows (Madachi *et al* 1995), rats (Reather *et al* 1972) and rabbits (Gilbert and Newton 1982). The detailed disposition kinetics of diminazene has also been established in buffalo calves when administered alone (Singh 1997).

Recently, the use of a new combination of diminazene diaceturate with local anaesthetic procaine, and antipyretic drug antipyrine has been recommended in clinical cases of trypanosomiasis in animals. The idea of combining procaine was to decrease the extravascular absorption of diminazene so that the effective concentration of drug is maintained for longer duration in body. Procaine, when combined with other chemotherapeutic drugs reported to form tissue depots in body from which drug is released slowly and effective concentration in blood and urine remain for prolonged periods e.g. procaine penicillin G (Goodman and Gilman 1985). Similarly antipyrine which is established as an antipyretic drug was added to relieve the patient from high fever. The high fever is reported to be one of the main clinical symptoms of trypanosomiasis (Verma 1973, Singh and Gaur 1983).

However, there is no report available on effect of procaine and antipyrine on pharmacokinetics and dosage regimen of diminazene when given concomitantly.

When multiple drugs are given concurrently, the complete knowledge of pharmacokinetic drug interactions is most important. It has been shown that when two or more drugs are used in combination, they alter the kinetic disposition of each other and can also affect the dosage regimen of drugs. Similar to antipyrine, other non narcotic analgesic antipyretic drugs such as paracetamol and salicylate are reported to alter the pharmacokinetic pattern and dosage regimen of 2nd and 3rd generation cephalosporins and aminoglycoside antibiotics (Sharma and Srivastava 1997, Srivastava and Chaudhary 1999).

Extent of plasma protein binding is reported to influence the distribution and elimination of drugs (Pilloud 1973, Wise *et al* 1980, Yamada *et al* 1981). Erythrocytic partitioning is considered as an important index because (I) the drug entered into erythrocytes may serve as reservoir, (ii) it is important for the treatment of intracellular infections.

Keeping these facts in view, the present investigation is planned with the following objectives :

1. To study the effect of procaine and antipyrine on pharmacokinetics and urinary excretion of diminazene in buffalo calves when given in combination.

2. To compute the appropriate dosage regimen of diminazene, keeping in view the effect of procaine and antipyrine.

CHAPTER II

REVIEW OF LITERATURE

Trypanosomiasis is widely distributed in different parts of world and results in great economic losses (Luckins 1992). In buffaloes the surra was first reported in India by Lingard (1896). Trypanosomiasis (Surra) caused by *Trypanosoma evansi* has a wide range of affecting domestic animals viz., horses, mules, cattle, buffaloes, sheep, goats and pigs (Hoare 1972) and wild animals (Gill 1991).

Diminazene aceturate has trypanocidal activity. In addition, it also has babesicidal and bacteriocidal action. Diminazene is considered as an ideal drug for control of trypanosomiasis in livestock (Gilbert and Newton 1982, Madachi *et al* 1995). Further, it also shows excellent therapeutic results, when used in control of babesiosis in horses, cattle, sheep, pigs and dogs (Bauer 1967, Joyner and Brocklesby 1973). Satisfactory antianaplasmosis (Bauer and Hochheimer 1975), antibacterial (against some species of bacteria like brucella and streptococci) (Brander 1991) activity of diminazene has also been established. Further in India, diminazene has been shown to be effective in natural *T. evansi* infection of buffalo (Hiregoudar and Avsathi 1971) and infections of cross bred calves (Verma *et al* 1976). Dimianzene has few advantages over other trypanocidal agents (I) it is effective against

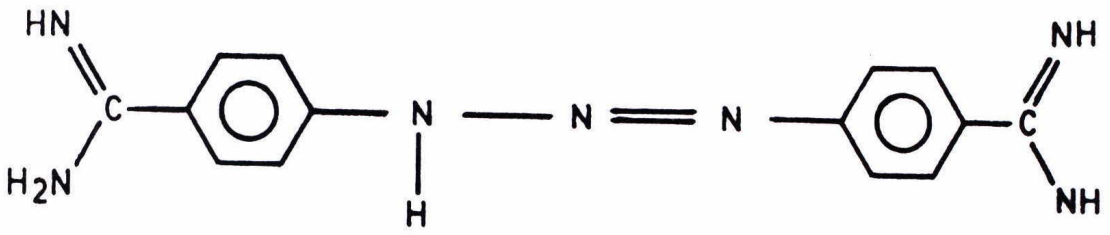
trypanosomes that are resistant to most other trypanocides (Williamson 1970), (ii) low incidence of resistance (Williamson 1976), (iii) low toxicity and higher therapeutic index than other trypanocides in most species of animals (Fairclough 1963, Williamson 1970).

Diminazene 4,4'-diazoamino dibenzamidine diaceturate is an aromatic diamidine. Diamidines are group of chemotherapeutic agents found to have trypanocidal activity. Aromatic diamidines are shown to have greater trypanocidal activity than alkyl diamidines (Brander 1991).

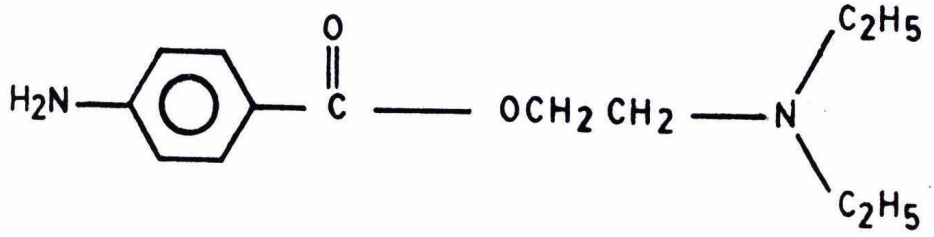
Procaine, p-amino diethyl aminoethyl benzoate is commonly used as local anaesthetic. But, it can also form poorly soluble salts or conjugate with other chemotherapeutic drugs and prolong their action (Goodman and Gilman 1985). For example, after intramuscular injection of procaine penicillin G, antibiotic is absorbed and released slowly, resulting the effective concentration of penicillin persist in blood and urine for prolonged periods.

Antipyrine, 2:3 dimethyl-1-phenyl pyrazolone 5-one shows antipyretic and analgesic effect. It relieves the patient from high fever. It has also weak antiseptic and anaesthetic action. It is oxidized to 4-hydroxy antipyrine (30-40 %) and excreted as glucuronide and sulphate. About 5 per cent is excreted unchanged. The fate of remainder is unknown. Structural formula of diminazene, procaine and antipyrine are shown in Fig. 1.

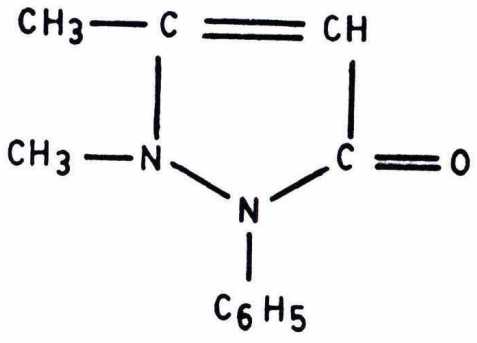
Fig. 1 Chemical structures of diminazene, procaine and antipyrine.



DIMINAZENE



PROCAINE



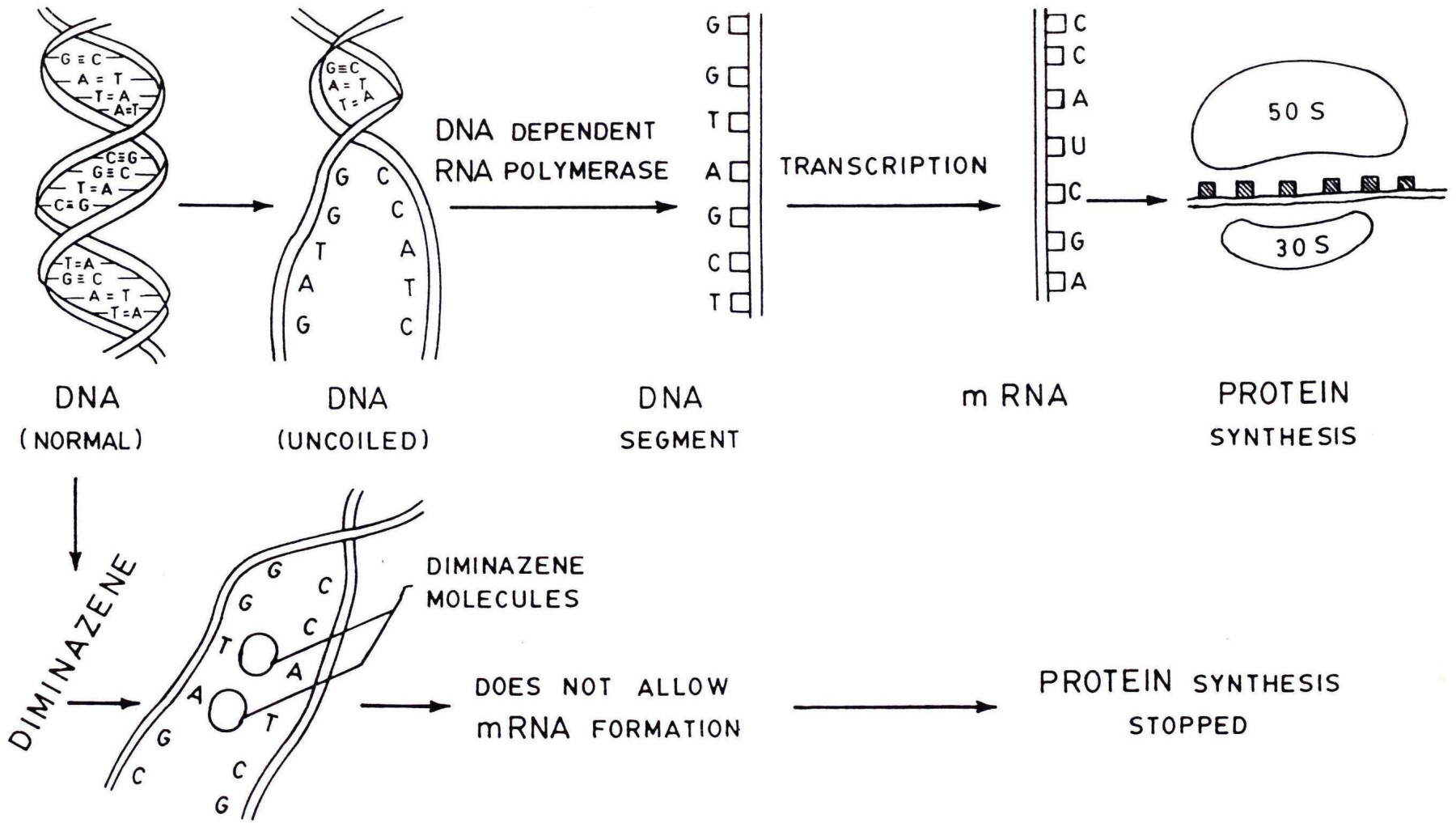
ANTIPYRINE

Mechanism of action of diminazene

Similar to other aromatic dimidines, the mode of action of diminazene is also related to interference with aerobic glycolysis of the parasite (Roberson 1982). All the aromatic diamidines are known to cause hypoglycemia in treated animals. Blood parasites, such as trypanosome depend upon host glucose for aerobic glycolysis.

Dimianzene aceturate has been established to selectively block kinetoplast DNA replication and to induce dyskinetoplasty (Newton 1975, Riou *et al* 1980). Diminazene is rapidly and irreversibly bound by trypanosome DNA-containing organelles, first in the kinetoplast and subsequently in the nucleus (Brack *et al* 1972a,b, Brack and Delain 1975). The ultraviolet microscopy of *T. brucei* isolated from rats before and after treatment with a curative dose of the drug revealed a brilliant blue fluorescence in the nucleus and kinetoplast of drug treated organism. The drug does not bind to DNA itself but across a small groove between the two complementary strands, resulting in distortion of the helical structure (Festy *et al* 1975). The relationship of the inhibition of kinetoplast DNA synthesis to trypanosome death and the significance of nuclear DNA binding of the drug are not clear (Riou *et al* 1980). The diagrammatic presentation of mode of action of diminazene has been shown in Fig. 2.

Fig. 2 Mode of action of diminazene.



MODE OF ACTION OF DIMINAZENE

In another study, it has been observed that administration of diminazene result in the release of trypanosomes from microcirculation to general circulation. It is suggested that drug does not kill trypanosomes directly but makes them accessible to other defense mechanism such as macrophage system (Maxie and Losos 1977).

Other effect of diminazene on trypanosome include an interaction with ribosomes *in vitro* and displacement of magnesium ions and inhibition of polyamines from the particles, modification of cytoplasmic and lysosomal membranes (Macadan and Williamson 1972), inhibition of phospholipid synthesis, basic amino acid transport and oxygen uptake (Hill and Hunter 1968, Gutteridge 1966, 1969).

Diminazene is reported to be actively absorbed by the parasites and host cells, leading to high concentration of drug in the cells within a short time and it is rapidly excreted from host system by kidney (Hawking and Sen 1960).

General pharmacokinetics

Pharmacokinetics is defined as behaviour of drug in body system, expressed in mathematical term which includes the rates of absorption, distribution, metabolism and excretion of drug in body system. An understanding of pharmacokinetics has two valid purposes. The principle of kinetic disposition ensure safety and we find a way to optimize the effective dosage regimen, nevertheless, *in vivo*, the efficacy of drug is modulated by

disease induced several alterations. Inspite of all the uncertainties, the scientific approach to recommend a dose of a chemotherapeutic agent is already based on its detailed pharmacokinetic study.

To study the pharmacokinetics of drug, the body is subjected to different compartments, these compartments are mathematical entities and have no physiological meaning (Riegelman *et al* 1968). Another way to calculate the pharmacokinetics is by non compartmental method.

To determine the pattern of kinetic disposition, the drug concentration in blood is plotted against time on semilogarithmic scale. The number of phases assigned the number of compartments if a mathematical model is to employ.

In one compartment open model, the semilogarithmic plot of blood drug concentration as a function of time shows a straight line. The rate of drug elimination from body is directly proportional to the concentration of drug in blood. The plasma drug level declines according to the equation :

$$Cp = Be^{-\beta t}$$

Two compartment open model is demonstrated with initial sharp decline followed by gradual departure of drug from the central compartment, while plotting the blood drug levels time profile. The early steep decline of plasma drug concentration is due to distribution of drug from central to peripheral compartment and gradual fall is mainly due to irreversible elimination (β). In two compartment open model, the distribution and

elimination processes are assumed to follow the first order kinetics and the plasma concentration of drug is expressed according to bi-exponential equation :

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

Following i.m. administration, if three distinct phases i.e. absorption, distribution and elimination are observed then pharmacokinetics is determined by two compartmental open model using triexponential equation :

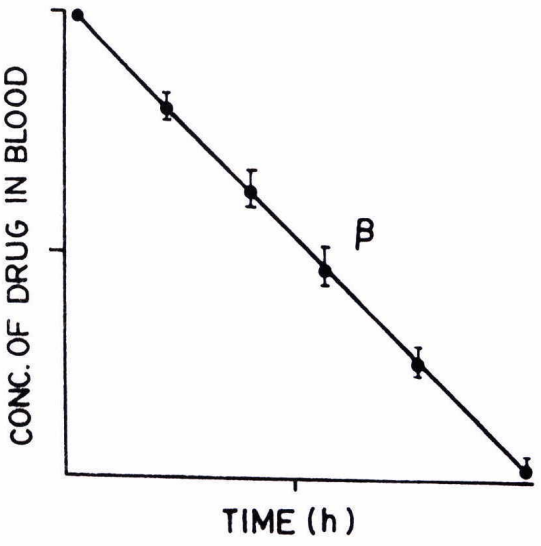
$$Cp = Ae^{-\alpha t} + Be^{-\beta t} - A' e^{-Kat}$$

The kinetic disposition of diminazene was determined by two compartment open model (Gummow *et al* 1994, Madachi *et al* 1995, Kellner *et al* 1985, Klatt and Hajdu 1976). Except these models, the distribution kinetics of some chemotherapeutic agents are also known to follow three or four compartment open model. Fig. 3, depict various pharmacokinetic models of drug after their single i.v. and i.m. administration.

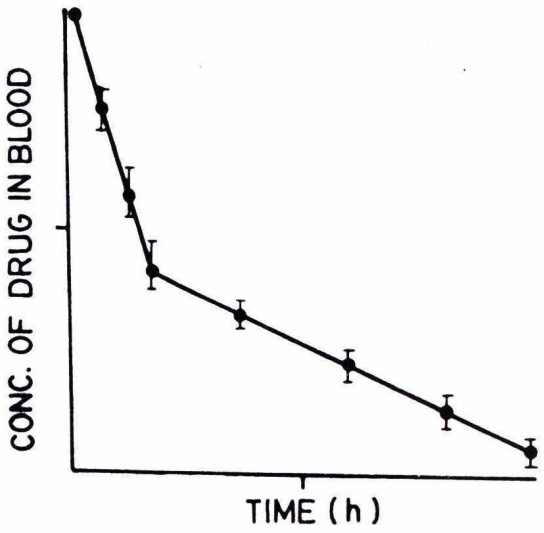
Some most important pharmacokinetics parameters are β, $V_{d(ss)}$ and Cl_B. Over all elimination rate constant (β) is important kinetic determinant as it is used to determine the values of elimination *half life, volume of distribution*, total body clearance and transfer of drug from central to peripheral compartment and vice versa. The apparent volume of distribution is defined as volume of body fluids required to dissolve the same amount of drug, as

Fig. 3 Diagramatic representation of various open compartment models of body.

AFTER I.V. ADMINISTRATION

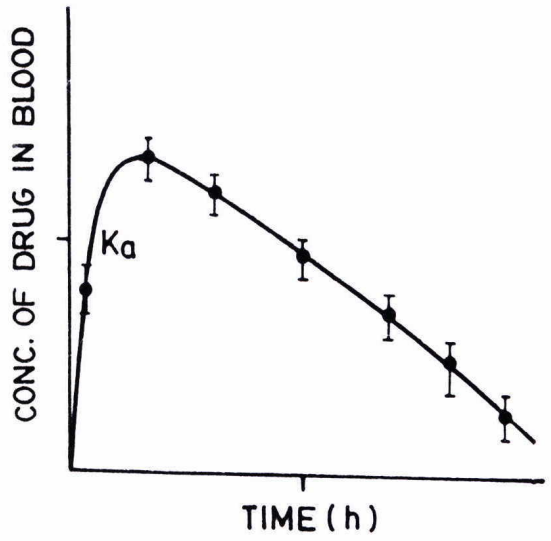


ONE COMPARTMENT



TWO COMPARTMENT

AFTER i.m. ADMINISTRATION



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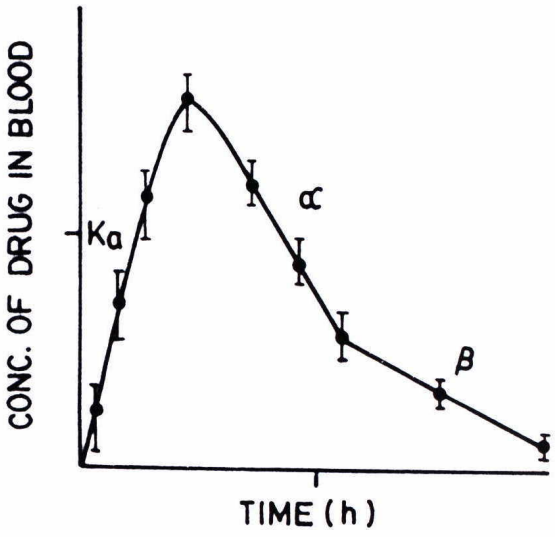


TWO COMPARTMENT

present in blood. It is important kinetic constant for computing the dosage regimen. Another useful parameter, total body clearance gives the sum of all clearance processes (Gibaldi and Perrier 1982). Various kinetic parameters along with their unit have been defined in Tables 1, 2 and 3.

Pharmacokinetics of diminazene

Pharmacokinetic data of antiprotozoan drugs have been extensively generated and published. In the present study the relevant work on pharmacokinetics of diminazene and interaction between drugs at pharmacokinetic level have been reviewed.

Reather *et al* (1972) compared the plasma levels and pharmacokinetics of diminazene in goats following i.m. and i.v. administration. Diminazene was given at dose of 2 $mg.kg^{-1}$ by i.v. route. After a gap of 6 weeks, diminazene was administered by i.m. route at dose of 3.5 $mg.kg^{-1}$. After i.v. administration, the drug disappearance from plasma followed triexponential phases. The total body clearance was 0.072 ± 0.007 $ml.kg^{-1}.h^{-1}$. After i.m. administration the peak plasma level (3.9-4.5 $\mu g.ml^{-1}$) was observed at 48 min. The decline of plasma drug concentration also followed triexponential equation and the systemic availability ranged from 43.5 to 63.8 per cent.

Gilbert and Newton (1982) determined the pharmacokinetic pattern of diminazene aceturate following its single intramuscular administration (3.5 $mg.kg^{-1}$) in uninfected and *T. congolense* infected rabbits. They have estimated the drug in plasma as well as in interstitial fluid at different time

Table 1. Definition of pharmacokinetic parameters related to absorption of drug

Parameter	Unit	Definition
A'	$\mu g.ml^{-1}$	Zero time plasma drug concentration intercept of regression line of absorption phase.
Ka	h^{-1}	Absorption rate constant
$t_{0.5}Ka$	h	Absorption half life
Ac	$\mu g.ml^{-1}$	The amount of drug in central compartment
A_T	$\mu g.g^{-1}$	The amount of drug in peripheral compartment
F	Per cent	The bioavailability of drug after extravascular administration
t_{max}	min or h	Time of peak plasma concentration
C_{max}	$\mu g.ml^{-1}$	Peak plasma concentration

Table 2. Definition of kinetic parameters related to distribution of drug

Parameter	Unit	Definition
Cp^o	$\mu g.ml^{-1}$	Plasma drug concentration immediately following intravenous injection of single dose.
A	$\mu g.ml^{-1}$	Zero-time plasma drug concentration intercept of regression line of distribution phase.
α	h^{-1}	Distribution rate constant
$t_{0.5}\alpha$	h	Distribution half life
K_{12}	h^{-1}	The rate of transfer of drug from central (blood) to peripheral (tissue) compartment
K_{21}	h^{-1}	The rate of transfer of drug from peripheral to central compartment
V_c	$L.kg^{-1}$	Volume of central compartment.
V_d	$L.kg^{-1}$	Volume of body fluids having the same concentration as in blood and calculated by various methods and is represented as : $V_{d(area)}$, based on total area under the plasma drug concentration time curve. $V_{d(\beta)}$, based on the volume of central compartments and elimination rate constants. $V_{d(ss)}$, based on average steady state plasma level $V_{d(ext)}$, calculated by extrapolation method.
AUC	$\mu g.ml^{-1}.h$	Total area of body which is under the plasma drug concentration time curve.
AUMC	$\mu g.ml^{-1}.h^2$	Total area under the first moment of plasma drug concentration time curve.
MRT	h	Mean residual time.
T/P	Ratio	Tissue/plasma ratio of drug concentration.

Table 3. Definition of kinetic parameters related to elimination fo drug

Parameter	Unit	Definition
B	$\mu g.ml^{-1}$	Zero time plasma drug concentration intercept of regression line of elimination phase.
β	h^{-1}	Overall elimination rate constant.
$t_{0.5}\beta$	h	Elimination half life.
$t_{0.9}\beta$	h	Time required for 10 per cent excretion of drug.
K_{el}	h-1	The first order elimination rate constant from central compartment
Cl_T	$ml.kg^{-1}.h^{-1}$	Total body clearance of drug
t_d	h	Duration of pharmacological effect.
D	$mg.kg^{-1}$	Priming dose
D'	$mg.kg^{-1}$	Maintenance dose

intervals. The peak plasma levels (1.1 $\mu g.ml^{-1}$) was detected within an hour of administration which showed a rapid initial fall. Thereafter the drug slowly reduced in blood over next six days. The pharmacokinetics of diminazene aceutrate was very similar in both infected and uninfected rabbits. In a similar experiment, Gilbert (1983) showed biphasic decline in plasma drug concentration of diminazene aceturate after its single i.m. administration (3.5 $mg.kg^{-1}$) in rabbits. The maximum blood and interstitial fluid concentration were recorded at 15 min and 3 h, respectively.

Kellner *et al* (1985) administered diminazene aceturate at dose rate of 3.5 $mg.kg^{-1}$ body weight by i.m. route to two healthy male calves. The blood levels of 4.6 and 4.7 $\mu g.ml^{-1}$ occurred at 15 and 45 min, respectively. Decrease in drug concentration in blood followed a biphasic curve with biological half life of 2 and 188 h.

Aliu *et al* (1993) studied the disposition kinetics and bioavailability of diminazene (3.5 $mg.kg^{-1}$) in five healthy heifers after a single i.v. and i.m. administration. Non linear regression analysis curves of diminazene were best described by triexponential equation. The volume of distribution, elimination half life and total body clearance were 1.91 ± 0.42 $L.kg^{-1}$, 37.1 h and 1.74 ± 0.40 $ml.min^{-1}.kg^{-1}$, respectively. Within 30 minutes of i.v. dose, erythrocyte/plasma partition ratio of diminazene was 0.30 ± 0.15. Diminazene was rapidly absorbed following i.m. administration. The highest concentration

(4.68 ± 1.12 $\mu g.ml^{-1}$) in plasma was attained within 10-15 minutes. The value of absorption half life and systemic availability was reported to be 0.60 h and 102.42 ± 7.25 per cent, respectively. The half life of terminal disappearance phase was 145.5 h.

The plasma and CSF concentration of diminazene in goats following its single i.m. injection at 3.5 $mg.kg^{-1}$ body weight was determined. The mean maximum concentration in CSF and plasma were 1.04 ± 0.81 and 4.31 ± 0.22 $\mu g.ml^{-1}$, respectively. The value of tmax at which maximum concentration occurred in CSF and plasma were 2 ± 0.09 and 0.41 ± 0.29 h, respectively. Values for area under the curve, overall clearance rate and steady state volume of distribution also differed significantly between CSF and plasma (Peregrine and Mamman 1993 and Mamman and Peregrine 1994).

Mamman *et al* (1993) compared the pharmacokinetics of diminazene aceturate in plasma, cerebrospinal fluid (CSF) and lymph of goats following its single injection at the dose rate of 3.5 $mg.kg^{-1}$. The concentration of diminazene in plasma was 2-3 times higher than that of CSF. The concentration in lymph were initially similar to those in CSF, but later to those in plasma. The C_{max} of diminazene in plasma (4.53 ± 0.41 $\mu g.ml^{-1}$) was significantly higher than that in CSF (0.96 ± 0.59 $\mu g.ml^{-1}$) and lymph (1.21 ± 0.58 $\mu g.ml^{-1}$). The total body clearance of diminazene determined for the plasma (0.69 ± 0.11 $ml.min^{-1}.kg^{-1}$) was greater than in CSF (0.23 ± 0.10 $ml.min^{-1}.kg^{-1}$) and lymph (0.13 ± 0.11 $ml.min^{-1}.kg^{-1}$). The mean residence time

of drug in plasma (57.27 ± 16.49 h) was shorter than in CSF (892.72 ± 12.34 h) and lymph (2432 ± 3080.39 h). The results indicate that although diminazene attains significantly higher levels in plasma than CSF and lymph, it persists longer in the CSF and lymph than in plasma.

In a comparative study of pharmacokinetics of diminazene in *T. congolense* infected and healthy female Boran cattle, the drug was injected in healthy and acute and chronic phase of infection as single i.m. dose of 3.5 $mg.kg^{-1}$ body weight (7 %). There was no significant difference between values of pharmacokinetic parameters obtained in chronic *T. congolense* infected cattle as compared to uninfected animal (Mamman *et al* 1993).

The biequivalence and pharmacokinetics of two commercial formulations of diminazene aceturate was evaluated when given intramuscularly to cattle (3.5 $mg.kg^{-1}$). Veribren was equivalent to berenil with respect to area under plasma concentration versus time curve, but not in terms of Cmax (maximum plasma drug concentration) and tmax (time to reach maximum plasma drug concentration). Two compartment open model best described the pharmacokinetic behaviour of diminazene in cattle. Peak concentration of diminazene (3.24 ± 0.16 $\mu g.ml^{-1}$) was reached at 49.8 ± 7.6 minutes. The values of $t0.5\alpha$ (1.93 ± 0.85 h), $t0.5\beta$ (222 h) and mean residence time (13.27 days) were also determined (Gummow *et al* 1994).

Sardar *et al* (1994) have determined the plasma concentration and pharmacokinetics of diminazene in female cross bred Jersey calves after its

i.v. administration (16 mg.kg^{-1} as 16 %). At 0.5 h, the plasma concentration was 30.25 ± 1.37 μg.ml^{-1} which declined to 11.83 ± 0.88 μg.ml^{-1} at 24 h. The apparent volume of distribution was 0.98 ± 0.120 L.kg^{-1}. The total body clearance and elimination half life were 0.229 ± 0.006 ml.kg^{-1}.min^{-1} and 48.064 ± 7.32 h, respectively.

In a study on pharmacokinetics of diminazene in buffalo species, Singh (1997) evaluated the therapeutic efficacy and alteration in disposition kinetics of diminazene in experimental trypanosomiasis in buffalo calves. Diminazene was given intramuscularly at dose rate of 3.5 mg.kg^{-1}. Following single i.m. administration the plasma level of diminazene at 1, 2, 4, 6, 12, 24 and 48 h intervals were 3.07 ± 0.220, 2.85 ± 0.171, 2.49 ± 0.134, 2.49 ± 0.166, 2.05 ± 0.,145, 1.95 ± 0.113 and 1.73 ± 0.056 μg.ml^{-1}, respectively. The plasma level of 0.3-0.6 μg.ml^{-1}, have been considered to be minimum effective concentration of diminazene against most trypanosome species.

Kaur (1998) studied the pharmacokinetics, urinary excretion and dosage regimen of diminazene in cross bred calves. The drug was administered (3.5 mg.kg^{-1}) by i.m. and i.v. routes. Following i.v. administration, at 1 min, the peak plasma concentration was 29.1 ± 6.55 μg.ml^{-1} which declined to 3.15 ± 0.27 μg.ml^{-1} at 120 h. After i.m. administration, the peak plasma concentration at 1 min was 2.64 ± 0.19 μg.ml^{-1} which rise to 7.42 ± 0.11 μg.ml^{-1} at 45 min. After i.v. administration, the distribution rate constant

(α), $V_{d(area)}$, $t_{0.5}\beta$ and $t_{0.5}\alpha$ were 7.824 ± 1.752 h^{-1}, 0.74 ± 0.07 $L.kg^{-1}$, 135.2 ± 13.7 h and 0.106 ± 0.016 h, respectively.

The pharmacokinetic data of diminazene following single i.m. dose (3.5 $mg.kg^{-1}$) in cattle and buffalo have been compared in Table 4.

To observe the influence of infection on disposition pattern of diminazene, Onyeyili and Anika (1989) administered diminazene aceturate (3.5 $mg.kg^{-1}$, i.v.) to Mongrel dogs before and after infection with *T. congolense*. The mean value of AUC of diminazene in healthy dogs (25.8 $\mu g.ml^{-1}.h^{-1}$) was significantly lesser than in diseased animals. The distribution half life of drug before and after the infection was 0.17 and 0.12 h, respectively. A safe dose of 0.2 to 3.5 mg diminazene per kg body weight has been recommended for control of *T. congolense* infection. Further, they have also reported that in dogs, diminazene should be used with caution because compound has narrow therapeutic index in this species.

Interaction between drugs

Drug interaction follow the simultaneous use of two or more drugs. With the increasing complexity of drug therapy and use of multiple drugs in the treatment of disease, the concept of drug interaction has became very important. In veterinary practice, it has become very common to use different drugs in combination such as antibiotics are frequently used in combination with antipyretic drugs. The drug interaction can be considered at two levels (a) pharmacodynamic drug interaction, (b) pharmacokinetic drug interaction. The

Table 4. Comparison of pharmacokinetics of diminazene (3.5 $mg.kg^{-1}$) in cattle and buffalo after single intramuscular administration

Kinetic contents	Unit	Cattle[a]	Buffalo[b]
A'	$\mu g.ml^{-1}$	3.17±0.97	0.76±0.25
Ka	h^{-1}	9.86±3.06	78.8±12.3
$t_{0.5}Ka$	h	0.121±0.040	0.010±0.002
B	$\mu g.ml^{-1}$	4.48±0.99	2.70±0.15
β	h^{-1}	0.0066±0.0005	0.009±0.018
$t_{0.5}\beta$	h	107.5±8.50	67.9±10.4
AUC	$\mu g.ml^{-1}.h$	687.3±166.0	360.1±76.1
AUMC	$\mu g.ml^{-1}.h^2$	104945.6±29858.9	65325.8±29464.5
MRT	h	155.3±12.3	141.7±0.18
td	h	194.4±15.4	730.2±591.6

[a]Kaur (1998)

[b]Singh (1997)

pharmacokinetic interaction has further been grouped into following types : (i) interaction during absorption phase, (ii) interaction during distribution and metabolism phase and (iii) interaction during excretion phase.

It has been shown that when drugs are given in combination, their pharmacokinetics may differ from their kinetic pattern when they are administered alone. Recently, a new preparation (Trypan), a combination of diminazene diaceturate with local anaesthetic procaine and a antipyretic drug antipyrine have been launched in the market and being used in clinical cases of trypanosomiasis in western countries. It is expected that local anaesthetic procaine and antipyretic drug antipyrine may alter the pharmacokinetics of diminazene. There is no report available on interaction between these three drugs, however, sufficient literature on pharmacokinetic interaction of other two drugs are available. Here some of such most relevant literature related to pharmacokinetic interaction between drugs have been reviewed.

Klatt and Hajdu (1976) compared the pharmacokinetics of diminazene by administering it alone (2.5 $mg.kg^{-1}$) and in combination with rolitetracycline. Following alone administration of diminazene, appreciable serum level (2.12 $\mu g.ml^{-1}$) was detected at 5 min. The peak plasma concentration (C_{max}) of 3.23 $\mu g.ml^{-1}$ was achieved at 10 min, which gradually declined to 1.15 $\mu g.ml^{-1}$ in 24 h. The values of elimination rate constant, elimination half life and volume of distribution were $0.11 \pm 0.006\ h^{-1}$, 63.0 h and 59.0 per cent, respectively. After

administration of diminazene (2.5 $mg.kg^{-1}$) in combination with rolitetracycline, the initial serum diminazene concentration was almost similar to its alone administration. The elimination rate constant, elimination half life and volume of distribution of diminazene when given in combination with rolitetracycline were $0.25 \pm 0.10\ h^{-1}$, 2.7 ± 0.75 h and 57 per cent, respectively.

Vorobev *et al* (1988) studied the effect of polyethylene glycol on azidine (diminazene analogue) when administered concomitantly in dairy cows. It was found that polyethylene glycol prolong the prophylactic effect of azidine.

In another set of studies, following simultaneous administration of DL-α-difluoromethylarnithine (DFMO) and berenil, the therapeutic and prophylactic activity of diminazene against trypanosoma infection in mice was noted by Onyeyili *et al* (1993). They reported that combination was curative and acted synergisically. But prophylactically, the combination had no advantage over diminazene aceturate alone.

It has been reported that concomitant administration of verapamil and berenil, resulted in enhancement of drug effect as shown by increased formation of dyskinetoplastic organisms, increased rates of clearing parasites from blood and enhanced survival of infected mice (Agbe and Yielding 1993). In the treatment of theileriosis, it was reported that the combination of oxytetracycline and berenil was more effective than oxytetracycline alone (Bandopadhyay *et al* 1994).

Odika *et al* (1995) reported that against the relapse infection of *Trypanosoma brucei. brucei* in rats, therapeutically the combination of diminazene aceturate (7 $mg.kg^{-1}$) and lithium chloride (10 $\mu g.kg^{-1}$) was more effective than diminazene aceturate alone and/or diminazene aceturate, lithium chloride and dexamethasone combination.

Anene *et al* (1997) have studied that simultaneous administration of difluoromethylornithine (DFMO) and diminazene aceturate was more effective because no relapses occurred in trypanosoma infected dogs.

In an extensive study on interaction between an antipyretic drug, paracetamol and a cephalosporin antibiotic, cefotaxime, Sharma and Srivastava (1997) reported that following concurrent administration of cefotaxime (10 $mg.kg^{-1}$, i.v.) and paracetamol (50 $mg.kg^{-1}$, i.m.) in cross bred calves, the therapeutic plasma concentration of cefotaxime ($\geq$ 0.2 $\mu g.ml^{-1}$) was maintained for longer duration as compared to its alone administration. The values of $t_{0.5}\beta$, $V_{d(area)}$ and Cl_B of cefotaxime, when administered alone were 2.54 h, 2.37 $L.kg^{-1}$ and 992.9 $ml.kg^{-1}.h^{-1}$, respectively which was altered to 3.74 h, 4.44 $L.kg^{-1}$ and 835.7 $ml.kg^{-1}.h^{-1}$, respectively when administered along with paracetamol.

In another set of experiment as explained in Table 5, the influence of paracetamol on pharmacokinetic behaviour of cefuroxime, a IIIrd generation cephalosporin was investigated in cow calves and buffaloes. Similarly in

Table 5. Influence of paracetamol on pharmacokinetics of cefuroxime[a]

Parameter	Unit	Cefuroxime (10 mg/kg)	Cefuroxime (10 mg/kg) + paracetamol (50 mg/kg)
		Cow calves	
$t_{0.5}\alpha$	h	0.06±0.01	0.14±0.03
$t_{0.5}\beta$	h	1.47±0.07	1.65±0.20
Vd(area)	$L.kg^{-1}$	0.32±0.08	0.33±0.11
Cl_B	$ml.kg^{-1}.h^{-1}$	146.9±29.0	128.2±23.0
		Buffalo calves	
$t_{0.5}\alpha$	h	0.10±0.01	0.10±0.01
$t_{0.5}\beta$	h	1.82±0.13	1.91±0.07
Vd(area)	$L.kg^{-1}$	0.68±0.09	0.55±0.12
Cl_B	$ml.kg^{-1}.h^{-1}$	253.9±17.1	194.2±35.9

[a]Srivastava and Chaudhary (1999)

Table 6, the influence of salicyclate, another antipyretic drug on pharmacokinetics of kanamycin, an aminoglycoside antibiotic has also been reported (Srivastava and Chaudhary 1999).

Shivprakash *et al* (1996) studied the effect of aspirin on pharmacokinetics of ciprofloxacin in rabbits. Seven days of aspirin (300 $mg.kg^{-1}$) pretreatment prolonged the elimination half life and reduced the total body clearance of ciprofloxacin (5 $mg.kg^{-1}$).

Manna *et al* (1994) studied the effect of oxytetracycline on the pharmacokinetic behaviour of paracetamol when administered concomitantly in goats. It was reported that elimination half life of paracetamol was increased when it was administered along with oxytetracycline, as compared to its alone administration.

Urinary excretion of diminazene

The urinary excretion of diminazene at dose rate of 3.5 $mg.kg^{-1}$ body weight was investigated in healthy male calves after i.m. administration (Kellner *et al* 1985). It was reported that after 7 days of treatment about 47.1 per cent of dose was excreted in urine and 7.1 per cent in faeces. Respective values were 72.2 per cent and 10.3 per cent after 20 days of treatment. The half life of diminazene in urine was similar to those of blood. The main product in urine was unchanged diminazene.

Table 6. Influence of salicylate on pharmacokinetics of kanamycin[a]

Parameter	Unit	Kanamycin (10 mg/kg)	Kanamycin and salicylate (44 mg/kg)
$t_{0.5}\alpha$	h	0.123±0.006	0.116±0.009
$t_{0.5}\beta$	h	1.94±0.11	2.05±0.019
$V_{d(area)}$	$L.kg^{-1}$	0.259±0.009	0.289±0.005
Cl_B	$ml.kg^{-1}.h^{-1}$	92.9±3.69	97.7±2.08

[a]Srivastava and Chaudhary (1999)

Aliu *et al* (1993) studied the excretion of drug in urine following administration of diminazene at dose rate of 3.5 $mg.kg^{-1}$ intramuscularly in healthy heifers. About 8.26 per cent of total dose was excreted in urine of heifer within the first 24 h of treatment. Gilbert and Newton (1982) studied the excretion pattern of diminazene aceturate following its single intramuscular administration (2.5 $mg.kg^{-1}$) in uninfected and *T. congolense* infected rabbits. Approximately 65 per cent of total administered dose was eliminated (44 % in urine and 21 % in faeces). The rate of urinary excretion was similar throughout this period while the excretion of drug in faeces was mainly after seven days.

Onyeyili and Anika (1989) adminsitered diminazene aceturate at dose rate of 3.5 $mg.kg^{-1}$ body weight to Mongrel dogs before and after infection with *T. congolense* and studied the excretion of drug in urine. The mean proportion of diminazene aceturate recovered in urine of infected dogs (25.1 %) was not significantly different from that of healthy dogs (26.8 %). It was concluded that infection with *T. congolense* has no great influence on urinary excretion of drug. However, in another experiment Gilbert (1983) reported that 40-50 and 8-20 per cent of diminazene was excreted in urine and faeces, respectively, seven days after its i.m. administration of 3.5 $mg.kg^{-1}$ in rabbits.

Kaur (1998) extensively studied the excretion of drug in urine in crossbred cow calves after its single i.m. administration (3.5 $mg.kg^{-1}$). Following administration the diminazene concentration in urine at 4, 8, 12 and

24 h time intervals were 58.3, 330.5, 142.6 ± 39.1 and 148.0 ± 13.2 $\mu g.ml^{-1}$, respectively. Approximately, 65 per cent of total administered dose was eliminated in urine within 24 h after drug administration.

Erythrocytic partitioning of diminazene

Singh (1997) has reported that at different blood concentrations of 10, 15 and 20 $\mu g.ml^{-1}$, drug penetrated into erythrocytes to the extent of 58.3, 51.3 and 49.7 per cent, respectively with an overall mean of 53.09 ± 2.64 per cent in buffalo calves.

Penetration of diminazene in the erythrocytes of cross bred cow calves was studied by Kaur (1998). At various blood concentrations ranging from 6.25 to 50 $\mu g.ml^{-1}$, the extent of penetration of diminazene into erythrocytes was 48.1 ± 4.87 per cent.

Plasma protein binding of diminazene

The *in vitro* binding of diminazene to plasma albumin of healthy heifers was studied by Aliu *et al* (1993). They reported that diminazene was bound to plasma albumin to the extent of 38.0 to 91.1 per cent.

Kaur (1998) studied the *in vitro* binding of diminazene to plasma proteins of cross bred calves. At different concentrations of 3.125, 12.5 and 25 $\mu g.ml^{-1}$, diminazene bound to plasma proteins to the extent of 51.8, 34.0 and 33.1 per cent, respectively with an overall mean of 32.3 ± 4.67 per cent.

CHAPTER III

MATERIALS AND METHODS

Experimental animals

Eleven healthy male buffalo calves of 1 to 1½ years and weighing between 50 to 136 kg were purchased from local market. Each animal was quarantined for 2 weeks. The animals were housed in clean barns with concrete floor. Animals were maintained on available green fodder of the season and wheat straw. They had free access to water. All the subjects were healthy at the time of experiment.

Drugs/chemicals

The list of important drugs and chemicals used in the present study is given below alongwith their sources/manufacturers

Sr.no.	Drug/chemical	Manufacturer
1	Trypan (a) Granules (b) Suspension	Chamberley,Germay Atarost, Germany
2	Berenil	Hoechst, India
3.	Trichloroacetic acid	SISCO, Research Laboratories pvt. Ltd. Mumbai, India.
4.	Hydrochloric acid	E. Merck (India) Ltd. Shiv Sagar Estate A, Dr.Annie Basant Road, Warli, Mumbai
5.	Sulphamic acid	s.d. Fine-chem Ltd. Mumbai, India
6.	Sodium nitrite	Qualigens Fine Chemicals, Mumbai, India
7.	NEDD	SISCO, Research Laboratories pvt. Ltd. Mumbai, India.

All other extrapure quality chemicals used in the present study were of analytical grade and obtained from reputed companies.

Trypan

The drug was courtesy gift from companies "Chamberley and Atarost" of Germany. It was supplied in two dosage forms (I) granular form and (ii) suspension. The composition of these dosage forms were, diminazene diaceturate (1.05 g), procaine (0.06 g) and antipyrine (1.25 g). In suspension concentration of diminazene diaceturate, procaine hydrochloride and antipyrine were 7, 0.4 and 8.3 per cent, respectively.

Experimental design

The experimental work was designed and undertaken on the following aspects :

I. Effect of procaine and antipyrine on pharmacokinetics of diminazene, when administered in granular form.

(a) Plasma levels

(b) Pharmacokinetics

(c) Feathered levels in central and tissue compartments

(d) Urinary excretion

(e) Calculation of dosage regimen

2. Effect of procaine and antipyrine on pharmacokinetics of diminazene, when administered in suspension form

   (a) Plasma levels

   (b) Pharmcokinetics

   (c) Feathered levels in central and tissue compartments

   (d) Urinary excretion

   (e) Calculation of dosage regimen

3. *In vitro* erythrocytic partitioning of diminazene

4. *In vitro* plasma protein binding of diminazene

Plan and Methodology

Grouping of animals

Eleven healthy male buffalo calves were divided into three groups of 4 animals each in group I and II and 3 animals in group III. The detailed study conducted on animals of these groups are presented in Table 7. The animals of group I were used to study the plasma levels, pharmacokinetics and urinary excretion of diminazene when administered along with procaine and antipyrine in granular form. The animals of group II were used to study the plasma levels, pharmacokinetics and urinary excretion of diminazene when administered along with procaine and antipyrine in suspension form. The animals of group III were untreated and used to study the *in vitro* erythrocytic partitioning and plasma protein binding.

Table 7. Experimental schedule to study pharmacokinetics and urinary excretion of diminazene in combination with procaine and antipyrine in buffalo calves

Group	Animal number	Drug used	Dose ($mg.kg^{-1}$)	Drug dosage form	Route	Study conducted
I	A_1,A_2, A_3, A_4	Trypan	7.86	Granules	i.m.	Plasma levels and pharmacokinetics
	A_1, A_2, A_3, A_4	Trypan	7.86	Granules	i.m.	Urinary excretion
II	B_1, B_2, B_3, B_4	Trypan	7.86	Suspension	i.m.	Plasma levels and pharmacokinetics
	B_1, B_2, B_3, B_4	Trypan	7.86	Suspension	i.m.	Urinary excretion
III	C_1, C_2, C_3	-	-	-	-	*In vitro* erythrocytic partitioning *In vitro* plasma protein binding

Trypan - 2.36 g granules of drug contain 1.05 g diminazene diaceturate; 0.06 g procaine; 1.25 g antipyrine

Administration of drug

To study the pharmacokinetics and urinary excretion of diminazene, the experiments animals were kept in metabolic stalls of standard size. The metabolic stalls were prepared in such a way that whole amount of urine voided by animals can be collected at any time without any spillage and contamination. The urine passed by animals were directly collected in bottle fixed underneath the floor. Constant supervision was provided to avoid any contamination of urine with faeces. The animals were acclimatized for the collection of blood and urine prior to the start of experiment.

The drug was administered by single i.m. injection in gluteal muscle, once animal was settled in metabolic stall.

Dosage and route of drug

Diminazene diaceturate was administered at the dose rate of 3.5 $mg.kg^{-1}$ by single intramuscular route in combination with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) in granules and suspension form both. In case of granules, the solution was freshly prepared as 15.73 per cent. By doing so, the concentration of diminazene diaceturate, procaine and antipyrine became 7, 0.4 and 8.3 per cent, respectively.

Collection of samples

(a) Blood

Blood samples were taken from jugular vein into heparinized glass test tubes at various predetermined time intervals. Plasma was separated at room temperature after centrifugation at 3000 rpm for 15 min. The time intervals for the collection of blood samples in different groups of animals were listed in Table 8.

(b) Urine

Urine was collected from the animals of groups I and II at various predetermined time intervals as shown in Table 8. At these time intervals, the volume of urine voided by individual animals was measured. 10 ml of urine was filtered and collected for drug analysis.

After collection, the samples of plasma and urine were stored at -20°C till analysis.

Estimation of diminazene

The concentration of diminazene in plasma and urine was estimated by diazotization reaction as explained by Klatt and Hajdu (1976) and subsequently modified by Srivastava and Bal (1995). The assay could detect minimum of 0.77 $\mu g.ml^{-1}$ of diminazene. The value of correlation coefficient was more than 0.97. The repeatability of this method was excellent and error

Table 8. Schedule for collection of blood and urine samples of buffalo calves after single intramuscular administration of diminazene in combination with procaine and antipyrine

Biological fluid	Time of collection of samples after drug administration
Blood	1, 2.5, 5, 7.5, 10, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144, 168 h
Urine	2, 4, 6, 8, 10, 12, 18 and 24 h

within day estimation was less than 4.5 per cent. The relevant details of this method are given below :

Reagents

1. Trichloracetic acid, 10 %
2. Hydrochloric acid, 1 N
3. Sodium nitrite, 0.5 %
4. Sulphamic acid, 1 %
5. Alpha-Naphthyl - ethylene diamino dihydrochloride (NEDD), 0.2 %

Procedure

1. Two ml of plasma was added to two ml of trichloroacetic acid (10 %). The mixture was thoroughly shaken and kept at room temperature for 5 min for complete deproteinization. The mixture was centrifuged for 15 min.
2. One ml of supernatant was taken into a test tube. To this 1 ml of hydrochloric acid (1N) was added. The diazotisation reaction was started with addition of 1 ml of sodium nitrite (0.5 %).The solution was mixed thoroughly and allowed to stand for exactly 3 minutes at room temperature.
3. To this mixture 1 ml of sulphamic acid (1 %) was added. Again mixture was shaken thoroughly and allowed to stand for 3 minutes at room temperature.

4. To this 1 ml of NEDD (0.2 %) was added. The final mixture was shaken and absorbance was recorded at 540 nm against blank.

5. Blank was simultaneously run in the same way using 2 ml of distilled water at place of sample.

6. The exact concentration of diminazene was calculated and corrected for the endogenous interfering substances in sample by preparing standard curve in plasma of untreated animals.

Recovery of diminazene

To study the recovery of diminazene in plasma of buffalo calves, different known concentrations of drug ranging from 2.5 to 20 $\mu g.ml^{-1}$ were added in plasma. The drug content in plasma was measured after 12 h of incubation. The results on recovery of diminazene is presented in Table 9. At lower concentration, the drug is recovered in good amount as compared to higher concentration.

Preparation of standard curve

To prepare the standard curve different known solution of diminazene in concentrations ranging from 0.78 to 50 $\mu g.ml^{-1}$ were prepared in plasma. These samples of plasma with known concentrations of diminazene were procured in a similar way as detailed above. For the preparation of standard curve in plasma, blood was collected from healthy and untreated buffalo calves of group III. The data of optical densities obtained for preparation of

Table 9. *In vitro* recovery of diminazene in blood of buffalo calves at different known blood concentrations

S. No.	Drug added in blood ($\mu g.ml^{-1}$)	Drug estimated in blood					Recovery (%)
		1	2	3	4	Mean ± SE	
1	20	6.64	5.53	5.10	9.25	6.63 0.93	33.1
2	10	4.32	2.96	4.27	4.10	3.91± 0.32	39.1
3	5	3.29	1.89	2.84	2.56	2.64±0.29	52.8
4	2.5	2.46	1.42	2.37	1.89	2.03±0.24	81.2

standard curve of diminazene in plasma is given in Table 10. The standard curve of diminazene in plasma of buffalo calves was linear in two phases i.e. (i) at lower concentration ranging from 0.78 to 3.12 $\mu g.ml^{-1}$ and (ii) at higher concentrations ranging from 6.25 to 50 $\mu g.ml^{-1}$. For the present study, the standard curve of lower concentrations was taken into consideration. Resulting, for calculating exact concentration of diminazene in plasma, two different factors depending upon O.D. of test sample were taken into account.

Pharmacokinetic analysis of data

The plasma concentration time profile of diminazene for each animal was used to determine disposition kinetics. Data was adequately described by two compartment open model. Pharmacokinetic analysis of the decline of drug concentration in plasma as a function of time yielded important hybrid parameters. Different pharmacokinetic parameters were analyzed according to the method of Gibaldi and Perrier (1982).

1. β, the overall elimination rate constant

$$\beta = 2.303 \times m$$

$$m \text{ (regression coefficient)} = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

Calculation of m is done by least square regression technique as detailed in Appendix.

Table 10. Optical densities for standard curve

Concentration of diminazene ($\mu g.ml^{-1}$)	Optical density* (O.D.)
0.78	0.041
1.56	0.078
3.12	0.097
6.25	0.135
12.5	0.210
25	0.415
50	0.840

*Values given are mean of 4 experiments

2. B, zero time plasma drug concentration intercept of the least square regression line of elimination phase.

$$B = \text{Antilog } (mX + C)$$

$$B = \text{Antilog of } C$$

3. α and A, hybrid rate constant associated with distribution of drug from central compartment and zero time plasma drug concentration intercept of regression line of distribution phase, respectively, α and A were calculated by resdiual concentrations as detailed in Appendix.

4. Ka and A', the hybrid rate constant of absorption of drug after intramuscular administration and zero-time plasma drug concentration intercept of regression line of absorption phase, respectively. Ka and A' were calculated by the method of residual yield as detailed in Appendix

5. $t_{0.5}(Ka)$, $t_{0.5}(\alpha)$ and $t0.5(\beta)$ absorption, distribution and elimination half-lives, respectively.

$$\text{Half life} = \frac{\ln 2}{\text{rate constant(s)}}$$

$$= \frac{0.693}{\text{rate constant(s)}}$$

6. $t_{0.9}\beta$, time required for 10 per cent excretion of drug.

$$t_{0.9}\beta = \ln 0.9/\beta$$

$$= 0.105/\beta$$

7. Cp^{o}, the expected concentration of drug in plasma at zero time.

$$Cp^{o} = A + B - A'$$

8. AUC, area under the curve, theoretical area of body which is covered with drug

For intramuscular study, $AUC = \frac{A}{\alpha} + \frac{B}{\beta} - \frac{A'}{Ka}$

9. AUMC, area under first moment of concentration time curve,

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} - \frac{A'}{Ka^2}$$

10. MRT, mean residence time,

$$MRT = \frac{AUMC}{AUC}$$

11. K_{el}, first order rate constant for elimination of the drug from the central compartment.

$$K_{el} = \frac{Cp^{o}}{AUC}$$

12. K_{21}, first order rate constant for transfer of drug from tissue to the central compartment.

$$K_{21} = \frac{A_\beta + B\alpha}{Cp^o}$$

13. K_{12}, rate constant for transfer of drug from central to tissue compartment.

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

14. Vc, the apparent volume of central compartment.

$$Vc = \frac{Dose}{Cp^o}$$

15. V_d, apparent volume of distribution.

(a) $V_{d(B)}$, based on zero time plasma drug concentration intercept of elimination phase.

$$V_{d(B)} = \frac{Dose\ (mg.kg^{-1})}{B}$$

(b) $V_{d(\beta)}$, based on volume of central compartment and elimination rate constant.

$$V_{d(\beta)} = \frac{Vc}{fc}$$

(c) $V_{d(ext)}$, calculated by extrapolation method

$$V_{d(ext)} = \frac{Vc\ (\alpha - \beta)}{(K_{21} - \beta)}$$

(d) $V_{d(ss)}$, based on average steady state plasma levels.

$$V_{d(ss)} = \frac{K_{12} + K_{21}}{K_{21}} . Vc$$

16. Cl_T, the total body clearance which is the sum of all clearance processes in the body

$$Cl_T = \frac{D}{AUC} \times 1000$$

17. Fc, the fraction of administered dose present in the central compartment.

$$Fc = \frac{\beta}{K_{el}}$$

18. T/P ratio, tissue to plasma ratio

$$\text{T/P ratio} = \frac{1}{fc} - 1$$

19. t_d, duration of pharmacological effect (Levy and Nelson, 1965)

$$t_d = \frac{2.3}{\beta} \log \frac{Ao}{A\ min}$$

Where Ao = dose, A min = minimum effective concentration

20. Ac, amount of drug in central compartment

$$Ac = \frac{Ao\ (\alpha - K_{21})}{\alpha - \beta} . e^{-\alpha t} - \frac{Ao\ (K_{21} - \beta)}{\alpha - \beta} . e^{-\beta t}$$

21. A_T, amount of drug in the peripheral compartment

$$A_T = \frac{K_{12}\ Ao}{\alpha - \beta} .e^{-\alpha t} + \frac{K_{12}\ Ao}{\alpha - \beta} . e^{-\beta t}$$

22. D', maintenance dose

$$D' = Cp(min)^{\alpha} . V_d(e^{\beta\tau} - 1)$$

23. D, loading dose

$$D = Cp\ (min)^{\alpha} . Vd\ (e^{\beta\tau})$$

Where τ is dosage interval

Erythrocytic partitioning

To study the *in vitro* erythrocytic partitioning, blood from healthy buffalo calves of group III was collected and different known concentrations of diminazene viz. 1.25, 2.5, 5, 10 and 20 $\mu g.ml^{-1}$ were added. Then the samples were placed for incubation at 37°C for 12 h. Plasma was separated after centrifuging the samples at 3000 rpm for 15 min at room temperature. Then the erythrocytes were washed with normal saline for 3 times, after that equal amount of water was added to them for complete hemolysis of erythrocytes. Drug was estimated in blood, plasma and erythrocytes.

In vitro plasma protein binding

In vitro binding of diminazene to plasma protein of healthy buffalo calves was determined according to the method of Kunin *et al* (1959). The dialyzing bags (pore size - 4A°) of convenient size were washed with distilled water and were soaked overnight in phosphate buffer (0.2M, pH = 7.4). The different concentrations of diminazene viz. 2 to 20 $\mu g.ml^{-1}$ were prepared in plasma. Each dialyzing bag was knotted on one end before filling 5 ml of plasma containing known amount of drug. The other end of bag was then securely tied. Each bag was then immersed in separate tubes containing 5 ml of phosphate buffer (pH 7.4; 0.2M). The tubes were kept at 37°C for 24 h with frequent shaking. At the end of incubation, buffer as well as content of cellophane bag were separately analyzed for concentration of diminazene.

For each concentration three separate sets of experiment were conducted. The extent of binding was calculated by the following equation :

$$\text{Per cent of diminazene bound to plasma proteins} = \frac{Cp' - CB}{Cp} \times 100$$

Where,

Cp' = concentration of drug in plasma after incubation

CB = concentration of drug in buffer after incubation

Cp = concentration of drug in plasma before incubation

Binding capacity of plasma proteins (β_i) and the dissociation constant of protein - drug complex (K_β) were also calculated (Pilloud 1973) by the following formula :

$$\beta_i Z_1 + K_\beta Z_2 + Z_3 = 0$$

$$Z_1 = PL,\ Z_2 = WL - T;\ Z_3 = WL-T$$

Where, L stands for free and T for total concentration of drug. P and W are the protein and water constants of plasma, respectively.

Statistical analysis

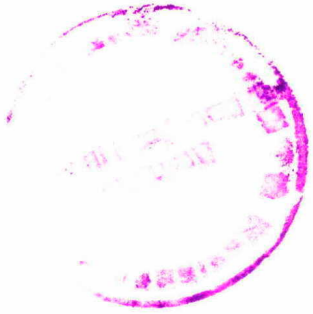
The difference between two means based on individual observations was determined by student's t-test. The significance was assayed at $P < 0.05$ and $P < 0.01$ levels (Singh *et al* 1991).

CHAPTER IV

RESULTS AND DISCUSSION

The trend of multiple drug therapy in human and veterinary practice, is increasing day by day. It is well understood that simultaneous administration of two or more drugs can markedly alter the pharmacokinetics of one or all drugs. Procaine and antipyrine are expected to change the pharmacokinetics of diminazene. To determine the therapeutic efficacy of a chemotherapeutic agent, the calculation of dosage regimen is of much importance. The therapeutic efficacy of diminazene is also expected to enhance to a remarkable extent when given in optimal dosages along with procaine and antipyrine. Keeping these facts in view, it was thought of quite interest to determine the effect of procaine and antipyrine on plasma levels, pharmacokinetics, urinary excretion and appropriate dosage regimen of diminazene in buffalo calves.

In the present study, diminazene diaceturate (3.5 mg..kg^{-1}), procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) were administered by single i.m. route, as "Trypan" (in granules and suspension form). The dosage of diminazene employed in this study is quite comparable to dosage given for



treatment of trypanosomiasis in field conditions (Abd-EL-Latif 1963; Castillow and Mojica 1968, Varia and Vaishnav 1970, Hiregouder and Avasthi 1971, Verma *et al* 1976, Mamman *et al* 1993).

Intramuscular administration of diminazene is most reliable means for managing even the severe cases of trypanosomiasis because (I) generally i.v. route is not too easy to use at farmer's door resulting i.m. route is preferred, (ii) once apparent equilibrium is maintained between blood and tissue i.v. administration is not of much significance for the transfer of drug from blood to tissues.

A. Plasma levels and pharmacokinetics of diminazene in combination with procaine and antipyrine

1. In granular form

Plasma levels of diminazene at various time intervals after its single intramuscular administration (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) as granules, in healthy buffalo calves are given in Table 11. The graphical representation of mean plasma concentration time profile, on a semilogarithmic scale are shown in Fig. 4.

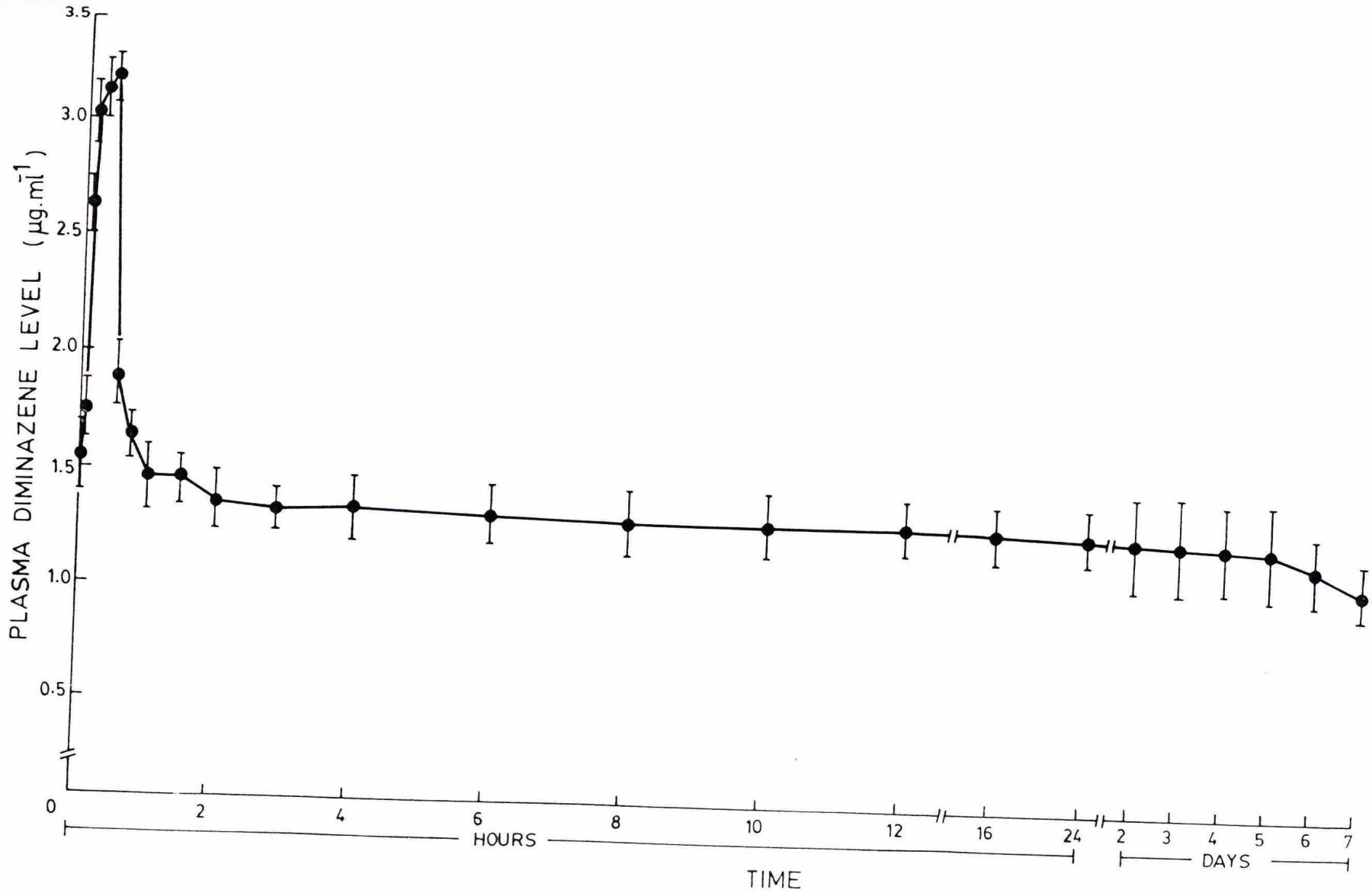
Appreciable level of diminazene (1.53 ± 0.15 $\mu g.ml^{-1}$) was detected at 1 min of injection. The peak drug concentration of 3.17 ± 1.05 $\mu g.ml^{-1}$ was achieved at 15 min which rapidly declined to 1.46 ± 0.13 $\mu g.ml^{-1}$ at 1 h. Thereafter the drug gradually disappeared to 1.05 ± 0.11 $\mu g.ml^{-1}$ at 168 h. The evaluation of results on observed plasma levels of diminazene indicated that

Table 11. Plasma levels of diminazene in buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time after drug administration	Animal number A_1	A_2	A_3	A_4	Mean ±SE
1 min	1.40	1.18	1.70	1.87	1.53±0.15
2.5	1.49	1.56	1.85	2.04	1.73±0.12
5	1.61	1.66	1.70	5.65	2.65±0.99
7.5	1.70	1.73	1.97	6.73	3.03±1.23
10	1.89	1.89	1.89	6.90	3.14±1.25
15	2.35	2.04	1.99	6.33	3.17±1.05
30	1.54	1.99	2.11	2.04	1.89±0.15
45	1.45	1.47	1.68	1.92	1.65±0.09
1 h	1.18	1.56	1.35	1.78	1.46±0.13
1.5	1.37	1.35	1.37	1.75	1.46±0.09
2	1.29	1.18	1.20	1.77	1.36±0.13
3	1.20	1.30	1.30	1.60	1.35±0.08
4	1.02	1.20	1.39	1.75	1.34±0.15
6	1.06	1.30	1.23	1.72	1.32±0.14
8	1.04	1.28	1.20	1.70	1.30±0.14
10	1.10	1.06	1.35	1.70	1.30±0.14
12	1.18	1.02	1.35	1.66	1.30±0.13
16	1.20	1.06	1.16	1.70	1.28±0.14
24	1.16	1.16	1.06	1.75	1.28±0.15
48	1.09	1.13	1.04	1.78	1.26±0.17
72	1.07	1.15	1.00	1.85	1.26±0.19
96	1.06	1.14	0.97	1.80	1.24±0.18
120	0.99	1.00	1.20	1.80	1.24±0.19
144	0.97	0.92	1.18	1.56	1.15±0.14
168	0.94	0.90	0.94	1.42	1.05±0.11

Values given are expressed as $\mu g.ml^{-1}$

Fig. 4 Graphical representation of plasma concentration time profile of diminazene in buffalo calves following single i.m. administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$).Values are presented as mean ± SE of four animals.


PLASMA DIMINAZENE LEVEL (µg.ml⁻¹)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2
4
6
8
10
12
16
24
2
3
4
5
6
7
HOURS
DAYS
TIME

the data can be best fitted to triexponential model and were described by an equation :

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} - A'e^{-Kat}$$

Where Cp is the concentration at time t, A, B and A' are zero time intercepts of distribution, elimination and absorption phases of plasma drug concentration time profile, respectively. α, β and Ka are the overall distribution, elimination and absorption rate constants, respectively and e represents the base of natural logarithm.

Various paharmacokinetic parameters calculated from the plasma concentration of diminazene after its i.m. administration, in relation to its absorption, distribution and elimination phases are given in Tables 12, 13 and 14, respectively. The values of C_{max} and t_{max} were 3.35 ± 1.18 $\mu g.ml^{-1}$ and 17.5 ± 4.33 min, respectively. The absorption rate constant and its half life were 9.24 ± 4.86 h^{-1} and 0.446 ± 0.219 h. The distribution rate constant and its half life were 2.76 ± 0.76 h and 0.314 ± 0.084 h, respectively.

The overall elimination rate constant 0.0014 ± 0.0001 h^{-1} whereas elimination half life was 538.3 ± 94.7 h. The area under curve (AUC) and total area under the first moment of plasma drug concentration time curve (AUMC) were 1017.7 ± 142.0 $\mu g.ml^{-1}.h$ and 826.2 ± 234.6 $mg.ml^{-1}.h^2$, respectively. The rate constants of drug transfer from central to peripheral compartment

Table 12. Absorption kinetics of diminazene in buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) alongwith procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Parameter[a]	Unit	Animal number				
		A_1	A_2	A_3	A_4	Mean ±SE
A'	$\mu g.ml^{-1}$	0.349	0.215	0.700	0.200	0.366±0.116
Ka	h^{-1}	5.32	7.32	1.02	23.3	9.24±4.86
$t_{0.5}Ka$	h	0.130	0.947	0.680	0.030	0.446±0.219
t_{max}	min	15	15	30	10	17.5±4.33
C_{max}	$\mu g.ml^{-1}$	2.35	2.04	2.11	6.90	3.35±1.18

[a]Kinetic parameters are described by Gibaldi and Perrier (1982)

Table 13. Distribution kinetics of diminazene in buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

		Animal number				
Parameters[a]	Unit	A_1	A_2	A_3	A_4	Mean ±SE
A	$\mu g.ml^{-1}$	2.23	1.58	3.61	12.9	5.08±2.64
α	h^{-1}	2.59	1.27	2.31	4.88	2.76±0.76
$t_{0.5\alpha}$	h	0.268	0.548	0.300	0.142	0.314±0.084
K_{12}	h^{-1}	1.586	0.658	1.684	4.223	2.037±0.764
K_{21}	h^{-1}	0.998	0.607	0.624	0.640	0.717±0.093
K_{12}/K_{21}	Ratio	1.58	1.08	2.70	6.60	2.99±1.25
AUC	$\mu g.ml^{1}.h$	858.8	697.8	1260.8	1253.5	1017.7±142.0
AUMC	$mg.ml^{-1}.h^{2}$	621.7	387.0	1478.8	817.6	826.2±234.6
Vc	$L.kg^{-1}$	1.140	1.335	0.880	0.240	0.898±0.239
$V_{d(B)}$	$L.kg^{-1}$	2.96	2.79	3.26	1.83	2.71±0.30
$V_{d(\beta b))}$	$L.kg^{-1}$	2.95	2.78	3.25	1.82	2.70±0.30
$V_{d(ext)}$	$L.kg^{-1}$	2.96	2.80	3.26	1.83	2.71±0.30
$V_{d(ss)}$	$L.kg^{-1}$	2.95	2.79	3.25	1.82	2.70±0.30
fc	Ratio	0.390	0.480	0.270	0.131	0.317±0.075
T/P	Ratio	1.59	1.08	2.70	6.58	2.98±1.24

[a]Kinetic parameters are as described by Gibaldi and Perrier (1982)

Table 14. Elimination kinetics of diminazene in buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Parameters[a]	Unit	Animal number				Mean±SE
		A_1	A_2	A_3	A_4	
Cp^o	$\mu g.ml^{-1}$	3.07	2.62	3.98	14.6	6.07±2.86
B	$\mu g.ml^{-1}$	1.18	1.25	1.07	1.91	1.35±0.19
β	h^{-1}	0.0014	0.0018	0.0009	0.0015	0.0014±0.0001
$t_{0.5}\beta$	h	502.2	385.0	813.4	452.9	538.3±94.7
$t_{0.9}\beta$	h	76.1	58.3	123.2	68.6	81.5±14.3
K_{el}	h^{-1}	0.0036	0.0038	0.0032	0.0116	0.0055±0.0020
$t_{0.5}Kel$	h	192.5	182.4	216.6	59.7	162.8±35.1
Cl_T	$ml.kg^{-1}.h^{-1}$	4.076	5.015	2.776	2.792	3.664±0.543
MRT	h	724.0	554.6	1172.9	652.2	775.9±136.8
td	h	894.8	696.0	1391.9	835.2	954.5±151.6

[a]Kinetic parameters are as described by Gibaldi and Perrier (1982)

(K_{12}) and from peripheral to central (K_{21}) were 2.037 ± 0.764 h^{-1} and 0.717 ± 0.093 h^{-1}, respectively. The rate of elimination of drug from central compartment (K_{el}) was 0.0055 ± 0.0020 h^{-1} whereas total body clearance (Cl_T) which is sum of all excretory processes was 3.66 ± 0.54 $ml.kg^{-1}.h^{-1}$. The mean residual time (MRT) and duration of pharmacological effect (td) were 775.9 ± 136.8 h and 954.5 ± 151.6 h, respectively.

The coefficient A' (0.349 $\mu g.ml^{-1}$), A (2.23 $\mu g.ml^{-1}$) and B (1.18 $\mu g.ml^{-1}$) and rate constants Ka (5.32 h^{-1}), α (2.59 h^{-1}) and β (0.0014 h^{-1}) of animal A_1 can be fitted in above triexponential equation as follows :

$$Cp = 2.23e^{-2.59t} + 1.18e^{-0.0014t} - 0.349e^{-5.32t}$$

The absorption and distribution rate constants of plasma drug concentration time profile of representative animal (A_1) are shown in Fig. 5.

In suspension form

Plasma levels of diminazene at various time intervals after its single intramuscular administration (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) as suspension in healthy buffalo calves are given in Table 15. The graphical presentation of mean plasma concentration time profile, on a semilogarithmic scale are shown in Fig. 6. Appreciable level of diminazene (1.50 ± 0.07 $\mu g.ml^{-1}$) was detected at 1 min of injection. The peak drug concentration of 3.77 ± 1.18 $\mu g.ml^{-1}$ was achieved at 10 minutes,

Fig. 5. Disposition curve depicting the absorption and distribution phases of plasma diminazene level in animal no. A_1, following its single i.m. administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). Absorption (Ka) and distribution (α) phases are represented by least square regression lines. The calculated points (0) of absorption and distribution phases were obtained by feathering technique. Constants A' and A are the zero time intercepts of absorption and distribution phases, respectively.

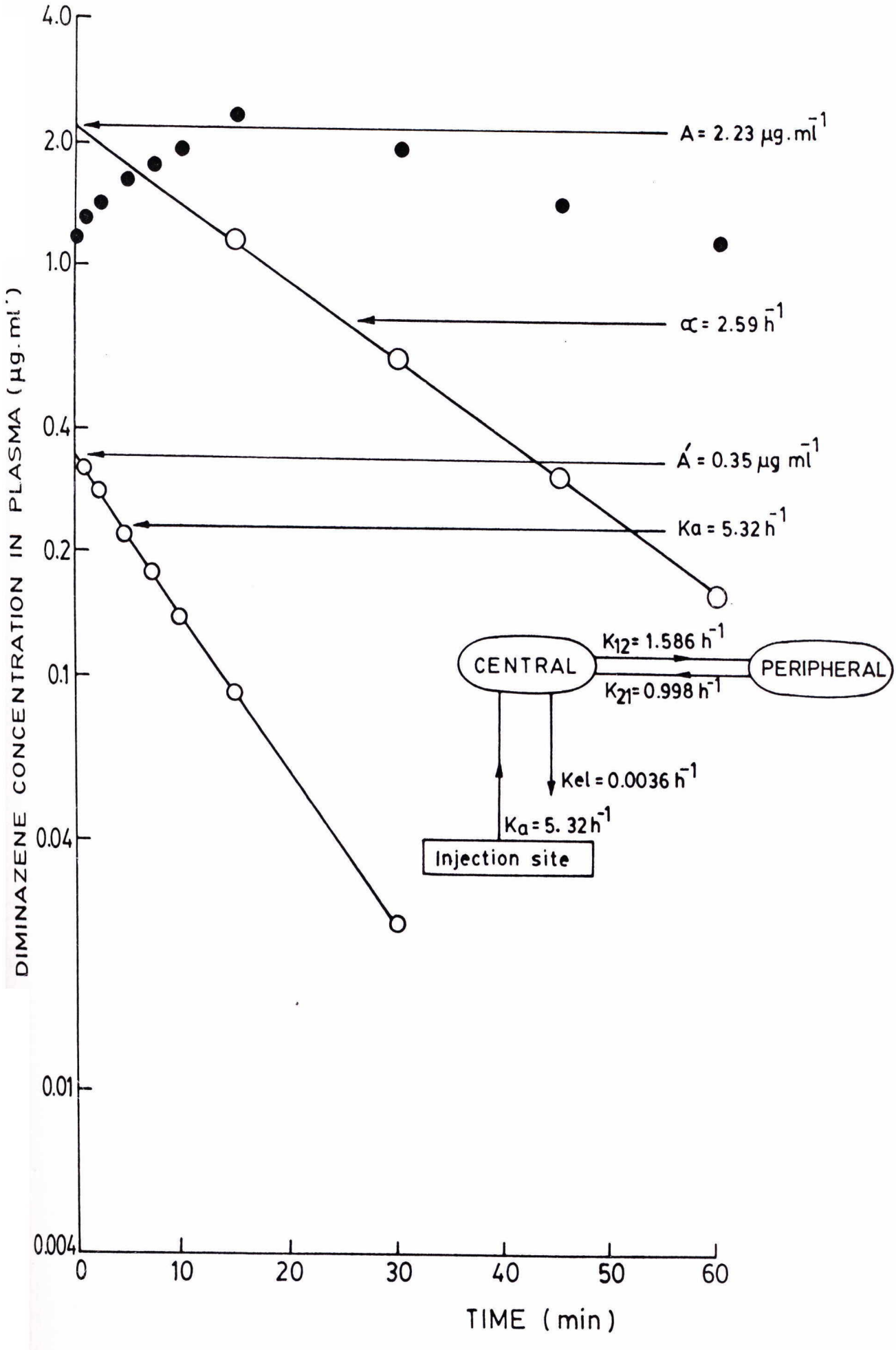
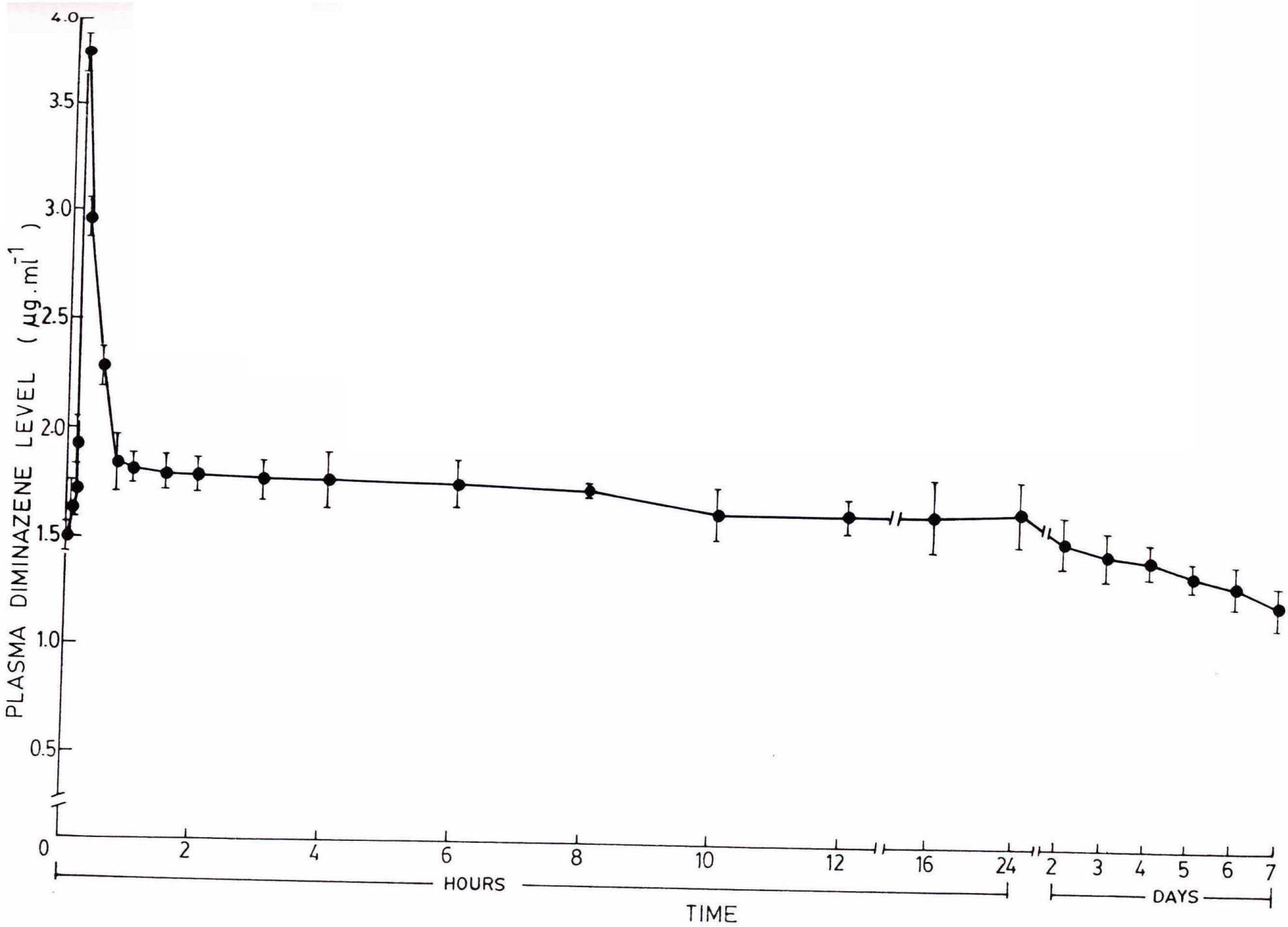

4.0
2.0
1.0
0.4
0.2
0.1
0.04
0.01
0.004
DIMINAZENE CONCENTRATION IN PLASMA (µg.ml⁻¹)
A = 2.23 µg.ml⁻¹
α = 2.59 h⁻¹
A' = 0.35 µg ml⁻¹
Ka = 5.32 h⁻¹
CENTRAL
PERIPHERAL
K12 = 1.586 h⁻¹
K21 = 0.998 h⁻¹
Kel = 0.0036 h⁻¹
Ka = 5. 32h⁻¹
Injection site
0
10
20
30
40
50
60
TIME (min)

Table 15. Plasma levels of diminazene in buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time after drug administration	Animal number				Mean±SE
	B_1	B_2	B_3	B_4	
1 min	1.37	1.63	1.37	1.66	1.50±0.07
2.5	1.51	1.70	1.42	1.92	1.63±0.11
5	1.63	1.70	1.47	2.08	1.72±0.13
7.5	1.87	2.11	1.56	2.16	1.92±0.13
10	1.80	5.99	1.66	5.65	3.77±1.18
15	2.39	1.89	1.70	5.88	2.96±0.98
30	1.54	1.82	2.18	5.65	2.78±0.96
45	1.47	1.78	1.89	2.18	1.84±0.13
1 h	1.70	1.70	1.89	2.00	1.82±0.07
1.5	1.62	1.70	1.89	1.99	1.80±0.08
2	1.60	1.82	1.75	2.00	1.79±0.08
3	1.60	1.60	1.90	1.98	1.77±0.09
4	1.59	1.70	1.62	2.18	1.77±0.13
6	1.73	1.66	1.56	2.10	1.76±0.11
8	1.73	1.70	1.70	1.85	1.74±0.03
10	1.45	1.94	1.45	1.75	1.64±0.12
12	1.51	1.85	1.51	1.70	1.64±0.08
16	1.42	1.99	1.89	1.28	1.64±0.17
24	1.66	2.06	1.63	1.32	1.66±0.15
48	1.49	1.89	1.42	1.35	1.53±0.12
72	1.32	1.79	1.50	1.28	1.47±0.11
96	1.20	1.60	1.50	1.50	1.45±0.08
120	1.20	1.50	1.50	1.35	1.38±0.07
144	1.11	1.49	1.56	1.23	1.34±0.10
168	0.99	1.42	1.42	1.18	1.25±0.10

Values given are expressed as $\mu g.ml^{-1}$

Fig. 6 Graphical representation of plasma concentration time profile of diminazene in buffalo calves following single i.m. administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). Values are presented as mean ± SE of four animals.


PLASMA DIMINAZENE LEVEL (µg.ml-1)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2
4
6
8
10
12
16
24
2
3
4
5
6
7
HOURS
DAYS
TIME

which rapidly decreased to 1.82 ± 0.07 $\mu g.ml^{-1}$ at 1 h. Thereafter gradually declined to 1.25 ± 0.10 $\mu g.ml^{-1}$ at 168 h. The experimental data of observed plasma concentration revealed that pharmacokinetics of diminazene in healthy buffalo calves after i.m. administration is described by triexponential model and adequately described by an equation :

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} - A'e^{-Kat}$$

The kinetic determinants which describe the absorption, distribution and elimination of diminazene in combination with procaine and antipyrine in healthy buffalo calves are presented in Tables 16, 17 and 18, respectively. The rate constants of absorption (Ka), distribution (α) and elimination (β) ranged between 8.81 to 32.6, 1.08 to 7.97 and 0.0006 to 0.0033 h^{-1}, respectively. The absorption half life ($t_{0.5}Ka$), distribution half life ($t_{0.5}\alpha$) and elimination half life ($t_{0.5}\beta$) were 0.057 ± 0.012, 0.400 ± 0.138 and 667.6 ± 248.0 h, respectively. The K_{12}, which represents the rate of transfer of drug from central to peripheral compartment and K_{21}, which represents the rate of transfer of drug from peripheral to central compartment were 2.063 ± 1.292 h^{-1} and 1.172 ± 0.370 h^{-1}, respectively. The rate of elimination of drug from central compartment (K_{el}) was 0.0046 ± 0.0021h^{-1} whereas total body clearance (Cl_T) which is sum of all excretory processes was 3.49 ± 1.24 $ml.kg^{-1}.h^{-1}$.

Table 16. Absorption kinetics of diminazene in buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Parameter[a]	Unit	Animal number				Mean ±SE
		A_1	A_2	A_3	A_4	
A'	$\mu g.ml^{-1}$	0.131	0.009	0.020	2.926	0.771±0.718
Ka	h^{-1}	8.81	32.6	10.3	11.2	15.7±5.64
$t_{0.5}Ka$	h	0.079	0.021	0.067	0.062	0.057±0.012
t_{max}	min	15.0	10.0	30.0	15.0	17.5±4.33
C_{max}	$\mu g.ml^{-1}$	2.39	5.99	2.18	5.88	4.11±1.05

[a]Kinetic parameters are described by Gibaldi and Perrier (1982)

Table 17. Distribution kinetics of diminazene in buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Parameters[a]	Unit	Animal number				Mean ±SE
		B_1	B_2	B_3	B_4	
A	$\mu g.ml^{-1}$	1.62	6.13	1.09	4.71	3.39±1.21
α	h^{-1}	2.80	7.97	1.08	1.10	3.24±1.62
$t_{0.5\alpha}$	h	0.247	0.087	0.638	0.628	0.400±0.138
K_{12}	h^{-1}	1.293	5.901	0.438	0.623	2.063±1.292
K_{21}	h^{-1}	1.509	2.053	0.648	0.480	1.172±0.370
K_{12}/K_{21}	Ratio	0.856	2.874	0.676	1.299	1.426±0.500
AUC	$\mu g.ml^{-1}.h$	525.1	824.7	2563.2	2086.9	1499.9±490.0
AUMC	$mg.ml^{-1}.h^2$	158.9	319.3	4186.7	3184.8	1962.4±1016.2
Vc	$L.kg^{-1}$	1.087	0.424	1.331	1.115	0.989±0.196
$V_{d(B)}$	$L.kg^{-1}$	2.02	1.65	2.23	2.57	2.12±0.19
$V_{d(\beta)}$	$L.kg^{-1}$	2.02	1.64	2.22	2.56	2.11±0.19
$V_{d(ext)}$	$L.kg^{-1}$	2.02	1.65	2.23	2.57	2.12±0.19
$V_{d(ss)}$	$L.kg^{-1}$	2.02	1.64	2.23	2.56	2.11±0.19
fc	Ratio	0.538	0.258	0.600	0.436	0.458±0.074
T/P	Ratio	0.858	2.876	0.660	1.293	1.421±0.502

[a]Kinetic parameters are as described by Gibaldi and Perrier (1982)

Table 18. Elimination kinetics of diminazene in buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Parameters[a]	Unit	Animal number				Mean ±SE
		B_1	B_2	B_3	B_4	
Cp^o	$\mu g.ml^{-1}$	3.22	8.25	2.63	3.14	4.31±1.31
B	$\mu g.ml^{-1}$	1.73	2.12	1.57	1.36	1.70±0.16
β	h^{-1}	0.0033	0.0026	0.0006	0.0006	0.0017±0.0007
$t_{0.5}\beta$	h	210.0	268.6	1132.3	1059.6	667.6±248.0
$t_{0.9}\beta$	h	31.8	40.7	171.6	160.5	101.1±37.5
K_{el}	h^{-1}	0.0061	0.0100	0.0010	0.0015	0.0046±0.0021
$t_{0.5}Kel$	h	113.6	69.3	693.0	462.0	334.4±148.3
Cl_T	$ml.kg^{-1}.h^{-1}$	6.666	4.244	1.365	1.677	3.488±1.240
MRT	h	302.7	387.2	1633.3	1526.1	962.3±357.5
td	h	379.6	481.8	2087.9	2087.9	1259.3±478.8

[a]Kinetic parameters are as described by Gibaldi and Perrier (1982)

The area under curve (AUC) and total area under the first moment of plasma drug concentration time curve (AUMC) were 1499.9 ± 490.0 $\mu g.ml^{-1}.h$ and 1962.4 ± 1016.2 $mg.ml^{-1}.h^2$ respectively. The mean residual time (MRT) and duration of pharmacological effect (td) were 962.3 ± 357.5 h and 1259.3 ± 478.8 h, respectively.

The coefficient A' (0.131 $\mu g.ml^{-1}$), A (1.62 $\mu g.ml^{-1}$) and B (1.73 $\mu g.ml^{-1}$) and rate constants Ka (8.81 h^{-1}), α (2.80 h^{-1}) and β (0.0033 h^{-1}) of animal B_1 can be fitted in above triexponential equation as follows :

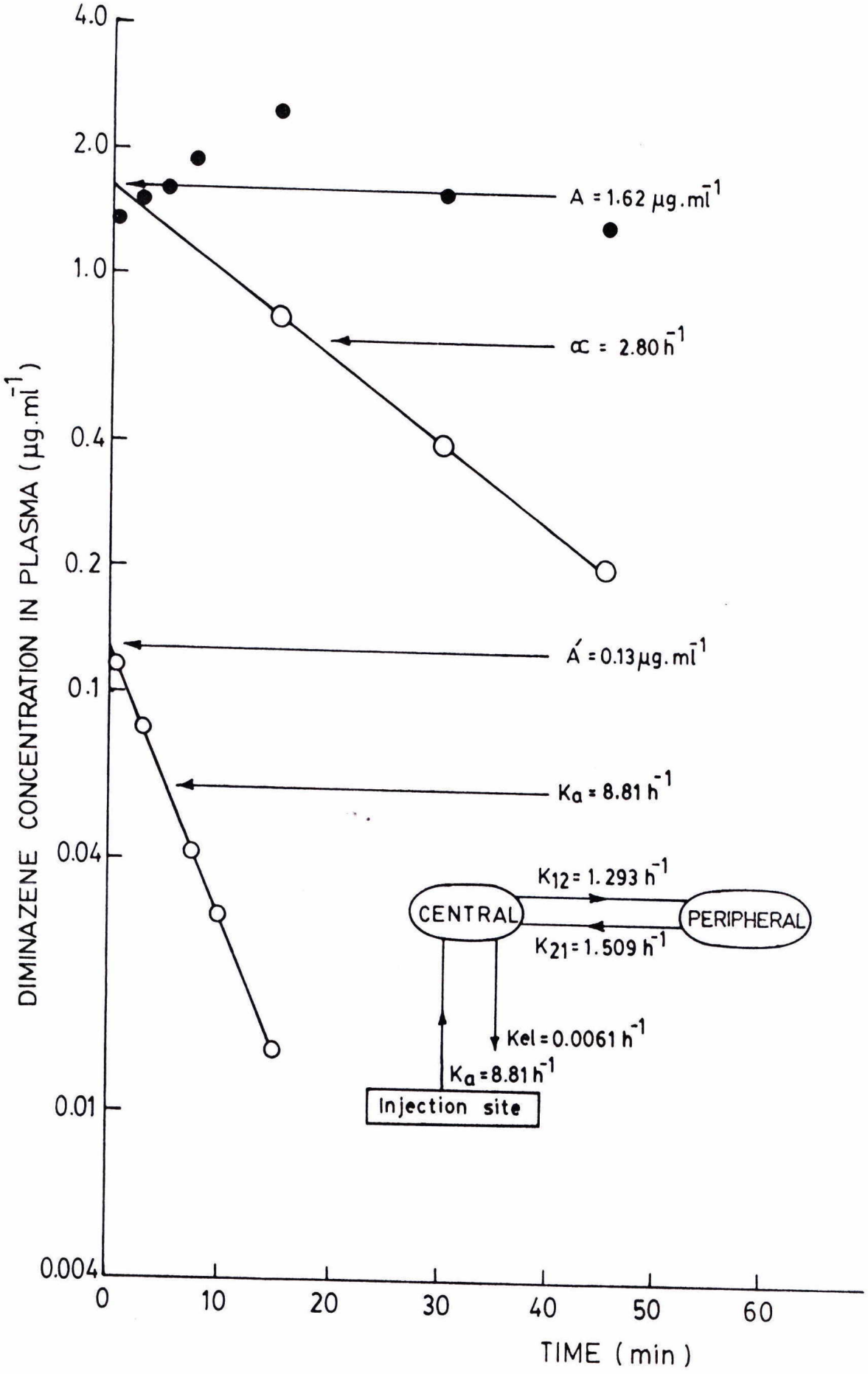
$$Cp = 1.62e^{-2.80t} + 1.73e^{-0.0033t} - 0.131e^{-8.81t}$$

The absorption and distribution rate constants of plasma drug concentration time profile of a representative animal of this group (B_1) are shown in Fig. 7.

Similar to present study, diminazene, when administered alone was also reported to follow two compartment open model in cattle (Klatt and Hadju 1976, Kellner *et al* 1985, Madachi *et al* 1995, Gummow *et al* 1994).

The peak plasma level obtained from both granules and suspension administration was 5-6 fold higher than minimum therapeutic plasma levels of diminazene and it was almost similar as reported following alone administration of diminazene in cows, heifers, goats and rabbits (Klatt and

Fig. 7. Disposition curve depicting the absorption and distribution phases of plasma diminazene level in animal No. B_1, following single i.m. administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). Absorption (Ka) and distribution (α) phases are represented by least square regression lines. The calculated points (0) of absorption and distribution phases were obtained by feathering technique. A and A' are the zero time intercepts of absorption and distribution phases, respectively.


4.0
2.0
1.0
0.4
0.2
0.1
0.04
0.01
0.004
0
10
20
30
40
50
60
TIME (min)
DIMINAZENE CONCENTRATION IN PLASMA (µg.ml⁻¹)
A = 1.62 µg.ml⁻¹
α = 2.80 h⁻¹
A' = 0.13 µg.ml⁻¹
Ka = 8.81 h⁻¹
K12 = 1.293 h⁻¹
K21 = 1.509 h⁻¹
CENTRAL
PERIPHERAL
Kel = 0.0061 h⁻¹
Ka = 8.81 h⁻¹
Injection site

Hajdu 1976, Gilbert and Newton 1982, Aliu *et al* 1993, Mamman and Peregrine 1994).

The time to obtain maximum concentration of diminazene in plasma (tmax) when given along with procaine and antipyrine in buffalo calves was 17.5 min suggesting quick absorption of diminazene from injection site but it was higher than the value (t_{max} 10 min) when diminazene was administered alone in buffalo calves (Singh 1997). In contrast, in different studies, the t_{max} of diminazene in cattle was reported to be 30 min (Klatt and Hajdu 1976), 36 min (Mamman *et al* 1993), 38 min (Kaur 1998) and 10-15 min (Aliu *et al* 1993).

Rapid appearance of diminazene in plasma and its high value of absorption rate constant Ka (9.24 ± 4.86 and 15.7 ± 5.64 h^{-1}) following its granules and suspension administration suggest that diminazene promptly enters into systemic circulation from extravascular injection.

The elimination half life of diminazene when given along with procaine and antipyrine was 8-10 fold higher as compared to its value of 67.88 ± 10.38 h in buffalo calves and 63 h in cattle when administered alone (Singh 1997, Klatt and Hajdu 1976). This suggests that diminazene remain for a very long time in body when administered along with procaine and antipyrine which is further confirmed by its low values of K_{el} and Cl_T. The results confirmed that procaine and antipyrine are responsible for very slow elimination of diminazene from body of buffalo calves.

Clinically, it is utmost important to determine the extent of penetration rather than rate of distribution. The calculated apparent volume of distribution (Granules 2.70 ± 0.30 $L.kg^{-1}$, suspension 2.11 ± 0.19 $L.kg^{-1}$) indicate that there is excellent penetration of diminazene through biological membranes in buffalo species when given in combination with procaine and antipyrine. Apparent volume of distribution was almost similar 2.99 ± 0.18 $L.kg^{-1}$) when diminazene was administered alone in buffalo calves (Singh 1997) but this value is lower in case of female calves and heifers in range of 0.94-1.91 $L.kg^{-1}$ (Aliu *et al* 1993, Sardar *et al* 1994). A small volume of distribution implies that the drug is confined within vascular bed while a larger volume (> 1.0 $L.kg^{-1}$) implies distribution throughout the body (Baggot 1977). High values of AUC and AUMC observed in present study further confirmed the excellent distribution of diminazene into body fluids and tissues of buffalo calves when given along with procaine and antipyrine. The values of AUC (360.10 $\mu g.ml^{-1}.h^2$) and AUMC (65.32 $mg.ml^{-1}.h^2$) were significantly lower when diminazene was administered alone (Singh 1997). So the present study ensures that this drug has excellent distribution and penetration through body fluids and tissues of buffalo calves.

B. Levels in central and peripheral compartment

To confirm the distribution pattern of drug in various body fluids and tissues, the plasma drug concentration time profile is feathered to compute

drug level in central and peripheral compartments. An attempt has also been made to describe application of compartment analysis for distribution pattern of diminazene (3.5 $mg.kg^{-1}$) between central and peripheral compartments when given along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$).

1. In granular form

The plasma concentration time profile of diminazene was feathered to derive the fractions of total drug present in central and peripheral compartment. The calculated concentration of drug in central (A_C) and peripheral (A_T) compartments are given in Tables 19 and 20, respectively. The results have been shown in Fig. 8.

2. In suspension form

The plasma concentration time profile of diminazene was feathered to derive the fractions of total drug present in central and peripheral compartment. The calculated concentration of drug in central and peripheral compartments are given in Table 21 and 22, respectively and shown in Fig. 9.

Drug is rapidly transferred from central to peripheral compartment as it is evidenced by high value of distribution rate constant and K_{12}/K_{21} ratio. The $t_{0.5}\alpha$ and K_{12}/K_{21} ratio were (granules 19 min, 2.99 suspension 24 min, 1.42), respectively. $t_{0.5}\alpha$ in cattle was 60 min when diminazene was administered alone (Klatt and Hajdu 1976).

As the drug rapidly transferred from central to peripheral compartment, an apparent equilibrium between blood and tissue was attained at 18 min for

Table 19. Level of diminazene in central compartment of buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time after drug administration	Animal number				Mean±SE
	A_1	A_2	A_3	A_4	
10 min	2.43	2.37	3.08	7.74	3.90±1.28
30	1.70	1.98	1.98	3.02	2.17±0.29
1 h	1.32	1.63	1.36	2.00	1.57±0.15
2	1.19	1.36	1.10	1.91	1.39±0.18
4	1.18	1.25	1.07	1.90	1.35±0.18
6	1.17	1.24	1.07	1.90	1.34±0.18
8	1.17	1.23	1.06	1.89	1.34±0.18
10	1.17	1.23	1.06	1.89	1.33±0.10
12	1.16	1.22	1.06	1.88	1.33±0.18
24	1.14	1.20	1.05	1.84	1.30±0.18
48	1.11	1.15	1.03	1.78	1.26±0.17
72	1.07	1.10	1.01	1.71	1.22±0.16
96	1.03	1.05	0.99	1.65	1.18±0.15
120	1.00	1.01	0.97	1.60	1.14±0.15
144	0.97	0.96	0.95	1.53	1.10±0.14
168	0.94	0.93	0.93	1.48	1.06±0.13
192	0.87	0.80	0.90	1.43	1.02±0.14
240	0.83	0.81	0.86	1.33	0.96±0.12
288	0.77	0.74	0.83	1.24	0.89±0.17
336	0.72	0.60	0.79	1.15	0.83±0.11
384	0.68	0.63	0.76	1.07	0.78±0.10
432	0.63	0.57	0.73	1.00	0.73±0.09
480	0.59	0.53	0.70	0.93	0.69±0.09

Values given are expressed as $\mu g.ml^{-1}$

Table 20. Levels of diminazene in peripheral compartment of buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time after drug administration	Animal number				Mean±SE
	A_1	A_2	A_3	A_4	
10 min	0.63	0.24	0.89	6.85	2.15±1.57
30	1.36	0.64	1.99	11.5	3.88±2.57
1 h	1.74	0.97	2.61	12.5	4.45±2.70
2	1.87	1.25	2.87	12.6	4.65±2.68
4	1.88	1.34	2.90	12.6	4.68±2.65
6	1.87	1.34	2.89	12.6	4.66±2.65
8	1.86	1.34	2.88	12.5	4.65±2.64
10	1.86	1.34	2.88	12.5	4.64±2.63
12	1.85	1.33	2.87	12.4	4.62±2.62
24	1.82	1.30	2.84	12.2	4.54±2.57
48	1.76	1.25	2.79	11.8	4.39±2.48
72	1.70	1.20	2.73	11.3	4.24±2.38
96	1.61	1.14	2.67	10.9	4.08±2.29
120	1.60	1.10	2.62	10.5	3.96±2.21
144	1.54	1.05	2.57	10.2	3.83±2.13
168	1.50	1.01	2.51	9.80	3.70±2.05
192	1.41	0.96	2.44	9.47	3.57±1.99
240	1.32	0.88	2.34	8.81	3.33±1.85
288	1.23	0.81	2.24	8.20	3.12±1.72
336	1.15	0.74	2.14	7.63	2.91±1.60
384	1.08	0.68	2.05	7.10	2.72±1.48
432	1.00	0.63	1.97	6.63	2.55±1.38
480	0.94	0.57	1.88	6.15	2.38±1.26

Values given are expressed as $\mu g.ml^{-1}$

Fig. 8. Levels of diminazene in central and peripheral compartments as a function of time. The curves are based on first order rate constants of diminazene following single i.m. administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). A scheme of two compartment open model showing the values of first order rate constant associated with the model is also given in inset.

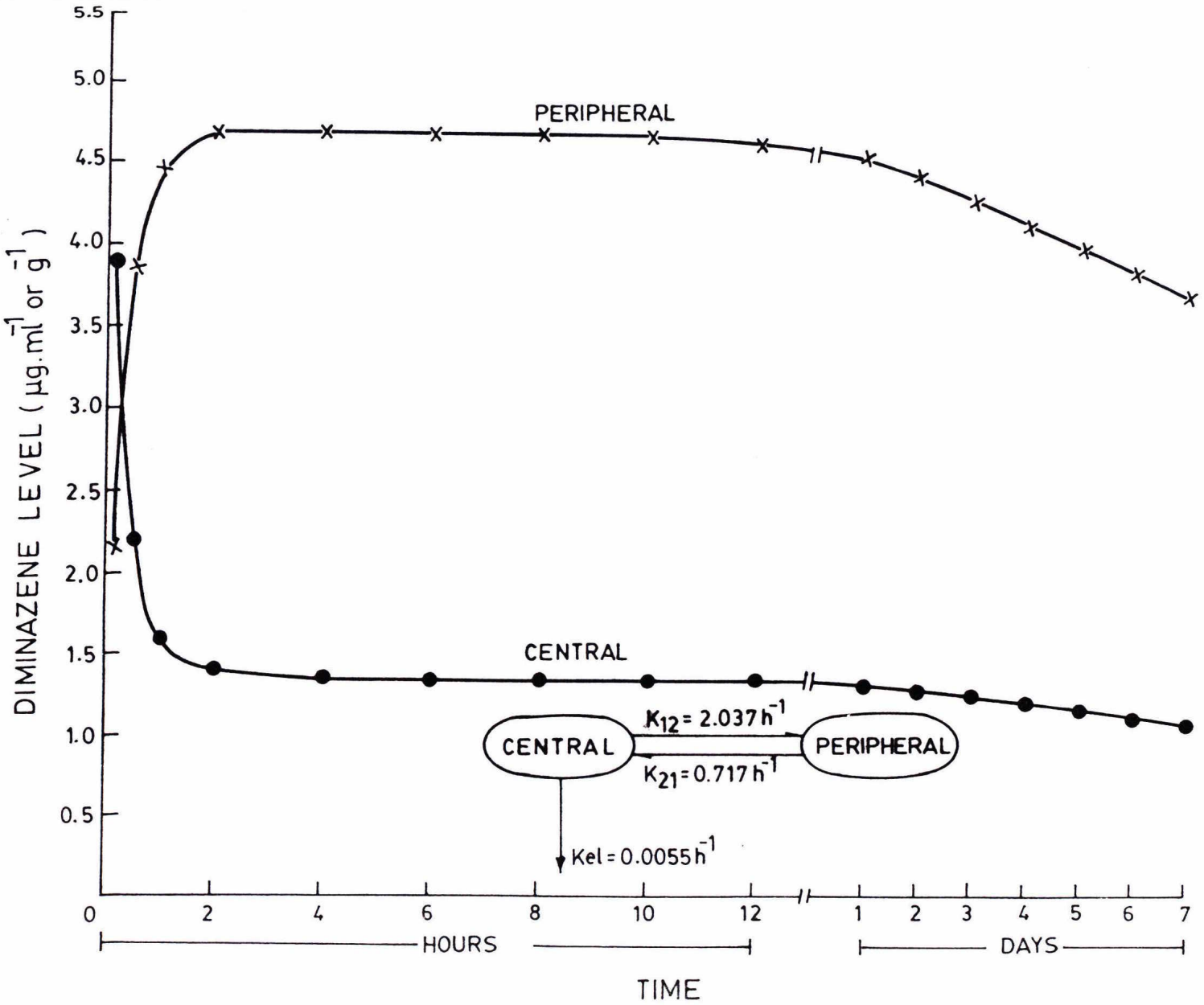

DIMINAZENE LEVEL (µg.ml^{-1} or g^{-1})
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
PERIPHERAL
CENTRAL
CENTRAL
PERIPHERAL
K_{12} = 2.037 h^{-1}
K_{21} = 0.717 h^{-1}
Kel = 0.0055 h^{-1}
0
2
4
6
8
10
12
1
2
3
4
5
6
7
HOURS
DAYS
TIME

Table 21. Levels of diminazene in central compartment of buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time after drug administration	Animal number				Mean± SE
	B_1	B_2	B_3	B_4	
10 min	2.68	2.83	2.46	2.85	2.95±0.30
30	2.10	2.23	2.18	2.39	2.22±0.06
1 h	1.82	2.12	1.92	1.95	1.95±0.06
2	1.73	2.11	1.69	1.56	1.77±0.11
4	1.71	2.10	1.58	1.38	1.69±0.15
6	1.70	2.09	1.56	1.36	1.68±0.15
8	1.69	2.08	1.56	1.36	1.67±0.15
10	1.68	2.07	1.56	1.35	1.66±0.15
12	1.67	2.06	1.55	1.35	1.65±0.15
24	1.60	1.99	1.54	1.34	1.62±0.13
48	1.48	1.87	1.52	1.32	1.55±0.11
72	1.47	1.76	1.50	1.30	1.50±0.09
96	1.26	1.65	1.48	1.28	1.42±0.09
120	1.17	1.55	1.46	1.26	1.36±0.08
144	1.08	1.46	1.43	1.24	1.30±0.08
168	0.99	1.37	1.41	1.22	1.25±0.09
192	0.92	1.29	1.40	1.22	1.20±0.10
240	0.78	1.14	1.36	1.18	1.11±0.12
288	0.70	1.00	1.33	1.15	1.04±0.13
336	0.57	0.89	1.29	1.12	1.00±0.13
384	0.49	0.78	1.25	1.09	0.90±0.17
432	0.42	0.69	1.22	1.06	0.84±0.18
480	0.35	0.60	1.18	1.02	0.79±0.19

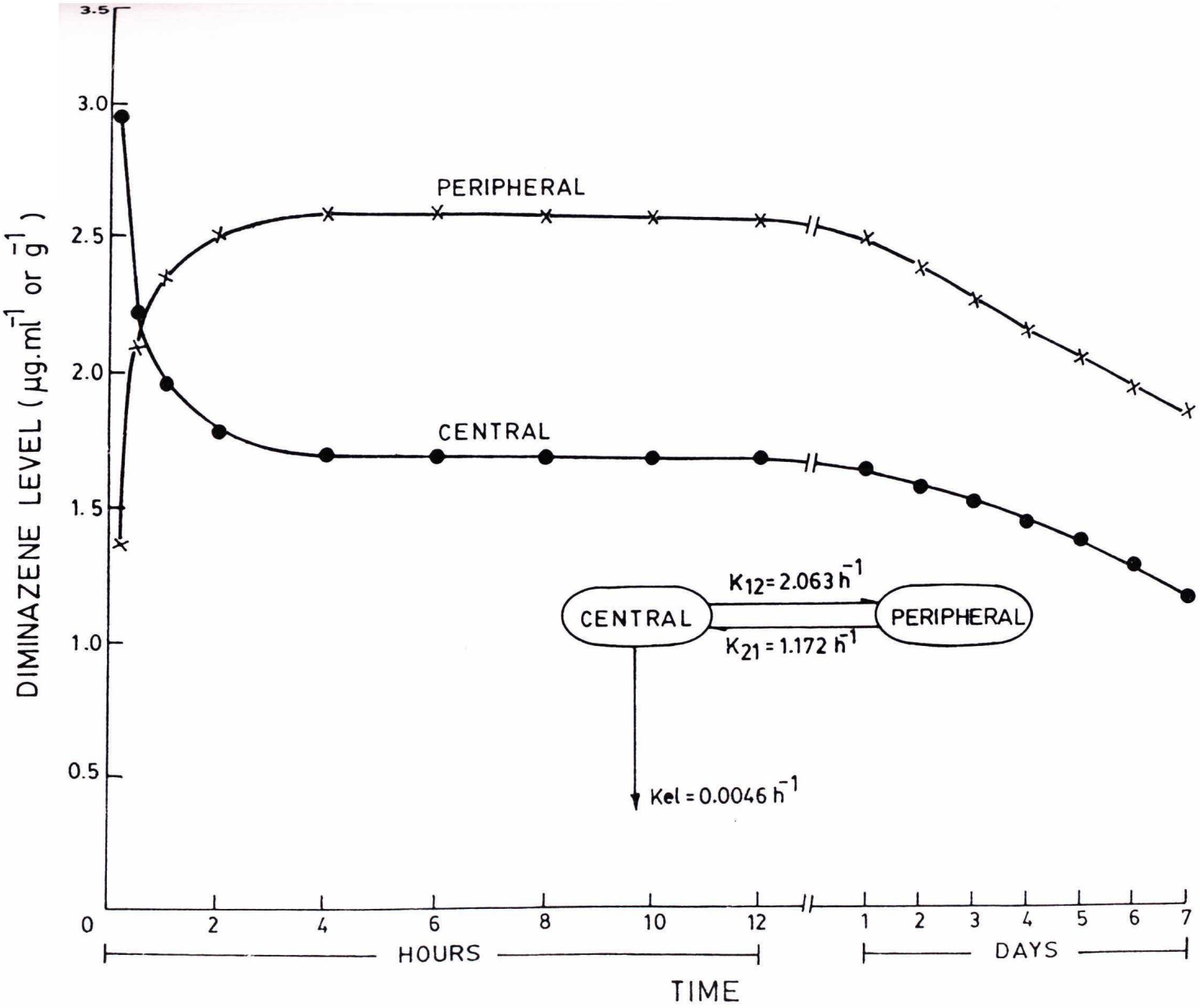
Values given are expressed as $\mu g.ml^{-1}$

Table 22. Level of diminazene in peripheral compartment of buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time after drug administration	Animal number				Mean± SE
	B_1	B_2	B_3	B_4	
10 min	0.54	4.40	0.17	0.29	1.35±1.01
30	1.12	5.99	0.44	0.75	2.07±1.31
1 h	1.39	6.09	0.70	1.18	2.34±1.25
2	1.47	6.08	0.94	1.57	2.51±1.19
4	1.47	6.05	1.04	1.74	2.57±1.16
6	1.46	6.01	1.05	1.76	2.57±1.15
8	1.45	5.98	1.05	1.76	2.56±1.14
10	1.44	5.95	1.05	1.76	2.55±1.14
12	1.43	5.92	1.05	1.76	2.54±1.13
24	1.37	5.74	1.04	1.74	2.47±1.09
48	1.27	5.39	1.03	1.71	2.35±1.02
72	1.17	5.07	1.01	1.69	2.23±0.95
96	1.08	4.76	1.00	1.66	2.12±0.89
120	1.00	4.47	0.99	1.64	2.02±0.82
144	0.92	4.20	0.97	1.61	1.92±0.77
168	0.85	3.95	0.96	1.59	1.83±0.72
192	0.79	3.70	0.95	1.58	1.75±0.67
240	0.67	3.27	0.92	1.54	1.60±0.58
288	0.57	2.89	0.90	1.50	1.46±0.51
336	0.49	2.55	0.87	1.45	1.34±0.45
384	0.42	2.25	0.85	1.41	1.23±0.39
432	0.36	2.00	0.82	1.37	1.14±0.35
480	0.30	1.75	0.80	1.33	1.04±0.31

Values given are expressed as $\mu g.ml^{-1}$

Fig. 9. Levels of diminazene in central and peripheral compartments as a function of time. The curves are based on first order rate constants of diminazene following single i.m. administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). A scheme of two compartment open model showing the values of first order rate constant associated with the model is also given in inset.


DIMINAZENE LEVEL (µg.ml⁻¹ or g⁻¹)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
PERIPHERAL
CENTRAL
K12= 2.063 h⁻¹
K21 = 1.172 h⁻¹
CENTRAL
PERIPHERAL
Kel = 0.0046 h⁻¹
2
4
6
8
10
12
1
2
3
4
5
6
7
HOURS
DAYS
TIME

granules and at 40 min for suspension. The calculated value of T/P ratio in granules and suspension administration were 2.98 and 1.42, respectively. From the calculated values it was found that drug level in tissue compartment was 1 to 3 fold higher than in central compartment.

At 24 hr, drug level in central and peripheral compartments were 1.30 and 4.54 $\mu g.ml^{-1}$ in granules and 1.62 and 2.47 $\mu g.ml^{-1}$ in suspension, respectively. At the end of 20 days, drug levels in central and peripheral compartments were (granules 0.69, 2.38 $\mu g.ml^{-1}$) and (suspension 0.79, 1.04 $\mu g.ml^{-1}$), respectively.

From the results, it is revealed that drug is not restricted to blood, it rapidly goes to body fluids and tissues and attain higher values in tissues than blood. Clinically, that drug is more preferred which has more penetration into tissues than blood.

C. Urinary excretion

Study of renal excretion is important to know the extent of excretion of drug through kidney. It is also useful to evaluate the pharmacological properties of drug since many drugs are concentrated by kidney, resulting a high concentration in urine.

In granular form

The concentration of diminazene in urine at different time intervals following its single intramuscular administration of its granules along with

procaine and antipyrine are given in Table 23. At different time intervals the concentrations of diminazene in urine ranged between 53.03-73.40 $\mu g.ml^{-1}$. The detailed data on urinary excretion of diminazene in buffalo calves were calculated and given in Table 24. A graphical presentation of urine concentrations of diminazene along with cumulative percent of total dose of drug excreted in urine are shown in Fig. 10. Approximately 47.2 per cent of total administered drug was recovered in urine at end of 24 h. About 26 per cent of drug was excreted in first 12 hours. During the first 2 hours only 5.48 ± 2.33 per cent of total administered drug was recovered in urine.

2. In suspension form

The concentrations of diminazene in urine at different time intervals following its single intramuscular administration of its suspension alongwith procaine and antipyrine are given in Table 25. At all time intervals, the concentration of drug in urine was many fold higher than the minimum effective concentration of diminazene (0.3-0.6 $\mu g.ml^{-1}$). The maximum drug concentration (75.62 ± 10.43 $\mu g.ml^{-1}$) in urine was estimated between 8-10 h intervals. The detailed data on urinary excretion of diminazene in buffalo calves were calculated and given in Table 26. A graphical presentation of urine concentration of diminazene along with cumulative per cent of total dose of drug excreted in urine are shown in Fig. 11. Approximately 74.90 per cent of total administered drug was recovered in urine at end of 24 h. About 40 per

Table 23. Level of diminazene in urine of buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) in combination with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time interval after drug administration (h)	Animal number				
	A_1	A_2	A_3	A_4	Mean ±SE
0-2	93.92	65.06	105.2	29.42	73.40±16.92
2-4	88.26	*	*	53.18	70.72
4-6	60.54	*	*	50.63	55.58
6-8	50.63	*	*	55.44	53.03
8-10	41.19	*	89.39	44.47	58.35±15.54
10-12	48.94	101.8	*	31.23	60.67±21.21
12-18	44.13	*	75.81	64.50	61.48±9.26
18-24	39.04	70.72	80.34	43.00	58.27±10.18

Values given are expressed as $\mu g.ml^{-1}$

*Urine was not voided by animals during the period

Table 24. Urinary excretion of diminazene in buffalo calves following single intramuscular administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time interval (h)	Concentration (µg)	Total amount of drug excreted (mg)	Per cent of total dose excreted	Time interval (h)	Cumulative amount excreted (mg)	Cumulative per cent of total dose excreted
0-2	73.40±16.92	23.08±11.01	5.48±2.33	0-2	23.08±11.01	5.48±2.33
2-4[a]	70.72	9.38	3.96	0-4	27.77±8.61	7.46±1.87
4-6[a]	55.58	16.21	5.60	0-6	35.87±3.84	10.26±1.89
6-8[a]	53.03	10.84	3.99	0-8	38.45±2.35	12.26±2.90
8-10	58.35±15.54	26.92±7.11	7.31±0.48	0-10	61.49±7.64	17.74±3.67
10-12	60.67±21.21	43.15±40.14	10.61±9.39	0-12	93.85±24.58	25.70±5.60
12-18	61.48±9.26	45.07±10.83	13.78±3.75	0-18	127.6±14.47	36.04±5.29
18-24	58.27±10.18	40.66±16.63	11.12±3.84	0-24	168.3±30.20	47.16±8.22

Values given are mean ± SE of the results obtained from 4 animals except
[a]Values of two animals.

Fig. 10. Urine concentrations (●—●) and cumulative excretion (⊕) of diminazene in buffalo calves following single i.m. administration of its granules (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). Each point represents the mean of 2-4 animals.

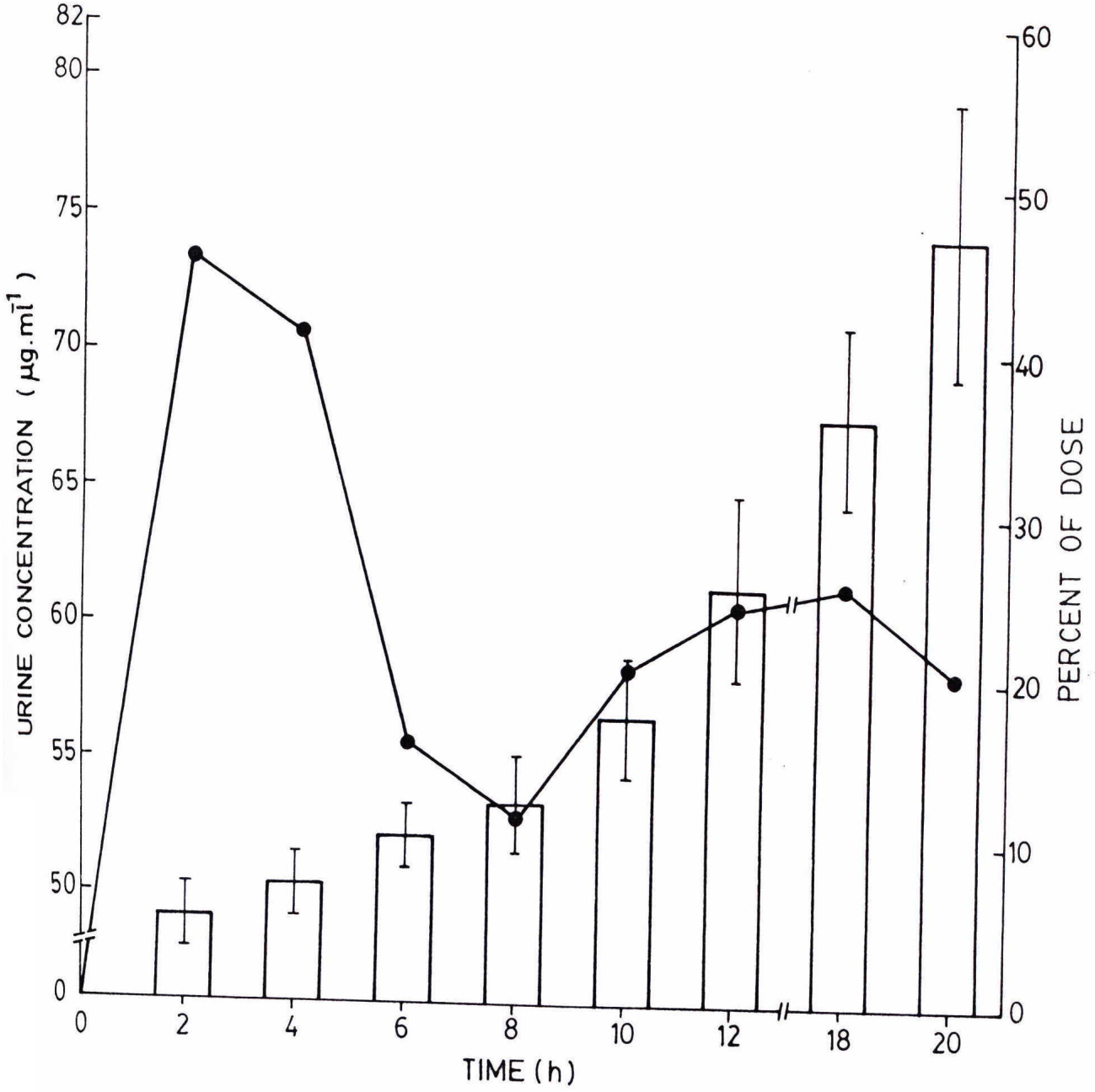

82
80
75
70
65
60
55
50
0
URINE CONCENTRATION (μg.ml⁻¹)
60
50
40
30
20
10
0
PERCENT OF DOSE
0
2
4
6
8
10
12
18
20
TIME (h)

Table 25. Levels of diminazene in urine of buffalo calves following single intramuscular administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$)

Time interval after drug administration (h)	Animal number				Mean ±SE
	B_1	B_2	B_3	B_4	
0-2	31.11	26.13	58.27	32.81	37.08±7.20
2-4	55.44	55.44	*	32.81	47.89±7.54
4-6	37.90	75.81	65.63	30.55	52.47±10.84
6-8	64.50	74.68	*	*	69.59
8-10	62.23	68.46	*	96.18	75.62±10.43
10-12	42.88	25.46	*	74.11	47.48±14.23
12-18	29.76	24.32	86.00	45.26	46.33±13.94
18-24	39.83	31.11	60.54	36.21	41.92±6.45

Values given are expressed as $\mu g.ml^{-1}$

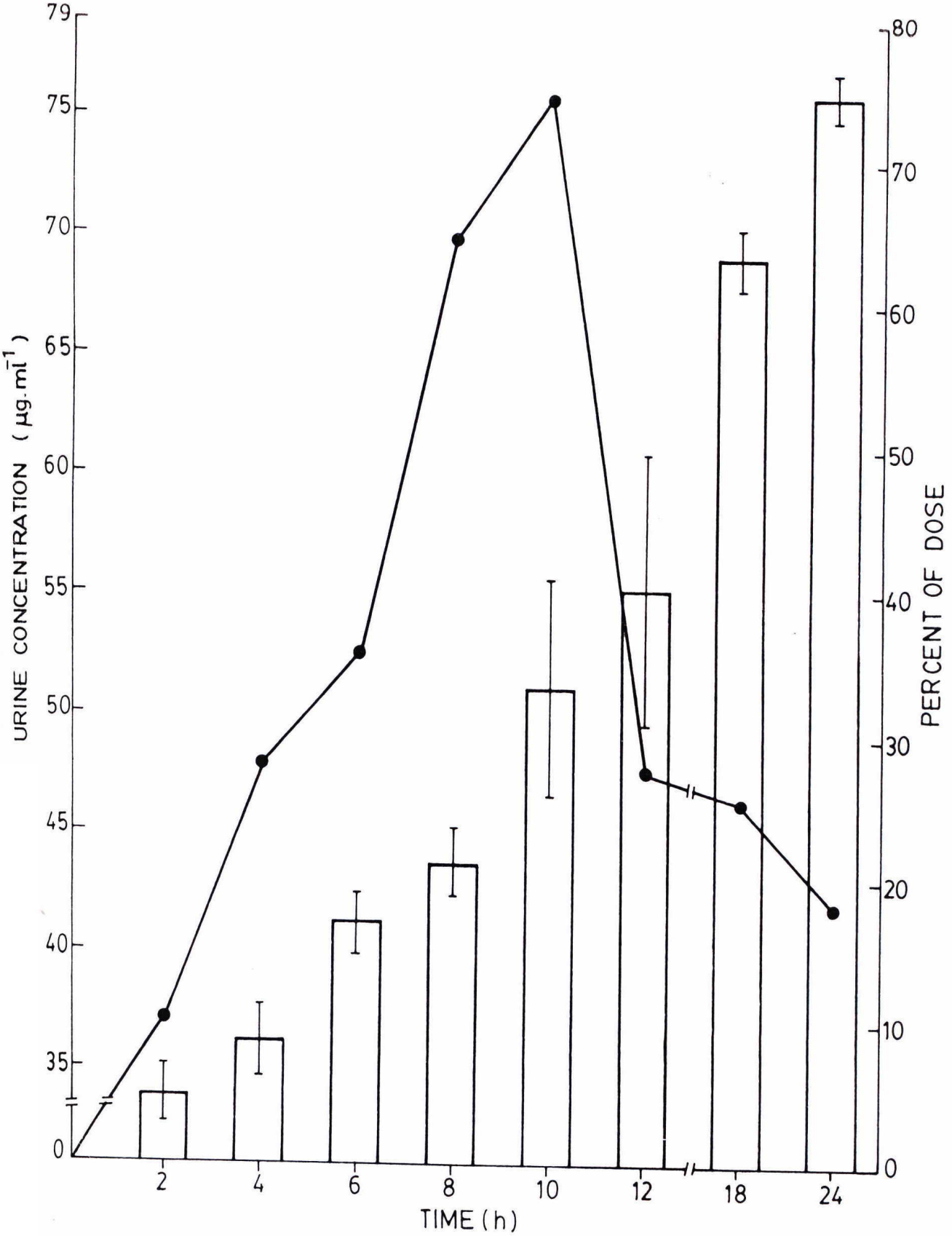
*Urine was not voided by the animals during the period

Table 26. Urinary excretion of diminazene in buffalo calves following single intramuscular administration of its suspension (3.5 mg.kg^{-1}) along with procaine (0.2 mg.kg^{-1}) and antipyrine (4.16 mg.kg^{-1})

Time interval (h)	Concentration (μg)	Total amount of drug excreted (mg)	Per cent of total dose excreted	Time interval (h)	Cumulative amount excreted (mg)	Cumulative per cent of total dose excreted
0-2	37.08±7.20	13.85±7.75	4.82±1.94	0-2	13.85±7.75	4.82±1.94
2-4	47.89±7.54	14.37±3.87	5.24±1.11	0-4	24.64±11.14	8.75±2.59
4-6	52.47±10.84	19.97±2.57	8.28±0.78	0-6	44.61±13.48	17.04±2.22
6-8	69.59	24.34	8.19	0-8	56.78±20.40	21.13±4.27
8-10	75.62±10.43	45.95±11.68	16.59±2.03	0-10	91.24±33.29	33.58±7.65
10-12	47.48±14.23	23.68±2.20	8.91±0.54	0-12	109.0±38.54	40.26±9.47
12-18	46.33±13.94	49.37±12.61	23.11±8.92	0-18	158.4±30.51	63.37±2.23
18-24	41.92±6.45	27.42±2.00	11.52±0.95	0-24	185.8±32.07	74.90±1.53

Values given are mean ± SE of the results obtained from 4 animals except
[a]Values of two animals.

Fig. 11. Urine concentrations (●—●) and cumulative urinary excretion (⊓) of diminazene in buffalo calves following single i.m. administration of its suspension (3.5 $mg.kg^{-1}$) along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$). Each point represents the mean of 2-4 animals.


URINE CONCENTRATION (µg.ml⁻¹)
PERCENT OF DOSE
TIME (h)
79
75
70
65
60
55
50
45
40
35
0
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
18
24

cent of drug was excreted in first 12 hours. During the first 2 hours only 4.82 ± 1.94 per cent of total administered drug was recovered in urine.

In case of suspension, there was more excretion of total administered drug than granules within 24 h in buffalo calves. The urinary excretion of diminazene within 24 h in cattle (Kaur 1998) and rabbit (Gilbert and Newton 1982) was reported to be 65 per cent of total administered dose. Contrary to this low value of urinary excretion had been reported in heifers and dogs 8.26 per cent and 26.8 per cent, respectively (Aliu *et al* 1993, Onyeyili and Anika 1989). The detailed mechanism of elimination of diminazene from animal body has not been conclusively elucidated.

D. Dosage regimen

The main and most important objective of present study was to compute the most appropriate dosage regimen of diminazene when given in combination with procaine and antipyrine. The satisfactory dosage regimen of diminazene may be computed by employing kinetic parameters as established in the present investigation. Primary and maintenance doses of granules and suspension of diminazene when given in combination with procaine and antipyrine in buffalo calves at different time intervals, to maintain the minimum therapeutic plasma concentration of 0.3, 0.45, 0.60 and 1.0 $\mu g.ml^{-1}$ are calculated and given in Tables 27 and 28, respectively. The dosage regimen of a drug predicted on the basis of pharmacokinetic study is intended only as

Table 27. Intramuscular dosage regimen of granules of diminazene when given in combination with procaine and antipyrine, calculated on the basis of kinetic parameters obtained in healthy buffalo calves, to maintain the specified plasma diminazene concentration

Time (days)	Dosage ($mg.kg^{-1}$)	Minimum effective concentration (MEC)*			
		0.30	0.45	0.60	1.0
3	D	0.9	1.3	1.8	3.0
	D'	0.1	0.1	0.2	0.3
4	D	0.9	1.4	1.8	3.1
	D'	0.1	0.2	0.2	0.4
5	D	0.9	1.4	1.9	3.2
	D'	0.1	0.2	0.3	0.5
6	D	1.0	1.5	2.0	3.3
	D'	0.2	0.3	0.3	0.6
7	D	1.0	1.5	2.0	3.4
	D'	0.2	0.3	0.4	0.7
8	D	1.1	1.6	2.1	3.5
	D'	0.2	0.4	0.5	0.8
9	D	1.1	1.6	2.2	3.6
	D'	0.3	0.4	0.6	0.9
10	D	1.1	1.7	2.2	3.8
	D'	0.3	0.5	0.6	1.1
11	D	1.1	1.7	2.3	3.9
	D'	0.4	0.5	0.7	1.2
12	D	1.2	1.8	2.4	4.0
	D'	0.4	0.6	0.8	1.3
13	D	1.3	1.9	2.5	4.2
	D'	0.4	0.7	0.9	1.5
14	D	1.3	1.9	2.6	4.3
	D'	0.5	0.7	1.0	1.6
15	D	1.3	2.0	2.7	4.5
	D'	0.5	0.8	1.0	1.7
20	D	1.6	2.3	3.2	5.3
	D'	0.7	1.2	1.5	2.6

Values are expressed as $\mu g.ml^{-1}$

D (Loading dose) = $Cp(min)^{\alpha} Vd(e^{\beta\tau})$

D' (Maintenance dose) = $Cp(min)^{\alpha} Vd(e^{\beta\tau}-1)$

Table 28. Intramuscular dosage regimen of suspension of diminazene in combination with procaine and antipyrine, calculated on the basis of kinetic parameters obtained in healthy buffalo calves, to maintain the specified plasma diminazene concentration

Time (days)	Dosage ($mg.kg^{-1}$)	Minimum effective concentration (MEC)*			
		0.30	0.45	0.60	1.0
3	D	0.7	1.1	1.4	2.4
	D'	0.1	0.1	0.2	0.3
4	D	0.7	1.1	1.5	2.5
	D'	0.1	0.2	0.2	0.4
5	D	0.8	1.2	1.6	2.6
	D'	0.1	0.2	0.3	0.5
6	D	0.8	1.2	1.6	2.7
	D'	0.2	0.3	0.4	0.6
7	D	0.8	1.3	1.7	2.8
	D'	0.2	0.3	0.4	0.7
8	D	0.9	1.3	1.8	3.0
	D'	0.2	0.4	0.5	0.8
9	D	0.9	1.4	1.9	3.1
	D'	0.3	0.4	0.6	1.0
10	D	1.0	1.5	1.9	3.3
	D'	0.3	0.5	0.7	1.1
11	D	1.0	1.5	2.0	3.4
	D'	0.4	0.6	0.8	1.3
12	D	1.1	1.6	2.1	3.6
	D'	0.4	0.7	0.9	1.5
13	D	1.1	1.7	2.3	3.8
	D'	0.5	0.7	1.0	1.7
14	D	1.2	1.8	2.4	4.0
	D'	0.6	0.8	1.1	1.9
15	D	1.2	1.9	2.5	4.2
	D'	0.6	0.9	1.2	2.1
20	D	1.4	2.1	2.9	4.8
	D'	0.8	1.2	1.6	2.7

Values are expressed as $\mu g.ml^{-1}$

D (Loading dose) = $Cp(min)^{\alpha} Vd(e^{\beta\tau})$

D' (Maintenance dose) = $Cp(min)^{\alpha} Vd(e^{\beta\tau}-1)$

a guide line and the effectiveness of drug in clinical condition still remained to be verified, since the final proof of effective plasma concentration of drug resides in clinical efficacy. The activity of drug depends on this plasma concentration during the course of therapy which should not fall certain effective concentration. For diminazene a concentration of 0.3 to 0.6 $\mu g.ml^{-1}$ is considered effective (Singh 1997). Keeping different factors in view, MEC (1.0 $\mu g.ml^{-1}$) more than higher range was taken into consideration.

When a fixed dose is repeated at constant interval, the minimum steady state ($C_{pmin}\alpha$) at the end of any dosing interval is calculated by the following equation :

$$C_{p(min)}^{\alpha} = \frac{D}{Vd(e^{-\beta\tau} - 1)}$$

If the minimum effective concentration of a drug is known, then after choosing a suitable and convenient dosage interval, one can calculate the priming (D) and maintenance (D') doses by the following formula :

$$D = MEC.\ Vd(e^{-\beta\tau} - 1)$$

$$D' = MEC.Vd(e^{-\beta\tau} - 1)$$

Taking the MEC as 1.0 μgml^{-1} and dosage interval as 7 days, the priming and maintenance doses of diminazene in buffalo calves would be 3.4 and 0.7 $mg.kg^{-1}$ in case of granules and 2.8 and 0.7 $mg.kg^{-1}$ in suspension

when given along with procaine and antipyrine, but when diminazene was administered alone its priming and maintenance doses were 14.5 and 11.5 $mg.kg^{-1}$, respectively (Singh 1997). It is clear from investigation that concomitant administration of procaine and antipyrine along with diminazene, decreases the dose rate of diminazene. As the therapeutic effect of this drug (both granules and suspension) remained in body for more than 20 days, by considering all the factors satisfactory intramuscular dosage regimen of diminazene when given along with procaine and antipyrine would be 4.3 $mg.kg^{-1}$ followed by 1.9 $mg.kg^{-1}$ at 15 days interval.

Finally, the calculated maintenance dose and dosage interval are supported by the duration of therapeutic level in plasma (td.). After injecting the priming dose, the duration for which the therapeutic level (1.0 $\mu g.ml^{-1}$) is maintained in plasma may be predicted from equation :

$$td = \frac{2.3}{\beta} \log \frac{Ao}{A\ min}$$

$$= \frac{\ln \frac{Ao}{A\ min}}{\beta}$$

Where Ao is dose administered and A min is minimum effective concentration. Substituting the values, the above equation reduces to 954.5 ±

151.6 and 1259.3 ± 478.8 h following i.m. administration of granules and suspension, respectively.

This pharmacokinetic approach to design the dosage regimen should prove more worthy and useful in evaluating the therapeutic efficacy of diminazene when given in combination with procaine and antipyrine in buffalo calves.

E. *In vitro* study of diminazene

1. Erythrocytic partitioning

Extensive data has been generated regarding penetration of various drugs into different types of isolated cells (Klempner and Styrt 1981). The penetration of diminazene into erythrocytes may serve as an important index because (i) the drug entered into erythrocytes may serve as reservoir for diminazene resulting the MEC can be maintained for longer duration (ii) in case of any intracellular infection, the drug will also be effective. The erythrocytic membrane is considered as a best suitable model for drug transfer study (Srivastava and Malik 1988).

Erythrocytic partitioning of diminazene was studied at different known blood concentrations following addition of diminazene ranging from 2.5 to 20 $\mu g.ml^{-1}$, the estimated levels of drug in blood, plasma and erythrocytes after incubation of 12 h, are presented in Table 29. Erythrocytic partitioning and other important parameters of erythrocytes partitioning were also calculated and presented in Table 30. At different blood concentrations ranging from 1.25

Table 29. *In vitro* level of diminazene ($\mu g.ml^{-1}$) in blood, plasma and erythrocytes of buffalo calves at different known blood concentrations

Known concentration of drug added in blood ($\mu g.ml^{-1}$)	Concentration measured ($\mu g.ml^{-1}$)		
	Blood	Plasma	Erythrocytes
20	5.87±0.77	9.47±0.69	2.37±0.71
10	4.29±0.02	5.04±0.34	1.73±0.45
5	3.06±0.22	3.83±0.06	1.37±0.43
2.5	2.01±0.04	2.78±0.05	0.99±0.09

Values given are mean ± SE of 3 experiments.

Table 30. *In vitro* erythrocytic partitioning studies on diminazene after adding different known concentrations ($\mu g.ml^{-1}$) in blood

Parameters	Unit	Concentration ($\mu g.ml^{-1}$)				
		20	10	5	2.5	1.25
Ke/p	Ratio	0.276±0.087	0.338±0.066	0.355±0.106	0.355±0.039	0.306±0.018
Kb/p	Ratio	0.616±0.036	0.855±0.053	0.799±0.046	0.867±0.033	0.802±0.003
Erythrocytic partitioning	%	17.6±1.71	25.1±3.71	25.8±5.84	26.2±2.14	23.4±2.84

Values given are mean ± SE of 3 animals

to 20 $\mu g.ml^{-1}$, the extent of partitioning was to the extent of 17.6 to 26.2 per cent.

The values of $K_{e/p}$ and $K_{b/p}$ ranged between 0.276 - 0.355 and 0.616 - 0.867, respectively. The diagrammatic relationship between $K_{e/p}$, $K_{b/p}$ and erythrocytic partitioning have also been presented in Fig. 12.

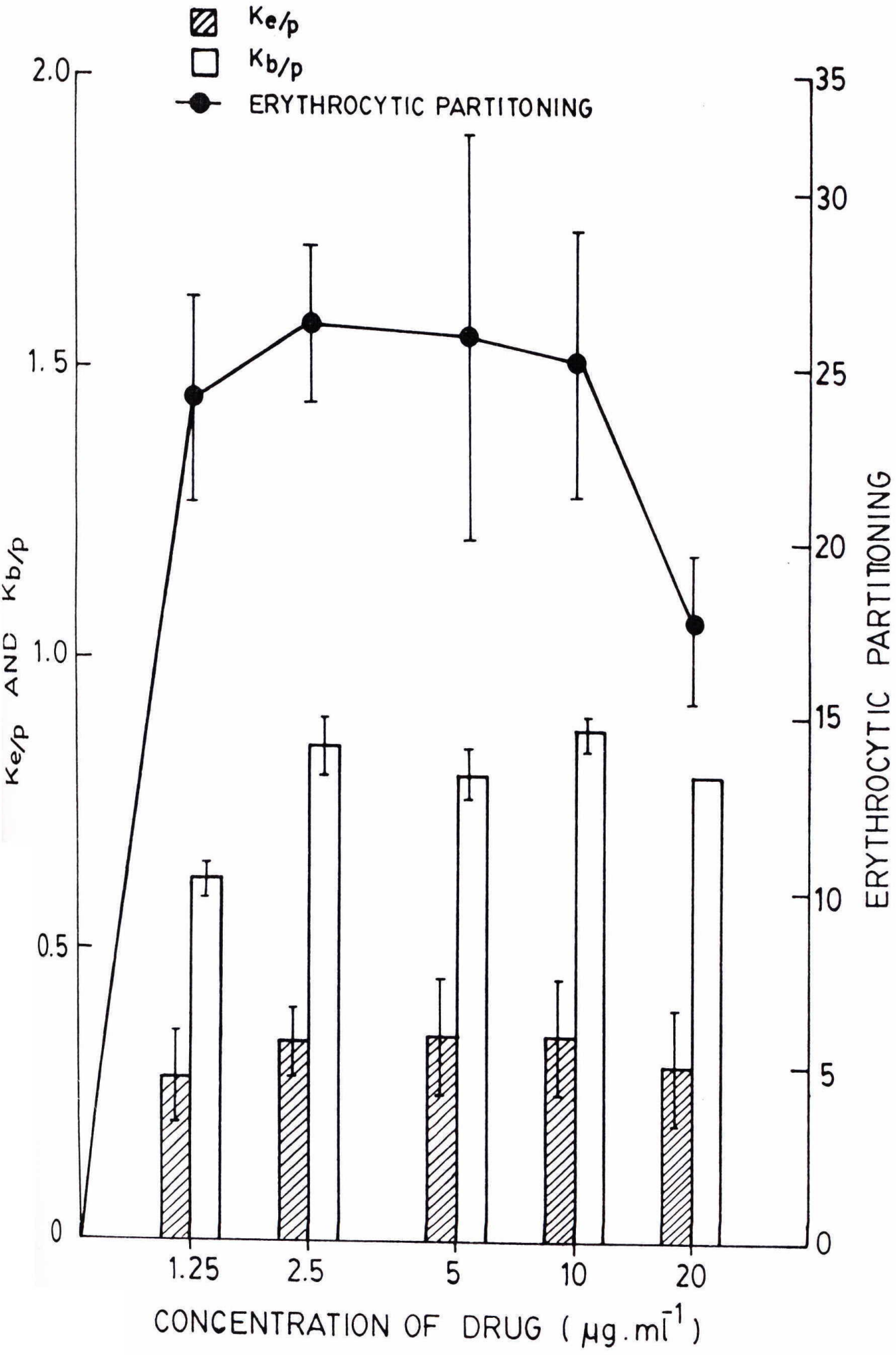
In contrast to present study, erythrocyte penetration of diminazene in cow calves was reported to the extent of 48.1 ± 4.87 per cent(Kaur 1998).

In buffalo species penetration of diminazene into erythrocytes to the extent of (23.6 ± 3.25 %) is considered good as value of PCV was 37 per cent. It had been reported that if a drug penetrates to be the extent of 3/4 of its PCV value, the penetration is considered to excellent (Srivastava and Malik 1987). The results of present investigation revealed that diminazene can be used for treatment of intracellular protozoan infection if it is sensitive to that. Although, there is no such report for diminazene but for antibiotics it has been established that antibiotics having good penetration into erythrocytes are preferred, because erythrocytes store the drug which is released slowly and help in maintaining therapeutic level for a longer time (Ziv 1980).

2. Plasma protein binding

The plasma protein binding of chemotherapeutic agent is an important index to assess the active fraction of the drug in blood and tissue compartments. As only unbound or free drug is mainly available at the

Fig. 12 The relationship between $K_{e/p}$ (▨), $K_{b/p}$ (□) and erythrocytic partitioning (●—●) of diminazene in buffalo calves at different known concentrations. Values given are mean ± SE of 3 animals.


Ke/p
Kb/p
ERYTHROCYTIC PARTITONING
2.0
1.5
1.0
0.5
0
35
30
25
20
15
10
5
0
Ke/p AND Kb/p
ERYTHROCYTIC PARTITONING
1.25
2.5
5
10
20
CONCENTRATION OF DRUG (μg.ml⁻¹)

receptor site for eliciting desired pharmacological action (Rolinson and Sutherland 1965, Kunin 1966, Peterson *et al* 1975) and also for distribution and renal clearance (Wise *et al* 1980, Craig and Gerber 1981,Yamada *et al* 1981), the extent of protein binding directly affects the pharmacokinetic determinants and therapeutic efficacy of drug.

In vitro concentrations of diminazene in plasma and buffer following dialysis at 37°C for 24 h are given in Table 31. The extent of *in vitro* plasma protein binding of diminazene at various known plasma concentrations viz., 20, 10, 5 and 2 $\mu g.ml^{-1}$ were determined and presented in Table 32. At different concentrations the diminazene bounds with plasma protein to the extent of 34.0 ± 1.40 per cent. Based on data of different experiments the association rate constant of diminazene (β_i; capacity of buffalo calves plasma protein to bind with diminazene) and dissociation rate constant (K_β) were also calculated and presented in Table 33. The values of β_i and K_β of diminazene ranged between 4.189×10^{-9} to 1.150×10^{-8} $mole.g^{-1}$ and 2.290×10^{-7} to 6.698×10^{-7} mole, respectively. A graphical presentation showing the relationship between free concentration of diminazene and a constant I_i has also been given in Fig. 13. It is evident from Fig. 13, that the slope of chosen plot is equal to β_i and its negative intercept with the ordinate is equal to K_β.

The results of plasma protein binding of present study are in accordance with the results of plasma protein binding of heifers and cross bred cow calves. The plasma protein binding of diminazene in heifers has

Table 31. *In vitro* concentration of diminazene in plasma and buffer following dialysis at 37°C for 24 h

Concentration of diminazene added ($\mu g.m^{-1}$)	Buffer			Plasma		
	1	2	3	1	2	3
2	0.62	0.72	0.60	1.35	1.41	1.42
5	1.62	1.49	1.35	3.07	3.16	3.15
10	3.25	3.17	3.45	6.17	6.22	6.04
20	6.08	6.38	6.48	13.17	12.95	12.10

The values are in $\mu g.ml^{-1}$ in three different experiments

Table 32. Extent (%) of *in vitro* binding of diminazene to plasma proteins of buffalo calves

Experiment no.	Concentration of diminazene ($\mu g.ml^{-1}$)			
	2	5	10	20
1	37.0	30.9	31.0	36.8
2	40.6	40.0	27.3	30.2
3	32.4	35.9	32.5	34.0
Mean ± SE	36.7±2.37	36.6±2.63	30.2±1.54	33.7±1.90

Overall mean ± SE = 34.0 ± 1.40

Table 33. *In vitro* binding of diminazene to plasma proteins of buffalo calves

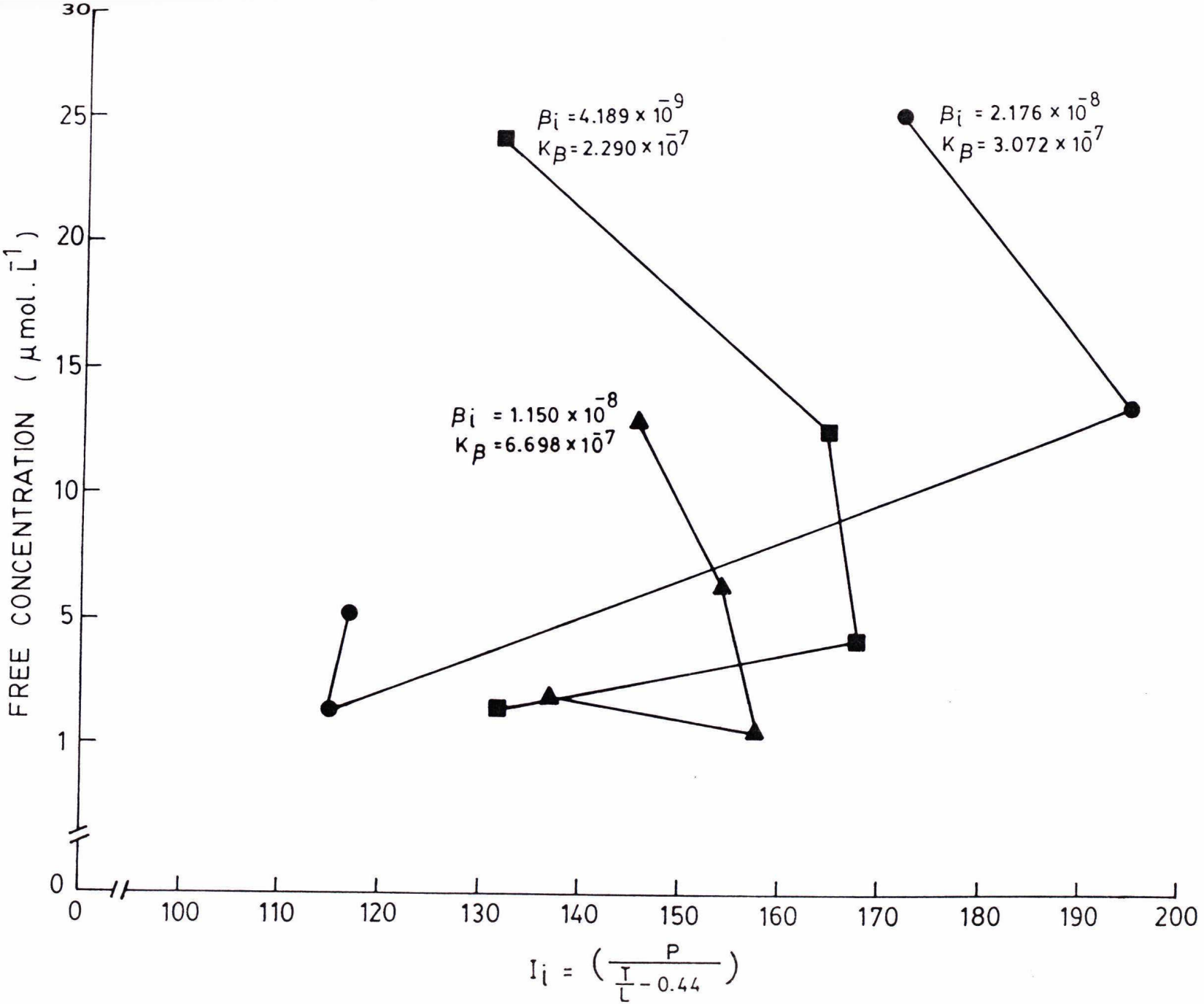
Coefficient	Unit	Experiment number			
		1	2	3	Mean ±Se
Ii	a	132.6	114.5	157.7	134.9±12.5
	b	167.5	117.1	137.0	140.5±14.7
	c	166.9	195.3	157.1	173.09±11.5
	d	132.2	172.2	147.9	150.8±11.6
m_i	$mole.g^{-1}$	1.819×10^{-9}	9.451×10^{-9}	4.993×10^{-9}	$5.421 \times 10^{-9} \pm 2.213 \times 10^{-9}$
β_i	$mole.g^{-1}$	4.189×10^{-9}	2.176×10^{-8}	1.150×10^{-8}	$1.248 \times 10^{-8} \pm 0.509 \times 10^{-8}$
$K\beta$	mole	2.290×10^{-7}	3.072×10^{-7}	6.698×10^{-7}	$4.020 \times 10^{-7} \pm 1.358 \times 10^{-7}$

For the calculation of β_i and $K\beta$ following standard reported values (Srivastava and Bal, 1995) were taken into consideration.
Protein content of plasma(P) = 8.5G/100 ml, water contents of plasma (W) = 0.94 ml/ml.

Fig. 13. Linearized plot of plasma protein binding of diminazene corresponding to equation, $b_1z_1 + b_2z_2 + z_3 = 0$. Ordinate shows the free concentration of diminazene. Abscissa represents the constants I_i, which is obtained mathematically by the equation :

$$I_i = \frac{P}{\frac{T}{L} - 0.94}$$

β_i was calculated by least square regression technique and its negative intercept with the ordinate was equal to K_β.


30
25
20
15
10
5
1
0
FREE CONCENTRATION (μmol. L⁻¹)
$\beta_i = 4.189 \times 10^{-9}$
$K_\beta = 2.290 \times 10^{-7}$
$\beta_i = 2.176 \times 10^{-8}$
$K_\beta = 3.072 \times 10^{-7}$
$\beta_i = 1.150 \times 10^{-8}$
$K_\beta = 6.698 \times 10^{-7}$
0
100
110
120
130
140
150
160
170
180
190
200
$I_i = \left(\frac{P}{\frac{T}{L} - 0.44}\right)$

been reported to be 38.0 to 91.1 per cent and in cross bred cow calves 32.3 ± 4.67 per cent (Aliu et al 1993, Kaur 1998). The moderate plasma protein binding of diminazene observed in the present study, suggests that a good percentage of drug is available in blood streams to elicit its pharmacological action at cellular site and hence diminazene can be effectively employed in buffalo species. Baggot (1977) had concluded that low to moderate extent (< 80 %) of protein binding have little or no influence on the kinetic disposition of drug, since the interaction between drug and plasma protein is readily reversible.

The determination of binding capacity of protein (β_i) and drug protein complex dissociation constant (K_β) allows complete quantitation of the system. The β_i and K_β were calculated according to the equation of Pilloud (1973). Free drug concentration was plotted as a function of l_i on semilogarithmic scales. The β_i was calculated by least square regression technique,

l_i was calculated as follows :

$$\text{Where } l_i = \frac{P}{\frac{T}{L} - W}$$

Where,

P = Protein contents of plasma (8.5 gm/100 ml)

T = Total concentration of drug

L = Free concentration of drug

W = Water content of plasma as 0.94 $ml.ml^{-1}$

From such semilogarithmic plot the relationship between β_i and K_β can easily be derived by law of mass action.

$$L = \beta_i \frac{P}{\frac{T}{L} - W} - K_\beta$$

Since binding was demonstrated to be reversible, application of the law of mass action is justified. After calculating the numerical value of β_i by the method of least square regression technique, value of K_β can be feathered from above equation.

On the basis of present results, it can be interpreted that (i) diminazene binds with plasma protein to moderate extent, (ii) the dissociation rate constant (K_β) is more than association rate constant (β_i) resulting the protein bound diminazene will be easily dissociated.

F. Comparison between two dosage form of Trypan (diminazene + procaine + antipyrine)

Based on present investigation, the data obtained on the plasma levels, pharmacokinetics parameters and urinary excretion of two preparations of Trypan i.e. granules and suspension have been compared in Tables 34, 35 and 36, respectively.

While comparing the plasma levels at various time intervals when given in combination with procaine and antipyrine in two different dosage forms i.e.

Table 34. Comparison of plasma levels of diminazene (3.5 $mg.kg^{-1}$) administered in two different dosage form of "Trypan" (diminazene + procaine + antipyrine) in buffalo calves

Time after drug administration	Form of dosage	
	Granules	Suspension
1 min	1.53±0.15	1.50 ±0.07
5	2.65±0.99	1.72 ±0.13
10	3.14±1.25	3.77 ±1.18
30	1.89±0.15	2.78 ±0.96
1 h	1.46±0.13	1.82 ±0.07
2	1.36±0.13	1.79 ±0.08**
4	1.34±0.15	1.77 ±0.13
6	1.32±0.14	1.76 ±0.11
8	1.30±0.14	1.74 ±0.03*
10	1.30±0.14	1.64 ±0.12
12	1.30±0.13	1.64 ±0.08
24	1.28±0.15	1.66 ±0.15
48	1.26±0.17	1.53 ±0.12
72	1.26±0.17	1.47 ±0.11
96	1.26±0.19	1.45 ±0.08
120	1.24±0.19	1.38 ±0.07
168	1.05±0.11	1.25 ±0.10

* Significantly ($P < 0.01$) different as compared to their corresponding values following administration of its granules.

** Significantly ($P < 0.05$) different as compared to their corresponding values following administration of its granules.

Table 35. Comparison of important pharmacokinetic parameters of diminazene (3.5 $mg.kg^{-1}$) administered in two different dosage form of "Trypan" (diminazene + procaine + antipyrine) in buffalo calves

Parameter[a]	Unit	Form of dosage	
		Granules	Suspension
Ka	h^{-1}	9.24±4.86	15.7±5.64
α	h^{-1}	2.76±0.76	3.24±1.62
β	h^{-1}	0.0014±0.0001	0.0017±0.0007
$t_{0.5}Ka$	h	0.446±0.219	0.057±0.012
$t_{0.5}\alpha$	h	0.314±0.084	0.400±0.138
$t_{0.5}\beta$	h	538.3±94.7	667.6±±248.0
t_{max}	min	17.5±4.33	17.5±4.33
C_{max}	$\mu g.ml^{-1}$	3.35±1.18	4.11±1.05
K_{el}	h^{-1}	0.0055±0.0020	0.0046±0.0021
$V_{d(ss)}$	$L.kg^{-1}$	2.70±0.30	2.11±0.19
MRT	h	775.9±136.8	962.3±357.5
Cl_T	$ml.kg^{-1}.h^{-1}$	3.66±0.54	3.48±1.292
K_{12}	h^{-1}	2.037±0.764	2.063±1.292
K_{21}	h^{-1}	0.717±0.093	1.172±0.370
K_{12}/K_{21}	Ratio	2.99±1.25	1.43±0.500
T/P	Ratio	2.98±1.24	1.42±0.502
td	h	954.5±151.6	1259.3±778.8

[a]Kinetic parameters are as described by Gibaldi and Perrier (1982)
None of the pharmacokinetic parameters differed significantly from each other.

Table 36. Comparison of data on urinary excretion of diminazene (3.5 $mg.kg^{-1}$) administered in two different dosage form of "Trypan" (diminazene + procaine + antipyrine) in buffalo calves

Parameter	Form of dosage	
	Granules	Suspension
Urine concentration ($\mu g.ml^{-1}$)		
0-2 h	73.4±16.9	37.1±7.20
10-12 h	60.7±21.2	47.5±14.2
18-24 h	58.3±10.2	41.9±6.45
Cumulative amount excreted (mg)		
0-12 h	93.8±24.6	109.0±38.5
0-24 h	168.3±30.2	185.8±32.1
Cumulaltive dose excreted (%)		
0-12 h	25.7±5.60	40.3±9.47
0-24 h	47.2±8.22	74.9±1.53*

*Significantly ($P < 0.01$) different as compared to their corresponding value obtained following administration of its granules.

granules and suspension, it was revealed that no significant difference in plasma concentrations at most of time intervals except at 2 and 8 hr after drug administration. At these two time intervals, the plasma concentration in suspension was significantly higher than granules.

Similarly there was no significant difference between pharmacokinetic parameters of two different dosage form of trypan.

Although the urine concentration of diminazene did not vary significantly in two dosage form, the cumulative urinary excretion (%) was higher in case of suspension as compared to granules.

G. Comparison between diminazene and Trypan (Diminazene + procaine + antipyrine)

The data on plasma levels and pharmacokinetics obtained in present investigation following administration of diminazene along with procaine and antipyrine (Trypan) and the established data in buffalo calves following administration of diminazene alone (Berenil) have been compared in Tables 37 and 38, respectively.

While comparing the plasma levels and pharmacokinetic parameters of diminazene administered along with procaine and antipyrine, as established in the present study with its alone administration, it was revealed that these data differ significantly from each other.

Table 37. Comparison of plasma levels of diminazene (3.5 $mg.kg^{-1}$) administered alone (as Berenil) and in combination with procaine + antipyrine (as Trypan) in buffalo calves

Time after drug administration	Diminazene[a] alone (Berenil	Diminazene + procaine+ antipyrine[b] (Trypan)
1 min	2.71±0.05	1.50 ±0.07*
5	3.90±0.06	1.72 ±0.13*
10	8.12±0.87	3.77 ±1.18*
30	3.72±0.10	2.78 ±0.96*
1 h	3.07±0.22	1.82 ±0.07*
2	2.85±0.17	1.79 ±0.08*
4	2.49±0.13	1.77 ±0.13*
6	2.49±0.17	1.76 ±0.11*
8	2.60±0.16	1.74 ±0.03*
10	2.34±0.13	1.64 ±0.12*
12	2.05±0.14	1.64 ±0.08
24	1.95±0.11	1.66 ±0.15
48	1.73±0.06	1.53 ±0.12
72	1.30±0.00	1.47 ±0.11
96	1.08±0.12	1.45 ±0.08**
120	1.00±0.07	1.38 ±0.07*
168	0.93±0.07	1.25 ±0.10**

[a]Data of Singh (1997)

[b]Data of suspension obtained after administration of Trypan

* Significantly ($P < 0.01$) different as compared to their corresponding values following administration of diminazene alone.

** Significantly ($P < 0.05$) different as compared to their corresponding values following administration of diminazene alone.

Table 38. Comparison of important pharmacokinetic parameters of (3.5 $mg.kg^{-1}$) diminazene administered alone (as Berenil) and in combination with procaine and antipyrine (as Trypan) in buffalo calves

Parameter[a]	Unit	Diminazene[a] alone (Berenil)	Diminazene + procaine+ antipyrine[b] (Trypan)
A'	$\mu g.ml^{-1}$	0.765±0.253	0.771±0.718
Ka	h^{-1}	78.85±12.33	15.7±5.64*
B	$\mu g.ml^{-1}$	2.72±0.15	1.70±0.16*
β	h^{-1}	0.0094±0.0018	0.0017±0.0007*
$t_{0.5}Ka$	h	0.010±0.002	0.057±0.012*
$t_{0.5}\beta$	h	67.88±10.38	667.6±248.0**
AUC	$\mu g.ml^{-1}.h$	360.1±76.1	1499.9±490.0**
AUMC	$mg.kg^{-1}.h^2$	65.32±29.46	1962.4±1016.2**
t_{max}	min	10.0±0.00	17.5±4.33
C_{max}	$\mu g.ml^{-1}$	8.13±0.87	4.11±1.05**
MRT	h	141.7±0.18	962.3±357.5**
$V_{d(B)}$	$L.kg^{-1}$	2.99±0.18	2.12±0.19**
t_d	h	730.2±591.6	1259.3±478.8

[a]Data of Singh (1997)
[b]Data of suspension obtained after administration of Trypan

* Significantly ($P < 0.01$) different as compared to their corresponding values obtained following administration of diminazene alone.
** Significantly ($P < 0.05$) different as compared to their corresponding values obtained following administration of diminazene alone.

If we compare the data of plasma levels upto 48 h, the plasma levels of diminazene were higher in case of its alone administration as compared to its combined administration (diminazene + procaine + antipyrine). But after that, the diminazene levels were higher in case of combined administration (1.25 ± 0.10 $\mu g.ml^{-1}$) than its alone administration (0.93 ± 0.07 $\mu g.ml^{-1}$). From the present result it is concluded that procaine and antipyrine significantly prolonged the maintenance of MEC of diminazene resulting the plasma levels of diminazene persisted for longer duration in body of buffalo calves.

While comparing the pharmacokinetic parameters, the absorption rate constant of diminazene when administered alone, was found higher (78.85 ± 12.33 h^{-1}) than its combined administration (15.7 ± 5.64 h^{-1}). It was further confirmed by shorter values of tmax (10 min) calculated after alone administration than combined administration (17.5 min). The elimination half lives ($t_{0.5}\beta$) of diminazene were 67.88 ± 10.38 and 667.6 ± 248.0 h, respectively following its alone and combined administration. Further, procaine and antipyrine were also found to decrease the elimination of diminazene from body of buffalo calves. This is again substantiated by the higher values of td (1259.3± 478.8 h) following combined administration than alone administration (730.2 ± 591.6 h).

CHAPTER V

SUMMARY

In the present study, the effect of procaine and antipyrine on pharmacokinetics and urinary excretion of diminazene was investigated in buffalo calves. Diminazene was administered at dose rate of 3.5 $mg.kg^{-1}$ along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) by single i.m. route in two dosage form i.e. granules and suspension. In addition, *in vitro* erythrocytic partitioning and *in vitro* plasma protein binding of diminazene was also studied in buffalo calves. Based on results obtained, an appropriate dosage regimen of diminazene, when given in combination with procaine and antipyrine was calculated in buffalo calves for their therapeutic use in the treatment of trypanosomiasis. The salient research findings are as follows :

1. Following intramuscular administration of diminazene along with procaine and antipyrine in its granules form, the appreciable concentration of diminazene 1.53 ± 0.15 $\mu g.ml^{-1}$ appeared at 1 min. The peak plasma level of 3.17 ± 1.05 $\mu g.ml^{-1}$ was achieved at 15 min of administration. The minimum effective plasma concentration was attained at 1 min and remain maintained upto 7 days.
2. The semilogarithmic plot of plasma concentration against time revealed three distinct phases i.e. absorption, distribution and excretion.

Different kinetic parameters viz., absorption half life (0.446 ± 0.219 h), distribution half life (0.314 ± 0.084 h), elimination half life (538.3 ± 94.7 h), apparent volume of distribution (2.70 ± 0.30 $L.kg^{-1}$), AUC (1017.7 ± 142.0 $\mu g.ml^{-1}.h$) and AUMC (826.2 ± 234.6 $mg.ml^{-1}.h^2$) were calculated by employing triexponential equation. The mean residual time, duration of pharmacological effect and T/P ratio were 775.9 ± 136.8 h, 954.5 ± 151.6 h and 2.98 ± 1.24, respectively.

3. Intramuscular administration of diminazene alongwith the procaine and antipyrine, in its suspension form, resulted in appreciable concentration of diminazene (1.50 ± 0.07 $\mu g.ml^{-1}$) at 1 min. The peak plasma level of 3.77 ± 1.18 $\mu g.ml^{-1}$ was achieved at 10 min of administration. The minimum effective concentration was attained at 1 min and remain maintained upto 7 days.

4. Similar to granual diminazene, the pattern of plasma diminazene concentration in suspension form also showed three distinct phases. The absorption half life, distribution half life, elimination half life, apparent volume of distribution, AUC and AUMC were calculated to be 0.057± 0.012 h, 0.400 ± 0.138 h, 667.6 ± 248.0 h, 2.11 ± 0.19 $L.kg^{-1}$, 1499.9 ± 490.0 $\mu g.ml^{-1}.h$ and 1962.4 ± 1016.2 $mg.ml^{-1}.h^2$, respectively. The mean residual time, duration of pharmacological effect and T/P were 962.3 ± 357.5 h, 1259.3 ± 478.8 h and 1.42 ± 0.50, respectively.

5. Following single i.m. administration of diminazene as granular and suspension form about 47 and 75 per cent, respectively of administered dose were eliminated in urine within 24 h.

6. In vitro erythrocytic partitioning of diminazene at different concentrations ranging from 1.25 to 20 $\mu g.ml^{-1}$, was in range of 17.6 to 26.2 per cent with an overall mean of 23.6 ± 3.25 per cent.

7. In vitro binding capacity of diminazene to plasma proteins (β_i) and dissociation constant of protein drug complex (K_β) of buffalo calves were calculated to be $1.248 \times 10^{-8} \pm 0.509 \times 10^{-8}$ $mole.g^{-1}$ and $4.020 \times 10^{-7} \pm 1.358 \times 10^{-7}$ mole, respectively. Diminazene at different concentrations ranging from 2 to 20 $\mu g.ml^{-1}$ bound to plasma proteins to extent of 34.0 ± 1.40 per cent.

8. On comparing the results, it is revealed that there was no significant difference in plasma levels and pharmacokinetic parameters of administration of two different dosage forms of diminazene i.e. granules and suspension. Although, the cumulative per cent of urinary excretion was higher in case of suspension as compared to granules but there was no difference in the urine concentration of diminazene. For evaluating the effect of procaine and antipyrine on pharmacokinetics of diminazene, the data of present investigation was compared with already established data following alone administration of diminazene. The results revealed that procaine and antipyrine causes slow

elimination of diminazene from body and prolongs the therapeutic effect of diminazene in body for more than 20 days. Further, when diminazene is administered along with procaine and antipyrine, its elimination half life was 8-10 fold increased as compared to its alone administration.

On the basis of present results, it is concluded that procaine and antipyrine significantly prolong the elimination half life and therapeutic effect of diminazene in body of buffalo calves. The most appropriate dosage regimen of diminazene in buffalo calves, when administered along with procaine and antipyrine would be 4.3 $mg.kg^{-1}$ followed by 1.9 $mg.kg^{-1}$ at 15 days interval.

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APPENDIX

Calculation of absorption, distribution and elimination constants and extrapolated coefficients of diminazene (3.5 $mg.kg^{-1}$) following its single intramuscular administration in healthy buffalo calves when given along with procaine (0.2 $mg.kg^{-1}$) and antipyrine (4.16 $mg.kg^{-1}$) are described below :

Weight of animal = 120 kg

Total dose of drug given = 420 mg

Elimination constant and its extrapolated coefficient

X Time (h)	Y' Observed plasma concentration ($\mu g.ml^{-1}$)	Y (log. Y')
24	1.16	0.064
48	1.13	0.053
72	1.15	0.060
96	1.14	0.056
120	1.00	0
144	0.92	-0.036

$\Sigma X = 504$ $\Sigma Y = 0.197$

$\bar{X} = 84$ $\bar{Y} = 0.032$

$\Sigma X^2 = 52416$ $\Sigma XY = 8.592$

$\frac{(\Sigma X)^2}{n} = 42336$ $\frac{\Sigma X \Sigma Y}{n} = 16.548$

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

Where n = number of observation

$m = -0.000789\ h^{-1}$

$\beta = m \times 2.303$

$\beta = -0.00180\ h^{-1}$

$Y = C + mx$ Equation a

$C = \bar{Y} - m\bar{X}$

$C = 0.098276$

taking X zero, the values of Y can be calculated from equation a.

$Y = \log B = 0.098276$

$B = \text{antilog } 0.098276$

$B = 1.2539\ \mu g.ml^{-1}$

Absorption constant and its extrapolated coefficient

The theoretical values were subtracted from observed plasma concentrations and residual values were obtained. Based on residual concentration Ka and A' were calculated as below :

X Time (min)	Observed plasma concentration ($\mu g.ml^{-1}$)	Calculated plasma concentration	Y' residual concentration	Y log (observed calculated)
1	1.18	1.0791	0.1009	-0.996
2.5	1.56	1.0791	0.4809	-0.317
5	1.66	1.0790	0.5810	-0.235
7.5	1.73	1.0789	0.6511	-0.186
10	1.89	1.0788	0.8112	-0.090
15	2.04	1.0787	0.9613	-0.017

$\Sigma X = 41$

$\bar{X} = 6.83$

$\Sigma X^2 = 413.5$

$\frac{(\Sigma X)^2}{n} = 280.16$

$\Sigma Y = -1.841$

$\bar{Y} = -0.306$

$\Sigma XY = -5.513$

$\frac{\Sigma X \Sigma Y}{n} = -12.580167$

$$m = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

$m = 0.0530\ min^{-1}$

$Ka = m \times 2.303$

$Ka = 0.122059\ min^{-1}$

$Ka = 7.32354\ h^{-1}$

$C = \bar{Y} - m\bar{X}$

$C = -0.66799$

Putting the value of C and X, A' is calculated in a similar way as B.

Distribution constant and its extrapolated coefficient

Based on residual concentration α and A were calculated in a similar way as Ka and A'.

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