SOME ASPECTS OF THE PHARMACODYNAMICS OF FLUKICIDAL AND RELATED DRUGS

A THESIS

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OF

DOCTOR OF PHILOSOPHY

BY

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PAPERS PUBLISHED BASED ON THE WORK OF THIS THESIS

1. The pharmacokinetics of oxyclozanide, rafoxanide and closantel : do salicylanilides really kill immature flukes?
   Mohammed-Ali, N.A.K. and Bogan, J.A. (1985).
   11th Conference of the World Association for the Advancement of Veterinary Parasitology, 5th - 9th August, 1985, Rio de Janeiro, Brazil.

2. Pharmacokinetic studies with triclabendazole/fenbendazole combination in sheep and cattle.
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3. Effect of feeding on the fate of orally administered phenylbutazone, trimethoprim and sulphadiazine in the horse.
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4. The analysis of the salicylanilides, oxyclozanide, rafoxanide and closantel in body fluids by high performance liquid chromatography.
   Mohammed-Ali, N.A.K. and Bogan, J.A. (In preparation)

5. Comparison of trimethoprim/sulphonamide combinations in sheep and cattle.
   Mohammed-Ali, N.A.K. and Bogan, J.A. (In preparation)

CONTENTS

SUMMARY

The pharmacokinetics of four trimethoprim/sulphonamide formulations (Trivetrin, Tribrissen, Duphatrim and Leotrox) available for treatment of bacterial infections in sheep were compared in five sheep after intramuscular administration at a total dose rate of 15 $mg.kg^{-1}$ of active ingredients (12.5 $mg.kg^{-1}$ sulphonamide plus 2.5 $mg.kg^{-1}$ trimethoprim).

The maximum concentrations of trimethoprim in plasma occurred at one hour after intramuscular administration of Trivetrin and Leotrox. After administration of Tribrissen and Duphatrim intramuscularly, trimethoprim was not detected in plasma ($< 0.1\ \mu g.ml^{-1}$).

The mean plasma elimination half-life of trimethoprim was 1.3 and 1 hour after administration of Trivetrin and Leotrox respectively.

The maximum concentrations of sulphadoxine, sulphadiazine, sulphadiazine and sulphatroxazole occurred at 2, 1, 1 and 1 hours after administration of Trivetrin, Tribrissen, Duphatrim and Leotrox respectively.

The mean elimination half-lives of sulphadoxine, sulphadiazine, sulphadiazine and sulphatroxazole were 4.8, 2.9, 2.6 and 9.30 hours after intramuscular administration of Trivetrin, Tribrissen, Duphatrim and Leotrox respectively.

No significant differences were found between the plasma concentrations of trimethoprim and sulphadoxine administered at 1 and 3 intramuscular sites.

Bioavailability of sulphadoxine and sulphatroxazole were 81 and 88% respectively.

The study revealed that administration of trimethoprim/sulphonamide combinations in solution type products (Trivetrin and Leotrox) produced better pharmacokinetic profile than those of suspension type products (Tribrissen and Duphatrim).

The degree of plasma-protein binding of sulphadoxine, sulphadiazine and sulphatroxazole was found to be 50, 14 and 70% respectively which appeared to be well correlated to their rates of elimination from plasma.

A detailed study was made of the pharmacokinetics of the commonly used flukicidal drugs, a new sulphonamide product, clorsulon, the salicylanilides rafoxanide, closantel and oxyclozanide, and a unique benzimidazole triclabendazole which has flukicidal activity with no nematodicidal activity.

All the flukicidal drugs studied showed markedly long plasma elimination half-lives and a high degree of binding to plasma-proteins.

Following administration of clorsulon to cattle subcutaneously as a 10 and 20% suspension at 4 $mg.kg^{-1}$ and orally at 7 $mg.kg^{-1}$, the drug was rapidly absorbed and the maximum concentrations in plasma occurred at 24, 12 and 24 hours at a mean concentration of 2.3, 4.3 and 4 $\mu g.ml^{-1}$ respectively. Mean elimination half-life of clorsulon in cattle was 29.9, 20.5 and 16.4 hours following administration of the drug subcutaneously as a 10 and 20% suspension at a dose rate of 4 $mg.kg^{-1}$ and orally at a dose rate of 7 $mg.kg^{-1}$ respectively.

Binding of clorsulon to bovine plasma-proteins was found to be 94%.

Following oral administration of rafoxanide at 7.5 $mg.kg^{-1}$ to fluke-free sheep, the drug was slowly absorbed and the maximum concentrations (23.6 $\mu g.ml^{-1}$) occurred three days after drug administration and was followed by very slow elimination with a mean plasma elimination half-life of 16.6 days.

Rafoxanide residues in bile, liver and muscle in sheep were found to be low relative to the concentration of the drug in plasma. A mean concentration of 0.2 $\mu g.ml^{-1}$ and 0.4 $\mu g.g^{-1}$ of rafoxanide was detected in bile and liver respectively after 28 days of drug administration at 7.5 $mg.kg^{-1}$ while in muscle, the drug was not detected ($< 0.1\ \mu g.g^{-1}$).

Rafoxanide was found to be 86.4 and 87.9% efficient against six and ten week old flukes when infected sheep were treated with 7.5 and 2.5 $mg.kg^{-1}$ rafoxanide respectively when these flukes were counted as adults at 14 weeks after infestation.

The study demonstrated that this putative efficacy may be misleading in that the drug may only be effective by virtue of the drug still being present when the immature flukes reach maturity. Clearly the current methods for testing for activity against immature flukes are inadequate when very persistent drug such as rafoxanide is being tested.

The pharmacokinetics of rafoxanide administered orally at 7.5 $mg.kg^{-1}$ to fluke-free sheep were found to be significantly different than those of infected sheep.

Absorption of closantel following oral administration at 7.5 $mg.kg^{-1}$ was also slow and the maximum concentration of 45.0 $\mu g.ml^{-1}$ occurred at two days after drug administration. Elimination of closantel was slow having a mean plasma elimination half-life of 14.5 days.

Residues of closantel in bile, liver and muscle obtained at various times after drug administration were low relative to those in plasma. Mean concentrations of 28.3, 0.7 $\mu g.ml^{-1}$; 1.7 and 0.7 $\mu g.g^{-1}$ closantel were detected in plasma, bile, liver and muscle one day after drug administration and these concentrations declined to 18.0, 0.0 $\mu g.ml^{-1}$; 1.2 and 0.4 $\mu g.g^{-1}$ 28 days respectively after administration of the drug.

Oxyclozanide was found to be more rapidly absorbed and the maximum concentrations in plasma (18 $\mu g.ml^{-1}$) occurred 24 hours after oral administration of the drug at a dose rate of 15 $mg.kg^{-1}$. Elimination of oxyclozanide was more rapid than that of rafoxanide and closantel with a mean plasma elimination half-life of 6.4 days.

Following the oral administration of triclabendazole to sheep at 10 $mg.kg^{-1}$, the drug was rapidly metabolised to the sulfoxide and the sulfone. The parent compound was not detected in the plasma samples at any time ($< 0.05 \mu g.ml^{-1}$), the maximum concentrations of triclabendazole sulfoxide and triclabendazole sulfone occurred at 36 and 48 hours after drug administration and were 12.8 and 13.0 $\mu g.ml^{-1}$ respectively.

Fenbendazole administered orally to sheep at 10 $mg.kg^{-1}$ was rapidly absorbed and was also metabolised to the sulfoxide (oxfendazole) and the sulfone (oxfendazole sulfone).

Following oral administration, the maximum plasma concentrations of fenbendazole, oxfendazole and the sulfone occurred at 24, 24 and 48 hours and they were 0.34, 0.45 and 0.21 $\mu g.ml^{-1}$ respectively.

Plasma elimination half-lives of triclabendazole sulfoxide, triclabendazole sulfone, fenbendazole, oxfendazole and oxfendazole sulfone were found to be 42.8, 25.0, 21.8, 23.7 and 35.0 hours respectively following administration of triclabendazole and fenbendazole alone to the same six sheep at a dose rate of 10 $mg.kg^{-1}$ of triclabendazole and of fenbendazole.

The pharmacokinetics of triclabendazole and fenbendazole were found not to be altered following administration of both drugs together from those when each drug was administered alone.

Triclabendazole was detected at a low concentration in bile (0.6 $\mu g.ml^{-1}$) in sheep treated with triclabendazole orally at 10 $mg.kg^{-1}$ while it was not detectable in plasma at that time. Triclabendazole sulfoxide and triclabendazole sulfone were found in bile in concentrations lower than those in plasma.

Plasma-protein binding of rafoxanide, closantel, oxyclozanide and triclabendazole and its metabolites (the sulfoxide and the sulfone) were found to be > 99%.

PRINCIPLES OF PHARMACOKINETIC ANALYSIS

Pharmacokinetics is the study and characterisation of the time course of drug absorption, distribution, metabolism and excretion.

The study of pharmacokinetics has been of increasing usefulness in recent years in designing suitable dosage regimens for the maintenance of adequate plasma or tissue concentrations. The relationship of the pharmacokinetics of drugs to the intensity and duration of their action (pharmacodynamics) has also become recognised as an important area of pharmacology. The increasing power of computers has led to much more rapid calculation of pharmacokinetic parameters thereby making their usefulness much greater.

Generally, pharmacokinetics is concerned with generating the best-fit equation for the plasma concentration/time curve after administration of single or repeated doses of a drug. From the calculated equation a number of important parameters can be derived, the absorption half-life, the elimination half-life, the apparent volume of distribution, etc. The theory of pharmacokinetics as applied to domestic animals has been well described in the recent book by Baggot (1977) and detailed background to the use of all the terminology used in this study can be found there. A glossary of terms as used in this study follows.

For most drugs the disposition curves can be fitted to a bi- (or less commonly a tri-) exponential term of the type.

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \ldots \text{etc.}$$

Where C is the plasma concentration at time t. A, B are plasma drug concentration intercepts, e is the base of natural logarithm and t is the time, and these are generated by iterative least-squares methods from the observed values.

GLOSSARY AND FORMULA OF THE PHARMACOKINETIC PARAMETERS

$C\ p^{o}$: The initial concentration of drug in plasma after intravenous administration of a single dose. It is expressed by $\mu g.ml^{-1}$.

$$C\ p^{o} = A + B$$

Where A is the extrapolated zero-time plasma drug concentration of the α phase. B is the extrapolated zero-time plasma drug concentration of the β phase.

$t\ 1/2\ \alpha$: The half-life of α phase expressed in units of time.

$$t\ 1/2\ \alpha = 0.693/\alpha .$$

$t\ 1/2\ \beta$: Half-life of the β phase expressed in time units.

The term can be defined as the time required for the body to eliminate one half of the particular drug and it is given by the expression $0.693/\beta$.

Vc : The apparent volume of the central compartment expressed in $ml.kg^{-1}$.

$$Vc = \frac{\text{Dose (i.v.)}}{C\ p^{o}}$$

Vd : The apparent volume of distribution and is expressed in $ml.kg^{-1}$. It can be defined as that volume of fluid which would be required to contain the amount of drug in the body if the drug was uniformly distributed at a concentration equal to that in the plasma. The apparent volume of distribution (Vd) can be calculated in a number of ways, by extrapolation, area, pseudo-equilibrium and steady state methods, with each method having limitations. These limitations have been discussed by Baggot (1978). Most commonly Vd is calculated by the area method using the following equations:-

$$Vd_{(area)} = \frac{Dose}{AUC.\beta}$$

$$= \frac{Dose}{A/\alpha + B/\beta}$$

AUC : Area under the plasma concentration versus time curve and is expressed in $\mu g.ml^{-1}.h$. It can be calculated by the trapezoidal method (Baggot 1977).

$$AUC = \int_{o}^{t} cp\ dt + \frac{Cp\ (t)}{\beta}$$

Where t = the time at which last sample was taken; Cp (t) = the concentration of the drug in the last sample. β = the

elimination rate constants

K_{12} : First-order distribution rate constants between the central and peripheral compartment, and is expressed in units of reciprocal time (min^{-1}, h^{-1}).

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

K_{21} : First-order distribution rate constants between the peripheral and central compartment.

$= (A.\beta) + (B.\alpha)/Cp^{o}$

K_{el} : First-order rate constant for elimination of the drug from the central compartment.

$= \alpha \beta / K_{21}$

CL_B : Body clearance. It is the volume of blood cleared of the drug expressed in $ml.kg^{-1}$ per units of time.

$= \beta . Vd_{(area)} . 1000.$

Bioavailability : It is the amount of the administered drug which entered the systemic circulation. It can be calculated from the area under plasma concentration/time curve (AUC) obtained after intramuscular (i.m.) or subcutaneous (s/c) or oral administration and that after intravenous administration (i.v.) of equal doses of the drug (Baggot 1977).

$$= \frac{\text{AUC (i.m., s/c, oral)}}{\text{AUC (i.v.)}}$$

STATISTICS

Student's "t" test for paired samples was used to test intra-individual significance.

One-way analysis of variance and two-way analysis of variance were used where appropriate.

Calculations of these statistics were done using a Minitab computer programme.

SECTION I

GENERAL INTRODUCTION

There is a continuous search for the development of new antibacterial agents with different modes of action due to the increasing problem of development of resistance of pathogenic bacteria to chemotherapeutic agents, both in man and animals. These antibacterial agents have to be highly effective against the resistant pathogens, safe to the host and possess good pharmacokinetic and pharmacodynamic properties.

The sulphonamide antibacterials were the first chemotherapeutic agents used for the treatment of bacterial infections in man. They were first discovered during an investigation with an azo dye called Prontosil (Gelmo 1908). Soon after that it was observed that in tissues, prontosil linkage split to yield a component "Sulphanilamide" to which the antibacterial activity is related. Sulphanilamide (Figure (1)) is the basic group of all sulphonamides and from this more than 5,000 variations have been synthesised although only a few are in clinical use.

The sulphonamides are bacteriostatic drugs which act by interfering with the uptake of para-amino benzoic acid (PABA) by the micro-organisms. PABA is an essential component of the enzyme folic acid which is required for the synthesis of nucleic acid. Thus its uptake will interfere with bacterial cell division.

For many years, it was thought that the antibacterial activity of sulphonamides might be improved when used with inhibitors of bacterial dihydrofolate reductase (Hitchings et al 1952a). Combination of sulphonamides with inhibitors of

dihydrofolate reductase will enhance the antibacterial activity of both drugs to include micro-organisms which are known to be resistant to the action of each drug when given alone (such as Proteus, Bordetella and Neisseria species). Furthermore their combination provides another advantage as less volume of drug will be required to produce therapeutic concentrations adequate for antibacterial activity thus reducing the risk of drug toxicity due to less drug residues in the body.

Inhibition of dihydrofolate reductase has been shown to be one of the properties of 2,4-diamino pyrimidines (Hitchings et al 1948; Falco et al 1949; Hitchings and Burchall 1965; Bushby and Hitchings 1968). Among these inhibitors trimethoprim (Figure (2)) has shown to have a broad spectrum antibacterial activity and with a high affinity of binding to the bacterial relative to the mammalian dihydrofolate reductase (Hitchings et al 1952b; Ellion et al 1954; Hitchings and Bushby 1961; Burchall and Hitchings 1965; Bushby and Hitchings 1968).

The activity of trimethoprim/sulphonamides was confirmed in vitro and in clinical trials using trimethoprim and different sulphonamides alone or in combination (Bushby and Hitchings 1968; Barnett and Bushby 1970; Rehm and White 1970; Bushby 1980; Powers et al 1980). In man, among the several possible sulphonamides, sulphamethoxazole has been chosen for the combination because of its high activity and its elimination half-life in man being approximately similar to that of trimethoprim (Bushby and Hitchings 1968; Barnett and Bushby 1970). In the veterinary field, although the combination (trimethoprim/sulphamethoxazole)

is also recognised to have improved effectiveness over sulphonamides alone, different sulphonamides have been used, sulphadiazine (Figure (3)) and sulphadoxine (Figure (4)), although there are variations in their elimination half-lives in different animal species (Rehm and White 1970; Craig and White 1976; Bushby 1980; Powers et al 1980). These combinations have been commercially available in different formulations, as injectable solutions or suspensions, or oral boluses, dispersible powders and as a paste. Recently trimethoprim has been combined with a sulphonamide, sulphatroxazole (Figure (5)) which is new to the veterinary market.

Surprisingly, little comparative work has been published on the different combination products and it was of interest to investigate the pharmacokinetics of sulphonamide/trimethoprim in different products and also to assess whether the suspension-type products or the products in solution gave better pharmacokinetic profiles.

During the period of this study, this aspect was completed successfully. However during this time a new important sulphonamide product, clorsulon, with major activity against liver flukes and little antibacterial activity became available.

Considering the wide problem of fascioliasis throughout the world and the economic importance of this group of drugs, the work of this study orientated itself towards a broad study of flukicidal drugs, especially, since as the work progressed this group of drugs has shown itself to have unique pharmacokinetic and pharmacodynamic features.

FIGURE (1)

STRUCTURE OF SULPHANILAMIDE

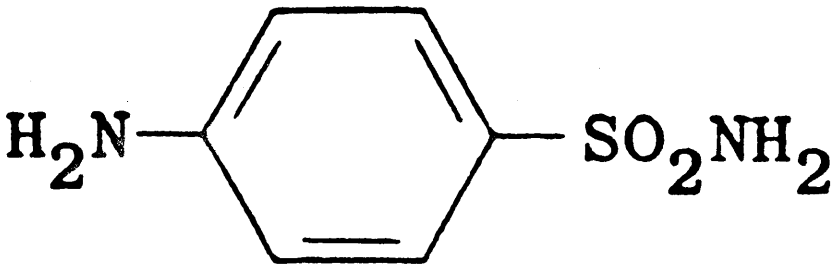

H2N
SO2NH2

FIGURE (2)

STRUCTURE OF TRIMETHOPRIM

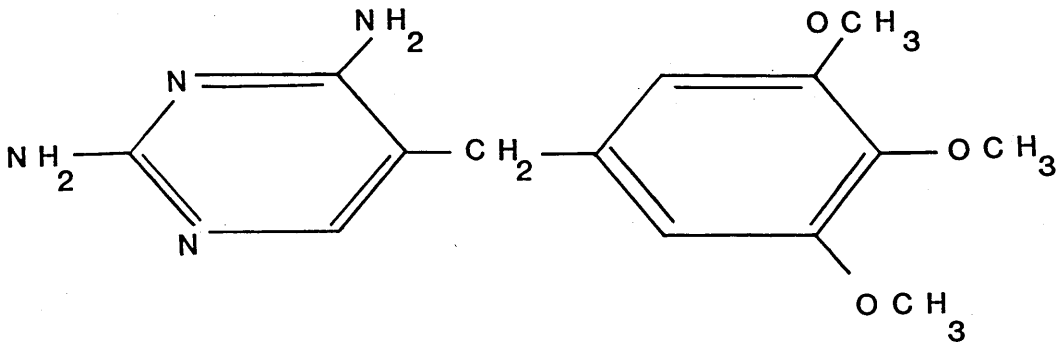

NH2
N
NH2
N
CH2
OCH3
OCH3
OCH3

FIGURE (3)

STRUCTURE OF SULPHADIAZINE

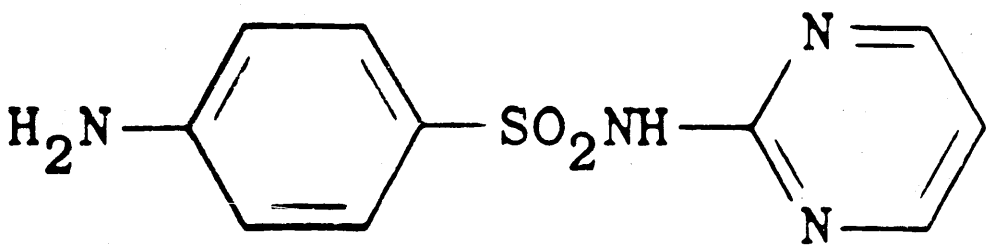

H2N
SO2NH
N
N

FIGURE (4)

STRUCTURE OF SULPHADOXINE

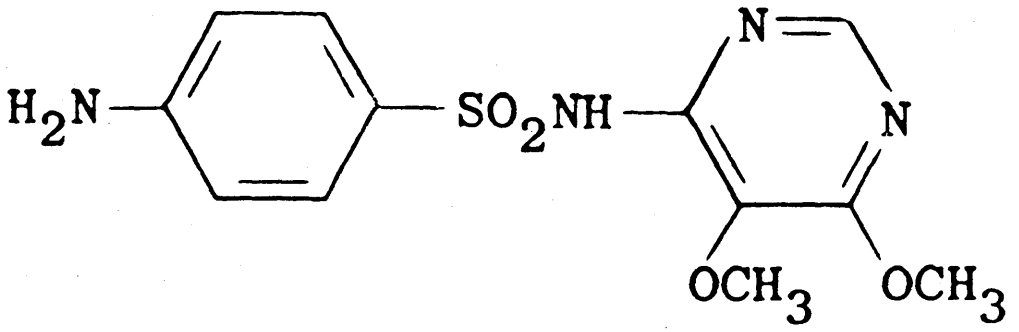
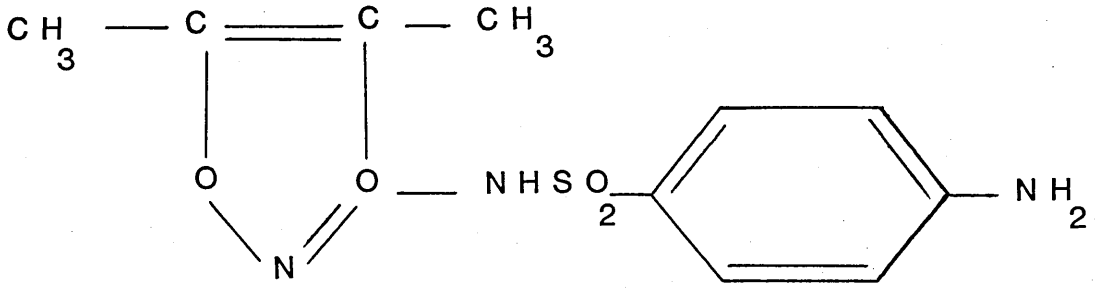

H2N
SO2NH
N
N
OCH3
OCH3

FIGURE (5)

STRUCTURE OF SULPHATROXAZOLE


CH3
C
C
CH3
O
O
NHSO2
NH2
N

PHARMACOKINETIC STUDIES OF TRIMETHOPRIM-SULPHONAMIDE COMBINATIONS IN SHEEP

(1) Pharmacokinetic studies of trimethoprim-sulphonamide combinations in sheep

(1) 1 Introduction

(1) 1.1 Activity

The combination of trimethoprim (2 : 4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine) and several sulphonamides have become widely used in the treatment of various bacterial and some protozoal diseases in man since 1968 and in animals since 1970 (Pugsley et al 1969; McGuinness 1969; Sander and Midtvedt 1970; Rehm and White 1970; Durr 1976; Bushby 1980).

The mixture of trimethoprim and one of the sulphonamides has been shown to be a broad spectrum preparation having activity against a wide range of gram-positive and gram-negative bacterial pathogens. These include Escherichia, Streptococcus, Proteus, Salmonella, Pasteurella, Shigella, Klebsiella, Staphylococcus, Corynbacterium, Pseudomonas, Clostridium and Bordetella spp (Bushby and Barnett, 1967; Barnett and Bushby 1970; Simmons 1970; Bassett 1971; Rasmussen 1971; Marks et al 1973; Wierup 1974; Batey and Smits 1976).

The activity of trimethoprim-sulphonamide combinations have been of particular value in the therapy of respiratory, urogenital and alimentary tract infections in human medicine (Gruneberg and Kolbe 1969; Hughes 1969; Lal et al 1970; Robertson et al 1973; Hughes et al 1975; Gower and Tasker 1976; Hills et al 1976; Smellie et al 1976) and in veterinary medicine

(Anderson 1967; Rehm et al 1968; McCaig 1970; Rehm and White 1970; Craig 1972; Powers et al 1980; Divers et al 1981; Miller et al 1984).

(1) 1.2 Mode of action

The trimethoprim-sulphonamide combination derives its activity from the fact that each drug inhibits a different step in the biosynthetic pathway used by microorganism in the synthesis of reduced folate cofactors.

Sulphonamides and trimethoprim are both antifolates for bacteria, interfering with important tetrahydro-folate co-enzymes but they have no or little effect on mammalian co-enzymes (Hitchings 1973). Sulphonamides compete with para-amino benzoic acid for the enzyme which converts this bacterial metabolite to folic acid. This step does not occur in animals because folate is a vitamin. Trimethoprim inhibits the enzyme dihydrofolate reductase which converts dihydrofolic acid into tetrahydrofolic acid. This step occurs in animals also, but at therapeutic concentrations, trimethoprim does not affect this conversion in animals because its affinity for the mammalian enzyme is 100,000 times less than those for the bacterial enzyme (Burchall and Hitchings 1965; Burchall 1973). The effect of this enzymatic sequential blockage of metabolism results in a marked synergistic action (Hitchings and Burchall 1965; Then and Angehrn 1974) and moreover a combination of the active substances has a bactericidal effect as compared to the bacteriostatic effect of each drug when given alone (Bohni 1969; Powers et al 1980). This synergy permits considerable reduction in the initial sulphonamide dose and increases the therapeutic effect (Gallien

1973). Also the combination will be less liable to the development of resistant strains (Bushby and Hitchings 1968).

Theoretically, since the efficacy and spectrum of all the sulphonamides are similar, the choice of any sulphonamide in the combination is based mainly on its pharmacokinetics being similar to those of trimethoprim. In man, sulphamethoxazole was chosen as the most appropriate sulphonamide since it is one of the more active sulphonamides (on a weight basis) and its rate of elimination by man is similar to that of trimethoprim (Barnett and Bushby 1970). In veterinary medicine, as the rate of excretion of these drugs (trimethoprim/sulphonamides) varies in different animal species the human combination (trimethoprim/sulphamethoxazole) may not be the sulphonamide of choice, although, in vitro, the sensitivity of the veterinary bacterial strains to the human preparation showed that only 1.5 percent of the veterinary strains were found to be resistant to the human preparation (Barnett and Bushby 1970; Romvary and Horvay 1976). However many sulphonamides have been used satisfactorily in the veterinary practice although they have different half-lives, some of which are the short-acting sulphonamide (sulphadiazine) (Mc Caig 1970; Cannon 1976; Craig and White 1976; Powers et al 1980; Sigel et al 1981 and the long acting sulphadoxine (Barnett and Bushby 1970; Rehm and White 1970). However the dosage intervals are changed to take account of the differing half-lives.

The minimum inhibitory concentration (MIC) of trimethoprim/sulphonamide singly and in various combination has

been determined *in vitro*, in a mixture of varying proportions. It was shown that for the majority of organisms, the maximum potentiation (measured by reduction in the MIC) occurred when the ratio is about 1 : 20 (trimethoprim : sulphonamide) (Barnett and Bushby 1970). This is because the activity of trimethoprim is usually considered to be 20-fold higher than that of sulphonamides (Gallien 1973). However in clinical practice in humans, it has been shown that the ratio of 1 : 5 (trimethoprim : sulphamethoxazole) in plasma exerts superior antibacterial activity than the ratio of 1 : 10 or 1 : 20 (Barnett and Bushby 1970; Gallien 1973; Then and Angehrn 1974). The differences between optimum ratios for *in vitro* and *in vivo* activities is thought to be due to the high concentration of trimethoprim in tissues relative to that in blood (Bushby and Hitchings 1968; Bushby 1980).

(1) 1.3 *Safety to host*

Toxicity of trimethoprim has been subjected to extensive studies. In mice, the oral LD_{50} is more than 2,000 $mg.kg^{-1}$ and it was not affected by the co-administration of sulphadiazine at equal doses. The intravenous LD_{50} for mice is about 200 $mg.kg^{-1}$ and in cats 100 $mg.kg^{-1}$ (Bushby and Hitchings 1968).

Toxicity studies in rats, monkeys and dogs showed that prolonged daily administration of trimethoprim (for 14 days) at doses up to 300 $mg.kg^{-1}$ per day had no obvious effect. At higher doses, the only toxic effect shown was maturation defects in haemopoiesis (i.e. effect on bone marrow). However another group of animals receiving the same dose rate but with a supplementary folate diet did not show such effects (Udall 1969).

Teratogenic studies in rats and rabbits showed that administration of trimethoprim/sulphamethoxazole combination on days 8 - 16 of pregnancy caused foetal malformation and reduced weight gain in the rats and does at doses of 160 and 640 $mg.kg^{-1}$ respectively (Udall 1969). The condition was easily prevented by supplementary folate diet.

Toxicity studies in cats and dogs, showed that administration of timethoprim-sulphadiazine combination at doses of 120 and 300 $mg.kg^{-1}$ per day respectively for 20 consecutive days did not cause any changes in bodyweight compared to non-treated animals. Marked anaemia and leucopenia were seen in cats in the last week following oral dosage of 300 $mg.kg^{-1}$ per day for 20 consecutive days (Craig and White 1976).

Administration of trimethoprim-sulphadiazine combination intravenously to horses at doses ranging from 13 - 62 $mg.kg^{-1}$ was safe and did not produce any adverse side effects (Miller and Szczech 1984).

In cattle and pigs, the single administration of trimethoprim/sulphadoxine intramuscularly or subcutaneously at doses up to 35 $mg.kg^{-1}$ was without any adverse effect (Rehm and White 1970).

(1) 1.4 Pharmacokinetics and Objective

The pharmacokinetic profile of trimethoprim in man and in dogs was described by Kaplan et al (1970). Craig and White (1976) reported on serum trimethoprim-sulphadiazine concentrations in dogs and cats given repeated oral and injectable doses of 30 $mg.kg^{-1}$ per day.

In sheep, dogs and calves, Piercy (1978) reported the distribution of trimethoprim and sulphadiazine in plasma, tissues and synovial fluids after oral and intravenous administration of 30 $mg.kg^{-1}$.

In goats (Nielsen and Rasmussen (1976a,b), in cattle Nielsen et al (1978) and in horses Rasmussen et al (1979) studied the kinetics of trimethoprim and sulphadoxine after oral and intravenous administration of the drug at different dose rates.

There is little published information on the kinetics of trimethoprim-sulphonamide combination in sheep following intramuscular administration of the commercial injectable preparations at the recommended dose rates. Therefore the present study was undertaken in order to study the kinetics of each active ingredient in sheep following the intramuscular administration of products containing trimethoprim in combination with sulphadoxine (Trivetrin), sulphadiazine (Tribrissen and Duphatrim) and sulphatroxazole (Leotrox), at the recommended dose rates.

The pharmacokinetics of trimethoprim/sulphadoxine (Trivetrin) were compared following the intramuscular administration of the drug at one and three sites.

Furthermore, trimethoprim/sulphadoxine (Trivetrin) and trimethoprim/sulphatroxazole (Leotrox) were administered intravenously in order to provide a more comprehensive description of the kinetics of both drugs in sheep.

The plasma protein-binding of each sulphonamide was also determined in vitro to relate the elimination half-lives of these drugs to the degree of protein-binding.

(1) 2 Determination of trimethoprim and sulphonamides in plasma

The first method developed for the determination of sulphanilamide in blood was in 1937 (Marshall et al 1937). The method was photometeric and based on diazotisation and coupling of the sulphonamide in acid solution to yield coloured product (N,N-dimethyl-1-naphthylamine (dimethyl-α-naphthylamine). The method had disadvantages of being not reproducible and sensitive and thus it was modified slightly by Bratton and Marshall (1939) who used a different colouring reagent (N-(1-naphthyl) ethylene diamine dihydrochloride which resulted in an increase in the sensitivity and reproducibility of the method, and besides, the developed colour by the reaction was more stable than that in the previous method. This method was furthermore modified by Reider (1972) to provide a method with greater rapidity of coupling and more sensitive and reliable, and since then this simple modified photometeric method (Reider 1972) has been widely used for the determination of sulphonamides (the free and total) in body fluids.

Bushby and Hitchings (1968) used a microbiological assay method for measuring the concentrations of trimethoprim and sulphonamide. The method has a limit of sensitivity of 0.03 and 0.01 $\mu g.ml^{-1}$ for trimethoprim and sulphonamide respectively. The disadvantages of microbiological methods, in general, are lack of precision and reproducibility. The same authors determined trimethoprim in body fluids using a u.v. absorption photometeric method.

Schwartz et al (1969) developed a sensitive flurometric

method for the analysis of trimethoprim in body fluids. The limit of sensitivity of the method is 20 $ng.ml^{-1}$.

In view of the discovery of high performance liquid chromatography and its usage in general as the most sensitive method by which drugs could be measured to a low concentration, many Hplc methods have been developed for the determination of sulphonamides (especially sulphamethoxazole) and trimethoprim in body fluids (Kram 1972; Poet and Pu 1973; Penner 1975; Cobb and Hill 1976; Vree et al 1978; Singletary and Sancilio 1980; Gochin et al 1981). Different Hplc methods have been used for the determination of sulphamethoxazole (Sharma et al 1976; Bye and Brown 1977; Bury and Mashford 1979) and trimethoprim separately (Sigel et al 1973; Kirchmeier and Upton 1978; Weinfeld and Macasieb 1979). All these techniques were very simple and sensitive to measure concentrations of trimethoprim and sulphonamides of 0.10 and 1.00 $\mu g.ml^{-1}$. Recoveries of the method were high (88 and 83% for trimethoprim and sulphonamide respectively).

In this study, in spite of the fact that the Hplc analytical method is considered to be more specific, the method of Reider (1972) and Schwartz et al (1969) was found to be sensitive, reliable and most convenient for the determination of sulphonamides and trimethoprim respectively in the plasma sample. Under the conditions in the laboratory, the limit of sensitivity of the methods was 2 and 0.10 $\mu g.ml^{-1}$ for different sulphonamides and trimethoprim respectively.

(1) 2.1 Materials and methods

(1) 2.1.1 Trimethoprim method of analysis

Trimethoprim was determined spectroflurometrically according to the method of Schwartz et al (1969). The limit of detection of the method is 0.10 $\mu g.ml^{-1}$.

(1) 2.1.2 Reagents

All reagents used were of "analar" grade and they were prepared using bidistilled water.

- 0.1 N Sodium carbonate solution (14.3 g $Na_2CO_3.10\ H_2O$ completed to 500 ml with bidistilled water.
- 0.01 N H_2SO_4
- 0.1 M Potassium permanaganate in 0.1 N sodium hydroxide (1.58 g $KMnO_4$ dissolved and completed to 100 ml with 0.1 N NaOH).
- 1 N H_2SO_4
- Chloroform (Rathburn Chemicals, Scotland).
- Formaldehyde (35%).

(1) 2.1.3 Procedure of analysis

The principle of the analytical procedure is extracting trimethoprim from plasma with chloroform at basic pH, back extracting into dilute H_2SO_4 and oxidising with $KMnO_4$ (in alkaline solution) to the fluorescent trimethyoxy-benzoic acid (TMBA), followed by removal of excess permanganate with formaldehyde and TMBA is then extracted into chloroform and its fluorescence measured.

Into a 50 ml glass stoppered test tube 8 ml of 0.1 N Na_2CO_3, 10 ml chloroform and 2 ml plasma samples were introduced using

glass bulb pipettes. The tubes were stoppered and shaken for four minutes on a slow rotary mixer and centrifuged for ten minutes at 3000 r.p.m.

In a 15 ml glass stoppered test tube 4 ml of 0.01 N H_2SO_4 was introduced to which 7 ml of the bottom clear chloroform extract was added, stoppered, shaken for ten minutes and centrifuged at 3000 r.p.m. for ten minutes.

In another 15 ml glass tube, 3 ml of the sulphuric acid extract and 2 ml of alkaline $KMnO_4$ solution were introduced. The extracts were thoroughly vortexed using a rotamixer (Griffin and George, London) and placed for ten minutes in a water bath at 60°C.

0.3 ml of formaldehyde was added and vortexed followed by 1 ml of 1 N H_2SO_4, vortexed and placed again in the water bath (60°C) for ten minutes. Thereafter the extracts were thoroughly vortexed several times and the tubes were cooled under running tap water to room temperature (18°C). 4 ml of chloroform was added to each tube, shaken for ten minutes and centrifuged at 3000 r.p.m. for ten minutes. The lower chloroform layer was transferred into 10 ml conical glass tubes and immediately its fluorescence was measured using an Aminco-Bowman spectrofluoremeter using an Excitation wave length of 275 nm and Emission wave length of 350 nm.

(1) 2.1.4 Preparation of standards and recoveries

A stock solution of an acid-free aqueous solution of trimethoprim was prepared as follows:-

In a 100 ml conical flask 100 ml trimethoprim (pure compound) was weighed and 7.7 ml 1 N Hcl was added. The drug

particles were dissolved with a glass rod and left at room temperature (18°C) for ten minutes to form the hydrochloride.

50 ml of bidistilled water was added and the flask was placed for five minutes in a water bath (60°C) until the compound was completely dissolved.

The mixture was then cooled and neutralised under magnetic stirring by adding 7.3 ml 1 N NaOH.

The mixture was transferred into a 100 ml volumetric flask and the wall of the conical tubes was rinsed with bidistilled water. The solution in the volumetric flask was completed to the mark by bidistilled water. The final solution contained 1000 $\mu g.ml^{-1}$ of trimethoprim in 0.9% NaOH. Known amounts of this stock solution were added to drug-free plasma and taken through the procedure of analysis for preparation of recoveries (of trimethoprim from plasma).

A stock standard solution of 10 $\mu g.ml^{-1}$ of trimethoxybenzoic acid (TMBA) in chloroform was prepared. Solutions containing 0.25, 0.50, 1, 2 and 2.5 $\mu g.ml^{-1}$ TMBA were prepared by dissolving appropriate amounts of the stock standard solution (TMBA) in chloroform. The fluorescence of these solutions was measured and used for the preparation of a calibration curve.

(1) 2.1.5 Calculations of percentage recovery

Recoveries of trimethoprim from the spiked plasma were calculated using the following formula:-

$$\text{Percentage recovery} = \frac{\text{Determined concentration of the measured substance (TMBA) in the final solution } (\mu g.ml^{-1})}{\text{Real concentration of the substance (trimethoprim) to be measured in the sample } (\mu g.ml^{-1})} \times 100 \times Q$$

Q = Theoretical concentration of TMBA ($\mu g.ml^{-1}$) in the final solution assuming complete extraction and 62% yield at the oxidation step.

Thus

$$Q = \frac{2}{4} \times \frac{212.2}{290.3} \times \frac{62}{100} \times \frac{3}{4} \times S \text{ (concentration of trimethoprim)}$$

Concentration of the drug (trimethoprim) in plasma samples was calculated using the formula:-

$$\text{Concentration } (\mu g.ml^{-1}) = \frac{Fa \times Cs}{Fs} \times F$$

Where Fa = Fluorescence reading of the analysed sample after subtraction of the sample blank.

Fs = Fluorescence reading of the standard after subtraction of the chloroform blank.

Cs = Concentration of the drug in the standard.

F = Correcting factor for the conversion yield in the oxidation step (trimethoprim to TMBA).

Under the working conditions, the percentage of conversion of trimethoprim to trimethoxy-benzoic acid (in the oxidation step) was found to be 62%.

The calibration curve was prepared by plotting the concentrations of TMBA in chloroform versus their fluorescence reading.

(1) 2.2 Sulphonamide method of analysis

The concentrations of the unchanged (free) sulphonamide were determined in the plasma samples using spectrophotometric method of Reider (1972) based on that of Bratton and Marshall (1939).

(1) 2.2.1 Reagents

All reagents were of analytical grade.

- McIlvain buffer (pH 5.5) = 43 ml 0.2 M aqueous solution of citric acid and 57 ml 0.4 M aqueous solution of sodium phosphate (Na_2HPO_4).
- Ethyl acetate.
- 8 N HCl.
- Acetone (redistilled in glass).
- 2 N HCl solution in acetone plus water.

This solution was prepared freshly and immediately before each series of analysis, by mixing 1 ml of 8 N HCl with 3 ml of acetone.

- 0.1% solution of sodium nitrite in a mixture of 3 : 1 acetone : distilled water.
- 5% solution of sulphamic acid in a mixture of 3 : 1 acetone : distilled water.
- 0.1% solution of α-naphthylethylenediamine dihydrochloride in a mixture of 3 : 1 acetone : distilled water.
- Methanol.

(1) 2.2.2 Procedure of analysis

1 ml McIlvain buffer (pH 5.5) was introduced into 15 ml glass stoppered tubes. 0.2 ml of the plasma samples and 5 ml of ethyl acetate was added to each tube. The tubes were stoppered, shaken for ten minutes on a slow rotary mixer and centrifuged for five minutes at 3000 r.p.m.

3 ml of the clear supernatant solution was transferred into 10 ml glass tubes and 0.5 ml of acetonic 2 N HCl (freshly prepared) was added, vortexed thoroughly on rotamixer.

0.5 ml of 0.1% acetonic solution of sodium nitrite was added, vortexed and left for six minutes at room temperature.

0.5 ml of 5% acetonic solution of sulphamic acid was added, vortexed and left for three minutes at room temperature.

0.5 ml of 0.1% acetonic solution of α-naphthylethylene-diamine dihydrochloride was added and vortexed.

Finally 0.5 ml of methanol was added and vortexed thoroughly until a homogenous liquid phase was formed.

The tubes were covered by aluminium foil and left for 20 minutes at room temperature. The extinction of the measured solution was measured spectrophotometrically using an absorption spectrometer (Pye Unicam SP8.500 UV/VIS spectrophotometer) at a wave length of 545 nm.

(1) 2.2.3 Preparation of standards and recoveries

Stock solution of 1000 $\mu g.ml^{-1}$ of each sulphonamide (pure compound) was prepared by dissolving 100 ml of the appropriate sulphonamide in a 100 ml volumetric flask in 1 ml of 1 N aqueous solution of NaOH and the flask was immediately filled up to the mark with a sorensen phosphate buffer (pH 8.0) (33.83 g $Na_2HPO_4.2H_2O$ and 1.36 g KH_2PO_4 completed with distilled water to 1 litre).

Stock standard solution containing 100, 80, 70, 50, 30, 20, 15, 10, 5 and 2 $\mu g.ml^{-1}$ of each sulphonamide were prepared by dissolving known amounts of the stock solution (1000 $\mu g.ml^{-1}$) in sorensen phosphate buffer (pH 8).

0.2 ml aliquots of drug-free plasma were mixed with 0.2 ml of the appropriate solution in buffer to yield concentrations of 100, 80, 70, 50, 30, 20, 15, 10, 5 and 2 $\mu g.ml^{-1}$ in plasma. Each plasma concentration was analysed by the standard method. The concentration of drug in the plasma was calculated by plotting a standard graph of concentration versus photometric reading.

(1) 3 Binding of sulphonamides to sheep plasma proteins

The extent of binding of sulphadoxine, sulphadiazine and sulphatroxazole was determined in vitro using the equilibrium dialysis method of Rehberg (1943).

Duplicate plasma samples containing 100 $\mu g.ml^{-1}$ of the appropriate sulphonamide were introduced into dialysis tubes (Visking tube size 1-8/32" Medicell International Ltd., London). The ends of the dialysis tubes were tied securely and washed under running tap water to remove any contamination.

The dialysis tube was introduced into a 50 ml glass-stoppered tube and filled partially with glass-beads. The tubes were stoppered and centrifuged for 35 minutes at 10°C at 3500 x g.

Plasma ultrafiltrates collected outside the dialysis tube were analysed for the concentration of the free sulphonamide using the standard method (Reider 1972).

(1) 3.1 Calculation of the percentage binding

The percentage binding of sulphonamide to plasma proteins was calculated using the formula:-

$$\text{Percentage binding} = 100 - \left[\frac{\text{Concentration of drug in plasma ultrafiltrate}}{\text{Concentration of drug in whole plasma}} \times 100 \right]$$

(1) 4 Experiments with trimethoprim-sulphonamide combination

(1) 4.1 Animals

Six experiments were performed on six clinically healthy sheep. The sheep were of mixed breed and sex, weighing between 30 - 70 kg and aged 1 - 2 years old.

The intervals between each experiment ranged from one to two weeks.

The animals were kept on concrete stalls covered with straw, fed hay and concentrates. Water was available ad libitum.

In some experiments five sheep were used, in others six. Throughout this series of experiments, the same sheep can be identified by animal number.

(1) 4.2 Administration of the drug

In each experiment, the combined preparation of trimethoprim/sulphonamide was administered to each animal at the recommended dose rate of 15 $mg.kg^{-1}$ bodyweight of the active ingredients (12.5 $mg.kg^{-1}$ sulphonamide + 2.5 $mg.kg^{-1}$ of trimethoprim).

(1) 4.3 Samples for analysis

Blood samples were withdrawn from the jugular vein using heparinised syringes "Monovette, Sarstedt" 20 gauge 1" needles.

Immediately after collection, the blood samples were centrifuged for ten minutes at 2500 r.p.m. and the plasma was transferred into 10 ml plastic tubes and stored at $-20^{o}C$ until analysed.

(1) 4.4 Experiments with trimethoprim-sulphadoxine

A group of six sheep received an intravenous administration

of trimethoprim-sulphadoxine (Trivetrin injectable solution 24% w/v [POM] Coopers) in the right jugular vein. Blood samples were collected from the left jugular vein before and at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 12.00, 24.00 and 27.00 hours after drug administration.

Two weeks later, the group of six sheep were divided into two groups. Group I sheep received the injectable preparation (Trivetrin 24% w/v [POM]) as an intramuscular injection at one site in the gluteal muscle. Group II received the intramuscular injection at three different sites in the gluteal muscle.

After two weeks, the injectable preparation was administered intramuscularly at three different sites to sheep in Group I and at one site to sheep in Group II.

Blood samples were collected before and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 27 hours after drug administration and the plasma was collected as described in (1) 4.3.

(1) 4.5 Experiments with trimethoprim-sulphadiazine

Two different preparations of the combined formulation of trimethoprim-sulphadiazine were used.

Five sheep received an intramuscular administration of trimethoprim-sulphadiazine (Tribrissen injectable suspension 24% w/v [POM] Coopers). Two weeks later, the sheep received the other injectable suspension "Duphatrim" 24% w/v (POM) Duphar.

Blood samples were collected before and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 27 hours after administration of each preparation.

(1) 4.6 Experiments with trimethoprim-sulphatroxazole

Five sheep received an intramuscular administration of

trimethoprim-sulphatroxazole (Leotrox injectable solution 24% w/v [POM] Leo). Blood samples were collected before and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 27, 32, 48 and 51 hours after intramuscular administration.

A further experiment was carried out using three sheep of the Finn-Dorset x breed, weighing between 40 - 42 kg and aged between 10-12 months. The sheep received an intravenous administration of trimethoprim-sulphatroxazole (Leotrox 24% w/v) in the left jugular vein. Blood samples were collected from the right jugular vein before and at 0.25, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 27, 32, 48 and 51 hours after the intravenous administration.

Treatment of blood samples was as described in (1) 4.3.

(1) 5 Results

(1) 5.1 Experiment with trimethoprim-sulphadoxine

After intravenous administration, the concentrations of trimethoprim and sulphadoxine in individual sheep and the mean concentration $\pm$ S.E.M. in plasma are shown in Table (1) 1. The concentrations of trimethoprim and sulphadoxine in plasma plotted against time on a semilogarithmic axis are shown in Figures (1) 1 and (1) 2 respectively.

The disposition curvesof trimethoprim and sulphadoxine which were analysed using the non-linear iterative least square curve-fitting programme CSTRIP (Sedman and Wagner 1976) was found to be best described by a two compartment open model fitted to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$. The values of the kinetic parameters of trimethoprim and sulphadoxine obtained from the

analysis are listed in Table (1) 2 and (1) 3 respectively.

Following the intramuscular administration, absorption of trimethoprim and sulphadoxine was rapid and the maximum concentrations of trimethoprim and sulphadoxine occurred at 1 and 2 hours respectively after the intramuscular administration at both one and three sites respectively (Tables (1) 4 and (1) 5). Figure (1) 3 shows mean concentration of sulphadoxine in plasma following administration of Trivetrin at one intramuscular site.

The plasma concentration/time curves of trimethoprim and sulphadoxine after intramuscular administration at one and three sites showed that both drugs were eliminated from plasma in accordance with a two compartment open model. Values of the kinetic parameters which describes the AUC, absorption and elimination phases of trimethoprim and sulphadoxine in sheep after intramuscular administration at one and three sites are shown in Table (1) 6.

The bioavailabilities of sulphadoxine and trimethoprim calculated as the ratio of the averaged AUC i.m./AUC i.v. were found to be 81 and 87% respectively.

(1) 5.2 Experiments with trimethoprim-sulphadiazine

The concentrations of sulphadiazine in plasma following intramuscular administration of Tribrissen and Duphatrim to five sheep are shown in Table (1) 7 and (1) 8 respectively.

The mean maximum concentrations of sulphadiazine in plasma of 42 and 33 $\mu g.ml^{-1}$ occurred at 1 hour after administration of Tribrissen and Duphatrim respectively (Figures (1) 3.

A two compartment open model was found to best fit the plasma concentrations time data of sulphadiazine following

administration of Tribrissen and Duphatrim.

Half lives in the absorption (t 1/2 α) and elimination (t 1/2 β) phases calculated from the computer generated pharmacokinetic constants (A, α, B, β) of sulphadiazine following intramuscular administration of Tribrissen and Duphatrim are illustrated in Table (1) 9.

Trimethoprim was not detected in the plasma samples at the limit of detection of the method (0.10 $\mu g.ml^{-1}$). However, samples between 0.5 and 6 hours contained "traces" of trimethoprim (i.e. < 0.10 $\mu g.ml^{-1}$).

(1) 5.3 Experiments with trimethoprim-sulphatroxazole

The plasma concentrations of trimethoprim and sulphatroxazole in each animal and the mean $\pm$ S.E.M. after intramuscular administration of Leotrox are tabulated in Table (1) 10.

The mean concentration of sulphatroxazole is represented graphically in Figure (1) 3.

The pharmacokinetic parameters for trimethoprim and sulphatroxazole administered intramuscularly to five sheep are illustrated in Table (1) 11.

Following intramuscular administration of Leotrox, the maximum plasma concentrationsof sulphatroxazole and trimethoprim occurred at 1 and 0.5 hours respectively.

Table (1) 12 and Figure (1) 4 shows the concentrations of sulphatroxazole in plasma following the intravenous administration.

Mean elimination half-life of sulphatroxazole after intravenous administration was found to be 11.70 ± 1.00 h. The kinetic parameters of sulphatroxazole are illustrated in Table (1) 13.

The percentage bioavailabilities of sulphatroxazole (calculated as the ratio of the mean AUC i.m./AUC i.v.) was found to be 88.

(1) 5.4 Binding of sulphonamides to sheep plasma proteins

Determination of the concentrations of the free sulphonamide in the plasma ultrafiltrates, obtained after dialysis, according to the method of Reider (1972) demonstrated that the percentage of binding of sulphadoxine, sulphadiazine and sulphatroxazole were 50, 14 and 70 percent respectively.

TABLE (1) 1

CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHADOXINE/TRIMETHOPRIM IN PLASMA FOLLOWING AN INTRAVENOUS ADMINISTRATION OF TRIVETRIN AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Time (hours)	Animal number 1	9	39	41	75	88	Mean ± S.E.M.
			Sulphadoxine				
0.25	52	64	72	80	65	71	67 ± 3.8
0.50	48	52	62	72	55	60	58 ± 3.4
0.75	45	49	55	64	53	48	52 ± 2.7
1.00	42	47	53	57	49	46	49 ± 2.0
1.50	39	45	42	46	45	43	43 ± 1.0
2.00	37	43	40	42	37	38	40 ± 1.0
2.50	34	37	31	40	35	35	35 ± 1.0
3.00	30	33	29	34	32	33	34 ± 0.8
4.00	26	30	24	27	29	21	26 ± 1.3
5.00	23	26	20	23	26	19	23 ± 1.2
6.00	19	21	13	20	22	16	19 ± 1.4
8.00	12	13	10	13	16	11	13 ± 0.8
12.00	5	3	5	8	11	17	8 ± 2.0
24.00	0	0	0	0	4	0	0
27.00	0	0	0	0	0	0	0
			Trimethoprim				
0.25	1.6	2.0	1.8	1.6	1.5	2.0	1.6 ± 0.20
0.50	1.0	1.0	1.4	1.0	0.8	0.7	1.0 ± 0.10
0.75	0.6	0.8	1.0	0.6	0.6	0.5	0.7 ± 0.07
1.00	0.3	0.5	0.7	0.4	0.3	0.3	0.4 ± 0.06
1.50	0.2	0.4	0.4	0.2	0.2	0.1	0.3 ± 0.05
2.00	0.2	0.2	0.3	0	0	0	0.1 ± 0.05
2.50	0	0.1	0.2	0	0	0	0
3.00	0	0	0.1	0	0	0	0
4.00	0	0	0	0	0	0	0
5.00	0	0	0	0	0	0	0
6.00	0	0	0	0	0	0	0
8.00	0	0	0	0	0	0	0
12.00	0	0	0	0	0	0	0
24.00	0	0	0	0	0	0	0
27.00	0	0	0	0	0	0	0

TABLE (1) 2

DISPOSITION KINETICS OF TRIMETHOPRIM IN SHEEP (n=6) AFTER SINGLE INTRAVENOUS ADMINISTRATION OF TRIVETRIN AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Pharmacokinetic parameters	Sheep number 1	9	39	41	75	88	Mean ± S.E.M.	Averaged Values
Cp^{o} ($\mu g.ml^{-1}$)	2.70	4.50	2.30	2.70	2.40	4.60	3.20 ± 0.40	2.50
A ($\mu g.ml^{-1}$)	2.00	2.80	0.40	1.00	1.70	2.80	1.80 ± 0.40	0.60
α (h^{-1})	3.00	5.60	1.30	3.70	2.80	2.30	3.00 ± 0.60	3.50
t 1/2 α (h)	0.20	0.10	0.50	0.20	0.20	0.20	0.20 ± 0.05	0.20
B ($\mu g.ml^{-1}$)	0.80	1.80	1.90	1.80	0.70	1.70	1.50 ± 0.20	1.80
β (h^{-1})	0.80	1.00	1.00	1.50	0.80	1.20	1.00 ± 0.10	1.40
t 1/2 β (h)	0.90	0.60	0.70	0.50	0.90	0.60	0.70 ± 0.06	0.50
AUC ($\mu g.ml^{-1}.h$)	1.70	2.00	2.30	1.50	1.50	1.20	1.70 ± 0.10	1.50
Vc ($ml.kg^{-1}$)	912.00	554.20	1079.30	923.70	1036.30	548.50	842.30 ± 93.70	1010.70
$Vd_{(area)}$ ($ml.kg^{-1}$)	1832.40	1095.00	1130.40	1172.90	2125.80	886.50	1373.80 ± 195.00	1199.40
K_{12} (h^{-1})	0.70	1.70	0.01	0.40	0.60	0.20	0.60 ± 0.20	0.30
K_{21} (h^{-1})	1.50	2.80	1.20	2.90	1.40	2.60	2.00 ± 0.30	3.00
K_{el} (h^{-1})	1.60	2.20	1.00	1.80	1.70	2.40	1.80 ± 0.20	1.60
CLB($ml.kg.^{-1}h^{-1}$)	1480.20	1209.30	1077.30	1705.00	1724.00	2005.00	1533.50 ± 139.00	1662.70

TABLE (1) 3

DISPOSITION KINETICS OF SULPHADOXINE IN SHEEP (n=6) FOLLOWING A SINGLE INTRAVENOUS ADMINISTRATION OF TRIVETRIN AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Pharmacokinetic parameters	Sheep number 1	9	39	41	75	88	Mean ± S.E.M.	Averaged values
Cp^{o} ($\mu g.ml^{-1}$)	75.60	66.30	69.40	77.80	83.50	78.00	75.00 ± 2.50	64.80
A ($\mu g.ml^{-1}$)	55.60	46.50	29.40	43.60	29.30	45.70	41.70 ± 4.20	30.50
α (h^{-1})	0.90	0.40	0.50	0.50	0.40	0.40	0.50 ± 0.07	0.40
t 1/2 α (h)	1.00	1.50	1.40	1.50	1.80	1.70	1.50 ± 0.10	1.60
B ($\mu g.ml^{-1}$)	53.80	35.80	40.00	34.30	31.50	20.40	36.00 ± 4.40	34.30
β (h^{-1})	0.20	0.10	0.20	0.10	0.09	0.03	0.10 ± 0.02	0.10
t 1/2 β (h)	3.70	4.80	4.00	5.70	8.00	2.50	4.80 ± 0.70	5.70
AUC ($\mu g.ml^{-1}.h$)	283.80	289.80	288.70	377.50	443.40	771.00	409.00 ± 75.40	355.40
Vc ($ml.kg^{-1}$)	165.30	187.90	180.00	160.70	149.70	160.30	169.50 ± 6.00	192.80
$Vd_{(area)}$ ($ml.kg^{-1}$)	189.00	263.30	250.00	290.00	327.40	526.80	305.40 ± 45.00	289.70
K_{12} (h^{-1})	0.10	0.10	0.08	0.10	0.08	0.20	0.10 ± 0.02	0.08
K_{21} (h^{-1})	0.50	0.40	0.40	0.30	0.20	0.20	0.30 ± 0.04	0.30
K_{el} (h^{-1})	0.20	0.20	0.20	0.20	0.10	0.09	0.20 ± 0.02	0.20
CLB($ml.kg.^{-1}h^{-1}$)	42.20	43.30	43.30	33.00	28.20	16.20	34.40 ± 4.30	35.20

TABLE (1) 4

CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHADOXINE/TRIMETHOPRIM IN PLASMA AFTER INTRAMUSCULAR ADMINISTRATION OF "TRIVETRIN" AT ONE SITE AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Time (hours)	Sheep number 1	9	39	41	75	88	Mean	± S.E.M.
				Sulphadoxine				
0	0	0	0	0	0	0	0	
0.5	20	7	28	26	16	25	20	± 3.0
1	28	17	39	34	28	33	30	± 3.0
2	34	23	35	30	37	34	32	± 2.0
3	32	27	28	26	33	26	29	± 1.0
4	27	22	23	21	31	21	24	± 2.0
6	22	16	15	15	29	14	19	± 2.0
8	20	11	10	12	20	8	14	± 2.0
12	13	5	4	7	12	2	7	± 2.0
24	0	0	0	0	0	0	0	
				Trimethoprim				
0	0	0	0	0	0	0	0	
0.5	0.5	0.3	0.5	0.4	0.3	0.5	0.4	± 0.04
1	0.6	0.5	0.8	0.2	0.4	0.6	0.5	± 0.08
2	0.3	0.4	0.4	0.2	0.3	0.3	0.3	± 0.03
3	0.2	0.2	0.3	0.1	0.3	0.1	0.2	± 0.03
4	0.1	0.1	0.2	0	0.2	0	0.1	± 0.03
6	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	
24	0	0	0	0	0	0	0	

TABLE (1) 5

CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHADOXINE/TRIMETHOPRIM IN PLASMA AFTER INTRAMUSCULAR ADMINISTRATION OF "TRIVETRIN" AT THREE SITES AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Time (hours)	Sheep number 1	9	39	41	75	88	Mean	± S.E.M.
Sulphadoxine								
0	0	0	0	0	0	0	0	
0.5	27	9	20	17	13	30	19	± 3.0
1	37	16	29	21	20	38	27	± 4.0
2	35	25	34	25	29	35	31	± 2.0
3	28	24	30	24	34	29	28	± 2.0
4	20	23	25	22	35	25	25	± 2.0
6	12	20	18	14	31	18	19	± 3.0
8	6	15	12	8	29	14	14	± 3.0
12	0	9	7	0	13	8	6	± 2.0
24	0	0	0	0	0	0	0	
Trimethoprim								
0	0	0	0	0	0	0	0	
0.5	0.5	0.4	0.6	0.2	0.3	0.5	0.4	± 0.05
1	0.6	0.5	0.7	0.4	0.4	0.6	0.5	± 0.04
2	0.3	0.4	0.5	0.3	0.3	0.3	0.4	± 0.03
3	0.2	0.3	0.3	0.1	0.2	0.2	0.2	± 0.03
4	0.1	0.1	0.2	0.1	0.1	0.1	0.1	± 0.01
6	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	
24	0	0	0	0	0	0	0	

TABLE (1) 6

VALUES OF THE KINETIC PARAMETERS OF SULPHADOXINE AND TRIMETHOPRIM FOLLOWING INTRAMUSCULAR ADMINISTRATION OF TRIVETRIN AT ONE SITE (I) AND AT THREE SITES (III) AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT (12.5 and 2.5 $mg.kg^{-1}$ SULPHADOXINE AND TRIMETHOPRIM RESPECTIVELY).

Animal number	t 1/2 α (h) I	t 1/2 α (h) III	t 1/2 β (h) I	t 1/2 β (h) III	AUC ($\mu g.ml^{-1}.h$) I	AUC ($\mu g.ml^{-1}.h$) III
			Sulphadoxine			
1	0.50	0.40	7.30	2.30	402.50	185.70
9	0.80	1.00	3.50	5.20	203.30	269.00
39	0.30	0.50	3.20	4.30	228.60	258.50
41	0.30	0.80	4.70	2.70	251.70	170.20
75	0.80	1.40	5.50	4.50	379.30	385.00
88	0.50	0.30	2.50	4.80	185.80	292.20
Mean ± S.E.M.	0.50 ± 0.09	0.70 ± 0.10	4.50 ± 0.70	4.00 ± 0.40	275.20 ± 37.00	260.00 ± 31.00
Averaged values	0.40	0.60	4.80	4.00	286.90	251.00
			Trimethoprim			
1	0.30	0.30	1.20	1.20	1.40	1.40
9	0.40	0.50	1.00	1.00	1.30	1.40
39	0.30	0.30	1.60	1.50	2.00	2.20
41	0.40	0.50	1.00	1.30	0.70	0.80
75	0.70	0.50	1.70	1.00	1.50	1.20
88	0.30	0.30	0.80	1.20	1.00	1.40
Mean ± S.E.M.	0.40 ± 0.06	0.40 ± 0.04	1.20 ± 0.10	1.20 ± 0.07	1.30 ± 0.10	1.40 ± 0.10
Averaged values	0.30	0.40	1.30	1.00	1.30	1.30

TABLE (1) 7

CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHADIAZINE IN PLASMA AFTER INTRAMUSCULAR ADMINISTRATION OF "TRIBRISSEN" AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADIAZINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Time (hours)	Sheep number 1	39	41	75	88	Mean	± S.E.M.
0	0	0	0	0	0	0	
0.5	47	39	42	38	38	41	± 2.0
1	42	42	43	41	39	42	± 1.0
2	31	35	33	32	34	33	± 1.0
3	25	27	22	26	26	25	± 1.0
4	18	19	15	21	19	18	± 1.0
6	11	12	7	13	10	11	± 1.0
8	8	9	3	9	6	7	± 3.0
12	4	5	0	5	3	3	± 1.0
24	0	0	0	0	0	0	

TABLE (1) 8

CONCENTRATIONS ($\mu g.mg^{-1}$) OF SULPHADIAZINE IN PLASMA AFTER INTRAMUSCULAR ADMINISTRATION OF "DUPHATRIM" AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADIAZINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Time (hours)	Sheep number 1	39	41	75	88	Mean	± S.E.M.
0	0	0	0	0	0	0	
0.5	22	7	41	37	34	28	± 6.0
1	31	15	40	41	38	33	± 5.0
2	26	27	30	33	30	29	± 1.0
3	17	28	23	26	23	23	± 2.0
4	14	23	16	19	16	18	± 2.0
6	9	14	8	11	9	10	± 1.0
8	5	8	4	7	6	6	± 1.0
12	0	4	0	4	3	2	± 1.0
24	0	0	0	0	0	0	

TABLE (1) 9

VALUES OF THE PHARMACOKINETIC CONSTANTS OF SULPHADIAZINE IN SHEEP (n=5) FOLLOWING INTRAMUSCULAR ADMINISTRATION OF TRIBRISSEN AND DUPHATRIM AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT (12.5 $mg.kg^{-1}$ SULPHADIAZINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Animal number	Pharmacokinetic parameters						
	A	α	t 1/2 α	B	β	t 1/2 β	AUC
	$\mu g.ml^{-1}$	h^{-1}	h	$\mu g.ml^{-1}$	h^{-1}	h	$\mu g.ml^{-1}.h$
Duphatrim							
1	- 40.40	2.20	0.30	40.40	0.30	2.70	137.40
39	- 51.00	1.00	0.70	51.00	0.20	3.00	180.20
41	- 58.80	3.80	0.20	58.80	0.30	2.00	161.40
75	- 47.90	4.20	0.20	47.90	0.20	3.00	205.70
88	- 44.60	4.00	0.20	44.60	0.20	2.90	177.00
Mean $\pm$ S.E.M.	-48.50 $\pm$ 3.00	3.00 $\pm$ 0.60	0.30 $\pm$ 0.10	48.50 $\pm$ 3.00	0.20 $\pm$ 0.02	2.70 $\pm$ 0.20	172.30 $\pm$ 11.30
Averaged values	- 51.20	2.10	0.30	51.20	0.30	2.60	166.00
Tribrissen							
1	- 43.00	2.00	0.30	43.00	0.20	3.40	190.20
39	- 47.00	5.00	0.10	47.00	0.20	3.50	228.20
41	- 68.20	3.00	0.20	68.20	0.40	1.80	155.50
75	- 46.20	5.00	0.10	46.20	0.20	3.60	229.00
88	- 51.20	4.00	0.20	51.20	0.20	2.80	193.30
Mean $\pm$ S.E.M.	- 51.00 $\pm$ 4.50	3.80 $\pm$ 0.60	0.20 $\pm$ 0.03	51.00 $\pm$ 4.50	0.20 $\pm$ 0.04	3.00 $\pm$ 0.30	199.00 $\pm$ 13.70
Averaged values	- 50.40	5.20	0.10	50.40	0.20	2.90	199.40

TABLE (1) 10

CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHATROXAZOLE/TRIMETHOPRIM IN PLASMA AFTER INTRAMUSCULAR ADMINISTRATION OF "LEOTROX" AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHATROXAZOLE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Time (hours)	Sheep number 1	39	41	75	88	Mean	± S.E.M.
			Sulphatroxazole				
0	0	0	0	0	0	0	
0.5	60	57	66	63	58	61	± 2.0
1	64	69	73	75	67	70	± 2.0
2	58	72	62	74	69	67	± 3.0
3	52	65	57	66	57	59	± 3.0
4	44	59	41	61	53	52	± 4.0
6	33	48	33	48	41	41	± 4.0
8	31	39	26	43	34	35	± 3.0
12	21	28	17	33	27	25	± 3.0
24	8	11	6	17	17	12	± 2.0
27	7	8	5	14	12	9	± 2.0
32	4	6	3	11	11	7	± 2.0
48	0	0	0	5	3	2	± 1.0
51	0	0	0	0	0	0	
			Trimethoprim				
0	0	0	0	0	0	0	
0.5	0.5	0.4	0.5	0.4	0.6	0.4	± 0.03
1	0.3	0.3	0.3	0.5	0.4	0.4	± 0.03
2	0.1	0.3	0.1	0.2	0.3	0.2	± 0.10
3	0	0.2	0	0	0.1	0.1	± 0.03
4	0	0	0	0	0	0	
6	0	0	0	0	0	0	
8	0	0	0	0	0	0	
12	0	0	0	0	0	0	
24	0	0	0	0	0	0	
27	0	0	0	0	0	0	
32	0	0	0	0	0	0	
48	0	0	0	0	0	0	
51	0	0	0	0	0	0	

TABLE (1) 11

VALUES OF PHARMACOKINETIC PARAMETERS OF TRIMETHOPRIM AND SULPHATROXAZOLE IN SHEEP FOLLOWING A SINGLE INTRAMUSCULAR ADMINISTRATION OF LEOTROX AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT (12.5 $mg.kg^{-1}$ SULPHATROXAZOLE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

Pharmacokinetic parameters	Animal number 1	39	41	75	88	Mean ± S.E.M.	Averaged values
			Trimethoprim				
A ($\mu g.ml^{-1}$)	- 0.90	- 0.70	- 0.90	- 1.30	- 0.90	- 0.90 ± 0.10	- 0.80
α (h^{-1})	7.70	1.80	7.70	2.30	6.00	5.00 ± 1.30	3.10
t 1/2 α (h)	0.10	0.40	0.10	0.30	0.10	0.20 ± 0.06	0.20
B ($\mu g.ml^{-1}$)	0.90	0.70	0.90	1.30	0.90	0.90 ± 0.10	0.80
β (h^{-1})	1.00	4.00	1.00	0.90	7.00	2.80 ± 1.20	7.00
t 1/2 β (h)	0.60	1.70	0.60	0.80	1.00	0.90 ± 0.20	1.00
AUC ($\mu g.ml^{-1}.h$)	0.70	1.30	0.70	0.80	1.20	0.90 ± 0.10	0.90
			Sulphatroxazole				
A ($\mu g.ml^{-1}$)	- 63.80	- 80.60	- 53.50	- 74.00	- 67.00	- 67.80 ± 4.60	- 70.00
α (h^{-1})	8.00	2.70	0.60	4.30	4.50	4.00 ± 1.20	4.80
t 1/2 α (h)	0.09	0.30	1.20	0.20	0.20	0.40 ± 0.20	0.10
B ($\mu g.ml^{-1}$)	63.80	80.60	53.50	74.00	67.00	67.80 ± 4.60	70.00
β (h^{-1})	0.09	0.08	0.09	0.06	0.06	0.08 ± 0.01	0.07
t 1/2 β (h)	8.00	8.30	7.70	11.80	11.00	9.30 ± 0.80	9.30
AUC ($\mu g.ml^{-1}.h$)	735.30	937.20	506.20	1241.80	1056.00	895.30 ± 128.00	924.70

TABLE (1) 12

CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHATROXAZOLE IN PLASMA AFTER A SLOW INTRAVENOUS ADMINISTRATION OF "LEOTROX" AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF THE ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHATROXAZOLE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM.

Time (hours)	Sheep number 91	92	99	Mean	± S.E.M.
0.25	66	86	81	78	± 6.0
0.50	73	82	70	75	± 3.0
0.75	79	80	66	75	± 4.0
1	77	75	63	72	± 4.0
1.5	75	69	58	67	± 5.0
2	74	63	56	64	± 5.0
2.5	66	60	53	60	± 4.0
3	65	54	50	56	± 4.0
4	64	48	45	52	± 6.0
5	63	42	41	49	± 7.0
6	62	42	40	48	± 7.0
8	50	35	34	40	± 5.0
12	43	26	23	31	± 6.0
24	24	12	13	16	± 4.0
27	21	10	8	13	± 4.0
32	16	8	7	10	± 3.0
48	7	0	0	2	± 2.0
51	6	0	0	2	± 2.0

TABLE (1) 13

DISPOSITION KINETICS OF SULPHATROXAZOLE IN SHEEP (n=3) FOLLOWING A SINGLE INTRAVENOUS ADMINISTRATION OF LEOTROX AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ BODYWEIGHT (12.5 and 2.5 $mg.kg^{-1}$ OF SULPHATROXAZOLE AND TRIMETHOPRIM RESPECTIVELY).

Pharmacokinetic parameters	Sheep number 91	92	99	Mean ± S.E.M.	Averaged values
Cp^{o} ($\mu g.ml^{-1}$)	89.70	92.40	89.30	90.50 ± 0.90	102.70
A ($\mu g.ml^{-1}$)	31.00	36.60	28.40	32.00 ± 2.40	10.00
α (h^{-1})	0.10	0.50	1.60	0.70 ± 0.40	1.30
t 1/2 α (h)	6.50	1.40	0.40	2.80 ± 1.80	0.60
B ($\mu g.ml^{-1}$)	77.80	55.80	61.00	64.90 ± 6.60	73.10
β (h^{-1})	0.05	0.06	0.07	0.06 ± 0.01	0.07
t 1/2 β (h)	13.90	11.10	10.00	11.70 ± 1.00	10.00
AUC ($\mu g.ml^{-1}.h$)	1589.00	966.30	888.60	1148.00 ± 222.60	1056.50
Vc ($ml.kg^{-1}$)	139.30	135.30	140.00	138.20 ± 1.40	150.30
$Vd_{(area)}$ ($ml.kg^{-1}$)	157.70	207.20	201.10	188.70 ± 15.60	169.70
K_{12} (h^{-1})	0.01	0.10	0.50	0.20 ± 0.10	0.10
K_{21} (h^{-1})	0.10	0.10	1.10	0.40 ± 0.30	1.10
K_{el} (h^{-1})	0.05	0.10	0.10	0.08 ± 0.01	0.80
CLB ($ml.kg^{-1}.h^{-1}$)	7.90	12.90	14.10	5.80 ± 3.80	11.80

FIGURE (1) 1

SEMILOGARITHMIC PLOT OF TRIMETHOPRIM CONCENTRATIONS ($\mu g.ml^{-1}$) IN PLASMA VERSUS TIME OBTAINED FOLLOWING A SINGLE INTRAVENOUS ADMINISTRATION OF TRIVETRIN AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

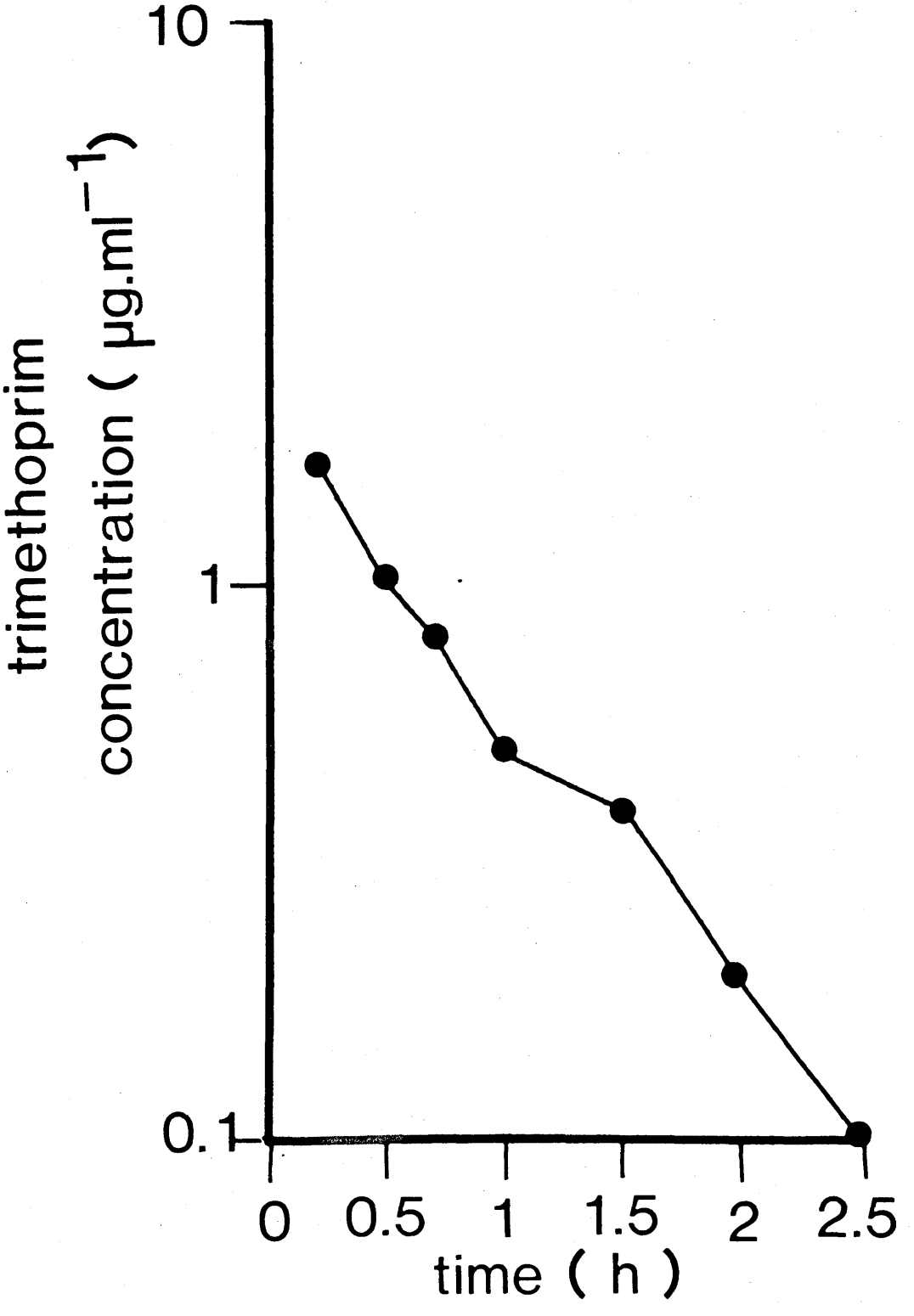

10
1
0.1
trimethoprim
concentration (µg.ml^{-1})
0
0.5
1
1.5
2
2.5
time (h)

FIGURE (1) 2

SEMILOGARITHMIC PLOT OF SULPHADOXINE CONCENTRATIONS ($\mu g.ml^{-1}$) IN PLASMA VERSUS TIME OBTAINED AFTER SINGLE INTRAVENOUS ADMINISTRATION OF TRIVETRIN AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHADOXINE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

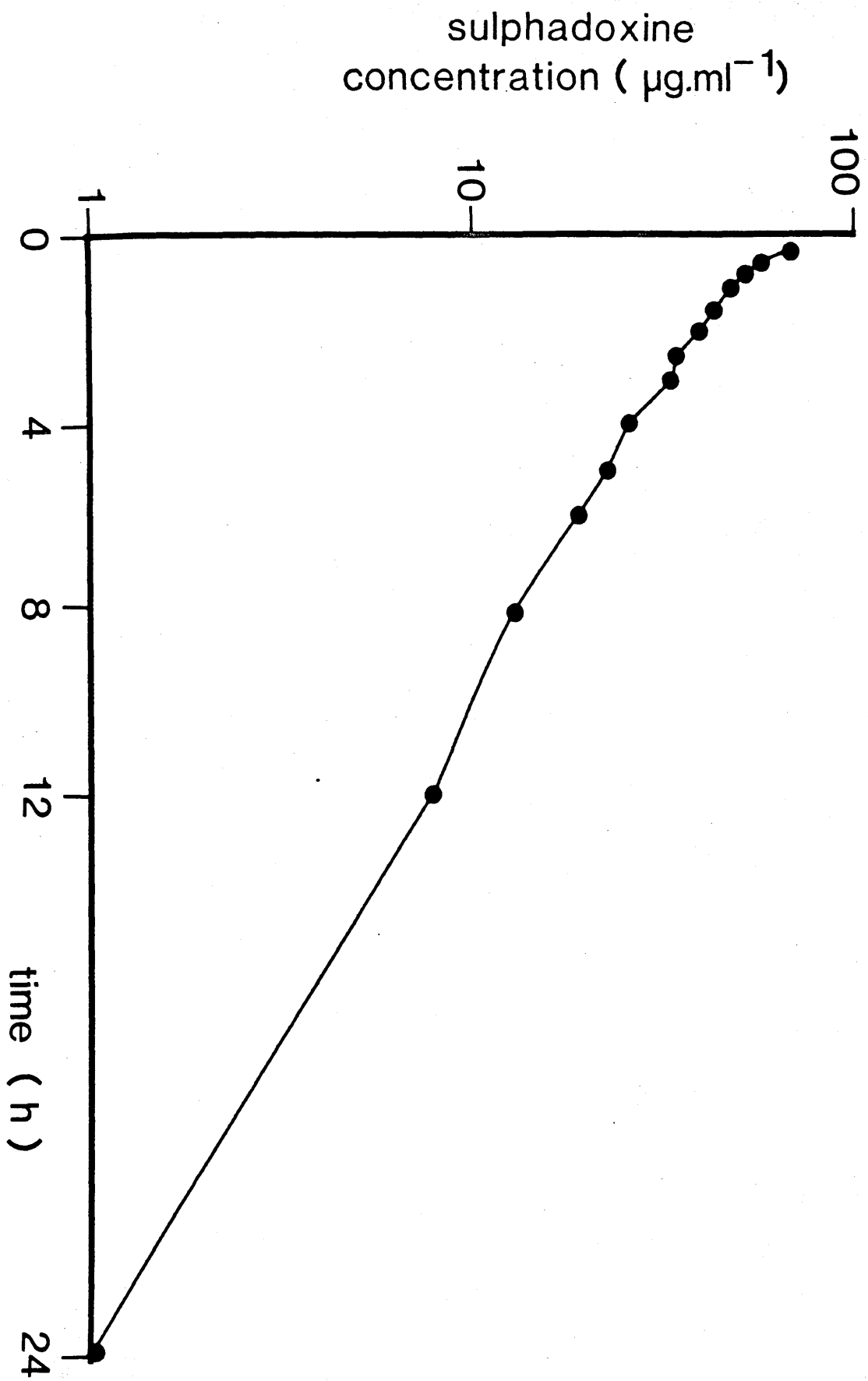

sulphadoxine
concentration (µg.ml⁻¹)
1
10
100
0
4
8
12
24
time (h)

FIGURE (1) 3

MEAN CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHADOXINE, SULPHADIAZINE, SULPHADIAZINE AND SULPHATROXAZOLE IN PLASMA OF SHEEP AFTER INTRAMUSCULAR ADMINISTRATION OF TRIVETRIN, TRIBRISSEN, DUPHATRIM AND LEOTROX AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHONAMIDE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).

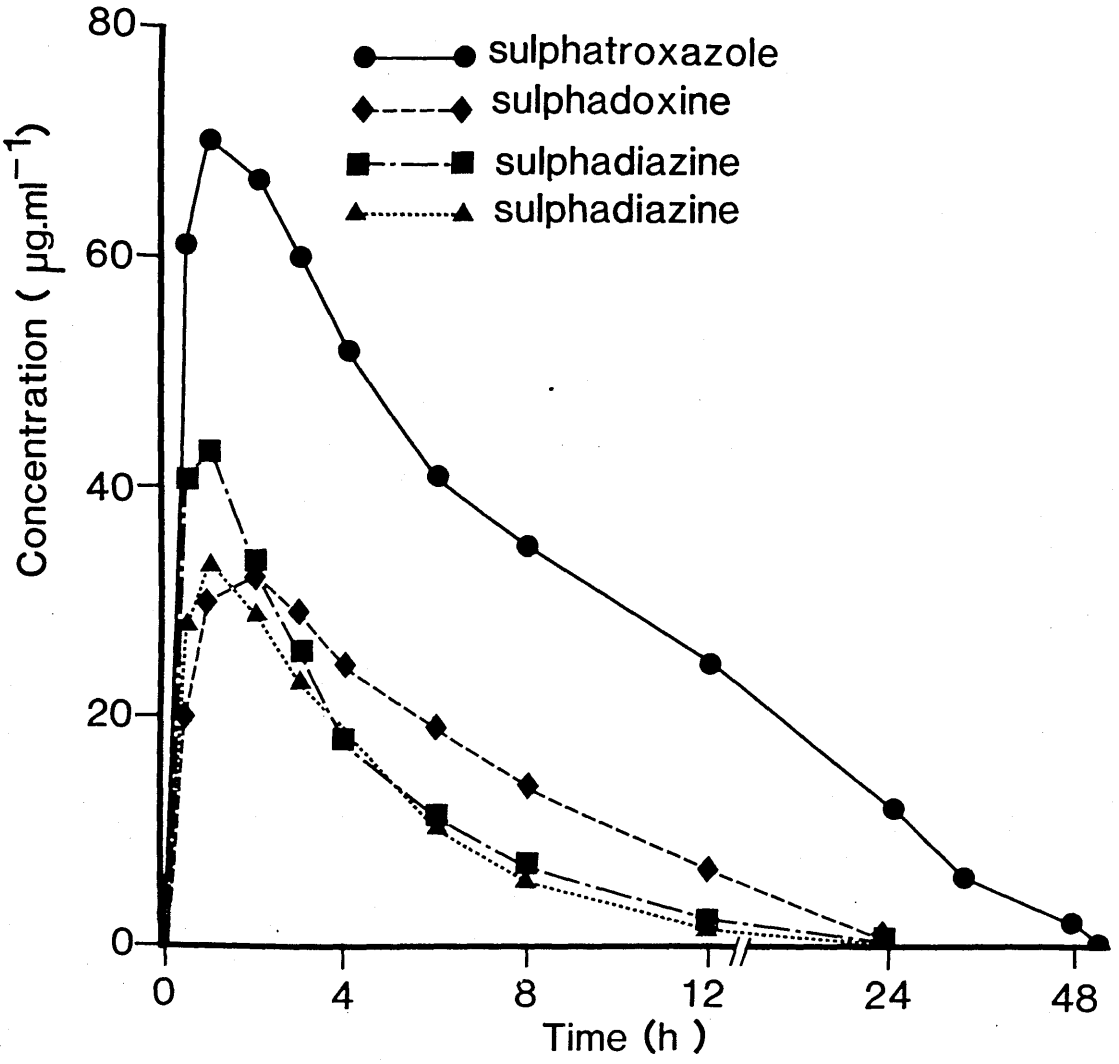
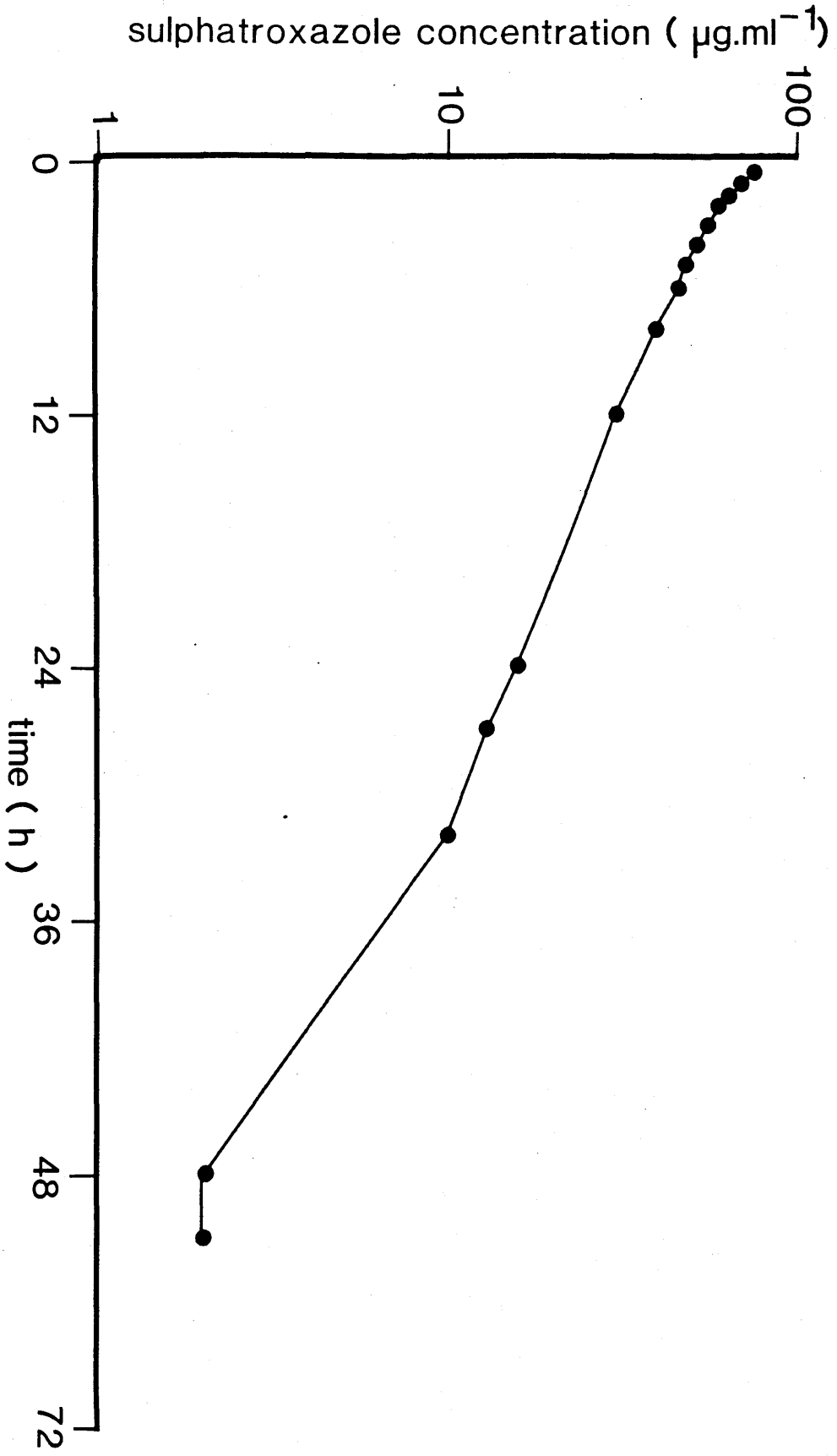

80
60
40
20
0
Concentration (µg.ml^{-1})
sulphatroxazole
sulphadoxine
sulphadiazine
sulphadiazine
0
4
8
12
24
48
Time (h)

FIGURE (1) 4

SEMILOGARITHMIC PLOT OF CONCENTRATIONS ($\mu g.ml^{-1}$) OF SULPHATROXAZOLE IN PLASMA VERSUS TIME AFTER AN INTRAVENOUS ADMINISTRATION OF LEOTROX AT A TOTAL DOSE RATE OF 15 $mg.kg^{-1}$ OF ACTIVE INGREDIENTS (12.5 $mg.kg^{-1}$ SULPHATROXAZOLE PLUS 2.5 $mg.kg^{-1}$ TRIMETHOPRIM).


sulphatroxazole concentration (µg.ml⁻¹)
1
10
100
0
12
24
36
48
72
time (h)

(1) 6 Discussion

The residence time of the different sulphonamides used in this study was found to be dependent on the degree of binding to plasma proteins. The short elimination half-lives (t 1/2β) of sulphadiazine (2.7 h) and sulphadoxine (4.5 h) corresponds well with the low degree of binding (14 and 50 percent respectively) and the prolonged residence of sulphatroxazole t 1/2 β = 9.3 h accords with its moderate degree of binding (70 percent). The percent binding of sulphadiazine to sheep plasma proteins was similar to that reported in cows and horses (Nielsen and Rasmussen 1977; Rasmussen et al 1979) and of sulphadoxine in cows (Nielsen and Rasmussen 1977).

The distribution and elimination curve of sulphadoxine and trimethoprim can best be described by the two compartment model and the biexponential equation:-

for sulphadoxine: $Cp = 41.70e^{-0.50t} + 36.00e^{-0.10t}$

for trimethoprim: $Cp = 1.80e^{-3.00t} + 1.50e^{-1.00t}$

Similarly the disposition curve of sulphadoxine was described by a two compartment model in goats following intravenous and oral administration (Nielsen and Rasmussen 1976b; Nielsen et al 1978) in cows (Davitiyananda and Rasmussen 1974) and in swine (Nielsen and Rasmussen 1975a). In horses, the disposition kinetics of sulphadoxine administered intravenously was in accordance with a three compartment model (Rasmussen et al 1979) while that of trimethoprim followed a two compartment model (Alexander and Collett 1975; Rasmussen et al 1979).

Following the intravenous administration, distribution and elimination of trimethoprim and sulphadoxine occurred at different rates. Distribution of trimethoprim occurred rapidly having a mean distribution half-life (t 1/2 α) of 12 minutes. This value is very similar to that reported in horses (10 minutes; Rasmussen et al 1979). Trimethoprim was eliminated from plasma rather rapidly (t 1/2 β = 42 minutes) and was similar to that reported in goats (30 - 40 minutes; Nielsen and Rasmussen 1972; 1976b) and considerably shorter than that in swine (2.5 h; Nielsen and Rasmussen 1975c) in dogs (3 h; Kaplan et al 1970) in horses (3 - 6 h; Alexander and Collett 1974, 1975; Rasmussen et al 1979) and man (9 - 15 h; Bushby and Hitchings 1968; Schwartz and Ziegler 1969; Schwartz and Reider 1970; Bergan and Brodwall 1972; Nolte and Buttner 1973; Andreasen et al 1978).

This short residence time of trimethoprim in plasma of sheep could lead to a limited antibacterial activity of the combination. However trimethoprim has been found to pass rapidly into tissues from plasma and attain concentrations in target tissues higher than those in plasma. This higher concentration of the drug in tissues is of value to its antimicrobial activity. This was confirmed by determination of the concentrations of trimethoprim in tissues and blood from goats and cows (Nielsen and Rasmussen 1975b) in pigs (Nielsen and Rasmussen 1975c) in dogs (Sigel et al 1981) and in mice (Bushby and Hitchings 1968). Furthermore calculation of the apparent volume of distribution Vd (area) provides an idea of the extent of distribution of a drug into tissues. The large Vd (area) of trimethoprim in sheep in this study (1.37 $l.kg^{-1}$) also confirms that trimethoprim is

well distributed into tissues. Similar values for Vd (area) were reported for goats (1.2 $l.kg^{-1}$; Nielsen and Rasmussen 1972) for swine (1.4 $l.kg^{-1}$; Nielsen and Rasmussen 1975c) for horses (1.1 and 1.5 $l.kg^{-1}$; Davitiyananda and Rasmussen 1974; Rasmussen et al 1979 respectively).

The elimination of trimethoprim in ruminants appears to be quicker than that in simple stomached animals and is probably due to differences in the metabolism of the drug in different animal species (Sigel et al 1973).

The rate of elimination of trimethoprim has been found to be age dependent. Young goats (two day old kids) eliminated trimethoprim 4 - 5 times slower (3 h) than the adult goats (40 minutes) (Nielsen and Rasmussen 1976b).

The metabolism of trimethoprim occurs mainly by oxidation, in the liver, by the hepatic microsomal enzymes (Nielsen and Rasmussen 1976b). Trimethoprim has been found to undergo degradation by ruminal microorganisms (Nielsen and Rasmussen 1976a; Nielsen et al 1978). This degradation reduces the activity of the drug when administered orally to ruminants and it is the reason for the inability to detect trimethoprim in plasma in sheep following oral administration (Piercy 1978). Microbial degradation is also the most likely explanation for the reduced amount of trimethoprim concentrations obtained in horse plasma after the animals have been fed (Bogan et al 1984).

Sulphadoxine was distributed more slowly than trimethoprim, into tissues having a mean distribution half-life ($t\ 1/2\ \alpha = 90 \pm 0.1$ minutes) which is very similar to that in

horses (80 minutes; Rasmussen et al 1979). Elimination of sulphadoxine also occurred at a slower rate than that of trimethoprim with a mean elimination half-life (t 1/2 β = 4.8 $\pm$ 0.7 h). The rate of elimination of sulphadoxine in sheep is considerably shorter than that obtained in goats (12 h; Nielsen and Rasmussen 1976b) in swine (8 h; Nielsen and Rasmussen 1975b) in cows (11 h; Davitiyananda and Rasmussen 1974) in horses (14 h; Rasmussen et al 1979) and in humans (123 - 205 h; Madsen and Iversen 1964; Stuller 1968; Bohni et al 1969).

Distribution of sulphadoxine into tissues was found to be less than that of trimethoprim. This is reflected by the small Vd (area) (0.31 $l.kg^{-1}$) of sulphadoxine in sheep administered intravenously at a dose rate of 12.5 $mg.kg^{-1}$. This value is similar to that found following intravenous administration at 40 $mg.kg^{-1}$ in goats (0.27 $l.kg^{-1}$; Nielsen and Rasmussen 1976b) in swine (0.35 $l.kg^{-1}$; Nielsen and Rasmussen 1975a) in cows (0.37 $l.kg^{-1}$; Davitiyananda and Rasmussen 1974) and in horses (0.39 $l.kg^{-1}$; Rasmussen et al 1979). Administration of sulphadoxine at higher doses of 120 $mg.kg^{-1}$ produced similar Vd (area) in horses to that after administration at 40 $mg.kg^{-1}$ dose rate (Rasmussen et al 1979).

The plasma concentrations/time data of trimethoprim/sulphadoxine following the intramuscular administration at one and three sites were in accordance with a two compartment model.

No significant differences ($P > 0.05$, Paired-t-test) were found between the plasma concentrations of trimethoprim and

sulphadoxine administered intramuscularly at one and three sites.

It has been suggested that, for insoluble oily bolus injections, an increase in the number of injection sites would cause an increase in the rate of drug absorption because the absorption of drug from the injection site is proportional to the surface area of the bolus and thus administration of the dose at more than one site should increase absorption of the drug because the surface area/volume increases (Bogan 1983). However, the results in this study showed that the rate of absorption of trimethoprim/sulphadoxine was not influenced by the increase in the number of injection sites. Bogan et al (1981) obtained higher plasma concentrations of levamisole at one hour after administration of the drug subcutaneously at five sites in the thoracic region than after administration of the drug at one site.

Injury and damage to tissues at the injection sites is thought to reduce the rate of drug absorption. Following intramuscular administration of Trivetrin (trimethoprim/sulphadoxine) and Tribrissen (trimethoprim/sulphadiazine) in pigs, Rasmussen and Svendsen (1976) observed marked tissue damage. If tissue damage had occurred following the intramuscular administration of Trivetrin to sheep, then the rate of absorption of the drug would be reduced due to the presence of necrotic and fibrous tissue as a consequence of tissue damage.

The disposition curve of sulphadiazine in plasma following the intramuscular administration of Tribrissen and Duphatrim was

in accordance with a two compartment model. Similar findings were reported in ewes following the intravenous administration of 100 $mg.kg^{-1}$ sulphadiazine (Youssef et al 1981).

Sulphadiazine was readily absorbed following the intramuscular administration of Tribrissen and Duphatrim having mean half-lives in the absorption phase t 1/2 (α) = 12 and 18 minutes respectively. Elimination of sulphadiazine from plasma occurred rapidly with mean elimination half-lives [t 1/2 (β)] of 3.0 and 2.7 hours following administration of Tribrissen and Duphatrim respectively. Following the intravenous administration of 100 $mg.kg^{-1}$, sulphadiazine was eliminated at a slower rate (t 1/2 β = 7.15 h) (Youssef et al 1981) than that obtained in this study after the intramuscular administration.

Trimethoprim was not detected in plasma samples following administration of the two suspensions intramuscularly (Tribrissen and Duphatrim). Trimethoprim was also not detected in plasma in sheep following the oral administration of Tribrissen at 30 $mg.kg^{-1}$ (Piercy 1978).

The failure to detect trimethoprim in plasma following intramuscular administration of suspension formulations puts in question the relative effectiveness of the suspension products as compared with the solution.

It may be argued that the drug is distributed rapidly to tissues such that with slow absorption from the injection site, plasma levels never reach detectable concentrations. However this is doubtful since there is always dynamic equilibrium between tissue and plasma and from other studies, the reason for

the rapid decline in plasma concentrations is rapid elimination rather than redistribution. Thus even in tissues it is very doubtful whether useful trimethoprim are achieved and certainly in plasma neither the optimal concentrations (1 : 5) of trimethoprim relative to sulphonamide are achieved nor are they maintained for the length of activity of the sulphonamide. Again it could be argued that trimethoprim does not require to be present all the treatment period to exert synergism with the sulphonamide. Nevertheless it is doubtful whether such large differences in measurable concentrations will not lead to relatively poor synergism as compared to other species. Undoubtedly, in sheep from these results, suspension formulations should be avoided, although this combination (trimethoprim/sulphadiazine) has been shown to have a good chemotherapeutic activity against many infectious diseases in ruminants (Rehm and White 1970; White and Withnell 1971) and in small animals (Craig and White 1976; Sigel et al 1981).

The pharmacokinetic profile of sulphatroxazole has not yet been reported in any animal species as the drug has been introduced only recently for the treatment of bacterial diseases. The drug is highly effective against a wide range of gram-positive and gram-negative bacteria sensitive to trimethoprim/sulphatroxazole combination. The drug is available as an injectable solution, bolus and dispersable powder for the treatment of bacterial infections of alimentary, respiratory and urinary tracts in sheep, cattle, pigs and horses (Data Sheet IVS/1985).

The plasma concentrations/time data of sulphatroxazole following intravenous and intramuscular administration of Leotrox were best fitted to two compartment term. Distribution of sulphatroxazole was relatively slower than that of sulphadoxine, following the intravenous administration, having a mean distribution half-life (t 1/2 α) 2.8 $\pm$ 1.8 h. This was followed by a long residence time (t 1/2 β = 11.7 $\pm$ 1.0 h).

Plasma concentrations of trimethoprim were not determined following the intravenous administration of Leotrox because it was assumed that they would not be different than those following the intravenous administration of Trivetrin, as the vehicle for these two formulations are similar (glycerol formal).

Following the intramuscular administration of Leotrox, trimethoprim and sulphatroxazole were rapidly absorbed having mean half-lives of 12 and 24 minutes respectively.

Trimethoprim was eliminated very rapidly t 1/2 β = 54 minutes while elimination of sulphatroxazole from plasma was rather slow with a mean elimination half-life of 9.30 $\pm$ 0.8 hours. Such long residence time of sulphatroxazole in plasma is probably related to its moderate plasma protein-binding (70%) as the bound drug is not readily available for tissue distribution, metabolism and excretion processes. Further evidence of this is from the low Vd (area) 188.70 $ml.kg^{-1}$ and the long time taken for tissue distribution (t 1/2 α = 2.8 hours).

The bioavailability of sulphatroxazole was found to be 87.5% after intramuscular administration. Surprisingly, the plasma kinetics of sulphatroxazole were similar after the initial

few minutes following administration of the drug intravenously or intramuscularly which will suggest that in cases when a rapid treatment is necessary the intramuscular route would very efficiently replace the intravenous route then reducing the risk of giving such an irritant combination via the intravenous route.

GENERAL INTRODUCTION

Fascioliasis is one of the most common diseases of domestic animals throughout the world and it is of great economic importance. Human infection with Fasciola hepatica "liver rot disease" occurs due to consumption of infected raw watercress and has been reported in Latin America, Great Britain, France, China, Algeria and other Mediterranean countries (Faust et al 1970; Brown and Neva 1983).

Two species are involved in the disease, Fasciola hepatica and Fasciola gigantica. In temperate areas F. hepatica is the most wide spread species in sheep and cattle but it is replaced by F. gigantica in many parts of Southern America, Indo-Pakistan and the Middle East. The epidemiology, pathogenesis and control of the two species are similar. Sheep and cattle become infested during grazing by ingestion of the encysted metacercariae on vegetation. The life cycle of F. hepatica is shown in Figure (2) 1. The disease manifests itself as three clinical forms. An acute form which is caused by the extensive haemorrhage and disruption of liver tissues due to the migration of immature flukes through the liver parenchyma within six weeks of infection. This form occurs mainly in sheep (and probably in goats). The infected animals might die suddenly without showing any obvious symptoms.

The sub acute form occurs due to the haemorrhage and damage of liver tissues and also due to anaemia caused by the presence of flukes in liver for a longer period.

A chronic form which occurs due to the presence of adult flukes in bile ducts for many weeks (beginning ten to twelve weeks after infection). In cattle the chronic form is more common and characterised by anaemia and gradual loss in general condition. In sheep, in most developed countries, the chronic form is now the most commonly encountered form principally because the use of drugs minimises the challenge to the animal such that the acute phase is not recognised.

Fascioliasis can also pre-dispose to clostridial infection (Clostridium novyi) due to the presence of damaged liver tissues caused by migrating flukes. This form ("Black disease") is serious and death often occurs among infected sheep. In most countries this form is becoming rarer because vaccination against this and other clostridial diseases is now routine.

The complete life cycle of F. hepatica has been elucidated in 1882 (Leuckart 1882; Thomas 1883) and since then there has been a continuous search for the production of satisfactory compounds for the control and treatment of the disease. Flukes need an intermediate host, a mud snail, (in Europe this is Lymnaea truncatula) to complete their life cycle. Control of snails is possible using molluscicides, including copper sulfate and the more recently introduced "Frescon" (N-tritylmorpholine) (Boyce et al 1967; Crossland et al 1969) but more general control of the intermediate host is by destroying the habitat of the snails by drainage. Routine control, however, is most commonly done by eradication of the fluke in the reservoir host using fasciolicidal drugs.

The fasciolicidal drugs can be separated into five groups according to their chemical structure.

(1) Halogenated hydrocarbons(carbon tetrachloride, hexachloroethane, tetrachlorodifluoroethane).

(2) Nitrophenolic and bisphenolic compounds (disophenol, niclofolan, nitroxynil, hexachlorophane, bithionol sulfoxide).

(3) Salicylanilide and aromatic amines (oxyclozanide, brotianide, rafoxanide, closantel, diamphenethide).

(4) Benzimidazoles (albendazole, triclabendazole).

(5) Sulphonamides (clorsulon).

An interesting feature of fasciolicidal drugs of each group (except albendazole) is the presence of halogen atom. However, it is unknown whether the halogen atom represents a common mechanism for the activity of fasciolicidal drugs.

Most of the fasciolicidal drugs introduced to the anthelmintic market (the chlorinated hydrocarbons and bisphenolic compounds) are mainly effective against the mature fluke and those which show activity against the immature flukes in liver parenchyma are invariably effective against this stage of the fluke only at doses near their toxic dosage rates (Boray et al 1967; Boray and Happich 1968). Efficacies of most the fasciolicidal compounds in routine use are represented in Table (2) 1.

Most of the fasciolicidal drugs in current use are the salicylanilides and the closely related substituted nitrophenols (Prichard 1978a). Members of this group have been proven to exert a high efficacy against mature liver flukes with less efficacy against the immature flukes (Broome and Jones 1966;

Davis et al 1966; Boray and Happich 1967, 1968; Colegrave 1968a, b).

The aromatic amine, diamphenethide (β, β'-bis (4-acetamidophenxyloxy) ethyl ether is unique amongst fasciolicidal drugs in having superior activity against very young fluke (one day to nine week-old fluke) (Armour and Corba 1972; Harfenist 1973; Rowlands 1973, 1974; Hughes et al 1974). However, because of its poor efficacy against adult fluke,its high dosage rate (100 $mg.kg^{-1}$) and its relatively high cost diamphenethide is usually used only when an acute fascioliasis is suspected. Indeed with the recent introduction of triclabendazole it is probable that this new anthelmintic will replace diamphenethide in the control of liver flukes down to one or two weeks-old.

The broad spectrum benzimidazole anthelmintic (albendazole) has been shown to have useful activity against mature liver flukes only at 1.5 times the dose used for nematodicidal activity (Knight and Colglazier 1977), however greater efficacy has been found in sheep by using the drug prophylactically in small daily doses of 3 $mg.kg^{-1}$ per day for 35 consecutive days (Rew and Knight 1980).

The need for a safe and an effective agent with a high activity against all stages of liver flukes has led to the discovery of a novel benzimidazole, triclabendazole, with specific activity against liver flukes (Boray et al 1983). The introduction of triclabendazole represents a breakthrough in the treatment of fascioliasis in sheep and cattle. The drug has a high safety index, low toxicity, low residue problems and an

excellent activity against all ages of F. hepatica from the early immature to the adult stages (Boray 1981; Boray et al 1983; Turner et al 1984).

The new member of sulphonamides series, clorsulon, has been shown to be highly effective against mature flukes with lesser efficacy against the young flukes (Mrozik et al 1977; Ostlind et al 1977).

All the other fasciolicidal drugs including the chlorinated hydrocarbons and bisphenolic compounds have lower activity and greater toxicity than the salicylanilides and the benzimidazoles (Boray et al 1967; Boray and Happich 1968).

The mode of action of most fasciolicidal drugs is by interfering with the energy metabolism in the parasite. All members of salicylanilide group act by stimulation of oxygen uptake and uncouple oxidative phosphorylation in intact flukes in vitro (Van Miert and Groeneveld 1969; Corbett and Goose 1971a,b; Van den Bossche 1972a; Yorke and Turton 1974; Cornish and Bryant 1976; Cornish et al (1977) and Prichard (1978a) obtained evidence of uncoupling in vivo. In addition it has been found that a wide range of salicylanilides inhibit succinate dehydrogenase activity in F. hepatica in vitro (Duwel and Metzger 1973; Metzger and Duwel 1973). The nitrophenolic and bisphenolic compounds, like the salicylanilides, are all potent uncouplers of phosphorylation. Generally the benzimidazoles are considered to act by a variety of effects. Inhibition of glucose uptake as primary energy-generating substrate thus starving the parasite to death (Van den Bossche 1972a; Van den Bossche and De Nollin 1973; De Nollin and Van den Bossche 1973), inhibition of the fumarate

reductase system (Coles 1977; Rew 1978; Barrowman et al 1984b) and it has been found that the benzimidazoles bind in vitro to tubulin thus preventing microtubule formation (Ireland et al 1979; Barrowman et al 1984a). The sulphonamide, clorsulon, has been shown to inhibit glucose utilisation and acetate and propionate formation by mature Fasciola hepatica in vitro (Schulman and Valentino 1980; Schulman et al 1982).

The relative economic importance of fascioliasis has been increased in recent years due to the difficulties in the prophylaxis and control of the disease.

In the U.K., fascioliasis is considered to be the most economically important disease affecting ruminants (Ross et al 1966; Reid et al 1967; Ross and Todd 1968). The disease is particularly significant in cattle because it causes loss in bodyweight in fattening stock and reduces milk yield in dairy cows (Ross 1970a; Black and Froyd 1972).

During a survey in 52 slaughter houses in U.K. in 1983 it was found that 2.7% and 8% of sheep and cattle livers respectively were affected by liver fluke (Lowndes 1984).

In the United States, none of the fasciolicidal drugs have been allowed for use because of residue problems. Only albendazole has been approved by FDA for therapeutic use in cases of fascioliasis. However the teratogenic effect of the drug and its prolonged withdrawal period in breeding herds reduce its application.

During the course of this study new compounds with specific activity against liver flukes were under development to be introduced for the treatment of fascioliasis. Such compounds are closantel, clorsulon and triclabendazole.

Surprisingly, little data has been published on the pharmacokinetic behaviour of fasciolicidal drugs. Therefore it was decided to study the kinetics of fasciolicidal drugs in current use and the newly developed fasciolicides thereby the course of study with the potentiated sulphonamides was completed and work directed toward the pharmacokinetics of fasciolicidal drugs.

TABLE (2) 1

ANTHELMINTIC EFFICACY OF SOME DRUGS USED FOR THE TREATMENT OF FASCIOLIASIS IN SHEEP.

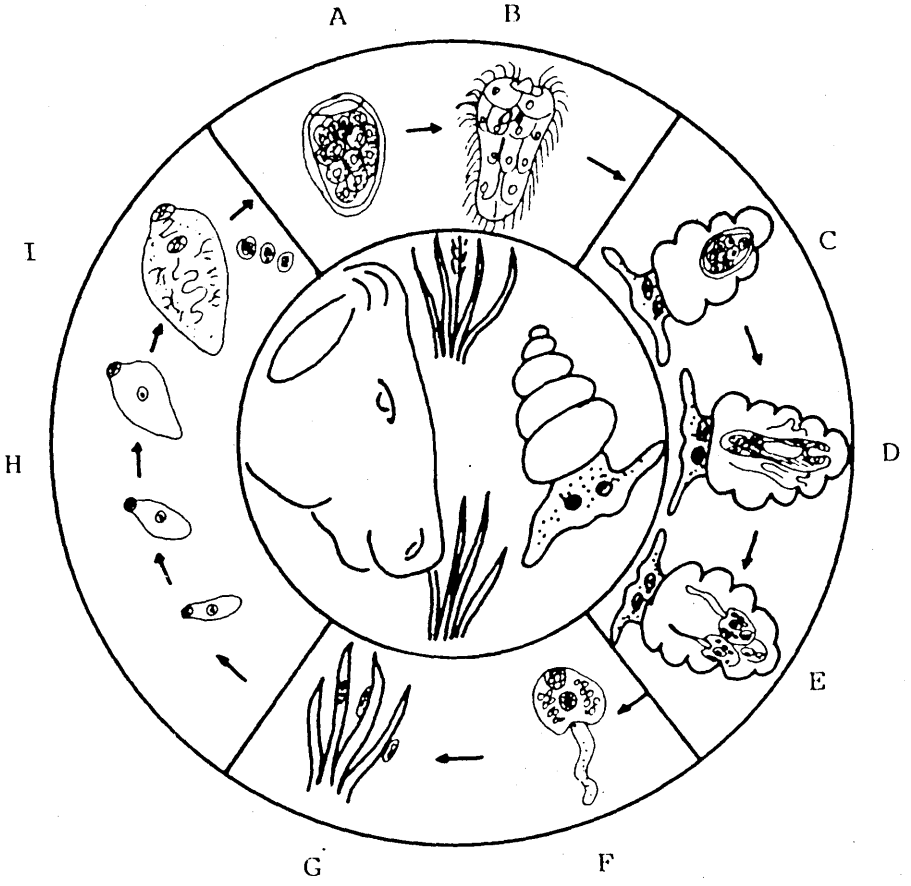
Anthelmintic (formulation)	Recommended dose rate ($mg.kg^{-1}$)	Maximum tolerated dose ($mg.kg^{-1}$)	Safety index at recommended dose	Age of flukes			Withdrawal period (days)	
				1 week	4-8 weeks	> 12 weeks	Meat	Milk
Oxyclozanide (drench, in-fed)	15	60	4.0	low	moderate	excellent	14	Nil
Rafoxanide (drench)	7.5	45	6.0	low	high/ excellent	excellent	28	Not used
Nitroxynil (subcutaneous injection)	10	40	4.0	low	high/ excellent	excellent	30	Not used
Diamphenethide (drench)	80 - 120	400	3.3-5.0	excellent	excellent	high/ excellent	7	Not used
Triclabendazole (drench)	10	200	20	excellent	excellent	excellent	28	Not used

low = < 50% high = 75 - 90%

moderate = 50 - 75% excellent = > 90%

FIGURE (2) 1

THE LIFE CYCLE OF *Fasciol a hepatica*



A. egg hatches on pasture. B. free swimming miracidium penetrates L.truncatula. C. sporocyst forms in snail. D. sporocyst produces several rediae. E. rediae produce cercariae. F. mobile cercariae settle on grass. G. metacercariae form. H. on ingestion by cow or sheep, metacercariae hatch and immature liver fluke migrate to and penetrate liver. I. immature liver fluke leave liver parenchyma after 6-8 weeks and settle in the bile duct.

Pharmacokinetics of orally administered drugs

The reason for studying the kinetics of a drug is to follow the time course of drug absorption, distribution, metabolism and excretion and to correlate these processes with the extent and duration of effectiveness of given drug (pharmacodynamics).

Usually anthelmintics are administered via the oral route due to the ease of handling by the farmer. Following oral administration, the concentration of the drug in plasma is determined to a great extent by the rate of absorption, distribution and elimination. After absorption of drug into the blood stream, a proportion of the drug may bind to plasma protein (mainly albumin) and the remainder, free drug, is dissolved in plasma. The free drug enters other tissues according to their blood supply or is eliminated by metabolism and/or excretion. The degree of distribution of a particular drug into various organs is determined by certain physicochemical properties of the drug (its degree of ionisation and the lipid solubility of the un-ionised molecule). The binding of drugs to plasma restricts their distribution to the sites of action, thereby giving higher concentration in plasma relative to that in tissues. As the bound drug is of high molecular weight, it is unavailable for glomerular filtration and its rate of excretion will be slowed. The process of binding is a reversible process and it is simplified by the following equation.

$$[P] + [D] \underset{K_2}{\overset{K_1}{\rightleftharpoons}} [PD]$$

Where [P] is concentration of free protein; [D] concentration of free drug and [PD] concentration of drug-protein complex. K_1 and K_2 are the association and dissociation processes rate constants which are usually extremely short.

According to this equation, if the dissociation of the complex (PD) is rapid there will be no significant effect on the rate of transmembrane movement since the free drug crossing cellular membrane would be rapidly replaced by a newly dissociating drug. Thus the drug-albumin complex serves as a reservoir of potentially active drug.

Binding of drugs to plasma proteins is usually thought to be of no great importance unless the extent of binding is sufficiently large such as to reduce the amount of drug available for distribution to tissues to a significant degree. The process of binding is usually reversible and generally occurs to a small extent. However, occasionally covalent binding occurs and the process of binding will be irreversible thus limiting the passage of such drug from blood to tissues. The extent of binding of a particular drug lies within a range from low (< 50 e.g. ampicillin, chloramphenicol) moderate, (50-80% e.g. salicylate, sulphasoxazole), high (> 90% e.g. phenylbutazone, digitoxin, rafoxanide).

Displacement of one drug by another from its binding site will result in increasing the proportion of free drug in the plasma and thus more drug will be available for tissue distribution leading to incease in the pharmacological action.

The extent of distribution of drug is another factor which also influences the concentration of drug in the plasma. An estimate of the extent of distribution is possible by determination of the apparent volume of distribution (Vd). This pharmacokinetic parameter (Vd) helps in relating the concentration of drug in plasma to the total amount of drug in the body at any time after pseudo-distribution equilibrium has been attained. In ruminant animals, because of the large volume of ruminal fluid which occupies up to 20% of animals' volume (Dobson 1967) an orally administered drug will attain low concentration in the rumen. The slow passage of ruminal content (solid form) also effects the rate of absorption of drugs especially the poorly soluble drugs e.g. rafoxanide, fenbendazole, triclabendazole. This effect is of value in extending their plasma kinetics. The concentration of drug in the rumen is influenced by the pH of the ruminal content (5.5 - 6.5), the lipid solubility and pka of given drug, adsorption of drug to ruminal contents, metabolic transformation of some drugs by ruminal microorganisms, rate of salivary flow, extent of drug binding to plasma proteins and the rate of elimination of the drug (biotransformation and excretion).

CHAPTER I

PHARMACOKINETIC STUDIES OF CLORSULON IN CATTLE

1. Pharmacokinetic studies of Clorsulon in cattle

1.1 Introduction

1.1.1 Efficacy

Clorsulon : 4 - amino - 6 - trichloroethenyl - 1,3 - benzendisulfonamide (Figure 2.1.1) is the most promising member of a series of sulphonamides that possess fasciolicidal activity. It was developed at Merck, Sharp and Dohme Inc., Rahway, US.A. (Mrozik 1976; Mrozik et al 1977). The drug has been shown to be a potent flukicide having high activity against mature and immature liver flukes in sheep and cattle (Mrozik et al 1977; Ostlind et al 1977; Malone et al 1984).

In sheep, single intraruminal doses of 15 and 30 $mg.kg^{-1}$ were found to be 97.2 and 99.7% effective against four and three week-old F. hepatica respectively. The efficacy of clorsulon against six week-old flukes was found to be 92.1% after a single intraruminal dose at 2.5 $mg.kg^{-1}$ (Mrozik et al 1977). Clorsulon given orally or intraruminally at 2.5 $mg.kg^{-1}$ was > 90% efficient against mature F. hepatica (16 week-old fluke) (Mrozik et al 1977; Ostlind et al 1977).

Intraruminal doses of 10 and 15 $mg.kg^{-1}$ clorsulon were found to be 96.9 and 99.5% effective against eight week-old F. hepatica respectively in calves. At low doses of 3.75 and 5 $mg.kg^{-1}$, clorsulon given either orally or intraruminally was 100% effective against mature fluke (> 14 week-old fluke) in calves (Ostlind et al 1977).

The activity of clorsulon against liver flukes in rats was also studied. Oral doses of 5 and 6.5 $mg.kg^{-1}$ were 90 and 100% effective respectively against mature F. hepatica (> 16 week-old)

(Schulman et al 1979; Schulman, Valentino, Cifelli and Ostlind 1982).

1.1.2 Mode of action

The mechanism of the fasciolicidal activity of clorsulon has been suggested to be due to inhibition of the glycolytic enzymes 3-phosphoglycerate kinase and phosphoglyceromutase which results in a block in glycolysis in Fasciola hepatica (Schulman and Valentino 1980).

In vivo investigation of the effect of clorsulon on F. hepatica phosphoglycerate kinase demonstrated that the drug was a competitive inhibitor of both 3-phosphoglycerate and ATP (Schulman, Ostlind and Valentino 1982) thereby depriving the parasite of its main source of metabolic energy.

Clorsulon has been observed to inhibit the pathway of glucose oxidation to acetate and propionate in mature F. hepatica in vitro thus resulting in an inhibition of the proposed sites of ATP formation (Schulman and Valentino 1980).

It has been reported that clorsulon binds to rat erythrocytes via carbonic anhydrase and reaches the parasite via ingestion of blood (Schulman et al 1979).

1.1.3 Safety to host

Toxicity studies in rats, mice and sheep have shown that clorsulon is a highly safe drug for use in domestic livestock (Ostlind et al 1977).

The LD_{50} in mice was found to be 761 $mg.kg^{-1}$ intraperitoneally and more than 10,000 $mg.kg^{-1}$ orally. In rats, clorsulon given at a single oral dose at 10,000 $mg.kg^{-1}$ showed no

gross toxic effects (Ostlind et al 1977).

Single oral and intraruminal doses of clorsulon at 200 and 400 $mg.kg^{-1}$ were well tolerated by fluke-free sheep without showing any toxic symptoms (Mrozik et al 1977; Ostlind et al 1977). Sheep harbouring eight week-old F. hepatica received a single intraruminal dose of clorsulon at 100 $mg.kg^{-1}$ without showing apparent toxic effects (Ostlind et al 1977).

1.1.4 Pharmacokinetics and objective

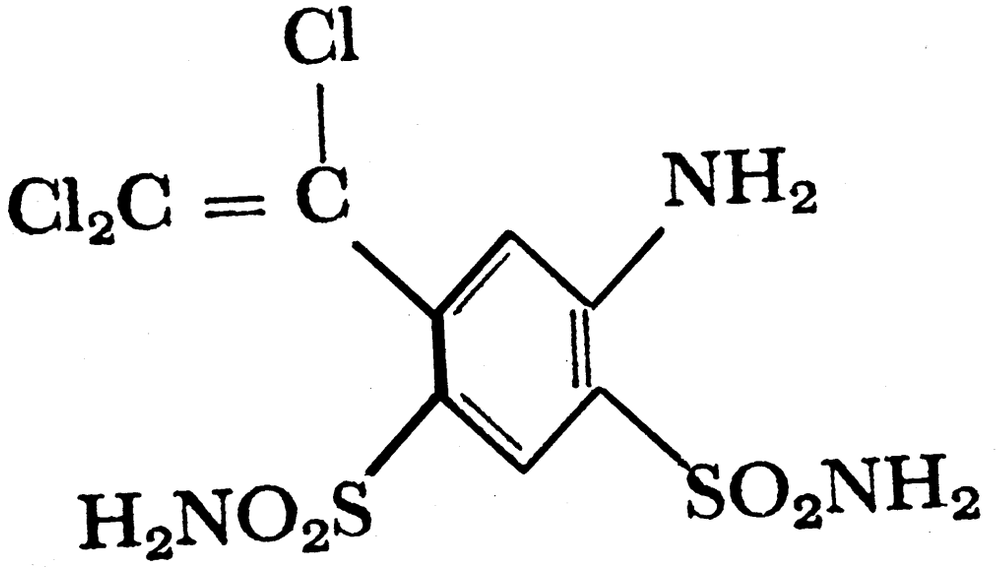
The kinetics of clorsulon have not yet been reported in domestic livestock and the only available data concerning clorsulon kinetics is in rats.

Following single oral doses of ^{14}C-clorsulon at 6.25 and 12.5 $mg.kg^{-1}$ approximately 75% of the circulating drug was present in the plasma and the remainder was in erythrocytes. The maximum concentration of the drug in whole blood occurred at four hours after dosing (Schulman et al 1979).

Since there is no published data about the kinetics of clorsulon in cattle therefore, the pharmacokinetic behaviour of clorsulon was studied in cattle. The extent of binding of clorsulon to plasma proteins was investigated to serve as a correlation between the pharmacokinetic behaviour and efficacy of the drug against liver flukes.

FIGURE 2.1.1

STRUCTURE OF CLORSULON


Cl
Cl2C = C
NH2
H2NO2S
SO2NH2

1.2 Determination of clorsulon in serum

A gas liquid chromatographic method (G.L.C.) has been published for the determination of clorsulon in blood and milk samples (Van den Heuvel et al 1977). The process of derivatisation prior to gas chromatography is lengthy and the overall recoveries are poor (~45%) from both blood and milk. Besides, the derivatisation techniques are difficult and more liable to operator error than most other techniques and unsuitable for routine analysis.

Current methods used for the determination of sulphonamides (anti-bacterial) are not suitable for the determination of this compound since the limit of detection of methods using the Bratton-Marshall reaction are sensitive only to about 2 $\mu g.ml^{-1}$ (Reider 1972). An alternative high performance liquid chromatography was found to be applicable and reliable for the determination of clorsulon in serum samples (mean recovery 88 $\pm$ 3.8%).

1.2.1 Materials and methods

1.2.1.1 Reagents

All reagents used were of "Analar" grade.

Di-ethyl ether

Glass-redistilled, ferrous sulphate washed and stabilised.

Ether was washed with ferrous sulphate solution (5% w/v $FeSO_4\ 7H_2O$) in proportion of 3 : 1 of ether : ferrous sulphate solution. Washing of ether with ferrous sulphate was necessary to remove peroxides. The ether was stabilised by adding 1 ml of 1 $mg.ml^{-1}$ pyrogallol (w/v in ether) to every 100 ml washed ether.

Ether was stabilised in order to prevent further peroxide formation if the ether was not used immediately.

Methanol

Redistilled before use.

Ammonium carbonate (0.025 M)

Phosphate buffer (pH 7.4)

50 ml 0.2 M K_2HPO_4 and 39.5 ml 0.2 M NaOH made up to 200 ml.

Water-permanganate redistilled

Used to prepare the reagents which was necessary for the removal of any impurities present in tap water.

1.2.1.2 HPLC apparatus and conditions

- Detector = Variable wave length ultraviolet spectrophometer model CE 2012 (Cecil Instruments, Cambridge, England).
- Column dimensions = Septum injector 100 x 5 mm (Shandon Southern Products, Runcorn, England).
- Column packing = Reverse-phase ODS-Hypersil, particle size 5 µm.
- Pump = Altex 110 A.
- Recorder = Vitatron 10 mV.
- Wave length = 266 nm.
- Chart speed = 0.5 cm.min.$^{-1}$.
- Flow rate = 0.7 ml.min.$^{-1}$.
- Mobile phase = Methanol : Ammonium carbonate (0.025 M).
  50 : 50.
- Injection volume = 5 µl.
- Retention time = circa 2.8 min.
- Limit of detection = 0.10 µg.ml^{-1}.

1.2.1.3 Procedure of analysis

Clorsulon was determined in serum samples by high performance liquid chromatography using di-ethyl ether extraction.

Aliquots of 1 ml of serum samples were introduced into a 50 ml thick-walled glass tube using a 1 ml glass bulb-pipette. 1 ml of phosphate buffer (pH 7.4) was added and followed by 25 ml of di-ethyl ether. The tubes were stoppered and shaken for ten minutes on a rotary mixer. 20 ml of the upper ether layer was transferred to 50 ml thin-walled glass tubes.

A further 25 ml diethyl ether was added to each sample in the first tubes, stoppered and shaken for ten minutes. 25 ml of the upper ether layer was removed and combined with the 20 ml ether extracts in the thin-walled tubes.

Ether extracts were evaporated on a dry-bath at 50°C under a stream of nitrogen to a volume of approximately 4 - 6 ml. They were then transferred to 10 ml conical tubes and the walls of the thin tubes were washed with 3 x 1 ml ether. The washings were combined each time with the ether extracts in the conical tubes.

The ether extracts were evaporated to dryness. Sides of the tubes were washed down with 1 - 2 ml ether and evaporated to dryness.

For HPLC analysis, the residue was reconstituted in 100 µl methanol and placed in an ultrasonic water bath for two minutes to aid dissolution. Residues of 5 µl were injected onto the HPLC column using a 10 µl microsyringe.

1.2.1.4 Preparation of standards and recoveries

Stock solution (100 $\mu g.ml^{-1}$) of clorsulon in phosphate buffer (pH 7.4) was prepared by dissolving 10 mg of pure compound in 100 ml pH 7.4 phosphate buffer.

Spikes containing 0, 0.2, 0.5, 1.0, 2.0 and 5.0 $\mu g.ml^{-1}$ were prepared by adding known amounts of the drug in stock solution to bovine drug-free serum. The spikes were taken through the analytical procedure with the actual plasma samples.

A standard solution of 10 $\mu g.ml^{-1}$ clorsulon in methanol was chromatographed at regular intervals to monitor any change in chromatographic sensitivity and to determine the recovery from the spiked serum samples as follows:-

From the peak heights (pk.ht) obtained, the percentage recovery of each concentration is determined using this formula:-

$$\text{Percentage recovery} = \frac{\text{pk.ht.sample}}{\text{pk.ht.standard}} \times \frac{\text{conc.standard } (\mu g.ml^{-1})}{\text{conc.prepared } (\mu g.ml^{-1})}$$

$$\times \frac{\text{vol. of residue (ml)}}{\text{vol. of serum used(ml)}} \times 100 \times \frac{100}{96} \text{ (Solvent losses)}$$

The percentage recoveries from each of the spiked serum were calculated (Table 2.1.1) and the concentrations in the serum samples analysed concurrently were adjusted for recovery using the calculated mean value for recovery from the spiked samples.

$$\text{Concentration } (\mu g.ml^{-1}) = \frac{\text{pk.ht.sample}}{\text{pk.ht.standard}} \times \frac{\text{conc.standard } (\mu g.ml^{-1})}{\text{vol. serum used (ml)}}$$

$$\times \frac{\text{Residue volume (ml)}}{\text{Recovery \%}} \times \frac{100}{96} \times 100$$

TABLE 2.1.1

RECOVERIES OF CLORSULON FROM SPIKED SERUM

Amount clorsulon added to drug-free serum (µg)	Amount clorsulon measured (µg)			Mean ± S.E.M.	Ratio assayed to added
0.2	0.2	0.2	0.1	0.2 ± 0.03	1.00
0.5	0.4	0.5	0.4	0.4 ± 0.03	0.80
1.0	1.0	0.8	0.9	0.9 ± 0.06	0.90
2.0	1.5	1.6	1.8	1.6 ± 0.08	0.80
5.0	4.9	4.5	4.7	4.7 ± 0.11	0.90

1.3 Experiments with clorsulon

1.3.1 Animals

Fifteen cows of mixed breed, weighing between 350 - 500 kg. were used in the study.

The cows were divided into three groups (five cows in each group), fed hay and concentrates, water was available ad libitum. The study was done in conjunction with Merck and Co., Inc. using animals belonging to the company.

1.3.1 Drug administration

Clorsulon in a 10% suspension (w/v) was administered subcutaneously to each of the five cows in Group I at a dose rate of 4 $mg.kg^{-1}$.

Group II cows received subcutaneous injection of a 20% (w/v) suspension at a dose rate of 4 $mg.kg^{-1}$.

Cows in Group III were dosed orally with clorsulon at a dose rate of 7 $mg.kg^{-1}$.

1.3.3 Sampling regimen

Blood samples were withdrawn from each animal from the jugular vein into vacutainers using 19 gauge needles immediately before and at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168 and 180 hours after administration of the drug.

1.3.4 Treatment of samples

The blood samples were left to stand (for one hour) at room temperature ($18^{o}C$). When the serum was separated it was transferred into 10 ml plastic tubes and stored at $-20^{o}C$ until analysed.

1.4 Binding of clorsulon to bovine plasma proteins

In vitro, the extent of binding of clorsulon to bovine plasma proteins was investigated.

Bovine drug-free plasma was spiked in duplicate, with clorsulon at 20 and 40 $\mu g.ml^{-1}$. The plasma samples were introduced into an Amicon centricon microconcentrator (Figure 2.1.2) and centrifuged using a fixed angle MSE centrifuge at 5000 x g for two hours.

Plasma ultrafiltrates were collected in a reservoir cap. The concentration of free clorsulon in the filtrates were analysed by high performance liquid chromatography as described in Section 1.2.1.

1.4.1 Calculations of percentage binding

The percentage binding of clorsulon to plasma proteins was calculated using the following formula:

$$\% \text{ bound} = 100 - \left[\frac{\text{Concentration of free drug in plasma ultrafiltrates}}{\text{Concentration of drug in whole plasma}} \times 100 \right]$$

1.5 Results

1.5.1 Concentration of clorsulon in serum

The concentration of clorsulon in serum of each cow and the mean serum concentration $\pm$ S.E.M. at each sampling time after subcutaneous administration of the 10% and the 20% suspension at 4 $mg.kg^{-1}$ and after the oral administration at 7 $mg.kg^{-1}$ are illustrated in Tables 2.1.2, 2.1.3 and 2.1.4 respectively.

The disposition curves, based on the mean concentrations of clorsulon in serum of cows in each group are shown in

Figure 2.1.3.

For each animal, the serum concentration/time curve was analysed using a non-linear iterative curve fitting programme (CSTRIP) of Sedman and Wagner (1976). The pharmacokinetic model of the disposition of clorsulon was also fitted to the averaged serum data. The mean values of individual parameters were found to be close to those obtained from the averaged data (Tables 2.1.5, 2.1.6 and 2.1.7).

The pharmacokinetic data of individual animals in each group were found to conform adequately to a two-compartment open model using the biexponential equation.

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

For each cow, the elimination half life (calculated from the terminal phase) and the area under serum concentration/time curve (AUC) were obtained from the same curve-fitting procedure (Sedman and Wagner 1976) (Tables 2.1.5, 2.1.6 and 2.1.7).

The elimination half life ($t_{1/2\beta}$) of clorsulon following the subcutaneous administration of the 10 and 20% suspensions at equal doses (4 $mg.kg^{-1}$) were found to be 29.9 $\pm$ 3.2 h and 20.5 $\pm$ 3.2 h respectively. Following the oral administration of clorsulon at 7 $mg.kg^{-1}$, the elimination half life ($t_{1/2\beta}$) was found to be 16.4 $\pm$ 1.6 h.

Clorsulon was well absorbed from the subcutaneous tissues to the peripheral circulation following the administration of the 10 and 20% suspensions to obtain maximum concentration in serum of 2.3 and 4.3 $\mu g.ml^{-1}$ at 24 and 12 hours post administration respectively. Thereafter the concentration of clorsulon in serum decreased exponentially. At 168 and 144 hours post

administration, the concentrations of clorsulon in serum fell below the limit of detection of the method (0.10 $\mu g.ml^{-1}$) after subcutaneous injection of the 10 and 20% suspensions respectively.

Following the oral administration, clorsulon was also well absorbed to attain a mean maximum concentration of 4.0 $\mu g.ml^{-1}$ 24 hours after administration. The concentrations of clorsulon in serum then declined slowly and clorsulon could not be detected at 120 hours post administration.

1.5.2 Binding of clorsulon to plasma proteins

A mean concentration of 1.2 and 2.3 $\mu g.ml^{-1}$ clorsulon was detected in plasma ultrafiltrates obtained from plasma spiked with 20 and 40 $\mu g.ml^{-1}$ clorsulon respectively.

According to the equation described in Section 1.4.1 clorsulon was found to be 94% bound to plasma proteins.

TABLE 2.1.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF CLORSULON IN SERUM AFTER SUBCUTANEOUS ADMINISTRATION OF CLORSULON (10% w/v) AT A DOSE RATE OF 4 $mg.kg^{-1}$ IN FIVE COWS.

Time (h)	Animal number 17	33	49	77	90	Mean ± S.E.M.
0	0	0	0	0	0	
12	1.7	2.5	2.3	1.6	3.1	2.2 ± 0.3
24	2.2	2.8	2.0	1.7	3.1	2.3 ± 0.3
36	1.8	1.9	1.6	1.4	1.7	1.7 ± 0.1
48	1.5	1.6	1.5	1.0	0.8	1.3 ± 0.2
60	1.0	1.3	0.7	0.4	0.5	0.8 ± 0.2
72	0.8	0.6	0.9	0.5	0.4	0.7 ± 0.1
84	0.6	0.8	0.8	0.4	0.2	0.6 ± 0.1
96	0.5	0.6	0.5	0.2	0.1	0.4 ± 0.1
108	0.4	0.5	0.5	0.4	0.1	0.4 ± 0.1
120	0.4	0.4	0.4	0.4	0.1	0.3 ± 0.1
132	0.3	0.2	0.2	0.2	0	0.2 ± 0.1
144	0.3	0.2	0.2	0.1	0	0.2 ± 0.1
156	0.1	0.1	0.2	0.1	0	0.1 ± 0.1
168	0	0.1	0.1	0.1	0	0
180	0	0	0	0	0	0

TABLE 2.1.3

CONCENTRATIONS ($\mu g.ml^{-1}$) OF CLORSULON IN SERUM AFTER SUBCUTANEOUS ADMINISTRATION OF CLORSULON (20% w/v) AT A DOSE RATE OF 4 $mg.kg^{-1}$ IN FIVE COWS.

Time (h)	Animal number 16	18	26	32	64	Mean	± S.E.M.
0	0	0	0	0	0		
12	1.4	7.7	3.2	2.9	6.0	4.3	± 1.1
24	1.3	4.1	3.5	2.6	6.1	3.5	± 0.8
36	0.9	2.5	2.5	1.4	4.6	2.4	± 0.6
48	0.6	1.6	1.5	0.6	2.2	1.3	± 0.3
60	0.4	0.9	1.3	0.4	1.4	0.9	± 0.2
72	0.2	0.4	0.8	0.2	0.7	0.5	± 0.1
84	0.1	0.3	1.0	0.1	0.6	0.4	± 0.2
96	0.1	0.2	0.4	0.1	0.2	0.2	± 0.1
108	0.1	0.1	0.4	0.1	0.2	0.2	± 0.1
120	0.1	0.1	0.4	0.1	0.1	0.1	± 0.1
132	0	0	0.4	0	0.1	0.1	± 0.1
144	0	0	0.2	0	0.1	0	
156	0	0	0.1	0	0	0	
168	0	0	0.1	0	0	0	
180	0	0	0	0	0	0	

TABLE 2.1.4

CONCENTRATIONS ($\mu g.ml^{-1}$) OF CLORSULON IN SERUM AFTER ADMINISTRATION OF CLORSULON ORALLY AT A DOSE RATE OF 7 $mg.kg^{-1}$ IN FIVE COWS.

Time (h)	Animal number 58	84	98	100	106	Mean	± S.E.M.
0	0	0	0	0	0		
12	1.7	3.0	2.2	1.7	1.5	2.0	± 0.3
24	4.6	2.6	3.6	3.8	5.9	4.0	± 0.6
36	3.7	1.5	1.9	2.6	4.5	2.9	± 0.6
48	1.8	1.3	1.3	1.5	2.2	1.6	± 0.1
60	1.6	0.8	0.7	0.9	1.2	1.0	± 0.1
72	1.1	0.4	0.3	0.5	1.0	0.7	± 0.2
84	0.7	0.4	0.3	0.3	0.5	0.4	± 0.1
96	0.4	0.2	0.1	0.1	0.3	0.2	± 0.1
108	0.3	0.2	0	0.2	0.1	0.2	± 0.1
120	0.1	0.1	0	0.1	0.1	0	
132	0.1	0.1	0	0	0.1	0	
144	0	0	0	0	0	0	
156	0	0	0	0	0	0	
168	0	0	0	0	0	0	
180	0	0	0	0	0	0	

TABLE 2.1.5

PHARMACOKINETIC CONSTANTS FOR CLORSULON IN COWS (n = 5)
FOLLOWING SUBCUTANEOUS ADMINISTRATION OF THE 10% SUSPENSION
AT A SINGLE DOSE RATE OF 4 $mg.kg^{-1}$.

Animal number	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	t 1/2 (α) (h)	t 1/2 (β) (h)	AUC $\mu g.ml^{-1}.h$
17	- 3.80	3.80	0.09	0.02	7.40	32.80	138.30
33	- 5.40	5.40	1.00	0.02	7.00	29.00	173.10
49	- 3.40	3.40	0.20	0.02	3.80	35.60	155.30
77	- 2.30	2.30	0.20	0.02	3.50	34.00	101.60
90	- 6.10	6.10	0.20	0.03	3.70	18.10	126.50
Mean	- 4.20	4.20	0.30	0.02	5.10	29.90	139.00
$\pm$S.E.M.	$\pm$ 0.70	$\pm$ 0.70	$\pm$ 0.20	$\pm$0.02	$\pm$ 0.90	$\pm$ 3.20	$\pm$ 12.30
Averaged values	- 3.90	3.90	0.10	0.02	5.10	29.70	140.00

TABLE 2.1.6

PHARMACOKINETIC CONSTANTS FOR CLORSULON IN COWS (n = 5) AFTER SUBCUTANEOUS ADMINISTRATION OF THE 20% SUSPENSION AT A SINGLE DOSE RATE OF 4 $mg.kg^{-1}$.

Animal number	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	t 1/2 (α) (h)	t 1/2 (β) (h)	AUC $\mu g.ml^{-1}.h$
16	- 2.30	2.30	0.30	0.03	2.70	22.90	67.30
18	- 9.70	9.70	0.10	0.04	4.90	17.10	170.70
26	- 5.00	5.00	0.10	0.02	4.70	31.50	193.60
32	- 9.20	9.20	0.10	0.05	5.50	12.90	98.50
64	-13.60	13.60	0.10	0.03	5.10	18.20	258.00
Mean	- 8.00	8.00	0.20	0.03	4.60	20.50	157.60
$\pm$ S.E.M.	$\pm$ 2.00	$\pm$ 2.00	$\pm$ 0.04	$\pm$0.05	$\pm$ 0.50	$\pm$ 3.20	$\pm$ 34.30
Averaged	- 7.20	7.20	0.20	0.03	3.10	20.10	177.40

TABLE 2.1.7

PHARMACOKINETIC CONSTANTS FOR CLORSULON ADMINISTERED ORALLY TO FIVE COWS AT A SINGLE DOSE RATE OF 7 $mg.kg^{-1}$.

Animal number	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	t 1/2 (α) (h)	t 1/2 (β) (h)	AUC $\mu g.ml^{-1}.h$
58	- 23.20	23.20	0.07	0.05	9.30	15.10	194.10
84	- 5.00	5.00	0.20	0.03	3.40	22.20	135.50
98	- 14.30	14.30	0.10	0.05	6.50	13.30	141.60
100	- 10.50	10.50	0.07	0.04	10.40	17.00	99.30
106	- 25.20	25.20	0.09	0.05	8.00	14.30	228.30
Mean	- 15.60	15.60	0.10	0.04	7.50	16.40	159.80
$\pm$S.E.M.	$\pm$ 3.80	$\pm$ 3.80	$\pm$ 0.02	$\pm$0.04	$\pm$ 1.20	$\pm$ 1.60	$\pm$ 23.00
Averaged	- 9.20	9.20	0.10	0.04	6.40	18.70	163.10

FIGURE 2.1.2

CENTRICON MICROCONCENTRATOR

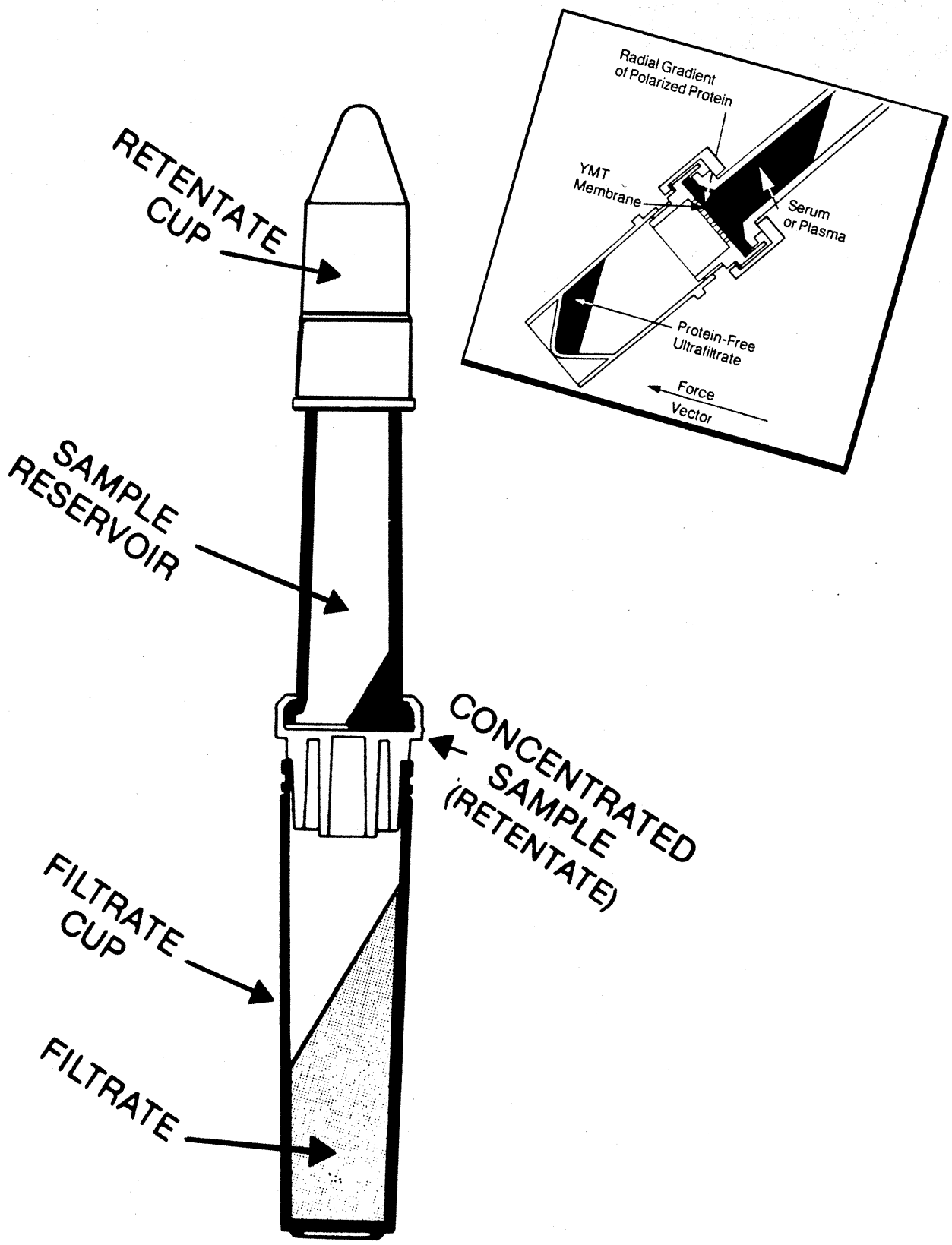
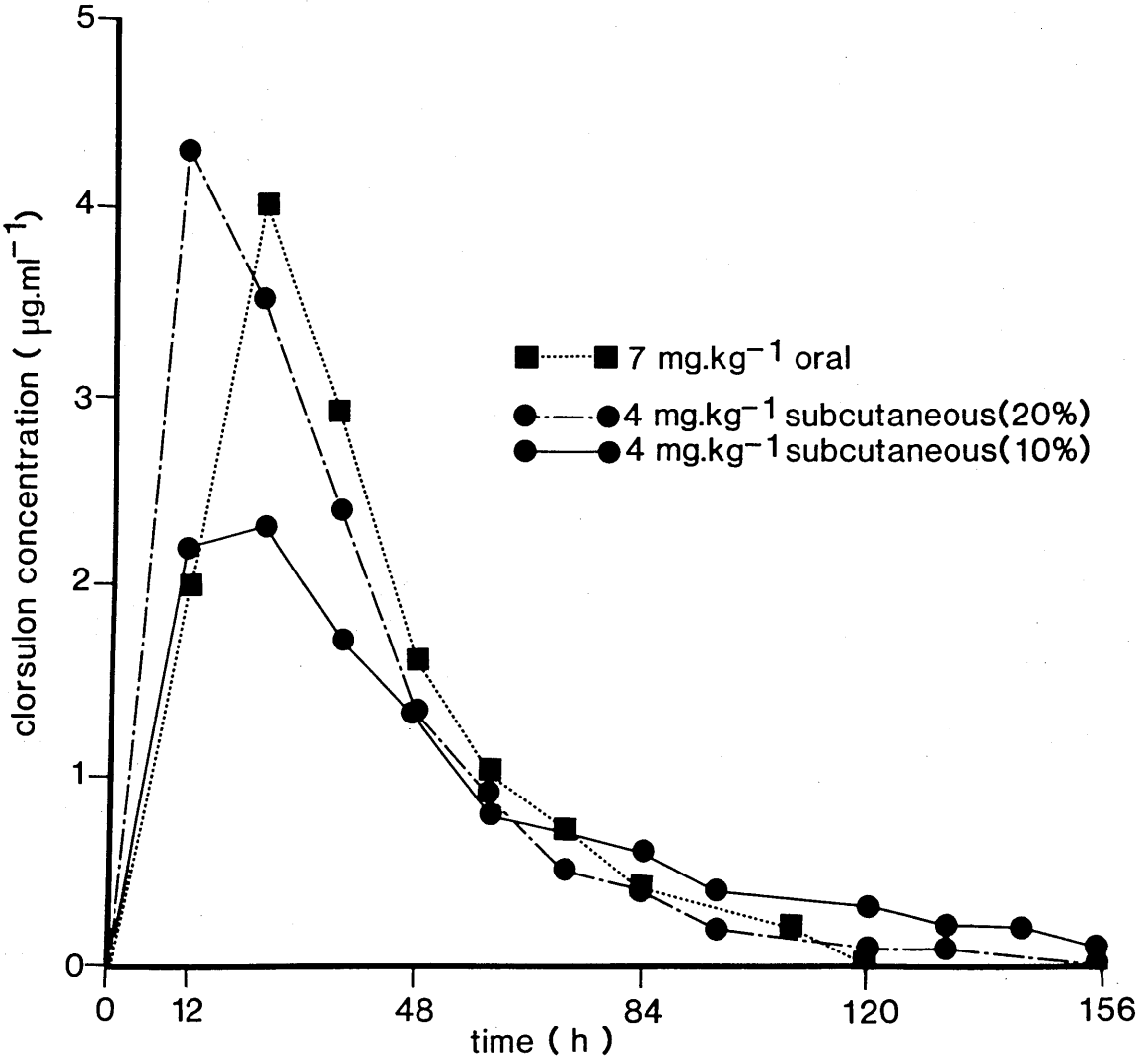

RETENTATE CUP
SAMPLE RESERVOIR
CONCENTRATED SAMPLE (RETENTATE)
FILTRATE CUP
FILTRATE
Radial Gradient of Polarized Protein
YMT Membrane
Serum or Plasma
Protein-Free Ultrafiltrate
Force Vector

FIGURE 2.1.3

MEAN SERUM CONCENTRATIONS ($\mu g.ml^{-1}$) OF CLORSULON AFTER SUBCUTANEOUS ADMINISTRATION OF 10% AND 20% SUSPENSIONS OF CLORSULON TO CATTLE AT A DOSE RATE OF 4 $mg.kg^{-1}$ AND AFTER ORAL ADMINISTRATION AT 7 $mg.kg^{-1}$.


clorsulon concentration (µg.ml^{-1})
5
4
3
2
1
0
0
12
48
84
120
156
time (h)
7 mg.kg^{-1} oral
4 mg.kg^{-1} subcutaneous(20%)
4 mg.kg^{-1} subcutaneous(10%)

1.6 Discussion

Flukicidal anthelmintics have been shown to bind strongly to plasma proteins and therefore they will be excreted slowly from the body (Broome and Jones 1966; Lee 1973).

The high binding of clorsulon (94%) to plasma proteins found in this study is probably the reason for the long elimination half life of the drug.

Although the mean concentrations of clorsulon in serum following the subcutaneous administration of the 20% suspension were found to be higher than those following the subcutaneous administration of the 10% suspension in a few early samples (from 12-36 hours), which appears to be due to rapid absorption in one animal in Group II (No.18), the disposition kinetics of the two suspensions appear to be similar in shape. Thirty-six hours post administration, the concentrations of clorsulon in serum were relatively close following administration of both suspensions.

Absorption of clorsulon following the subcutaneous administration of equal doses of both suspensions was found to be relatively rapid. Mean absorption half lives ($t_{1/2\,\alpha}$) of 5.1 ± 0.9 h and 4.6 ± 0.5 h were found after the subcutaneous administration of the 10 and 20% suspensions respectively.

The slow elimination of clorsulon [$t_{1/2\,(\beta)} = 29.9 \pm 3.2$ h] following subcutaneous administration of the 10% suspension appears to be of value for a high activity against liver fluke infections, since the longer elimination of the drug from the body was found to be associated with a higher flukicidal activity (Lee 1973).

In relating the flukicidal activity with the pharmacokinetic behaviour of the drugs, it was found that the duration of exposure of liver flukes to the drug is as important as the high concentration of the drug in the body (Corba et al 1979). This is not unexpected of a drug whose putative mode of action involves inhibition of metabolic enzymes.

The elimination half lives of clorsulon [t 1/2 (β)] following the subcutaneous administration of the 20% suspension was found to be not significantly higher ($P > 0.05$) than those following the subcutaneous administration of the 10% suspension at equal dose rates (4 $mg.kg^{-1}$).

The disposition kinetics of clorsulon after the oral administration at 7 $mg.kg^{-1}$ is characterised by a slow absorption phase having a mean absorption half life [t 1/2 (α)] of 7.5 $\pm$ 1.2 h which was followed by a single rapid elimination phase with a mean half life [t 1/2 (β)] of 16.4 $\pm$ 1.6 h.

When the AUCs of clorsulon for each cow following the subcutaneous administration of 4 $mg.kg^{-1}$ of the 10 and 20% suspension, were corrected to 7 $mg.kg^{-1}$ (the orally administered dose rate), and the AUC after the oral administration for each animal were subjected to one way analysis of variance, it was found that the AUC after the subcutaneous administration of the 10% suspension was significantly higher ($P > 0.05$) than those following the subcutaneous administration of the 20% suspension and after the oral administration.

The mean bio-availability of clorsulon determined from the ratio of the corrected mean AUC following the subcutaneous injection of the 10 and 20% suspension relative to that after the

oral administration was found to be 152.4 and 98.3% respectively.

The results of the present study shows that administration of clorsulon subcutaneously or orally to cattle produces relatively similar patterns of disposition kinetics. It would also appear that the subcutaneous administration of the 10% suspension at a dose rate of 4 $mg.kg^{-1}$ produced high serum concentrations and for longer periods than after the subcutaneous administration of the 20% suspension and the oral administration. This prolonged residence time of clorsulon in the body is of great value for a high flukicidal activity.

CHAPTER 2

PHARMACOKINETIC STUDIES OF RAFOXANIDE IN SHEEP

2. Pharmacokinetic studies of rafoxanide in sheep

2.1 Introduction

2.1.1 Efficacy

In 1969 Mrozik et al reported the discovery of a new fasciolicidal compound, rafoxanide, one of the halogenated salicylanilides.

Rafoxanide (Flukanide, Ranizole MS & D) is the generic name of 3,5-Diiodo-3'-chloro-4-(p-chloro-phenoxy)-salicylanilide. The chemical structure is shown in Fig. 2.2.1. It was synthesised at the Merck Sharp and Dohme Research Laboratories, New Jersey, USA, by reacting 3,5-diiodosalicylic acid with 3-chloro-4-(p-chlorophenoxy)-aniline. Raxofanide is a colourless crystalline solid compound insoluble in water, moderately soluble in organic solvents and sensitive to light.

Rafoxanide was introduced to the anthelmintic market as an oral suspension, given at 7.5 $mg.kg^{-1}$ (Flukanide MS & D 3.0% w/v), as an injectable formulation given at 3 $mg.kg^{-1}$ (Flukanide 7.5% w/v), and as a paste and in combination with thiabendazole (Ranizole MS & D).

Since its appearance and general use, rafoxanide has proved to be an effective fasciolicide against Fasciola hepatica and Fasciola gigantica in sheep (Mrozik et al 1969; Boray 1969; Armour and Corba 1970; Campbell et al 1970; Ross 1970; Campbell and Hotson 1971; Roncalli, Fernandez and Barbosa 1971; Edwards and Parry 1972b; Horak, Snijders and Louw 1972; Snijders, Horak and Louw 1973; Annen 1974; Campbell and Brotowidjoyo 1975), cattle (Snijders, Horak and Louw 1971; Knapp and Presidente 1971; Snijders, Louw and Serrano 1971; Presidente and Knapp 1972;

Campbell and Narelle 1972; Snijders and Horak 1975; Whitelaw and Fawcett 1981; Dobbins and Wellington 1982) and in rats (Duwel and Metzger 1973).

Activity of rafoxanide against the immature flukes in the liver parenchyma has been studied. Armour and Corba (1970) have reported efficacies of 98, 99 and 98% against four week old flukes when rafoxanide was administered orally at 7.5, 10 and 15 $mg.kg^{-1}$ respectively, while Ross (1970) found that rafoxanide was 45 and 67% efficient when administered intraruminally to four month old lambs at 7.5 and 10 $mg.kg^{-1}$ respectively. Efficacy of > 50 - 90% was reported by Mrozik et al (1969) when rafoxanide was given to sheep at 25 - 50 $mg.kg^{-1}$ of bodyweight.

As bovine fascioliasis is mainly a chronic disease the activity of rafoxanide against the immature fluke in cattle has not been tested. However recently Dobbins and Wellington (1982) have reported efficacies of 58 and 60% against six and eight week old flukes respectively in calves receiving the injectable preparation at the recommended dose rate of 3 $mg.kg^{-1}$.

Activity of rafoxanide against gastrointestinal nematodes has also been tested. In sheep and cattle rafoxanide given at dosage of 5 - 10$mg.kg^{-1}$ was highly effective against the adult abomasal worms Haemonchus contortus and Haemonchus placei (Egerton and others 1970; Snijders, Horak and Louw 1971; Horak, Snijders and Louw 1972; Snijders, Horak and Louw 1973; Snijders and Horak 1975). This activity of rafoxanide against Haemonchus spp provides a potent treatment against haemonchosis and fascioliasis in areas where mixed infection with both parasites

takes place.

Rafoxanide was also effective against the hook worm Chabertia ovina and Gaigeria pachyscelis in sheep (Horak, Snijders and Louw 1972) and against all the parasitic larval stages of the sheep nasal bot Oestrus ovis (Horak, Louw and Raymond 1971; Roncalli, Barbosa and Fernandez 1971; Snijders, Horak and Louw 1973).

Mrozik et al (1969) found that rafoxanide was effective against Hymenolepis nana and H. diminuta in rodents and against Schistosoma mansoni in rats.

Clioxanide, another salicylanilide, has been shown to be less effective when given intraabomasally than intraruminally (Boray and Roseby 1969; Campbell and Brotowidjoyo 1975). The effect of "rumen by pass" on the activity of rafoxanide against liver flukes was studied by Campbell and Brotowidjoyo (1975). They found no differences in activity when rafoxanide was administered orally, intraruminally or intraabomasally.

Campbell et al (1970) have emphasised that the potency of rafoxanide is affected by the type of formulation used. The formulations tested consisted of the polyethylene glycol, silica gel, bentonite and tragacanth suspension. The formulations were administered orally to sheep at 15 $mg.kg^{-1}$ of bodyweight. The results showed that all the formulations used were partially active but that the silica gel formulation was fully active at this dosage. The reasons why rafoxanide should act more consistently when administered bound to silica gel are not clear.

2.1.2 Mode of action

Like the other salicylanilide fasciolicides, rafoxanide is known to be a potent uncoupler of oxidative phosphorylation in animal tissues. It interferes with the production of ATP in the intact fluke (Corbett 1974; Bryant, Cornish and Rahman 1976; Coles 1977; Prichard 1978b).

Rafoxanide reduced the concentration of ATP in Fasciola hepatica both in vivo and in vitro. In vivo flukes recovered from sheep treated with rafoxanide showed reduction of total nucleotide concentrations, depleted ATP pools and elevated AMP pools. In vitro flukes incubated with rafoxanide showed a decreased ATP, ADP and AMP concentrations, while the concentration of total nucleotides was not altered (Bryant, Cornish and Rahman 1976).

Duwel and Metzger (1973); Metzger and Duwel (1973); Cornish and Bryant (1976) have reported that rafoxanide inhibited succinate dehydrogenase activity in Fasciola hepatica in vitro.

Rafoxanide has also been shown to exert an inhibitory effect on ^{32}Pi incorporation in Ascaris mitochondria (Van den Bossche 1972) and to inhibit the succinate dehydrogenase and fumarate reductase systems, in vitro in Haemonchus contortus.

2.1.3 Safety to host

Rafoxanide used at the therapeutic dose rate of 7.5 $mg.kg^{-1}$ has proved to be a safe fasciolicidal drug (Mrozik et al 1969; Sutherland and Batty 1971; Snijders, Louw and Serrano 1971).

It has a safety index of approximately six. Although the recommended dose rate in sheep and cattle is 7.5 $mg.kg^{-1}$, no undesirable effects have been seen when using single or repeated

doses of rafoxanide. No toxicity signs were observed in sheep when rafoxanide was given in single doses of 60-100 $mg.kg^{-1}$ (Mrozik et al 1969) and in cattle when rafoxanide was administered at 5-14 times the recommended dose rate (Schroder 1982).

Rafoxanide given in multiple dosages ranging from 0 - 37.5 $mg.kg^{-1}$ to lambs (Swan and Schroder 1981) and to calves at 150 $mg.kg^{-1}$ repeated three times at monthly intervals caused no adverse effects (Mrozik et al 1969).

The LD_{50} of rafoxanide for rats is approximately 2300 $mg.kg^{-1}$ while in sheep and cattle the LD_{50} has not been determined (Roberson 1977).

Sheep and cattle infected with liver fluke are more susceptible to rafoxanide toxicity than are the non-infected ones. Thus the therapeutic index of the drug is dependent on the severity of the infection. No deaths occurred in non-infected sheep receiving dosages of 200 $mg.kg^{-1}$ while deaths occurred in naturally infected sheep receiving the same dose (Mrozik et al 1969).

Signs of rafoxanide toxicity were seen in animals receiving an overdosage of rafoxanide (Pienaar 1977; Prozesky and Pienaar 1977). Blindness and pronounced mydriasis are the most obvious signs of rafoxanide toxicity shown by sheep given single doses of 100 $mg.kg^{-1}$ or more (Mrozik et al 1969, Prozesky and Pienaar 1977; Guilhon et al 1971) and by calves dosed at 45-60 $mg.kg^{-1}$ (Schroder 1982) and by dogs receiving 3-11 oral doses of 100 $mg.kg^{-1}$ per day (Brown et al 1972). These signs of toxicity

are similar to the descriptions of sheep suffering from progressive retinal degeneration (bright blindness) which is thought to be caused by ingestion of bracken Pteridium aquilinum (Watson and others 1972) or Helichrysum argyrosphaerum toxicity in sheep (Basson and others 1975).

Thus in view of these possible differential diagnoses, it is advisable that any diagnosis of rafoxanide toxicity be confirmed by a plasma-rafoxanide assay (Swan and Schroder 1981).

2.1.4 Pharmacokinetics and objective

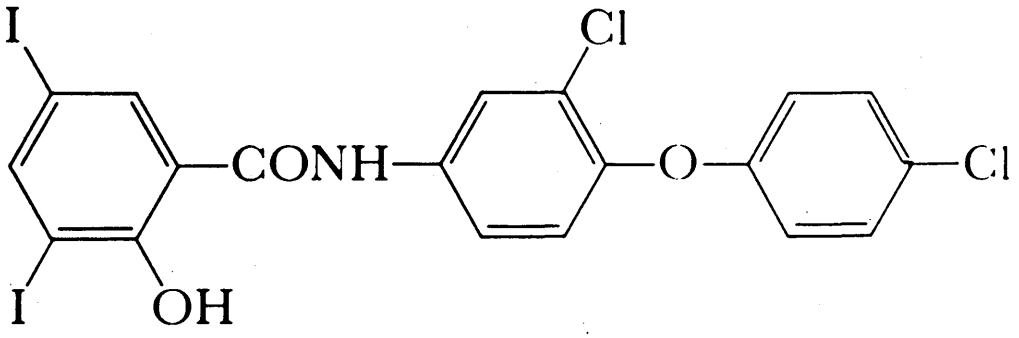
Pharmacokinetic studies of rafoxanide in sheep and cattle have been less extensive than efficacy studies. In fact there is no publication concerned with the kinetics of rafoxanide in sheep plasma and the work done by Dedek et al (1976, 1977, 1978) was concerned mainly with the degradation, excretion and metabolism of rafoxanide in sheep and cattle using radiolabelled rafoxanide (^{131}I).

From the toxicological point, Swan and Schroder (1981) measured the plasma concentration of rafoxanide in lambs, treated with different dosages of rafoxanide, up to 17 days after administration. Because of the inaccuracy of the assay method used, their results give little confidence. The pharmacokinetic behaviour of rafoxanide was therefore examined in sheep plasma with particular emphasis being placed on plasma protein binding. Residues of rafoxanide in tissues in sheep were also examined.

It was also of interest to compare the pharmacokinetics of rafoxanide given alone with those of rafoxanide when given along with thiabendazole.

FIGURE 2.2.1

STRUCTURE OF RAFOXANIDE


I
Cl
CONH
O
Cl
I
OH

2.2 Determination of rafoxanide in plasma

Rafoxanide is insoluble in water, moderately soluble in organic solvents and very sensitive to light.

Few analytical procedures have been published for the determination of rafoxanide in body fluids or in tissues.

After the oral administration of radiolabelled rafoxanide to sheep and cattle (Dedek et al 1976, 1977, 1978) the drug was measured by counting the total radio-activity produced. The disadvantage in using such technique is that large amounts of sample are required for the analytical procedure (50 ml or g) and the method fails to distinguish between parent compound and metabolites.

A gas chromatographic method was developed by Talley et al (1971) for the determination of rafoxanide in plasma by electron capture of its di-trimethyl-silyl derivatives. The method appeared to be sensitive with a detection limit of 0.01 $\mu g.ml^{-1}$. Preparation of plasma samples for the analysis was based mainly on denaturation of plasma proteins (using 95% ethanol) and the drug was then detected by a combined gas chromatography-mass spectrometry. Such facilities were not available in the department and also the method is unsuitable for routine use.

Swan and Schroder (1981) used a photometric method to determine rafoxanide in plasma. Their analytical method was not reliable (stated in Section 2.6).

2.2.1. Materials and methods

2.2.1.1 Reagents

All reagents used were of Analytical Grade.

Di ethyl ether

Redistilled, ferrous sulphate washed and stabilised.

(Section 1.2.1.1)

Methanol

Redistilled before use.

Phosphate-citrate buffer (pH 6)

62.1 ml M/5 disodium hydrogen sulphate ($Na_2 HPO_4$) and

37.9 ml M/10 citric acid.

Ammonium carbonate (0.05 M)

Water-permanganate redistilled (Section 1.2.1.1)

2.2.1.2 HPLC equipment and conditions

Detector

Reference channel variable wave length UV detector,

Model CE 2012 (Cecil Instruments, Cambridge, England).

Pump

Gilson module 302.

Recorder

Vitatron 10 mV.

Absorbance

0.05 a.u.f.s.

Wave length

282 nm.

Column

Septum injector (100 mm length x 5 mm i.d.) packed with a Reverse-phase ODS-Hypersil particle size 5 µm.

Chart speed

1 cm.min.$^{-1}$

Flow rate

0.8 ml.min.$^{-1}$

Retention time

Circa 3.85 min.

Injection volume

5 µl.

Mobile phase

Methanol : Ammonium carbonate (0.05 M) 80 : 20.

Limit of detection

0.10 µg.ml^{-1}

2.2.1.3 Procedure of analysis

Rafoxanide was determined in plasma samples and plasma ultrafiltrates by an HPLC method. Di-ethyl ether was used for the extraction.

1 ml plasma samples was introduced into a 50 ml glass stoppered tubes, using a 1 ml glass bulb pipette, to which 1 ml of phosphate-citrate buffer (pH 6) was added.

20 ml ether was added to each tube, using an automatic dispenser, the tubes were stoppered and shaken on a slow rotary mixer for 15 minutes.

15 ml of the upper ether layer were transferred to 50 ml thin-walled glass tubes.

A further 20 ml ether were added to the first tubes and shaken for 15 minutes. 20 ml of the upper ether layer was transferred and combined with the 15 ml ether extracts in the thin-walled tubes.

Extracts were evaporated on a dry bath at 50°C under nitrogen to approximately 5-6 ml. They were then transferred into 10 ml conical glass tubes and the walls of the thin tubes were washed with 1 ml ether several times and combined each time with the extracts in the conical tubes which were evaporated to dryness. The tubes were washed down with approximately 0.5-1 ml ether and placed on the dry bath until dryness.

Residues were dissolved in 100 µl methanol and placed in an ultrasonic water bath for 1 - 2 minutes. 5 µl of residues were injected onto the HPLC column using a 10 µl microsyringe.

Samples which appeared to contain higher concentrations were dissolved in volumes of methanol larger than 100 µl. When low concentrations were expected in samples, more than 1 ml of sample was used for the extraction of rafoxanide.

2.2.1.4 Preparation of standards and recoveries

Rafoxanide, like most salicylanilide fasciolicides, is insoluble in water and therefore a stock solution was made up in methanol by dissolving 100 mg of the pure compound in 100 ml methanol to make up a stock solution containing 1000 $\mu g.ml^{-1}$.

As rafoxanide is photo-labile this stock solution was made up freshly each time before the analysis and wrapped in aluminum foil and stored in a refrigerator for further protection from light.

Spikes containing 0.5, 1.0, 10.0 and 20.0 $\mu g.ml^{-1}$ of rafoxanide in plasma are prepared by adding an appropriate amount of drug in methanol and taken through the whole extraction procedure.

Percentage recovery of each spike and the concentrations of rafoxanide in plasma samples were calculated using the equations in Section 1.2.1.4.

Table 2.2.1 shows percentage recoveries of rafoxanide in the spiked plasma.

2.2.1.5 Evaluation of the method

Rafoxanide binds highly to plasma proteins (Duwel and Metzger 1973; Roberson 1977, Dedek et al 1976; Swan and Schroder 1981) and thus it was decided to investigate the effect of protein binding on the extraction procedure of rafoxanide from plasma samples.

A series of spikes were made up by adding known amounts of rafoxanide to drug free plasma and to aqueous solution (water).

1 ml of each spike was introduced into 50 ml stoppered glass thick tubes to which 1 ml of phosphate-citrate buffer (pH 6) was added followed by the addition of 20 ml ether. The tubes were shaken for 15 minutes on a rotary mixer.

17 ml of the upper ether layer was transferred into 50 ml thin-walled glass tubes and kept as Fraction I.

A further 20 ml ether was added to the first tubes and after shaking 20 ml of the upper ether extracts was transferred into a clean 50 ml thin-walled tubes (Fraction II).

Another 20 ml ether was added and the extracts were transferred into a further 50 ml thin-walled tubes

(Fraction III).

Each fraction was evaporated on a dry bath at 50°C under nitrogen stream to 5 - 6 ml and then transferred into 10 ml conical tubes and the walls of the thin tubes were washed three times with 1 ml ether and transferred to the conical tubes after each washing. They were then evaporated to dryness.

Residues of each fraction were dissolved in 100 µl methanol for injection onto the HPLC column.

Table 2.2.2 shows the percentage recoveries obtained in each fraction.

Recovery of rafoxanide from water was high (mean 98%) and recovery from plasma was less (mean 75%) (Table 2.2.1) but satisfactory.

TABLE 2.2.1

RECOVERIES OF RAFOXANIDE FROM SHEEP PLASMA.

Rafoxanide added to blank plasma ($\mu g.ml^{-1}$)	Rafoxanide measured (μg)			Mean	± S.E.M.	Ratio assayed to added
0.5	0.4	0.4	0.3	0.4	± 0.02	0.80
1.0	0.6	0.7	0.7	0.7	± 0.02	0.70
5.0	3.4	3.5	3.7	3.5	± 0.08	0.70
10.0	7.0	6.5	7.5	7.0	± 0.30	0.70
20.0	16.0	15.6	14.0	15.0	± 0.60	0.75

TABLE 2.2.2

PERCENTAGE RECOVERIES OF RAFOXANIDE FROM DRUG-FREE PLASMA AND AQUEOUS SOLUTION.

Amount rafoxanide added (µg)	Amount rafoxanide measured in fraction (µg)			Total rafoxanide recovered (µg)	% Recovery
	I	II	III		
Drug free plasma					
20	8.4	3.6	2.5	14.5	73
30	12.4	5.6	3.8	21.8	73
40	16.0	7.6	5.6	29.2	73
50	22.0	10.4	7.2	39.6	79
					Mean: 75%
Aqueous solution					
20	18.2	1.3	0.1	19.6	98
30	29.7	0.1	0.0	29.8	99
40	28.6	1.2	0.0	39.8	100
50	42.4	5.0	0.2	47.6	95
					Mean: 98%

2.2.2 Experiments with rafoxanide

2.2.2.1 Animals

Five sheep of different breed and sex, aged between 1 - 3 years, parasite free were used in the study.

The animals were divided into two groups, three sheep in Group (I) and two sheep in Group (II). Each group was housed separately in concrete stalls bedded with straw. They were fed hay and concentrates, while water was available ad libitum.

The sheep were weighed one day before the beginning of the experiment.

2.2.2.2 Administration of the drug

Group (I) was dosed with rafoxanide (3.0% w/v with 17.6% w/v thiabendazole "Ranizole"). Group (II) were dosed with rafoxanide (Flukanide 3.0% w/v). The animals were drenched orally using a syringe placed on the back of the tongue. Each sheep received rafoxanide at a dose rate of 7.5 $mg.kg^{-1}$. Thiabendazole was given to Group II sheep at a dose rate of 44 $mg.kg^{-1}$.

Table 2.2.3 shows animals' weight, suspension and volume of drug administered to each sheep.

2.2.2.3 Sampling regimen

10 ml blood samples were withdrawn by venepuncture into heparinised "Monovette" syringes immediately before drug administration (pre sample) and after 1, 2, 3, 7, 8, 9, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105 and 112 days after the administration.

2.2.2.4 Sample treatment

Blood samples were centrifuged at 2500 rpm for ten minutes and the plasma was transferred into 10 ml plastic tubes and stored at -20°C until analysed.

2.3 Binding of rafoxanide to plasma proteins

The binding of rafoxanide to plasma proteins was examined in vitro. Filtrates were obtained from blank sheep plasma fortified with known amounts of rafoxanide at different concentrations using the following different techniques.

2.3.1 Materials and methods

2.3.1.1 Ultrafiltration technique

If a protein solution containing a known amount of drug is exposed under pressure to a dialysis membrane, the protein free filtrate containing free drug only will collect outside the dialysis membrane. Knowledge of the initial concentration of drug in the plasma before filtration and the concentration of drug in the ultrafiltrate will allow the calculation of binding (Rehberg 1943).

5 ml of drug free plasma fortified with rafoxanide at 10, 20 and 30 $\mu g.ml^{-1}$ were introduced, in duplicate, into dialysis tube (Visking tube size 1-8/32" Medicell International Ltd., London, England). The ends of the tubes were folded several times and tied with a cotton string and washed under running tap water to remove any contamination. The dialysis tube was introduced into a 50 ml glass tube which was filled partially with glass beads. The tubes were centrifuged at 2500 - 3000 r.p.m. at 10°C for 30 - 35 minutes. The filtrates collected outside the dialysis membrane were transferred into 5 ml plastic tubes and stored at

-20°C until analysed.

2.3.1.2 Ultrafree drug filter

The technique is mainly based on the ultrafiltration technique which involves the use of special filter membranes with different pore size to allow the separation of plasma proteins from ultrafiltrates.

Two millilitres of the fortified plasma samples were filtered through an Amicon centricon microconcentrator (Amicon Corporation, Scientific Division, Upper Mill, Stonehouse, Gloucestershire) which had a molecular weight cut off 10,000 (Fig. 2.1.2).

The filtrates collected in a reservoir cap were stored at -20°C until analysed by a high performance liquid chromatographic method.

2.3.1.3 Equilibrium dialysis technique

A common procedure for the measurement of binding, this procedure involves the equilibrium of a volume of protein solution in a dialysis tube with a known amount of a solution of the drug. Thus, at equilibrium, measuring the concentration of free drug outside the dialysis tube will allow calculation of the amount of drug which was bound to proteins.

Five to seven millilitres of the fortified sheep plasma were dialysed against 10 ml phosphate buffer pH 7.4 at room temperature (18°C) and at 37°C. 1 ml of the dialysed solution containing the free drug (outside the dialysis membrane) was collected and deep frozen until analysis.

2.3.2 Method of analysis

A high performance liquid chromatographic method was used for the analysis of the ultrafiltrates as described in Section 2.2.1.

2.3.3 Calculation of percentage binding

The percentage binding of rafoxanide is calculated using the formula described in Section 1.4.1.

2.4 Determination of rafoxanide residues in bile and tissues

2.4.1 Materials and methods

Residues of rafoxanide were determined after oral administration of rafoxanide (Flukanide 3.0% w/v) to sheep at the therapeutic dose rate of 7.5 $mg.kg^{-1}$.

Twenty-eight days after dosing the sheep was slaughtered. Blood, bile, liver and muscle samples were collected and the concentrations of rafoxanide were determined.

2.4.2 Determination of rafoxanide in bile

1 ml of the bile sample was extracted into 20 ml di-ethyl ether. The ether extract was evaporated to dryness on a dry bath at 50°C under nitrogen. The residue was dissolved in 100 µl methanol and injected onto the HPLC column.

Preparation of a standard curve was made by extracting blank bile, obtained from untreated sheep, fortified with rafoxanide at different concentrations. The mean recovery obtained from the extraction of the fortified bile used for the calculation of the concentration of rafoxanide in bile sample as described in Section 1.2.1.4. Mean percentage recoveries obtained from the extraction of fortified bile were 91 $\pm$ 5.00 S.E.M. (Table 2.2.4).

2.4.3 Determination of rafoxanide in tissues

The HPLC method used for the determination of rafoxanide in tissues was modified slightly from that developed by Janssen Pharmaceutical Research Laboratories, B-2340 Beerse, Belgium, for residues of closantel in tissues.

Method

0.5 g of minced meat and liver was weighed into 15 ml glass stoppered tubes to which 3 ml of acetonitrile was added.

The tissue was homogenised using an Ultra-Turrax homogeniser (Janke and Kunkel, Ika-werk, Stanfen, F.R.G.). The homogenates were vortexed for 30 seconds and centrifuged at 2500 x g for 15 minutes. The supernatant was transferred into 10 ml thin-walled glass tubes and acidified with 3 ml of 0.4% v/v formic acid solution in water and vortexed for a few seconds.

The extracts were pumped through a pre-wetted (with water) "SEP-PAK" C18 cartridge using a 10 ml syringe. The solvent was displaced by pumping a further 0.5 ml of acetonitrile and the compound was eluted with 3 ml acetonitrile, collected in a 10 ml conical glass tubes and evaporated to dryness on a dry bath at 50°C under a stream of nitrogen.

Residues were suspended in 100 µl methanol and injected onto the HPLC column.

Analysis of each sample was done in duplicate. Drug free liver and muscle were fortified with different concentrations of rafoxanide and carried through the extraction procedure along with the samples. The method was reproducible (mean percentage recovery 89 $\pm$ 6.00) (Table 2.2.4). The HPLC conditions were the same as described in Section 2.2.1.2. The method had a detection

limit of 0.10 $\mu g.g^{-1}$.

Rafoxanide was also determined in the blood sample according to the method described in Section 2.2.1.

TABLE 2.2.3

THE EXPERIMENTAL DESIGN AND DOSAGE REGIMEN OF RAFOXANIDE ADMINISTERED ORALLY TO EACH SHEEP.

Sheep No.	Weight (kg)	Suspension	Dose rate rafoxanide ($mg.kg^{-1}$)	Total dose rafoxanide (mg)	Volume suspension administered (ml)
39	55	Ranizole	7.5	412.5	13.75
90	58	Ranizole	7.5	435.0	14.50
91	42	Ranizole	7.5	315.0	10.50
92	40	Flukanide	7.5	300.0	10.00
99	40	Flukanide	7.5	300.0	10.00

TABLE 2.2.4

RECOVERIES OF RAFOXANIDE FROM SHEEP BILE AND TISSUES.

Amount rafoxanide added (µg)	Amount rafoxanide measured (µg)			Mean	± S.E.M.	Ratio assayed to added
	Bile					
0.2	0.2	0.2	0.2	0.2	± 0.00	1.00
0.4	0.3	0.3	0.4	0.3	± 0.03	0.83
0.6	0.5	0.5	0.6	0.5	± 0.03	0.90
						Mean 91 ± 5.00 S.E.M.
	Liver and muscle					
0.3	0.2	0.3	0.3	0.3	± 0.03	1.00
0.5	0.4	0.5	0.4	0.4	± 0.03	0.80
0.7	0.6	0.7	0.5	0.6	± 0.06	0.86
						Mean 89 ± 6.00 S.E.M.

2.5 Results

2.5.1 Concentration of rafoxanide in plasma

The mean plasma concentrations of rafoxanide in individual sheep and the mean concentrations ± S.E.M. following oral administration of rafoxanide at 7.5 $mg.kg^{-1}$ bodyweight are presented in Table 2.2.5. Figure 2.2.2 shows mean plasma concentration of rafoxanide versus time curve.

The maximum concentration of rafoxanide in plasma of each sheep occurred 2-3 days post dosing and the mean maximum concentration was 23.6 $\mu g.ml^{-1}$. Thereafter the mean concentrations declined gradually to the limit of detection (0.10 $\mu g.ml^{-1}$) 16 weeks after the administration.

The averaged plasma concentration/time data was fitted to a computer model using an iterative least-square method derived from the method of Sedman and Wagner (1976). To estimate the variability of the parameters within the five sheep, the method was fitted to the individual plasma data as well (Table 2.2.6).

The sum of the weighted squared deviation between the data point and computer calculated points for each sheep were found to be best expressed as a tri-exponential term of the form:-

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

Area under the plasma concentration/time curve (AUC) and the half life in the "absorption", "distribution" and "elimination" phases were also computed (Table 2.2.6). A mean elimination half life ($t\ 1/2\ \gamma$) of 16.6 ± 1.2 days was found.

In comparing the AUC and elimination half life obtained from the mean plasma concentration for each sampling time versus time in both groups, it was determined that there was no significant differences ($P > 0.05$) between the group receiving rafoxanide alone (Flukanide) or in combination with thiabendazole (Ranizole). Therefore the results were combined and considered as one group from which all the pharmacokinetic parameters were computed.

2.5.2 Binding of rafoxanide to plasma proteins

Filtrates obtained from plasma fortified with rafoxanide at 10, 20, and 30 $\mu g.ml^{-1}$ contained a mean concentration of 0.2, 0.4 and 0.4 $\mu g.ml^{-1}$ of rafoxanide respectively when the dialysis tubing ultrafiltration technique was used.

Rafoxanide was not detected in the filtrates obtained by the dialyis equilibrium and ultrafree drug filter technique.

According to the limit of detection of the method (0.10 $\mu g.ml^{-1}$) and the highest concentration of rafoxanide used (30 $\mu g.ml^{-1}$) therefore the percentage binding, calculated using the equation in Section 1.4.1, will be:-

$$\% \text{ bound} = > 100 - \left(\frac{0.1}{30} \times 100\right)$$

$$= 100 - 0.3$$

$$= > 99$$

2.5.3 Determination of rafoxanide residues in bile and tissues

Concentrations of rafoxanide in the plasma and bile samples of the sheep killed 28 days after rafoxanide administration were 7.0 and 0.2 $\mu g.ml^{-1}$ respectively. In liver a mean concentration

of 0.4 $\mu g.g^{-1}$ of rafoxanide was detected while in the muscle sample rafoxanide was not detected at the limit of detection of the method 0.10 $\mu g.g^{-1}$.

TABLE 2.2.5

PLASMA CONCENTRATION ($\mu g.ml^{-1}$) OF RAFOXANIDE IN SHEEP AFTER A SINGLE ORAL ADMINISTRATION AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ OF BODYWEIGHT.

Time (days)	Sheep No. 39	90	91	92	99	Mean	± S.E.M.
Baseline	0	0	0	0	0		
1	27.0	7.7	14.7	20.6	11.8	16.4	± 3.4
2	26.0	10.0	23.0	30.0	16.8	21.2	± 3.6
3	27.8	16.0	21.4	29.6	22.8	23.6	± 2.5
7	23.4	9.2	21.4	24.0	20.0	19.6	± 2.7
8	18.3	9.3	20.3	21.0	16.8	17.2	± 2.0
9	18.2	8.6	18.6	20.5	21.2	17.4	± 2.3
10	15.7	10.4	18.0	19.5	14.8	15.7	± 1.6
14	13.3	8.7	14.6	15.4	13.5	13.0	± 1.2
21	10.4	5.3	13.8	12.4	12.2	10.8	± 1.5
28	8.3	4.7	10.7	10.2	9.8	8.8	± 1.0
35	5.6	3.5	9.3	9.8	7.4	7.0	± 1.2
42	3.7	2.3	7.0	5.6	6.0	5.0	± 0.9
49	2.8	1.2	5.3	4.0	3.5	3.4	± 0.7
56	1.0	1.6	3.8	2.8	2.8	2.4	± 0.5
63	1.5	1.0	2.8	2.5	2.3	2.0	± 0.3
70	0.3	0.5	2.4	1.8	1.8	1.4	± 0.4
77	0.5	0.5	2.0	1.0	1.5	1.0	± 0.3
84	0.4	0.3	1.4	0.8	0.9	0.8	± 0.2
91	0.3	0.2	1.3	0.8	1.4	0.8	± 0.2
98	0	0	0.7	0.4	0.8	0.4	± 0.2
105	0	0	0.3	0.3	0.5	0.2	± 0.1
112	0	0	0	0	0		

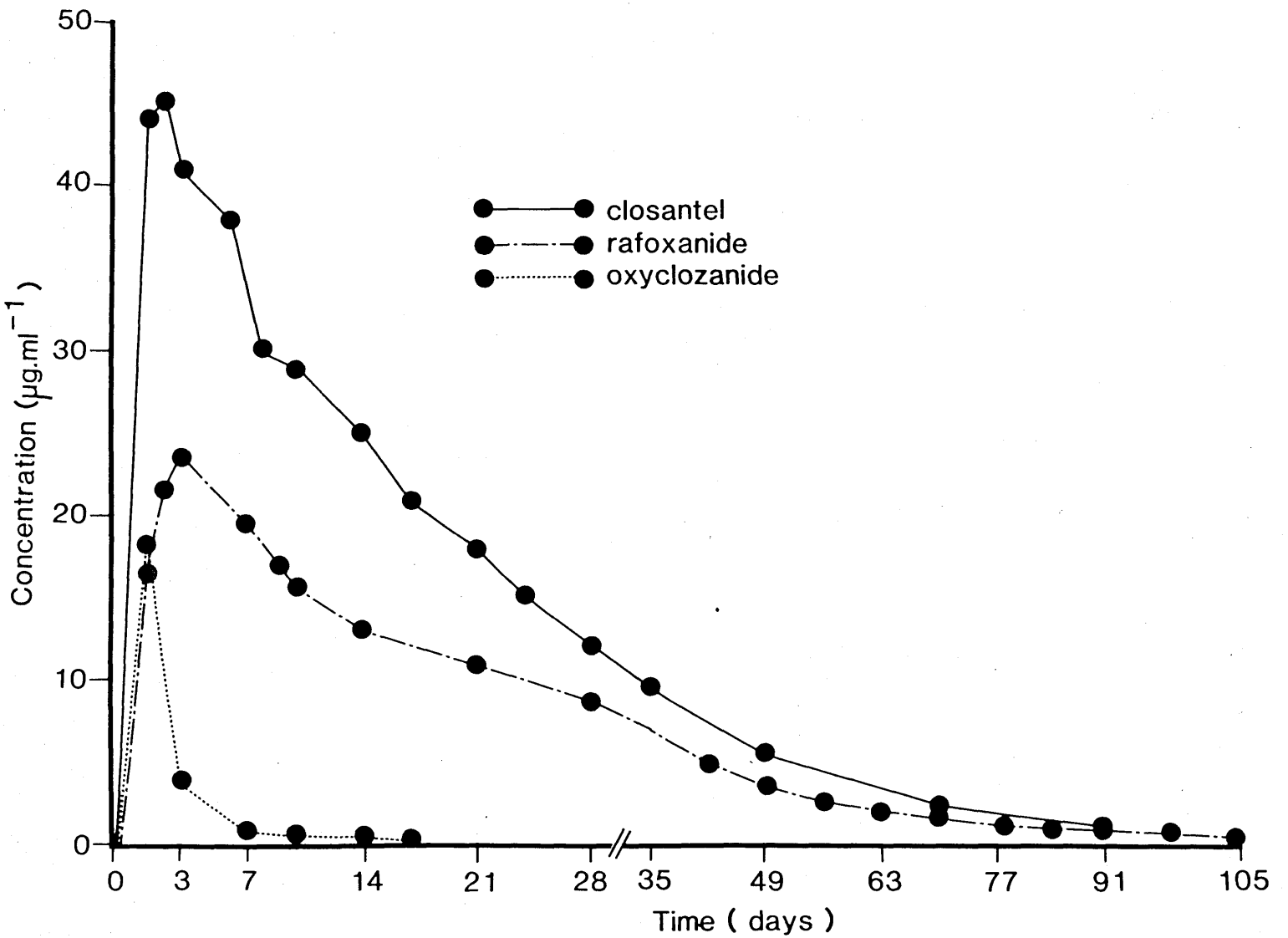
TABLE 2.2.6

VALUES OF PHARMACOKINETIC PARAMETERS OF RAFOXANIDE DETERMINED BY A THREE COMPARTMENT MODEL IN FIVE SHEEP FOLLOWING ORAL ADMINISTRATION AT 7.5 $mg.kg^{-1}$ BODYWEIGHT .

Animal no.	t 1/2 (α) day	t 1/2 (β) day	t 1/2 (γ) day	AUC $\mu g.ml^{-1}.day$
39	0.2	12.8	17.9	594.0
90	0.9	9.7	13.0	305.6
91	0.6	7.9	17.7	721.0
92	0.5	10.2	14.8	704.8
99	0.8	13.3	19.3	698.8
Mean	0.6	10.8	16.6	605.0
$\pm$ S.E.M.	$\pm$ 0.1	$\pm$ 1.0	$\pm$ 1.2	$\pm$ 78.6
Averaged values	0.5	10.8	15.4	577.0

FIGURE 2.2.2

MEAN CONCENTRATIONS ($\mu g.ml^{-1}$) OF RAFOXANIDE, CLOSANTEL AND OXYCLOZANIDE IN THE PLASMA OF SHEEP FOLLOWING ORAL ADMINISTRATION OF RAFOXANIDE (7.5 $mg.kg^{-1}$), CLOSTANTEL (7.5 $mg.kg^{-1}$) AND OXYCLOZANIDE (15 $mg.kg^{-1}$) RESPECTIVELY.


50
40
30
20
10
0
Concentration (µg.ml⁻¹)
closantel
rafoxanide
oxyclozanide
0
3
7
14
21
28
35
49
63
77
91
105
Time (days)

2.6 Discussion

After the oral administration, rafoxanide was absorbed slowly from the gastrointestinal tract to the blood stream and was detected in the plasma at high concentration at 24 hours, reached a mean maximum concentration of 23.6 $\mu g.ml^{-1}$ 2-3 days after the administration. Thereafter the decline in plasma concentrations was biphasic.

Once rafoxanide was absorbed , it was reversibly bound to plasma proteins. The process of binding influences the distribution and fate of drugs, that are extensively bound and subsequently acts to further prolong the elimination time of the drug from the peripheral plasma. Only the unbound or free drug is available to distribute out of the vascular system and be subjected to elimination and excretion processes.

By inspection of the estimated curve (Fig. 2.2.2) one term was associated with the absorption phase ($t\ 1/2(\alpha) = 0.6 \pm 0.1$ days), one with a relatively rapid elimination phase ($t\ 1/2\beta = 10.8 \pm 1.0$ days) and one with a slower phase ($t\ 1/2\ (\gamma) = 16.6 \pm 1.2$ days). It is probable that the slower elimination phase is due to elimination of extensively protein bound rafoxanide and this may be due to the elimination of plasma albumin in sheep (plasma albumin turnover rate = 400-480 h, Holmes et al 1968). The relatively rapid phase will be due to various influences on less extensively and/or less strongly bound drug in plasma being exposed to metabolism and/or excretion etc.

The relationship of the volume of distribution to the kinetics of the elimination of drug from the body is of interest.

The factors of interaction between drug and the normal constituents of the body that determine the volumes of distribution also play a role in influencing the rate at which a drug is eliminated from the body. Whatever the route or process of elimination, the rate is in general at least roughly proportional to the concentration of drug reaching the site of elimination.

Substances of high molecular weight or substances almost completely bound to plasma albumin will disappear only slowly from the vascular system and will have small volumes of distribution.

The apparent volume of distribution (Vd) is a pharmacokinetic constant which can be calculated from the total plasma concentration and can be of value in assessing whether a drug distributes well or poorly from the plasma into tissue sites. It is normally calculated in litres.kg $^{-1}$. Drugs distributing evenly throughout the animal's body have values of Vd of 1 l.kg^{-1}, poorly distributed drugs have Vd values < 1 l.kg^{-1}. The higher the value of Vd (> 1 l.kg^{-1}) the more extensive the localisation of drugs in sites other than plasma water. However, the value of Vd cannot give any information about the localisation of the sites of concentration.

There are two problems in calculating Vd for salicylanilides. Firstly, Vd is normally calculated from intravenous data. It can be calculated from intramuscular or oral administration data provided the amount of absorption is known. Unfortunately, salicylanilides are too toxic to be given intravenously. Secondly, Vd is calculated from a two-compartment

model and, as the pharmacokinetic data for salicylanilides shows, these data are best fitted to a three-compartment model. Thus for rafoxanide, assuming absorption is 100% and using the data points in the rapid elimination phase i.e. from three to 21 days (Fig. 2.2.2), Vd (area) was calculated to be 331 $ml.kg^{-1}$. This indicates that rafoxanide is very poorly distributed to tissues, consistent with a highly plasma protein bound drug. If rafoxanide is less than 100% absorbed, then this calculated value of Vd will give an overestimate of Vd.

Swan and Schroder (1981) measured rafoxanide plasma concentrations in lambs after administration of rafoxanide at different dosages (0, 7.5, 15, 22.5 and 37.5 $mg.kg^{-1}$). In all tested groups, the maximum concentration of rafoxanide in plasma was reached at 24 hours after dosing except in one group treated at 15 $mg.kg^{-1}$ in which the maximum concentration of rafoxanide in plasma occurred later at 48 hours after treatment. The lambs were 3-4 months old. The incomplete report of their results gives little confidence in their measurements as concentrations of 17.6 $\mu g.ml^{-1}$ of rafoxanide were detected in the plasma of untreated lambs.

In cattle, Dedek et al (1978) measured the serum concentration of rafoxanide after the oral administration of ^{131}I-rafoxanide at 5 $mg.kg^{-1}$ dose rate. In their experiment, the first sample was taken six days after treatment and no earlier samples were collected. Therefore they certainly missed the time at which the maximum concentration had occurred.

The rate of elimination of rafoxanide was found to be

dependent on the dosage of drug administered. Elimination half-lives of seven and 10.5 days were found following the oral administration of ^{131}I-rafoxanide to cattle at 5 and 12 mg.kg^{-1} bodyweight respectively (Dedek et al 1976, 1977), although Swan and Schroder (1981) did not report increasing elimination half lives with increasing dosage.

Rafoxanide is very often administered with the nematodicide, thiabendazole. The results showed no significant differences ($P < 0.05$) in the kinetics of rafoxanide administered alone or concurrently with thiabendazole. This was not surprising since it has been found that ^{14}C-thiabendazole eliminated from plasma within twenty hours after administration of thiabendazole (44 mg.kg^{-1}) in sheep (Tocco et al 1964). Weir and Bogan (1985) have also shown thiabendazole kinetics to be rapid in sheep with metabolism to the 5-hydroxy-metabolite although much less rapidly and extensively than in cattle.

Failure to detect rafoxanide in plasma filtrates obtained using the ultrafree drug filter indicates an extensive binding of rafoxanide to plasma proteins (> 99%). Since the molecular weight of rafoxanide is 626.01 therefore it should have been eluted through the filter membrane which had a molecular weight exclusive of 10,000.

The use of the dialysis tubing ultrafiltration technique for the determination of the percentage of binding has been shown to be unreliable. Variations in the pore size of the dialysis membrane might allow the passage of some bound drug through the membrane pores and give misleading results. Contamination of the dialysis tube during the introduction of the spiked plasma and washing the

tube before dialysis might also add another error factor. These possibilities might be the reason for the detection of rafoxanide at concentrations of 0.3 and 0.4 $\mu g.ml^{-1}$ by the dialysis tubing ultrafiltration technique.

The percentage of plasma protein binding of other drugs which are known to be less tightly bound to plasma proteins (< 99%) were also examined in vitro using the ultrafree drug filter technique. Among these drugs were sulphadiazine and albendazole sulfoxide. Each compound was added to drug-free plasma at different concentrations and then filtered through an Amicon centricon microconcentrator as described in Section 2.3.1.2. Ultrafiltrates obtained were analysed for the concentration of the free drug using Reider's method (1972) for the determination of free sulphadiazine and a high performance liquid chomatographic method for the determination of albendazole sulfoxide (Bogan and Marriner 1980). The percentage of binding of sulphadiazine and albendazole sulfoxide was found to be 14 and 49 respectively.

In view of the different results obtained for the determination of percentage of binding of rafoxanide to plasma protein using different techniques, it was found that using the ultrafree drug filter was the most reliable technique since there was less opportunity of any contamination and error which might lead to inaccurate results.

After absorption of rafoxanide from the gastrointestinal tract, a small quantity of the compound had entered the bile. A comparison of the low concentration of rafoxanide in sheep bile

(0.2 $\mu g.ml^{-1}$) to the higher concentration of 8.8 $\mu g.ml^{-1}$ in plasma which occurred 28 days post administration and the firm binding of rafoxanide to plasma proteins might support the conclusion that this drug exerts its effect towards liver flukes by the consumption of host blood rather than the concentration of the drug around the flukes in the bile duct.

Furthermore, it has been found that young flukes in liver parenchyma primarily feed on hepatic cells and blood (Dawes 1961; Pearson 1963) whereas the adult flukes in bile ducts feed mainly on blood rather than on biliary epithelium (Jennings et al 1956; Pearson 1963; Rowlands 1969; Brown and Neva 1983). Thus rafoxanide will inhibit the young flukes in liver parenchyma, via ingestion of liver tissues containing small amounts of the drug, and as they grow adult and migrate to bile ducts they will be exposed to more lethal doses of the blood bound drug.

From the safety point of view, animals treated with rafoxanide are not allowed to be used for human consumption for 28 days after administration in the U.K.

In this study no rafoxanide was detected in sheep muscle at the limit of detection of the method of 0.10 $\mu g.g^{-1}$ 28 days post dosing. Therefore consumption of muscle will be safe after 28 days. However the concentration of rafoxanide in liver tissues (0.4 $\mu g.g^{-1}$) might make the consumption of liver from treated animals unsafe for human consumption after such a period. Moreover the presence of rafoxanide in plasma at such a high concentration (mean of 8.8 $\mu g.ml^{-1}$) should also preclude the use of treated animal blood in the making of black pudding and other blood products at 28 days after administration. It could be that

the concentrations found in liver tissue were, in fact, due to blood trapped in the liver at post-mortem and the differences found between muscle and liver being due to the highly vascular nature of liver. Nevertheless whether the concentrations found reflect the 'correct' liver concentrations or not, is unimportant, since they will reflect the approximate amount which could be eaten by a consumer.

CHAPTER 3

EXAMINATION OF THE EFFICACY OF RAFOXANIDE AGAINST SIX WEEK-OLD *Fasciola hepatica* IN SHEEP

3. Examination of the efficacy of rafoxanide against six week-old Fasciola hepatica in sheep

3.1 Introduction

The anthelmintic efficacy of rafoxanide against immature Fasciola hepatica has been described by several authors (Mrozik et al 1969; Armour and Corba 1970; Ross 1970; Horak, Snijders and Louw 1972; Edwards and Parry 1972a, b).

The efficacy trials are usually carried out by treating the animals six weeks after experimental infestation with Fasciola hepatica metacercariae and necropsies are delayed to allow time for the flukes to mature so that they can be readily identified and counted. The possibility exists that the efficacy of rafoxanide against six week-old fluke might be attributed to the long residence time of the drug in plasma (Chapter 2) and that as the flukes become adult they will be exposed to the continued action of rafoxanide by ingesting the bound drug. Therefore the following experiment was designed to test this possibility.

3.2 Materials and Methods

3.2.1 Animals

Nine sheep fluke-free, of different breed, sex, aged between 1 - 3 years and weighing between 30 and 60 kg were assigned to three groups of three sheep on age, weight and breed basis.

The sheep were kept indoors in concrete stalls covered by straw. Hay, concentrates and water were available ad libitum.

All sheep were drenched orally by 450 viable Fasciola hepatica metacercariae on Day 0 and the animals were kept under observation throughout the experiment.

After six weeks of the infestation, Group 1 sheep were dosed orally, by syringe, with rafoxanide (Flukanide, 3.0% w/v MS & D) at 7.5 $mg.kg^{-1}$ dose rate.

Ten weeks post administration of metacercariae Group 2 sheep were dosed with a dose which was estimated would produce the plasma concentration remaining in Group 1 sheep at 10 weeks. This was done by sampling Group 1 sheep from weeks 6 to 10. The dose calculated was 2.5 $mg.kg^{-1}$ (see results section for data).

Group 3 were not treated and kept as controls.

Blood samples were taken from all sheep at 0, 1, 2, 3, 4, 7, 14, 21, 28, 42, 49, 56, 63, 70, 84 and 98 days after infestation.

Blood samples were withdrawn into heparinised syringes (Monovette, Sarstedt) and the plasma was separated as described in Section 2.2.2.4.

The plasma samples obtained from all sheep were used for biochemical analysis as a possible measure of damage to liver tissues caused by liver fluke infestation which is thought to increase the concentrations of certain plasma enzymes (see discussion section for details).

The plasma samples obtained from treated animals were analysed for the concentrations of rafoxanide in order to compare the pharmacokinetics of rafoxanide in the infected sheep to those in the non-infected sheep (Chapter 2). Additional blood samples were collected at the same times in tubes containing EDTA for haematological estimation for percentage of eosinophilic count and determination of haemoglobin values which were considered to be altered during liver fluke infection (see discussion section for details).

Faecal samples were taken from the rectum at intervals throughout the trial for examination for fluke eggs.

After 14 weeks of infestation all sheep were necropsied and the livers of each sheep were collected separately and examined for flukes.

Pieces of gluteal muscle were also taken from each sheep at slaughter time and kept at -20°C for determination of rafoxanide residues.

3.2.2 Estimation of the viability of Fasciola hepatica metacercariae

Metacercariae encysted on cellophane were initially purchased from the Agricultural Research Institute, Compton, Berks.

Initially, prior to the sheep experiment, the viability of metacercariae was estimated both *in vitro* and *in vivo*.

3.2.2.1 In vitro

Twenty-four metacercariae were scraped from the cellophane strips and collected on a solid watch glass plate.

The metacercariae were washed twice with pepsin solution (prepared as detailed below) and then incubated in 50 ml pepsin solution for 2 - 3 hours at 22°C.

During this time the thick outer coatings of the metacercariae were digested and the young flukes were seen under the microscope contracting away from the inner cyst wall.

The metacercariae were then washed with warm distilled water and incubated in 50 ml of trypsin-bile solution (preparation details below) for three hours at 22°C. This process causes lysis of the cyst shell and the immature fluke can emerge into

the solution (Figure 2.3.1).

The numbers of flukes to emerge successfully were counted and this figure used to determine the overall percentage viability which was found to be 75%.

Preparation of Reagents

All reagents used were of analytical grade.

Pepsin solution

Pepsin	0.5 g
NaCl	0.8 g
1/20 N HCl	100 ml

Trypsin-bile solution

Trypsin	0.4 g
NaCl	0.8 g
Na HCO_3	1.0 g
OX bile	20 ml
Dist.water	80 ml

3.2.2.2 In vivo

Fifty embryonated metacercariae were scraped from the cellophane strips and placed in a solid watch glass plate containing 2 - 3 ml of 0.1% detergent solution in water (Lissapol, ICI Ltd., Macclesfield, Cheshire). This detergent solution was used to prevent the sticking of the metacercariae to the glass plate.

The numbers of metacercariae were checked using a stereoscopic microscope (Leitz Ltd.).

The solution containing the metacercariae was drawn into a 1 ml syringe to which a short cannula (1.19 mm i.d.) was

attached.

Two rats (Sprague-Dawley breed) were lightly anaesthetised by placing them in a jar containing cotton soaked with trichloroethylene for a few minutes. When the rats showed no physical reflexes, they were held from the back of the neck and the cannula was introduced into the oesophagus and the solution was administered.

The syringe was rinsed with the detergent solution and the washings were administered to the rats as above.

Three weeks later, the rats were killed and the livers were removed. This time was allowed to see the gross pathological lesions in the liver which is associated with the migration of young flukes.

Both livers (Fig. 2.3.2) showed marked fibroblastic canals which were caused by the migration of the immature flukes through liver parenchyma.

3.2.3 Post mortem findings

The livers from all sheep in Groups 1, 2 and 3 were cut carefully into 5 - 10 mm slices and all visible flukes were removed and stored for counting. The gall bladders from livers with no visible flukes were retained and the bile from the treated animals was collected and stored at -20°C for analysis.

One to two liver slices were squeezed by hand and kept at -20°C for determination of rafoxanide residues. The remaining liver slices were incubated in a bucket containing water at 42°C for 2 - 3 hours.

The slices were then removed from the warm water, washed and squeezed manually and the remaining materials in the bucket were washed onto sieves with 317 μm pore size.

The contents of these sieves were collected and examined microscopically. The number of liver flukes present was determined by counting the oral suckers recovered.

Analysis of plasma, bile, liver and muscle samples were as described in Sections 2.2.1, 2.4.2 and 2.4.3 respectively.

The plasma enzyme concentrations were measured using test kits (BCL, Boehringer Mannheim House, East Sussex).

FIGURE 2.3.1

In vitro ASSESSMENT OF VIABILITY OF *Fasciola hepatica* METACERCARIAE. A : ENCYSTED METACERCARIAE, B : METACERCARIAE EMERGING FROM THE CYST AFTER INCUBATION IN PEPSIN AND TRYPSIN-BILE SOLUTIONS, C : YOUNG FLUKES, D : EMPTY CYST.

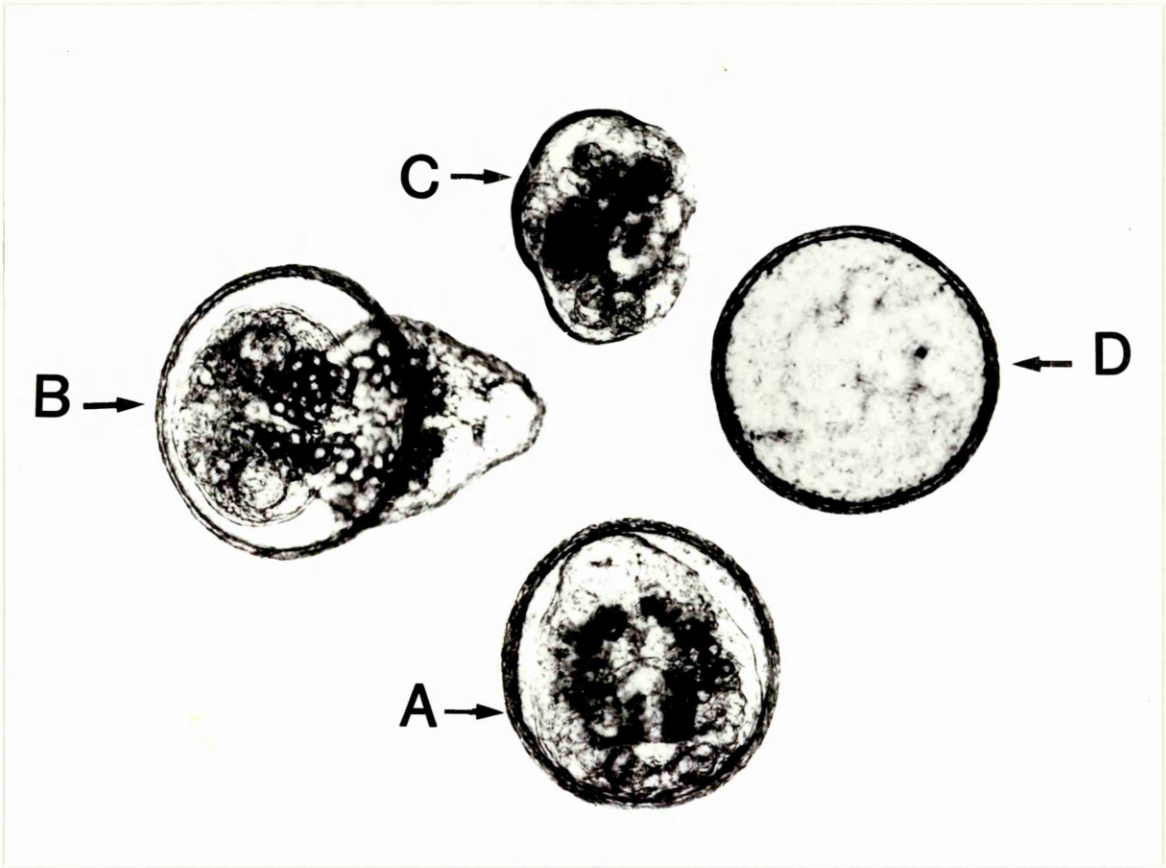

C
B
D
A

FIGURE 2.3.2

LIVER OF RATS RECOVERED AT AUTOPSY (3 WEEKS AFTER EXPERIMENTAL INFESTATION WITH 50 Fasciola hepatica METACERCARIAE) SHOWING MARKED FIBROBLASTIC CANALS CAUSED BY MIGRATION OF IMMATURE FLUKES.

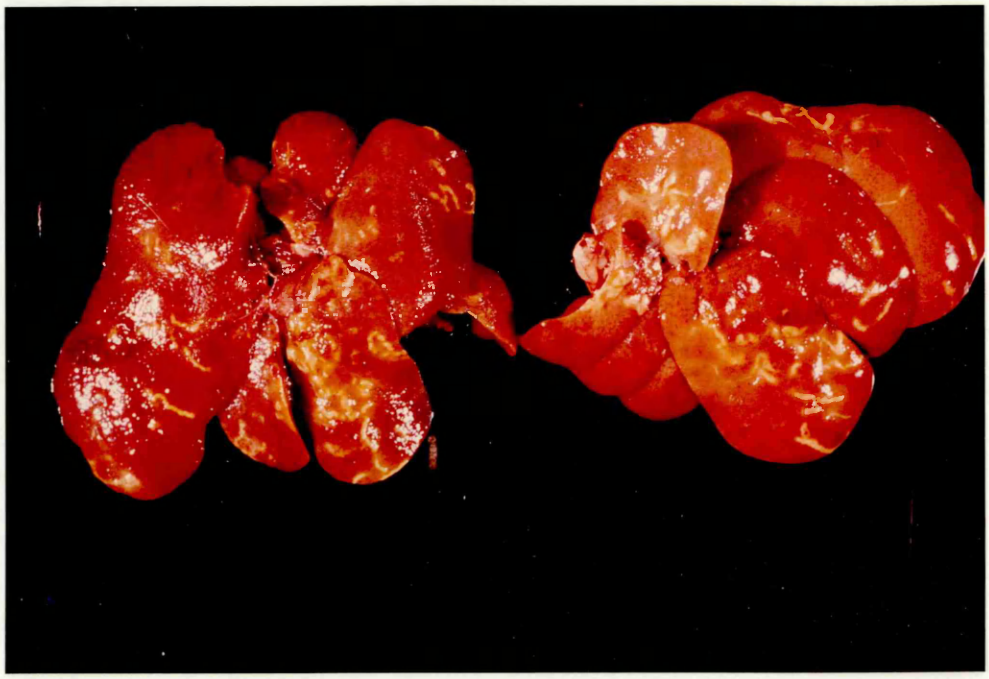
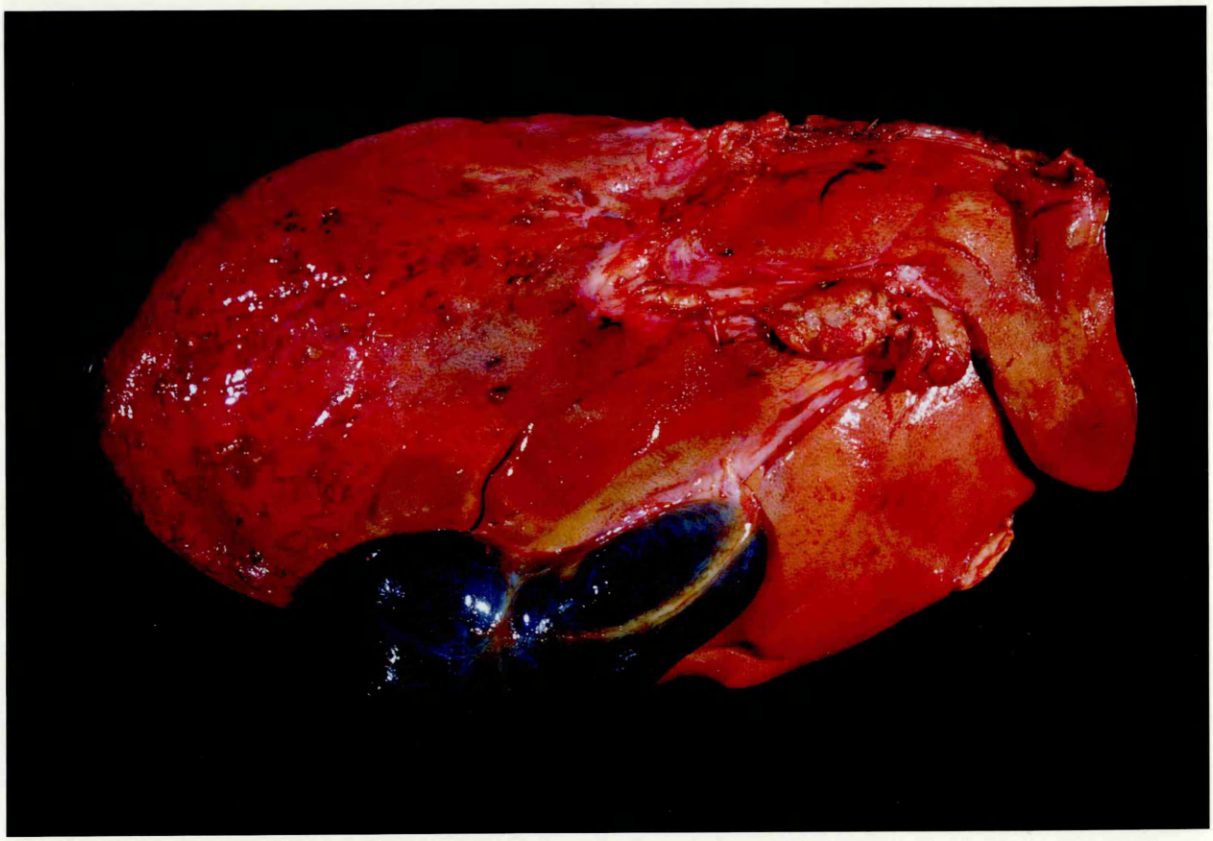


FIGURE 2.3.3.

LIVER FROM INFECTED AND UNTREATED SHEEP RECOVERED AT AUTOPSY (14 WEEKS AFTER EXPERIMENTAL INFESTATION WITH 450 Fasciola hepatica METACERCARIAE) SHOWING FIBROBLASTIC CANALS AND SOME DEGREE OF CIRRHOSIS.



3.3 Results

3.3.1 Efficacy trial

The treatment schedule, number of fluke eggs, number of flukes recovered at autopsy and the percentage efficacy are summarised in Table 2.3.1.

All the control animals were highly infected with Fasciola hepatica and the fluke populations were of different size and length. All livers had some degree of cirrhosis (Figure 2.3.3). Macroscopically some of the treated livers were badly damaged and some had normal parenchyma but portions of the bile ducts were fibroblastic and calcareous.

Rafoxanide was found to be 86.40 and 87.88% effective against six and ten week-old flukes when administered at 7.5 and 2.5 $mg.kg^{-1}$ respectively.

3.3.2 Determination of rafoxanide in plasma

The concentrations of rafoxanide in individual sheep and the mean $\pm$ S.E.M. concentration in plasma in both treated groups are shown in Table 2.3.2.

Rafoxanide was absorbed through the gastrointestinal epithelium to the circulation and was detected in plasma six hours post dosing at a mean concentration of 6.3 $\mu g.ml^{-1}$ and rose to attain the mean maximum concentration of 43.0 $\mu g.ml^{-1}$ after three days of administration (Figure 2.3.4). Thereafter, a relatively fast decline in the plasma concentration/time profile occurred from three to 17 days after dosing and followed the same pattern as shown by the non-infected sheep. Elimination of rafoxanide after three weeks was slow and produced a mean

concentration of 3.4 $\mu g.ml^{-1}$ at 56 days after drug administration.

The plasma kinetics of rafoxanide in Group 2 sheep (treated at 2.5 $mg.kg^{-1}$) were similar to those seen in Group 1 (Figure 2.3.4). The maximum plasma concentration occured three days post dosing at a mean concentration of 14.2 $\mu g.ml^{-1}$ followed by a relatively rapid decline down to 7.5 $mg.kg^{-1}$ 14 days post administration. Thereafter the decline was slower and the concentrations decreased slowly to 3.7 $\mu g.ml^{-1}$ 28 days after dosing.

Significant differences ($P < 0.05$) were found between the mean plasma concentrations in the infected (Group 1) and non-infected sheep. The AUC of each sheep in Group 1, 2 and in non-infected sheep were analysed using one-way analysis of variance. The AUC for sheep in Group 2 were multiplied by three to provide a direct comparison to those in Group 1 and in non-infected sheep. It was found that the AUC in infected sheep (Group 1 and 2) were significantly higher ($P < 0.05$) than those in the non-infected sheep.

3.3.3 Residues of rafoxanide in plasma, bile, liver and muscle

Rafoxanide was detected in the plasma samples, obtained at slaughter, at a mean concentration of 3.4 and 3.7 $\mu g.ml^{-1}$ after administration of rafoxanide at 7.5 and 2.5 $mg.kg^{-1}$ respectively (Table 2.3.2).

Mean concentrations of 0.1 and 0.3 $\mu g.g^{-1}$ were found in liver samples after administration of rafoxanide at 7.5 and 2.5 $mg.kg^{-1}$ respectively (Table 2.3.3).

Rafoxanide was not detected at the limit of detection of the method (0.10 $\mu g.ml^{-1}$) in any bile and muscle samples (Table 2.3.3) obtained at slaughtering time from treated animals.

3.3.4 Biochemical estimation

The mean concentrations of ALT, AST, AP and γGT in plasma are shown in Fig. 2.3.5, 2.3.6, 2.3.7 and 2.3.8 respectively.

Plasma ALT activity did not differ significantly from that of the control group throughout the trial.

The mean maximum plasma concentration of AST and AP occurred four to six and six weeks after infestation respectively and declined to normal after treatment in Group 1 sheep.

Significant elevations of plasma concentration of γGT were seen before treatment in Group 1 and 2 sheep and declined slowly after treatment but rose again only in Group 1 sheep after 12 weeks of infestation. Typical rise in γGT activity was seen in the control group and reached a maximum concentration of 116 iU. l^{-1} nine weeks after infestation and then declined to levels still higher than the normal levels.

TABLE 2.3.1

ANTHELMINTIC EFFICACY OF RAFOXANIDE AFTER ORAL ADMINISTRATION AGAINST SIX AND TEN WEEK-OLD Fasciola hepatica IN SHEEP.

Sheep No.	Dose rate $mg.kg^{-1}$	Time of dosing after infection (weeks)	No.of flukes recovered	No.of eggs (/g)	Efficacy + (%)
90	7.5	6	21	Negative	
95	7.5	6	3	1	86.40
107	7.5	6	3	7	
11	2.5	10	2	4	
15	2.5	10	4	1	87.88
44	2.5	10	18	Negative	
30	*	*	83	Negative	
45	*	*	64	15	
66	*	*	51	23	

* Control (infected and untreated)

$$+ \% \text{ efficacy} = \frac{(\text{Control mean} - \text{treated mean})}{\text{control mean}} \times 100$$

TABLE 2.3.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF RAFOXANIDE IN PLASMA OF SHEEP TREATED AFTER 6 AND 10 WEEKS OF INFESTATION AT 7.5 AND 2.5 $mg.kg^{-1}$ DOSE RATE.

Time	Sheep number			Mean ± S.E.M.	Sheep number			Mean ± S.E.M.
	90	95	107		11	15	44	
pre	0	0	0		0	0	0	
6 h	4.0	8.0	7.0	6.3 ± 1.2	-	-	-	- -
12 h	14.0	13.8	21.6	16.5 ± 2.6	6.2	6.3	6.0	6.2 ± 0.1
1 d	24.8	24.2	37.7	29.0 ± 4.4	11.8	11.5	9.0	10.8 ± 0.1
2 d	32.2	36.8	45.3	38.0 ± 3.8	14.5	14.5	13.2	14.0 ± 0.4
3 d	32.4	42.0	54.3	43.0 ± 6.4	14.3	14.0	14.3	14.2 ± 0.1
7 d	24.6	33.4	40.0	32.7 ± 4.5	10.8	12.5	12.6	12.0 ± 0.5
10 d	23.0	26.7	34.8	28.2 ± 3.5	-	-	-	- -
14 d	21.7	24.5	29.3	25.2 ± 6.5	9.0	8.0	5.4	7.5 ± 1.0
17 d	18.6	23.0	31.4	24.3 ± 3.8	7.0	6.0	4.0	5.7 ± 0.8
21 d	14.0	19.2	21.6	18.3 ± 2.2	6.4	5.0	3.2	4.8 ± 0.9
28 d	13.7	13.8	16.0	14.5 ± 0.7	4.5	4.2	2.4	3.7 ± 0.6
35 d	10.4	10.0	11.8	10.7 ± 0.5	-	-	-	- -
42 d	4.4	6.2	5.4	5.3 ± 0.5	-	-	-	- -
49 d	3.7	4.0	4.7	4.0 ± 0.3	-	-	-	- -
56 d	3.0	3.5	3.8	3.4 ± 0.2	-	-	-	- -

Sheep 90, 95, 107 treated at 7.5 $mg.kg^{-1}$ when flukes were 6 weeks old.

Sheep 11, 15, 44 treated at 2.5 $mg.kg^{-1}$ when flukes were 10 weeks old.

TABLE 2.3.3

CONCENTRATIONS ($\mu g.ml^{-1}$ or $\mu g.g^{-1}$) OF RAFOXANIDE IN BILE, LIVER AND MUSCLE OF SHEEP AFTER ORAL ADMINISTRATION AT 7.5 AND 2.5 $mg.kg^{-1}$ DOSE RATE.

Sheep no.	Sample: Bile * ($\mu g.ml^{-1}$)	Liver * ($\mu g.g^{-1}$)	Muscle * ($\mu g.g^{-1}$)
90	ND	0.1	ND
95	ND	0.1	ND
107	ND	0.1	ND
11	ND	0.5	ND
15	ND	0.3	ND
44	ND	0.2	ND

* Each value is mean value of duplicate samples.

ND None detected.

Sheep 90, 95, 107 treated at 7.5 $mg.kg^{-1}$ when flukes were six weeks old.

Sheep 11, 15, 44 treated at 2.5 $mg.kg^{-1}$ when flukes were ten weeks old.

FIGURE 2.3.4

MEAN CONCENTRATIONS ($\mu g.ml^{-1}$) OF RAFOXANIDE IN THE PLASMA OF SHEEP (GROUP 1, n=3) TREATED 6 WEEKS AFTER INFESTATION WITH *Fasciola hepatica* METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND AT 10 WEEKS AT A DOSE RATE OF 2.5 $mg.kg^{-1}$ (GROUP 2, n=3).

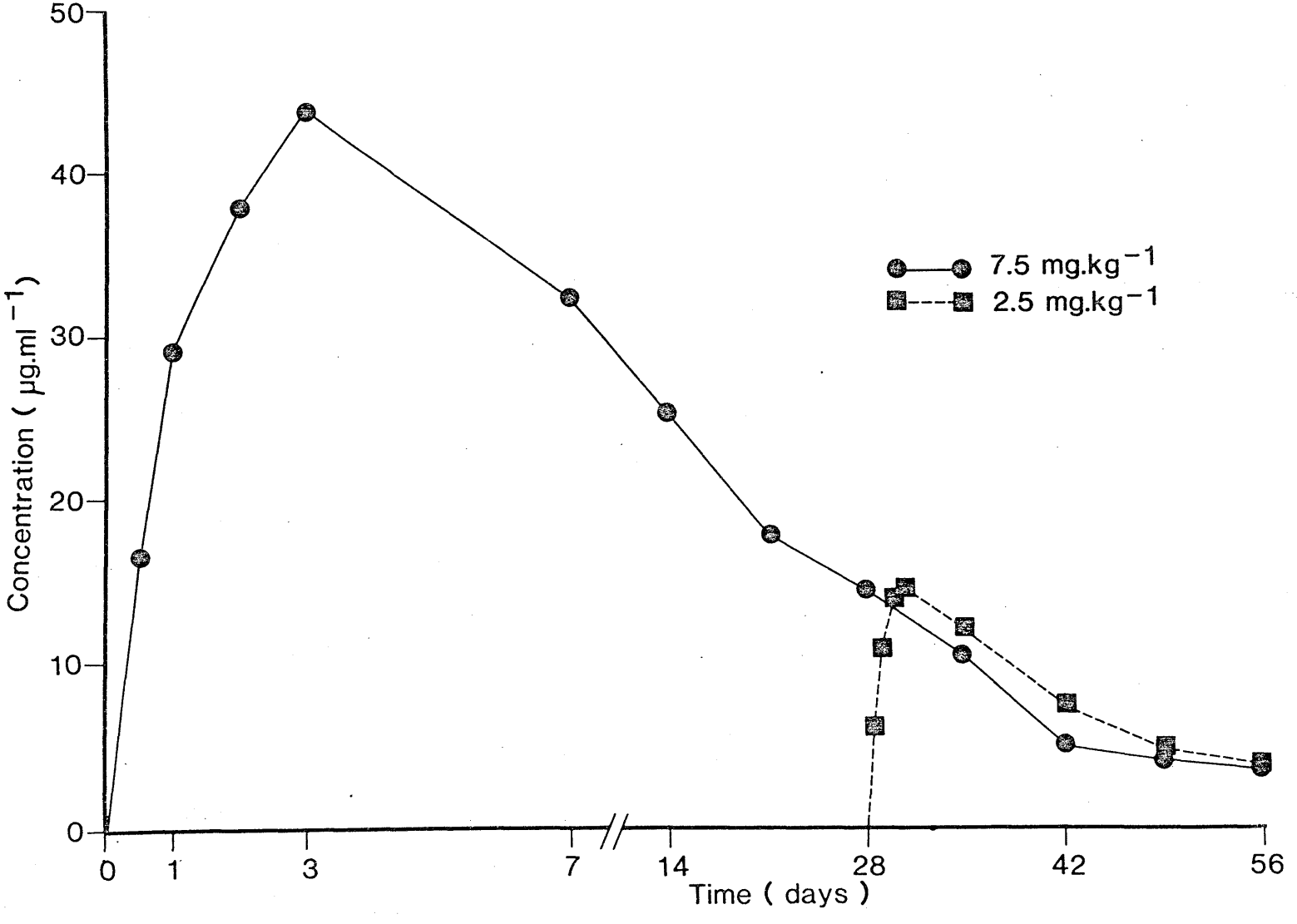

50
40
30
20
10
0
Concentration (µg.ml^{-1})
7.5 mg.kg^{-1}
2.5 mg.kg^{-1}
0
1
3
7
14
28
42
56
Time (days)

FIGURE 2.3.5

MEAN CONCENTRATIONS ($iu.l^{-1}$) OF ALANINE AMINOTRANSFERASE (ALT) OBTAINED IN PLASMA OF SHEEP IN GROUP 1 (n=3) TREATED WITH RAFOXANIDE 6 WEEKS AFTER INFESTATION WITH Fasciola hepatica METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND IN GROUP 2 (n=3) TREATED AFTER 10 WEEKS OF INFESTATION AT A DOSE RATE OF 2.5 $mg.kg^{-1}$ AND IN GROUP 3 (n=3) INFECTED AND UNTREATED.

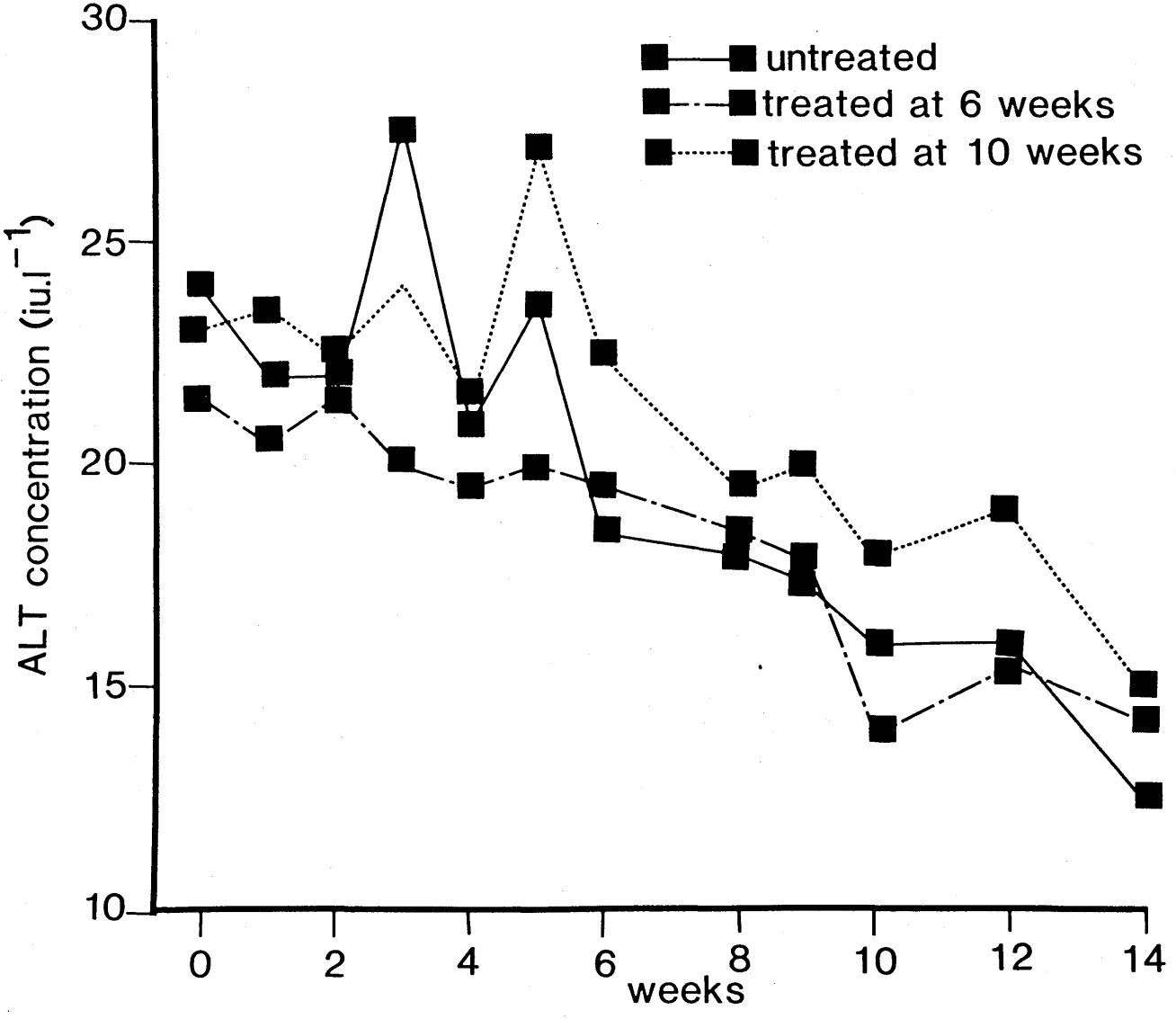

30
25
20
15
10
ALT concentration (iu.l^{-1})
untreated
treated at 6 weeks
treated at 10 weeks
0
2
4
6
8
10
12
14
weeks

FIGURE 2.3.6

MEAN CONCENTRATIONS ($iu.l^{-1}$) OF ASPARTATE AMINOTRANSFERASE (AST) OBTAINED IN THE PLASMA OF SHEEP IN GROUP 1 (n=3) TREATED WITH RAFOXANIDE 6 WEEKS AFTER INFESTATION WITH *Fasciola hepatica* METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND IN GROUP 2 (n=3) TREATED 10 WEEKS AFTER INFESTATION AT A DOSE RATE OF 2.5 $mg.kg^{-1}$ AND IN GROUP 3(n=3) INFECTED AND UNTREATED.

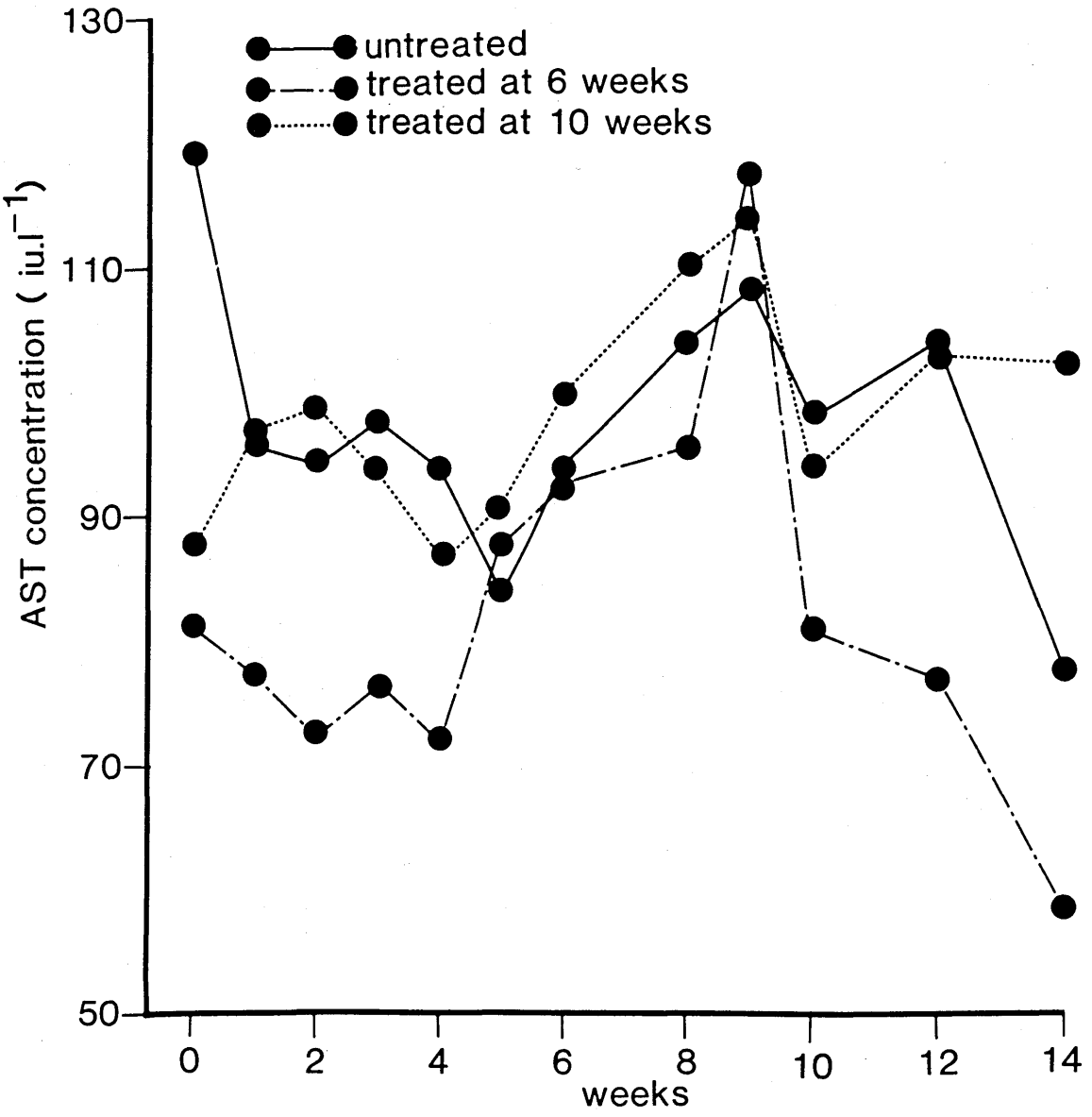

untreated
treated at 6 weeks
treated at 10 weeks
AST concentration (iu.l^{-1})
130
110
90
70
50
0
2
4
6
8
10
12
14
weeks

FIGURE 2.3.7

MEAN CONCENTRATIONS ($iu.l^{-1}$) OF ALKALINE PHOSPHATASE (Ap) IN THE PLASMA OF SHEEP IN GROUP 1 (n=3) TREATED WITH RAFOXANIDE 6 WEEKS AFTER INFESTATION WITH Fasciola hepatica METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND IN GROUP 2 (n=3) TREATED AFTER 10 WEEKS OF INFESTATION AT A DOSE RATE OF 2.5 $mg.kg^{-1}$ AND IN GROUP 3 (n=3) INFECTED AND UNTREATED.

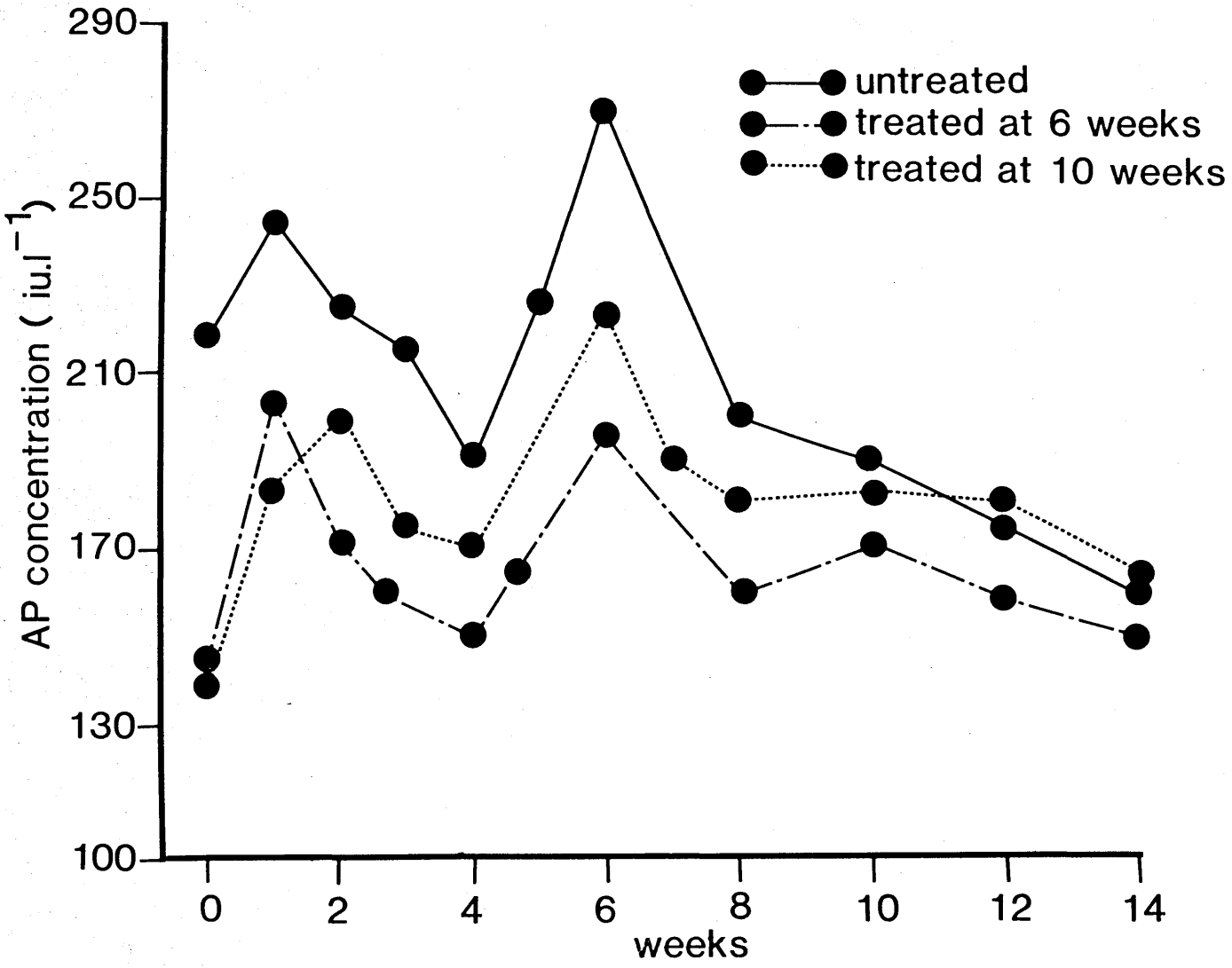
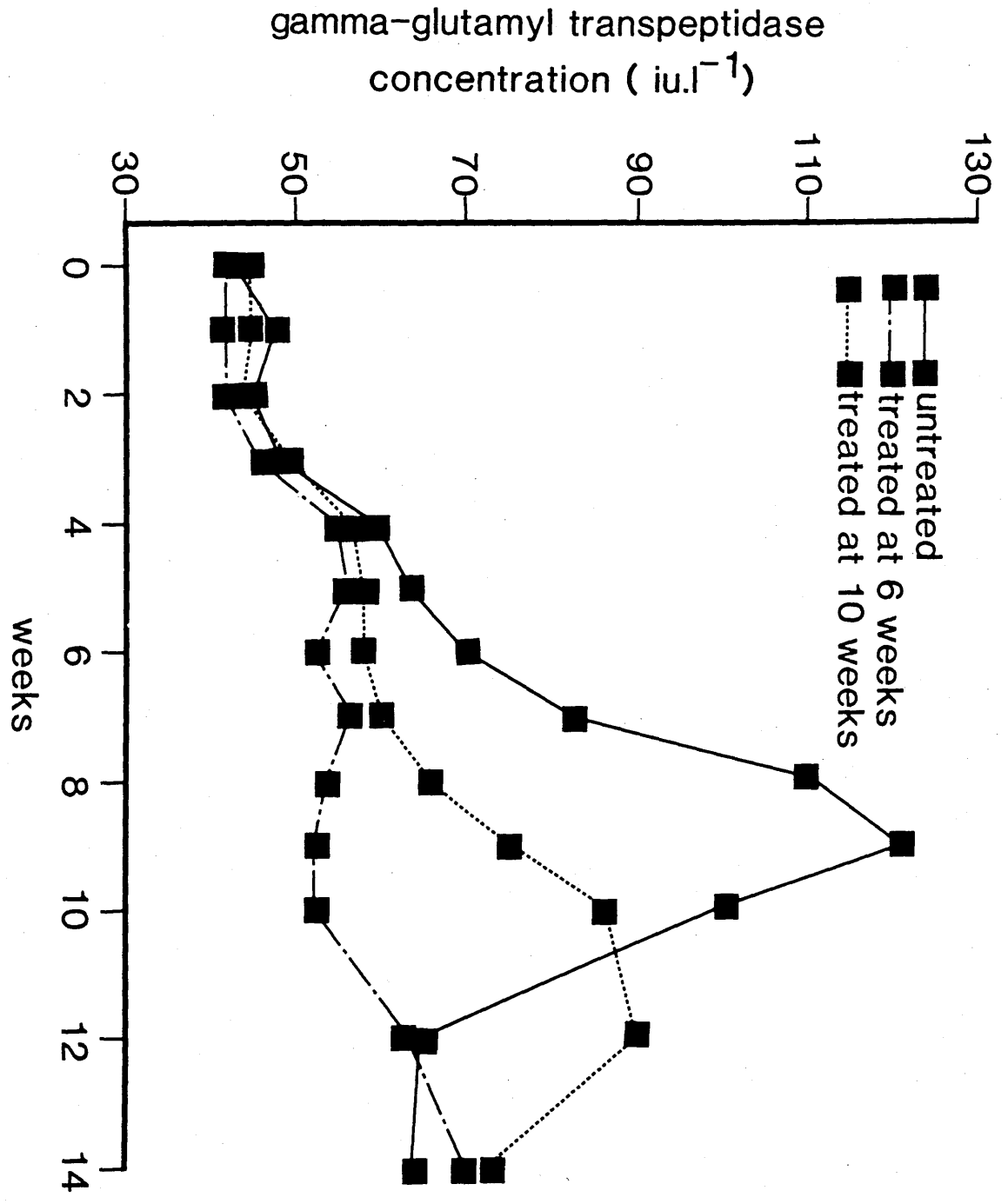

290
250
210
170
130
100
AP concentration (iu.l^{-1})
0
2
4
6
8
10
12
14
weeks
untreated
treated at 6 weeks
treated at 10 weeks

FIGURE 2.3.8

MEAN CONCENTRATIONS ($iu.l^{-1}$) OF GAMMA-GLUTAMYL TRANSPEPTIDASE (γGT) IN THE PLASMA OF SHEEP IN GROUP 1 (n=3) TREATED WITH RAFOXANIDE 6 WEEKS AFTER INFESTATION WITH *Fasciola hepatica* METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND IN GROUP 2 (n=3) TREATED AFTER 10 WEEKS OF INFESTATION AT A DOSE RATE OF 2.5 $mg.kg^{-1}$ AND IN GROUP 3 (n=3) INFECTED AND UNTREATED.


gamma-glutamyl transpeptidase
concentration (iu.l^{-1})
30
50
70
90
110
130
weeks
0
2
4
6
8
10
12
14
untreated
treated at 6 weeks
treated at 10 weeks

3.5 Discussion

It has been shown that the anthelmintic activity of most of the flukicidal anthelmintics is influenced by their strong affinity for plasma proteins. The process of binding acts to prolong the half life of the drug in the animal's body and might provide a possible route by which these drugs are delivered to the parasite and exert their activity (Broome and Jones 1966; Lee 1973; Guerrero *et al* 1982). For example, closantel has been shown to have a residual activity against *Haemonchus contortus* for 60 days after drug administration (Hall *et al* 1981), rafoxanide prevented the reinfection with the larval stage of *Oestrus ovis* in sheep for 11 days after administration (Horak, Louw and Raymond 1971).

In this experiment, efficacy of rafoxanide (86.40%) at 7.5 $mg.kg^{-1}$ against six week-old fluke after oral administration was in general agreement with those obtained after oral administration of the drug [99%; Armour and Corba (1970) and 97%; Guilhon *et al* (1970)], and with those after intraruminal administration [87%; Ross (1970); 95%; Campbell and Hotson (1971) and 88.9%; Horak, Snijders and Louw (1972)].

Presidente and Knapp (1972) found that rafoxanide given at 7.5 $mg.kg^{-1}$ was 58.8% efficient against six week-old flukes in calves. Lower efficacy (37.9%) was reported in cattle by Snijders and Horak (1975).

The differences in efficacy between sheep and cattle is probably due to the greater amount of connective tissues in cattle liver parenchyma whereas sheep have less connective

tissues (soft parenchyma) which presents no barrier to the flukes. Also the short half life of rafoxanide (7 days) found in cattle following the administration of 5 $mg.kg^{-1}$ (Dedek et al, 1977) indicates a more rapid elimination of rafoxanide in cattle. Since acute fascioliasis is not such a major problem in cattle as in sheep, the aim of treatment against fascioliasis in cattle is mainly toward the adult flukes.

Against ten week-old fluke rafoxanide given at 2.5 $mg.kg^{-1}$ showed similar efficacy (87.88%) to that obtained after intraruminal administration (2.5 $mg.kg^{-1}$) against F. gigantica in cattle (Snijders, Horak and Louw, 1971). When, however, in this trial the dosage that was received by sheep harbouring ten week-old flukes was increased from 2.5 to 7.5 $mg.kg^{-1}$, the efficacy of rafoxanide would be higher than 87.88% as those stages of flukes which are adult at this stage are more susceptible to the action of rafoxanide.

The experiment would have been better designed if one of the sheep dosed after six weeks of infestation had been killed shortly after treatment to assess the actual efficacy of rafoxanide against the immature liver flukes. But, unfortunately, the restricted number of sheep used in this trial made this assessment not possible. Also, obtaining a true count of immature flukes at about six to seven weeks is very difficult (and is the reason why all other authors have allowed surviving flukes to mature before counting) and would also have necessitated the killing of equivalent numbers of control animals. However, fortunately, the results of efficacy of rafoxanide against six and ten week-old liver fluke obtained in

this trial was as expected and confirmed the hypothesis upon which this experiment was based, namely that rafoxanide has little efficacy against younger flukes and that the efficacy reported by previous workers is due to continuing efficacy as the flukes reach maturity and become blood-suckers.

Flukes recovered at autopsy from sheep treated after six and ten weeks of infestation were smaller in size (Fig. 2.3.9) when compared to flukes recovered from control sheep. It is possible that rafoxanide may be slow acting and caused considerable stunting in the growth of the flukes and hence reduce their pathogenicity.

Knowing the concentration of rafoxanide in the flukes recovered from the group treated six and ten weeks after infestation would provide further information which is helpful to interpret whether rafoxanide had affected the immature or the mature flukes. Unfortunately, however, it was not possible to measure rafoxanide in the flukes due to the small number of flukes recovered from both groups which proved to be insufficient for the analytical procedure.

An experiment was also considered to establish whether plasma protein-bound rafoxanide accumulates in flukes or whether biliary concentrations are involved.

It is easily possible to label albumin using ^{131}I. Therefore giving rafoxanide to a sheep given labelled albumin would produce the possibility of measuring accumulated rafoxanide and simultaneously ^{131}I in flukes and the concentrations should be well correlated if flukes take plasma albumin. Unfortunately,

however ^{131}I has a short half life and it was not possible to devise a regimen to allow rafoxanide to equilibrate with the albumin in a sufficiently short time before the ^{131}I decayed.

Changes in plasma enzyme concentration due to liver damage during the course of natural and experimental fascioliasis in sheep, goats and cattle have been reported by many authors (Sewell 1967; Pinkiewicz and Madej 1967; Genchi and Locateli 1969; Thorpe 1968; Thorpe and Ford 1969; Gurlap and Weissenburg 1969; Hughes et al 1974; Anderson et al 1981; Martone et al 1983; Kumar and Pachaur 1984; Rowlands et al 1985).

Certain of these enzymes exist in high concentrations in hepatic cells and might be regarded as being good indicators of liver cell damage e.g. elevation in alkaline phosphatase (AP) concentration occurs due to lack of biliary excretion, elevation in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations due to disruption of hepatic parenchymal cells and increase in plasma concentrations of gammaglutamyl transpeptidase (γGT) due to impaired synthesis by the liver i.e. damage affecting the biliary epithelium (Kelly 1974).

The significant ($P < 0.05$) elevation in the concentration of AST, AP and γGT in plasma of all sheep seen six weeks after infestation demonstrates the usefulness of such enzymes in assessing the nature of damage occuring in liver tissues. ALT activity changes were not significant ($P > 0.05$), this enzyme occurring in very low concentrations in ruminants.

Liver flukes are considered as a haematophagous parasite and animals suffering from fascioliasis show some degree of anaemia, which is of a normocytic normochromic type (Stephenson 1947;

Jennings et al, 1955, 1956; Pearson 1963; Holmes et al 1967; Rowlands 1969; Cawdery 1976; Brown and Neva 1983).

In Group 2 sheep (treated ten weeks after infestation) the values of haemoglobin were found to be significantly lower ($P < 0.05$) than those in the control group whereas in Group 1 sheep (treated six weeks after infestation) the values were not significantly changed ($P < 0.05$) (Figure 2.3.10).

An increase in the eosinophil count as a response to the damage of liver tissues caused by migration of the flukes in liver parenchyma has also been reported in cases of fascioliasis (Kelly 1974). In all sheep, the percentage of eosinophil count (Fig. 2.3.11) was not significantly changed ($P < 0.05$).

It is interesting that there were significant changes in the plasma concentrations and AUC between the infected and non-infected animals. The differences in the plasma concentrations and AUC may be due to the use of different sheep. However, the sheep were not markedly fat or thin in either group sufficient to explain such a large difference. In fact, one of the sheep in the infected group (No.90) was used in the previous experiment (as non-infected). The plasma concentrations were found to be significantly higher ($P < 0.05$) when the animal was infected.

However, by inspection of the plasma concentrations of rafoxanide/time profile, it can be concluded that liver fluke infection did not alter the overall shape of the plasma concentration/time curve of rafoxanide in sheep. The reason for higher concentrations in the infected group may be due to the fluke infection causing a reduced rate of hepatic metabolism.

However, this might be expected to evidence itself as a more prolonged elimination rather than higher maximum concentrations. The infected group will be relatively deficient in plasma protein but this should lead to reduced rather than higher concentrations (the analytical method measures free and protein-bound drug). Alternatively better absorption may be occurring. Infection, leading to inappetance, may be prolonging the gut passage time allowing a longer time for absorption.

Rumen by-pass has been shown to affect the anthelmintic activity of benzimidazole drugs against resistant strains of important species of gastrointestinal nematodes in sheep (Kelly et al, 1977). Clioxanide, another member of salicylanilide fasciolicides. has been shown to be less effective against liver flukes and H. contortus when given intraabomasally (Boray and Roseby 1969; Symons and Roseby 1969; Campbell and Brotowidjoyo 1975), whereas rumen by-pass did not affect the anthelmintic activity of rafoxanide in sheep (Campbell and Brotowidjoyo 1975). However using an efficacy experiment for this purpose is a rather crude way of investigation since individual variation in efficacy is large.

The plasma rafoxanide concentrations/time curve shown by both infected groups, shows no evidence that rumen by-pass had occurred since rafoxanide was detected in only low concentrations in the earlier samples (six hours after dosing). Absorption from the abomasum is relatively faster than that from the rumen. Therefore if rumen by-pass had occurred a bi-phasic pattern of absorption would be seen i.e. the drug would be detected in higher concentrations in samples earlier than three days.

Residues of rafoxanide obtained in this trial are in agreement with those found in non-infected sheep (see Section 2.5.3). Rafoxanide was not detected at the limit of detection (0.10 $\mu g.ml^{-1}$) in any bile sample obtained from the sheep at slaughter. This result further confirms the previous suggestion that the concentration of rafoxanide around the mature flukes inhabiting bile ducts is probably not the way by which this anthelmintic reaches the parasite but that rafoxanide bound to plasma proteins is being ingested by the flukes in liver and bile ducts as flukes in bile ducts feed mainly on blood rather than on tissues (Rowlands 1969; Jennings et al 1956; Pearson 1963).

Livers of sheep in Group 1 and 2 contained a mean concentration of 0.1 and 0.3 $\mu g.g^{-1}$ of rafoxanide respectively. In all muscle samples no rafoxanide was detected at the limit of detection (0.10 $\mu g.g^{-1}$) which is also similar to those findings in the previous experiment.

FIGURE 2.3.9

FLUKES RECOVERED AT AUTOPSY (14 WEEKS AFTER INFESTATION WITH 450 Fasciola hepatica METACERCARIAE FROM A : SHEEP INFESTED AND UNTREATED, B : SHEEP TREATED WITH RAFOXANIDE AT 7.5 $mg.kg^{-1}$ 6 WEEKS AFTER INFESTATION, C : SHEEP TREATED AT 2.5 $mg.kg^{-1}$ AFTER 10 WEEKS OF INFESTATION.

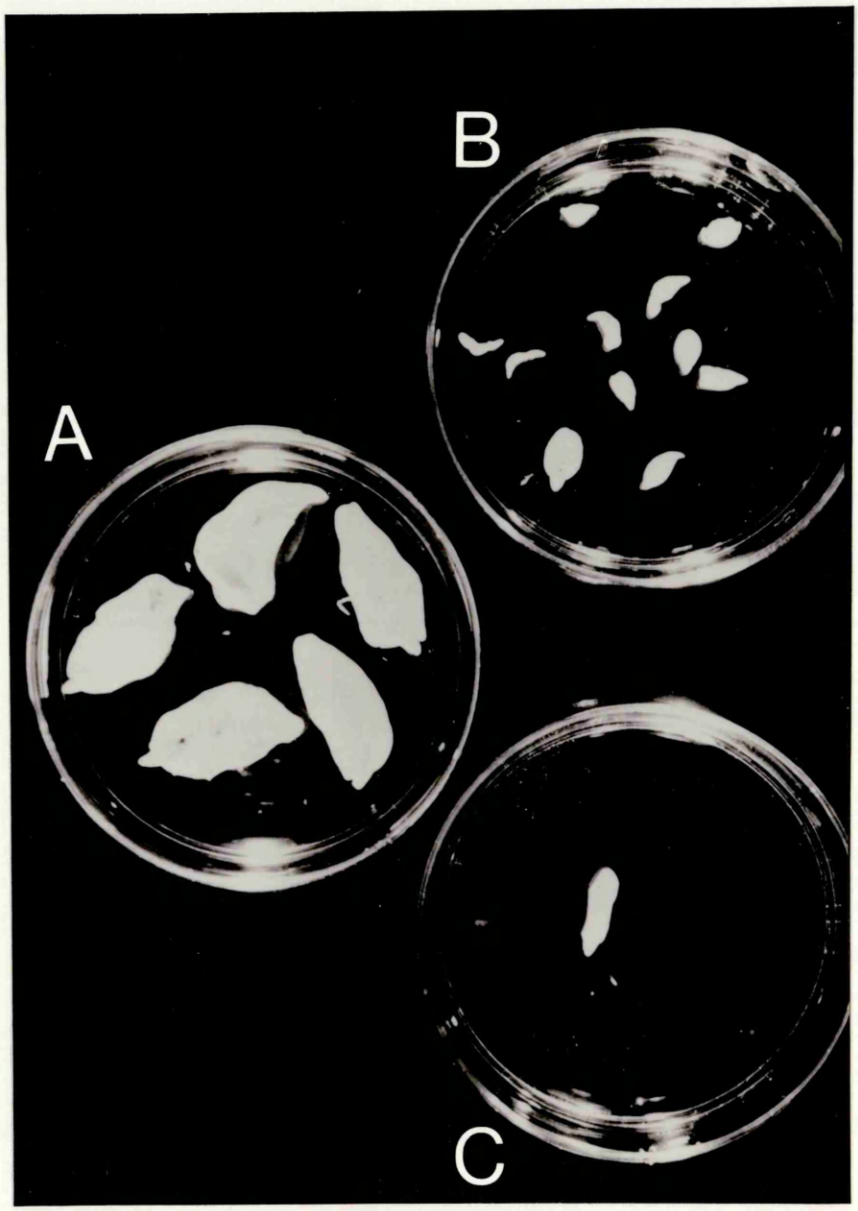

B
A
C

FIGURE 2.3.10

MEAN VALUES OF HAEMOGLOBIN ($g.dl^{-1}$) MEASURED IN SHEEP IN GROUP 1 (n=3) TREATED SIX WEEKS AFTER INFESTATION WITH *Fasciola hepatica* METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND IN GROUP 2 (n=3) TREATED AFTER 10 WEEKS OF INFESTATION AT A DOSE RATE OF 2.5 $mg.kg^{-1}$ AND IN GROUP 3 (n=3) INFECTED AND UNTREATED.

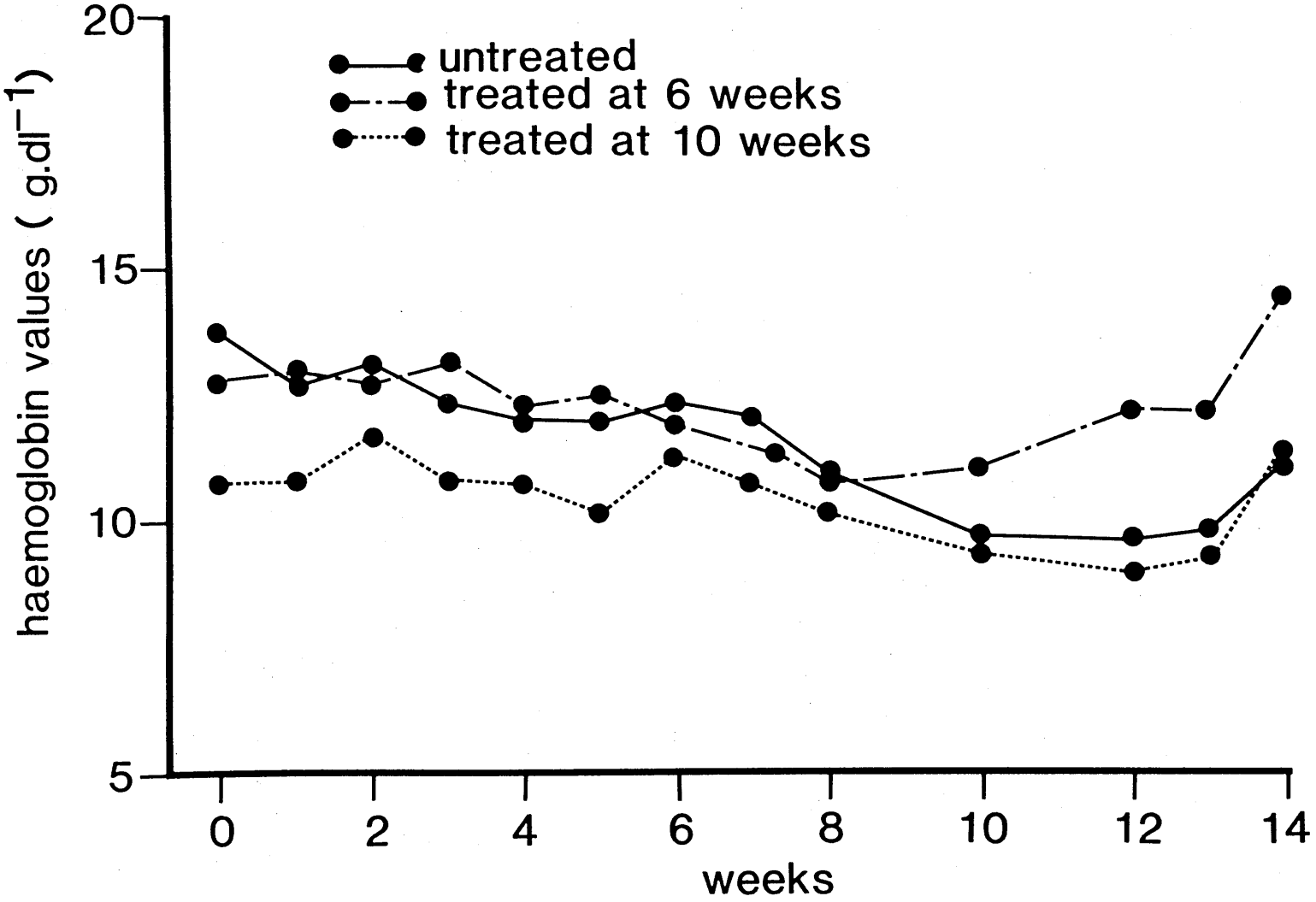
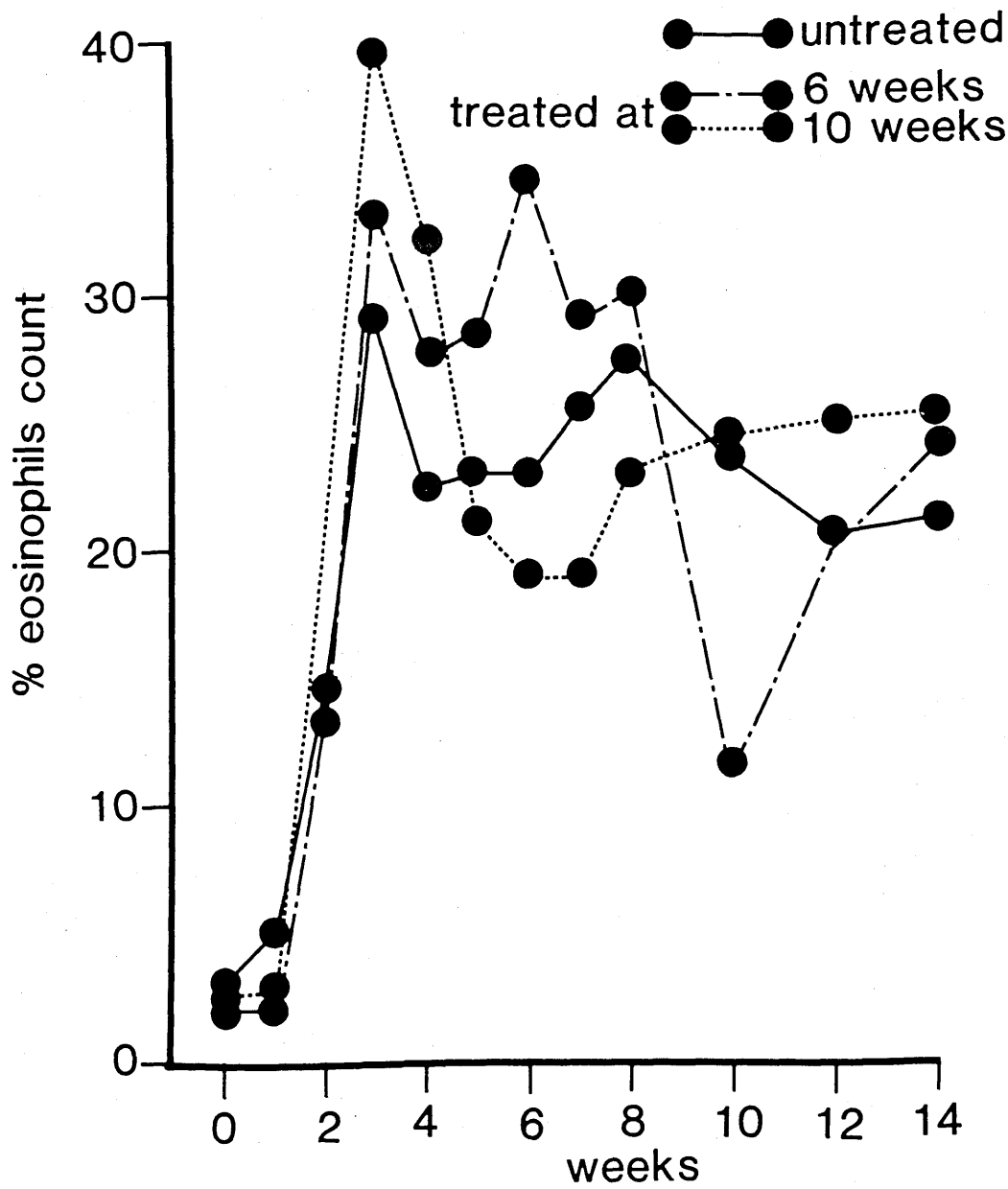

untreated
treated at 6 weeks
treated at 10 weeks
haemoglobin values (g.dl^{-1})
20
15
10
5
0
2
4
6
8
10
12
14
weeks

FIGURE 2.3.11

MEAN EOSINOPHILIC COUNT (%) OBTAINED IN SHEEP IN GROUP 1 (n=3) TREATED SIX WEEKS AFTER INFESTATION WITH Fasciola hepatica METACERCARIAE AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ AND IN GROUP 2 (n=3) TREATED AFTER 10 WEEKS OF INFESTATION AT A DOSE RATE RATE OF 2.5 $mg.kg^{-1}$ AND IN GROUP 3 (n=3) INFECTED AND UNTREATED.


untreated
treated at
6 weeks
10 weeks
% eosinophils count
40
30
20
10
0
0
2
4
6
8
10
12
14
weeks

CHAPTER 4

PHARMACOKINETIC STUDIES OF CLOSANTEL IN SHEEP

4. Pharmacokinetic Studies of Closantel in Sheep

4.1 Introduction

4.1.1 Efficacy

Closantel : N-{5-chloro-4-[(4-chloro-phenyl) cyanomethyl]-2-methyl phenyl}-2-chlroxy-3, 5-diiodobenzamide, is a new injectable and orally active salicylanilide anthelmintic discovered by Janssen and Sipido (1977). The structure of closantel is shown in Fig. 2.4.1. Closantel has been shown to be efficacous against important species of nematodes of sheep and cattle e.g. H. contortus (Van den Bossche et al 1979; Hall et al 1980, 1981; Boisvenue et al 1983), mature and immature forms of Fasciola hepatica and Fasciola gigantica in sheep and cattle (Van den Bossche et al 1979; Dobbins and Wellington 1982), bovine and ovine arthropods, Dermatobia hominis, Hypoderma spp and Oestrus bovis (Van den Bossche et al 1979; Chaia et al 1981); Canine hookworm Ancylostoma caninum (Guerrero et al 1982) and against the larval stage of the tapeworm Taenia pisiformis in rabbits (Chevis et al 1980). Closantel has been shown to be active against adult and migrating larvae of Strongylus vulgaris in horses (Guerrero et al 1983).

4.1.2. Mode of Action

The mechanism of action of closantel against F. hepatica has been described as disruption of phosphorylation of mitochondria which occurs both in vivo and in vitro (Van den Bossche et al 1979; Kane et al 1980). Closantel did not uncouple phosphorylation in rat heart and liver mitochondria in vivo when rats were injected intramuscularly with closantel at doses of 5 $mg.kg^{-1}$ (Van den Bossche et al 1979).

In flukes incubated *in vitro* and flukes recovered from treated sheep, closantel diminished ATP synthesis, increased carbohydrate mobilisation and increased end product formation especially of succinate (Kane *et al* 1980). Such results are consistent with the hypothesis of the uncoupling activity of most salicylanilides (Van den Bossche 1976) and thus a similar mechanism of activity may also be responsible for the varied activity of closantel against some nematode and arthropod species. Evidence of an anticholinestrase action of closantel was shown in dogs (Holenweger and Taroco 1982). It is not clear if this action is important in the activity of closantel against parasites.

4.1.3. *Safety to Host*

Closantel administered orally or intramuscularly at 5 and 10 $mg.kg^{-1}$ of bodyweight appeared to be well tolerated by sheep (Hall *et al* 1980, 1981). No undesirable signs were shown in one month old foals treated orally five times at two month intervals with closantel at doses of 5, 10, 20 and 40 $mg.kg^{-1}$ (Guerrero *et al* 1983).

The injectable formulation of closantel was shown to be safe in dogs injected intramuscularly at doses up to 20 $mg.kg^{-1}$ of bodyweight (Guerrero *et al* 1982) and in calves at 2.5, 8.0 and 12.5 $mg.kg^{-1}$ (Dobbins and Wellington 1982; Chaia *et al* 1981).

4.1.4 *Pharmacokinetics and Objective*

There are few published data on the anthelmintic activity of closantel as the drug has only recently been marketed (not in the United Kingdom) and no studies have been published concerning the

pharmacokinetic behaviour of closantel in sheep. Therefore it was interesting to investigate the pharmacokinetics of closantel in sheep with a special emphasis on the plasma protein binding and tissue residues.

4.2 Determination of Closantel in Plasma

There is currently no published method for the determination of closantel in body fluids. However it was found that the same method previously developed for the extraction of rafoxanide into di-ethyl ether was also applicable to closantel and thus this method was used for the determination of closantel in plasma samples.

4.2.1 Materials and Methods

4.2.1.1 Procedure of Analysis

Closantel was extracted from plasma samples into di-ethyl ether and determined by an Hplc method as described in Chapter 2, Section 2.2.2.3 using U.V. detection.

Residues were dissolved in 100 µl methanol and injected onto the Hplc column. The limit of detection of the method was 0.10 $\mu g.ml^{-1}$. Closantel was detected at a wave length of 254 nm. Using the Hplc conditions described in Section 2.2.1.2 closantel had a retention time of 3.85 minutes.

4.2.1.2 Preparation of Standards and Recoveries

A stock solution of closantel was made up in methanol as described in Chapter 2, Section 2.2.1.4.

It was found that closantel decomposes in solution when exposed to light so all samples containing closantel were protected from light by using brown glass tubes and wrapping the tubes with aluminum foil.

Spikes containing 2, 5, 10, 14 and 20 $\mu g.ml^{-1}$ of closantel in plasma were prepared by adding known amounts of the drug. These were taken through the procedure of analysis together with the actual samples.

Table 2.4.1 shows percentage recoveries of closantel from the spiked plasma.

Calculation of the percentage recoveries in the spikes and concentration of the drug in the samples was performed using the equations described in Chapter 1, Section 1.2.1.4.

4.2.2 Experiments with Closantel

4.2.2.1 Animals and Drug Administration

Five sheep of different breed and sex were used in this experiment. The sheep were dosed orally with closantel at a dose rate of 7.5 $mg.kg^{-1}$ of bodyweight. The suspension was a ready formulation for oral administration at a concentration of 15% w/v.

Table 2.4.2. shows individual sheep weight and volume of closantel administered orally to each sheep.

4.2.2.2 Samples for Analysis

Blood samples were withdrawn from the jugular vein into heparinised syringes (Monovette, Sarstedt) using 20 gauge 1" needles, immediately before the administration of the drug (pre sample) and at 1, 2, 3, 6, 8, 10, 14, 17, 21, 24, 28, 31, 35, 49, 70 and 91 days after the administration. On the outset a sampling period of 28 days was used but early analysis of the samples indicated that sampling should be continued.

4.2.2.3 Treatment of the Samples

Treatment of blood samples was as described in Section 2.2.2.4. All samples were wrapped with aluminium foil and deep frozen at -20°C until analysed.

4.3 Binding of Closantel to Plasma Proteins

The extent of binding of closantel to plasma proteins was determined in vitro using an ultra free drug filter (Amicon centricon microcentrator) (Fig.2.1.2).

Drug-free plasma containing 10 and 20 $\mu g.ml^{-1}$ closantel was introduced into the filter to which a reservoir cap was connected. The filter was then centrifuged using a fixed Angle (MSE) centrifuge at 5000 x g for two hours. The concentration of the free closantel in the filtrates was determined by a high performance liquid chromatography as described in Chapter 2, Section 2.2.1 and the percentage bound was calculated as described in Chapter 1, Section 1.4.1.

4.4. Determination of Closantel in Bile and Tissue

Residues of closantel were determined in bile and tissues of three sheep dosed orally with closantel in an experimental preparation (10% w/v) at a dose rate of 7.5 $mg.kg^{-1}$.

The sheep were slaughtered 1, 7 and 28 days after the administration. Table 2.4.3 shows the dose of closantel administered to each sheep.

Before slaughter blood samples were drawn from the animals and the plasma was separated and deep frozen until analysed.

Bile and pieces of liver and gluteal muscle were taken at slaughter and kept at -20°C until analysed.

4.4.1 Materials and Methods

An Hplc method was used for the determination of closantel in bile, liver and muscle as described in Chapter 2, Section 2.4.3. Duplicate samples were taken through the extraction procedure and each sample was analysed for the amount of closantel present. Blank bile, liver and muscle was carried through the extraction procedure. The method was reproducible, accurate and sensitive to 0.10 $\mu g.ml^{-1}$ and 0.10 $\mu g.g^{-1}$ and it was convenient for rapid routine determination. Table 2.4.4 shows recoveries of closantel in bile and tissues. Plasma samples were analysed as described in Chapter 2 Section 2.2.1.

TABLE 2.4.1

PERCENTAGE RECOVERIES OF CLOSANTEL IN SHEEP PLASMA

Closantel added to blank plasma (µg)	Closantel measured (µg)			Mean	± S.E.M.	Ratio Assayed to added
2	1.6	1.7	1.6	1.6	± 0.10	0.80
5	3.7	3.8	4.0	3.8	± 0.10	0.76
10	7.8	9.0	8.6	8.5	± 0.30	0.85
14	11.8	10.5	11.0	11.0	± 0.30	0.79
20	17.0	17.8	16.8	17.0	± 0.30	0.85

TABLE 2.4.2

THE DOSE OF CLOSANTEL ADMINISTERED ORALLY TO EACH OF THE FIVE SHEEP

Sheep No.	Weight (kg)	Dose rate ($mg.kg^{-1}$)	Volume administered (ml)
818	41	7.5	2.05
598	38	7.5	1.90
435	41	7.5	2.05
XNT	31	7.5	1.55
430	39	7.5	1.95

TABLE 2.4.3

DOSE OF CLOSANTEL ADMINISTERED TO THE THREE SHEEP

Sheep No.	Weight (kg)	Dose rate ($mg.kg^{-1}$)	Volume (ml)	Days from administration to slaughter
106	64	7.5	4.80	1
105	62	7.5	4.65	7
111	60	7.5	4.50	28

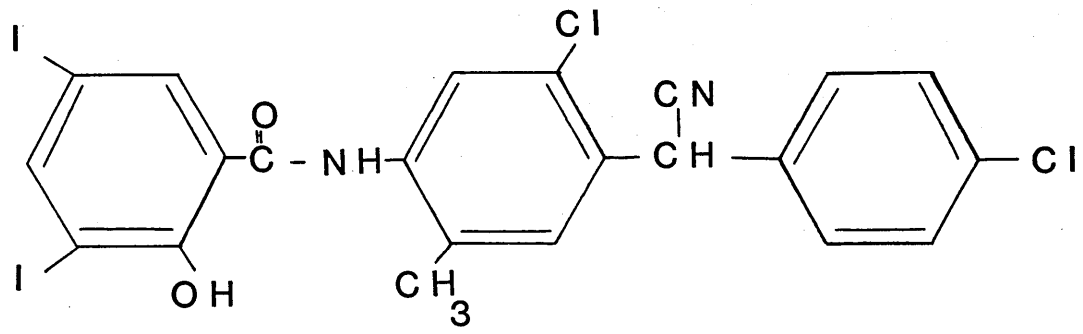
TABLE 2.4.4

RECOVERIES OF CLOSANTEL IN BILE, LIVER AND MUSCLE

Amount drug added (µg)		Amount drug measured (µg)	Ratio of Assayed to Added
		liver and muscle	
0.2	n = 10	0.2	1.00
0.5		0.4	0.80
0.8		0.7	0.88
1.0		0.9	0.90
2.0		1.9	0.95
		Bile	
0.4	n = 5	0.3	0.75
0.6		0.5	0.83
1.0		0.8	0.80

FIGURE 2.4.1

STRUCTURE OF CLOSANTEL


I
I
O H
O
C
N H
C l
C H 3
C N
C H
C l

4.5 Results

4.5.1 Determination of Closantel in Plasma

The mean plasma concentration/time profile of closantel obtained after the oral administration is represented in Fig. 2.2.2. Table 2.4.5. presents plasma concentrations of closantel in each sheep and the mean concentration ± S.E.M.

The individual and the mean plasma concentration of closantel expressed as a function of time was modelled using an iterative least square method (Sedman and Wagner 1976). The data was found to be well fitted by a tri-exponential term. The reciprocal of the observed plasma concentration was applied as a weighting factor and an improvement in the goodness of fit was described by a tri-exponential model rather than by a bi-exponential model (Table 2.4.6).

After oral administration of closantel, the drug was absorbed to obtain a maximum concentration of 45 $\mu g.ml^{-1}$ at two days post administration, thereafter the plasma concentration declined triexponentially to a mean of 0.9 $\mu g.ml^{-1}$ at 91 days after dosing.

Individual values of AUC and half life in the "distribution" and "elimination" phases were obtained by computing the experimental data (Table 2.4.7). A mean elimination half life (t 1/2 γ) and AUC were found to be 14.5 ± 2.3 days and 1035.4 ± 212.4 $\mu g.ml^{-1}$.day respectively.

4.5.2 Binding of Closantel to Plasma Proteins

Closantel (molecular weight 663.07) was not detected in ultrafiltrates of plasma containing different concentrations of

the drug. Since the highest concentration of closantel used was 20 $\mu g.ml^{-1}$ and the limit of detection of the method is 0.10 $\mu g.ml^{-1}$ therefore using the equation described in Chapter 1 Section 1.4.1 it would appear that closantel bound > 99% to plasma protein.

4.5.3 Determination of Closantel in Bile and Tissues

The concentrations of closantel in sheep plasma, bile, liver and muscle, slaughtered at different times after the oral administration of closantel at a dose rate of 7.5 $mg.kg^{-1}$ of bodyweight is shown in Table 2.4.8.

The concentrations of closantel were considerably higher in the plasma, liver and muscle samples of sheep slaughtered seven days after administration than those obtained in the sheep slaughtered one day after dosing.

Closantel was detected in plasma, liver and muscle samples 28 days post-administration while in bile, closantel was not detected at that time at the limit of detection of the method (0.10 $\mu g.ml^{-1}$).

TABLE 2.4.5

PLASMA CONCENTRATIONS ($\mu g.ml^{-1}$) OF CLOSANTEL AFTER ADMINISTRATION OF AN ORAL SUSPENSION (15% W/V) AT A DOSE RATE OF 7.5 $mg.kg^{-1}$ BODYWEIGHT TO FIVE SHEEP.

Time (days)	Sheep no. 818	XNT	435	598	430	Mean ± S.E.M.
Baseline	0	0	0	0	0	
1	48.2	40.0	35.3	64.0	34.0	44.3 ± 5.5
2	39.0	42.0	46.0	60.0	37.0	45.0 ± 4.0
3	43.0	36.0	49.0	45.0	33.0	41.2 ± 3.0
6	43.0	35.0	33.0	47.0	31.0	38.0 ± 3.0
8	36.0	33.0	27.0	-	24.0	30.0 ± 2.5
10	35.0	31.0	24.0	35.0	21.0	29.0 ± 2.8
14	29.0	24.0	19.0	36.0	15.0	25.0 ± 3.7
17	25.0	23.0	13.0	33.0	11.0	21.0 ± 4.0
21	21.0	18.0	11.0	28.0	12.0	18.0 ± 3.0
24	18.0	17.0	8.0	24.0	9.0	15.2 ± 3.0
28	15.0	12.0	5.0	22.0	6.0	12.0 ± 3.0
31	14.0	12.0	4.0	21.0	5.0	11.2 ± 3.0
35	14.0	10.0	2.0	18.0	4.0	9.6 ± 3.0
49	8.0	5.0	0.4	13.0	1.7	5.6 ± 2.3
70	4.0	2.0	0.2	5.0	0.2	2.3 ± 1.0
91	1.4	0.5	0.2	2.6	0.0	0.9 ± 0.5

TABLE 2.4.6

A COMPARISON OF THE COMPUTER GENERATED VALUES OF R-SQUARE BETWEEN TWO AND THREE COMPARTMENT MODEL FOLLOWING ORAL ADMINISTRATION OF CLOSANTEL.

Animal number	Type of compartment	Values of R-square
430	2	0.97845
	3	0.98991
435	2	0.92432
	3	0.99186
598	2	0.80319
	3	0.97243
818	2	0.95200
	3	0.97736
XNT	2	0.98498
	3	0.99542

TABLE 2.4.7.

VALUES OF HALF LIFE IN "ABSORPTION", "DISTRIBUTION" AND "ELIMINATION" PHASE (DAYS) AND AREA UNDER PLASMA CONCENTRATION TIME CURVE (AUC $\mu g.ml^{-1}$. days) OF CLOSANTEL DETERMINED BY A TRIEXPONENTIAL MODEL IN SHEEP AFTER ORAL ADMINISTRATION OF 7.5 $mg.kg^{-1}$.

Animal No.	t 1/2 (α) day	t 1/2 (β) day	t 1/2 (γ) day	AUC $\mu g.ml^{-1}$.days
430	0.30	4.60	9.30	571.80
435	0.60	4.00	10.50	630.00
598	0.20	1.60	20.70	1704.00
818	0.13	6.80	18.80	1285.00
XNT	0.20	7.00	13.00	986.00
Mean	0.30	4.80	14.50	1035.40
$\pm$ S.E.M.	$\pm$ 0.08	$\pm$ 1.00	$\pm$ 2.30	$\pm$ 212.40

TABLE 2.4.8

CONCENTRATIONS ($\mu g.ml^{-1}$ or $\mu g.g^{-1}$) OF CLOSANTEL IN SHEEP PLASMA, BILE, LIVER AND MUSCLE AFTER ADMINISTRATION OF CLOSANTEL AT A DOSE RATE OF 7.5 $mg.kg^{-1}$.

Sheep No.	Time from dosing to slaughter (days)	Concentration of closantel $\mu g.ml^{-1}$		$\mu g.g^{-1}$	
		Plasma	Bile	Liver	Muscle
106	1	28.3	0.7	1.7*	0.7*
105	7	35.7	0.5	2.3*	1.0*
111	28	18	ND	1.2*	0.4*

* Each value represents the mean of 2.

ND None detected.

4.6 Discussion

Closantel, like the other salicylanilides, was found to be highly bound to plasma proteins (> 99%). The high efficacy of many salicylanilides and substituted phenols against blood sucking parasites such as H. contortus and hookworm may be related to their attachment to plasma protein. They probably are released to kill the parasite after ingestion of the blood. For example, in an efficacy study, a single administration of closantel had sufficient residual effect to prevent the development of infective Dermatobia hominis larvae in calves during a period of three weeks (Chaia et al 1981) and of H. contortus up to 60 days after administration in sheep (Hall et al 1981). Disophenol, which is a fasciolicide used in ruminants, has been shown to have a prolonged period of protection against the establishment of adult H. contortus in sheep while the drug had no apparent effect on the early fourth stage larvae in arrested development as those stages are not blood suckers (Gordon 1974; Sinclair and Prichard 1975).

Following the oral administration, the rate of absorption of closantel was relatively slow and the maximum plasma concentration occurred within 1 - 2 days after dosing (Fig.2.2.2). This slow absorption of drugs seems to be a characteristic feature in ruminants which is due to mixing of the drug with the large ruminal content and then progressive delivery of the drug to the absorption sites in the gastrointestinal tract distal to the rumen. The low solubility of closantel in the aqueous phase probably also contributes to the slow absorption

phase.

On the basis of the data presented (Table 2.4.7) a two phase elimination could be distinguished. A relatively rapid initial phase (t 1/2 (β) = 4.8 $\pm$ 1.0 days) and a slower terminal phase (t 1/2 (γ) = 14.5 $\pm$ 2.3 days).

The rapid elimination phase is usually considered as a "distribution" phase where drug is equilibrated with tissues as well as being metabolised and excreted. The terminal phase is equivalent to the rate of metabolism and excretion. Nevertheless in the case of closantel it would be surprising if the "distribution" phase takes such a long time to occur since, for most drugs, this phase lasts only a few minutes to hours.

It is possible to consider that initially after absorption binding to plasma protein is less strong (possibly ionic bonding) and that the initial phase is due to distribution of the rapidly dissociated free drug from blood to the peripheral compartment and the slower phase due to utilisation and destruction of the plasma protein along with strongly associated drugs. Also other evidence against the initial elimination phase being a "distribution" phase is from the low value of distribution of closantel Vd (area) = 155.4 $ml.kg^{-1}$ which indicates that only a small proportion of closantel was distributed to extravascular tissues. This finding is further supported by the low concentration of closantel found in the bile, liver and muscle samples (Table 2.4.8) obtained from animals at different intervals after drug administration, although there was considerable animal variation.

Furthermore, the low levels of closantel in bile compared with those in plasma (Table 2.4.8) is further evidence which confirms that ingestion of blood by F. hepatica provides the most likely pathway by which closantel and most other salicylanilides are presented to the parasite rather than via the biliary concentration. Moreover the binding of closantel to plasma proteins considerably prolongs the residence time of the drug in the bloodstream resulting in a residual effect which also explains the prolonged activity of closantel against blood sucking ecto- and endo-parasites.

CHAPTER 5

PHARMACOKINETIC STUDIES OF OXYCLOZANIDE IN SHEEP

5. Pharmacokinetic studies of oxyclozanide in sheep

5.1 Introduction

5.1.1 Efficacy

A number of drugs have been available for the treatment of fascioliasis in cattle and sheep since the 1920s. The drugs were mostly chlorinated hydrocarbons (carbon tetrachloride, hexachloroethane) and they have proved to be efficacious against adult flukes in the bile duct (Cawdery 1972; Cawdery and Donnelly 1972) but they are all liable to produce unpredictable toxic effects with occasional deaths and require certain precautions during their use (Kendall and Parfitt 1962; Boray et al 1967; Boray and Happich 1968).

In 1966 Imperial Chemical Industries discovered a new drug oxyclozanide, which, although its chemical structure has similarities to the chlorinated hydrocarbons, it belongs to the group of salicylanilides, a structural grouping which appears to possess specific flukicidal activity (see rafoxanide and closantel).

Oxyclozanide (Zanil) is a white crystalline solid substance with the chemical name of 3,3′,5,5′,6-penta-chloro-2′-hydroxysalicylanilide. The chemical formula is shown in Figure 2.5.1.

It is virtually insoluble in water but moderately soluble in the common organic solvents. Oxyclozanide is formulated as an aqueous suspension of 10 μ particle size for oral treatment of sheep and cattle at a therapeutic dose rate of 15 and 10-15 $mg.kg^{-1}$ respectively.

At the recommended dose rate (15 $mg.kg^{-1}$) oxyclozanide was

found to be > 95% effective against adult Fasciola hepatica and Fasciola gigantica in sheep, goats and cattle (Walley 1966; 1970; Boray et al 1967; Boray and Happich 1968; Froyd 1969; Harrow 1969; Cotteleer and Fameree 1971; Campbell and Narelle 1972; Kadhim et al 1973; Foreyt and Todd 1973).

The activity of oxyclozanide against adult liver fluke is approximately equal to that of the previously introduced fasciolicide carbon tetrachloride, hexachloroethane and hexachlorophene but its therapeutic safety margin exceeds that of the other drugs (Boray et al 1967; Boray and Happich 1968; Armour 1983).

Like the other halogenated hydrocarbons and salicylanilide fasciolicides, efficacy of oxyclozanide against the flukes increases with increasing age of the flukes. Although, in vitro, experiments showed that both adult and immature fluke, investigated under the same conditions, were susceptible to the action of oxyclozanide, in vivo the dose rates had to be increased approximately to its maximum tolerated dose to achieve high activity against immature flukes (Broome and Jones 1966; Boray et al 1967; Boray and Happich 1968). This resistance of the immature fluke to oxyclozanide was thought to be due to protein binding of the compound in blood which bathes the immature flukes in the liver parenchyma (Broome and Jones 1966).

5.1.2 Mode of action

It is well known that the substituted phenols and salicylanilides are potent uncouplers of oxidative phosphorylation (Williamson and Metcalf 1967) and symptoms of

uncoupling of the host metabolism have been shown for several fasciolicides (Van miert and Groeneveld 1969; Metzger and Duwel 1973; Duwel and Metzger 1973; Druckrey and Metzger 1973; Van den Bossche 1976). This is the reason for using the nitrophenols as anti-obesity drugs in man.

Oxyclozanide has been shown to uncouple rat liver mitochondria and stimulate oxygen uptake by the intact fluke (Corbett and Goose 1971a,b) in isolated Hymenolepis diminuta mitochondria (Yorke and Turton 1974). Such activities are considered to be due to the uncoupling of oxidative phosphorylation.

Succinate dehydrogenase complex in liver fluke has also been identified as a possible site of action of oxyclozanide. Within a few hours oxyclozanide inhibited succinic dehydrogenase, decreased ATP and increased pyruvate concentration of liver fluke (Metzger and Duwel 1973). Similar results were observed by Schacht et al (1971) from the application of another salicylanilide, resorantel (4-bromo-2,6-dihydroxy-benzanilide) in Hymenolepis diminuta.

All of these activities are secondary consequences of uncoupling of oxidative phosphorylation.

5.1.3 Safety to host

For curative treatment of adult F. hepatica infections, especially in cattle, oxyclozanide has proved to be successful and with a reasonable safety index at the lowest dose rates which are highly effective against flukes in the bile duct (Walley 1966; 1970; Jones 1966; Kelsey 1966; Vaughan 1966; Boray et al 1967; Boray and Happich 1968; Froyd 1969; Oakley et al 1984).

In rats the oral LD_{50} is 1000 $mg.kg^{-1}$ (Broome and Jones 1966). In sheep Boray and Happich (1968) and Walley (1970) found a safety index of 4. In cattle the drug was shown to be safe in doses up to 30 $mg.kg^{-1}$ of bodyweight (Boray et al 1967, Boray and Happich 1968).

Sheep, goats and cattle tolerated single and repeated administrations of oxyclozanide at 10-20 $mg.kg^{-1}$ (Jones 1966; Vaughan 1966; Walley 1966; 1970; O'Brien 1970).

Pregnant ewes and cows treated at doses up to 20 $mg.kg^{-1}$, on one or many occasions, showed no adverse effect during the gestation period and all gave birth to normal offspring (Vaughan 1966; Walley 1970). Lactating ewes dosed, when their lambs were 1-10 weeks old, with oxyclozanide at 15 $mg.kg^{-1}$ showed no reduction in milk yield or in liveweight gain of their lambs (Walley 1970). Milking cows receiving the therapeutic dose rate of 15 $mg.kg^{-1}$ showed temporary reduction in milk yield by 3-18% over 48 hours. The milk yield returned to normal by the third day after treatment.

Bulls treated at 15 $mg.kg^{-1}$ showed slight temporary loss of libido; sperm studies carried out at 24 hours, 72 hours and two weeks after treatment showed no apparent change.

Toxicity signs appeared in sheep and cattle which had received 30-40 $mg.kg^{-1}$ of oxyclozanide. The toxic symptoms were softening of the faeces, increased frequency of defecation, slight depression and inappetence. At 60 $mg.kg^{-1}$ the toxic effects increased progressively and deaths occurred up to eight days after treatment (Boray et al 1967; Boray and Happich 1968).

Post-mortem examination showed inflammation of intestine especially duodenum, the contents of the rumen and reticulum were very fluid, and some petechial haemorrhages in the trachea and endocardium.

Treatment of sheep, goats and cattle with a combination of oxyclozanide and other chemotherapeutic agents (sulphamezathine, organophosphates, oxibendazole and tetramisole) showed no adverse toxicity (Walley 1970).

The above findings indicate that oxcylozanide is a well tolerated and efficient treatment for cattle and sheep.

5.1.5 Pharmacokinetics

Little attention has been given to the pharmacokinetic behaviour of most fasciolicidal drugs while extensive studies have been carried out on their activity against adult and immature flukes and concerning their toxicity. Since the introduction of oxyclozanide there has been no published data on the kinetics of oxyclozanide in sheep and cattle.

In an efficacy study Froyd (1969) measured the concentration of oxyclozanide in cattle serum at 19 hours only after administration at a fixed dose of 10 $mg.kg^{-1}$ bodyweight. The concentrations varied from 8.9-24.7 $\mu g.ml^{-1}$.

A milk residue study in ewes showed that following administration of a single oral dose of 30 $mg.kg^{-1}$ of oxyclozanide to ewes shortly after parturition, the maximum concentrations in the range 0.27 to 1.02 $\mu g.ml^{-1}$ occurred during the 12 hours after treatment (Oakley et al 1984).

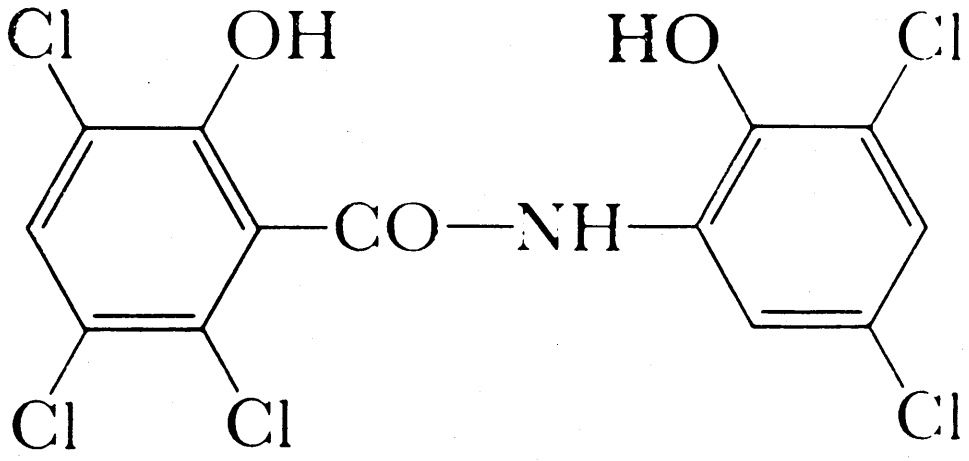
5.1.6 Objective

Oxyclozanide is one of the major fasciolicides in current use in ruminants in the U.K. It is of particular use in lactating cows as there are no restrictions on the use of milk from cows treated with oxyclozanide.

Oxyclozanide, whose pharmacokinetics have not been reported either in sheep or cattle, provides an interesting comparison with closantel and rafoxanide since although all three are salicylanilides, oxyclozanide has lower efficacy against immature fluke (Boray and Happich 1968; Walley 1970). Therefore the pharmacokinetic behaviour of oxyclozanide in sheep plasma and the degree of binding of oxyclozanide to plasma proteins were investigated.

FIGURE 2.5.1

STRUCTURE OF OXYCLOZANIDE


Cl
OH
HO
Cl
CO—NH
Cl
Cl
Cl

5.2 Determination of oxyclozanide in plasma

A spectrophotometric method has been used for the determination of oxyclozanide in plasma samples (Froyd 1969). The disadvantage in using a photometric method is that of being non-specific and false results might be obtained from other interfering substances. In the method of Froyd (1969) oxyclozanide was extracted into chloroform from strongly acidified plasma and back extracted into borate buffer and the sample reacted with a colouring reagent suitable for spectrometric determination.

Oakley et al (1984) used a high performance liquid chromatographic method for the determination of oxyclozanide in milk samples. The method is based on that described by Froyd (1969).

Neither Froyd (1969) nor Oakley et al 1984 gave any evidence about the reliability of their method of analysis. The method used for rafoxanide and closantel was found to be applicable to oxyclozanide and produced reliable and satisfactory results (mean recovery 96 $\pm$ 1.6%). Thus oxyclozanide was determined in the plasma samples and plasma ultrafiltrates according to the analytical method used for the determination of rafoxanide and closantel in plasma samples as described in Chapter 2 Section 2.2.1.

5.2.1 Materials and methods

5.2.1.1 Methods of analysis

An HPLC method was used for the determination of oxyclozanide in the plasma samples as described in Chapter 2, Section 2.2.1 using di-ethyl ether extraction.

For the analysis of plasma oxyclozanide samples, the same HPLC conditions were used (Section 2.2.1.2) except that oxyclozanide was detected at a wave length of 254 nm, the mobile phase was Methanol : Ammonium carbonate (0.05 M) 70 : 30 and a flow rate of 0.5 ml.minute^{-1} was used in order to separate the oxyclozanide peak from that of the solvent. Under these conditions oxyclozanide had a retention time of 5.50 minutes.

5.2.1.2 Preparation of standards and recoveries

The preparation of standard solutions of oxyclozanide was as described in Chapter 2, Section 2.2.1.4.

Oxyclozanide was added to drug-free plasma in different concentrations and taken through the extraction procedure whenever an extraction was made.

Calculations of percentage recovery and the concentrations of oxyclozanide in plasma samples were as described in Chapter 1 Section 1.2.1.4. Table 2.5.1 shows percentage recoveries of oxyclozanide from spiked sheep plasma.

5.2.2 Experiments with oxyclozanide

5.2.2.1 Animals and drug administration

Five parasite-free Finn-Dorset lambs aged between six and eight months, were used in the experiment. They were housed in stalls bedded with straw and fed hay and concentrates. Water was available ad libitum.

The lambs were dosed orally with oxyclozanide (Zanil Drench fine suspension PML 3.4% w/v) at the recommended dose rate of 15 mg.kg^{-1} bodyweight. The suspension was administered using a syringe placed on the back of the lamb's tongue.

Animal weight and volume of drug administered is shown in Table 2.5.2.

5.2.2.2 Sampling regimen

Blood samples were withdrawn by venepuncture into 10 ml heparinised syringes (Monovette, Sarstedt) just before dosing the lambs and at 1, 2, 3, 6, 7, 8, 10, 14, 17, 21, 24 and 28 days after dosing.

The plasma was obtained as described in Chapter 2, Section 2.2.2.4 and deep frozen at -20°C until analysed.

5.3 Binding of oxyclozanide to plasma proteins

In vitro binding of oxyclozanide to sheep plasma proteins was tested. Oxyclozanide was added in known amounts (5 and 10 $\mu g.ml^{-1}$) to drug-free plasma and filtered through an Amicon microconcentrator which had a molecular weight cut off 10,000. The concentrations of free drug were determined in the plasma ultrafiltrates using the analytical method described in Chapter 2 Section 2.2.1.

TABLE 2.5.1

PERCENTAGE RECOVERIES OF OXYCLOZANIDE FROM SHEEP PLASMA

Oxyclozanide added to drug free plasma (µg)	Oxyclozanide measured (µg)			Mean	± S.E.M.	Ratio assayed to added
0.2	0.2	0.2	0.2	0.2	± 0	1.00
0.6	0.5	0.6	0.6	0.6	± 0.02	1.00
2.0	1.8	1.9	1.9	1.9	± 0.02	0.95
10.0	8.5	9.6	9.7	9.3	± 0.30	0.93
20.0	18.2	19.0	18.2	18.5	± 0.30	0.93

TABLE 2.5.2

DOSE OF OXYCLOZANIDE ADMINISTERED TO FIVE LAMBS

Animal number	Weight (kg)	Dose rate ($mg.kg^{-1}$)	Volume administered (ml)
49	39	15	17.20
77	38	15	16.76
80	34	15	15.00
85	33	15	14.56
88	37	15	16.32

5.4 Results

5.4.1 Determination of oxyclozanide in plasma

Plasma concentrations of oxyclozanide in each sheep and the mean ± S.E.M. concentration at each sample time are shown in Table 2.5.3.

A maximum mean plasma concentration of 18.0 $\mu g.ml^{-1}$ occurred at the first sampling time (24 hrs) post administration followed by a rapid decline to a mean concentration of 3.6 $\mu g.ml^{-1}$ within 72 hours after dosing. Thereafter the decline was slower and oxyclozanide was not detected at the limit of detection of the method (0.10 $\mu g.ml^{-1}$) 21 days post dosing (Figure 2.2.2).

For each sheep, the pharmacokinetic analysis of oxyclozanide plasma data after oral administration was calculated using the CSTRIP computer programme of Sedman and Wagner (1976).

For each sheep and for the mean concentrations, a three-compartment curve was found to be superior to the two-compartment curve fit when the computer generated plasma oxyclozanide/time curves were compared with the experimental data.

Values of area under the curve of plasma oxyclozanide concentration verus time (AUC) from time 0 to 21 days was computed from the plasma concentration/time equation for each animal using the same method (Sedman and Wagner 1976). A mean AUC of 51.50 ± 8.20 $\mu g.ml^{-1}$. day was found.

A mean elimination half life, based on the slope of the terminal portion of the plasma oxyclozanide curve was found to be 6.40 ± 0.8 days. Mean and individual values of pharmacokinetic parameters are shown in Table 2.5.4.

5.4.2 Binding of oxyclozanide to plasma proteins

Oxyclozanide was not detected in plasma ultrafiltrates at the limit of detection of the method (0.10 $\mu g.ml^{-1}$) which demonstrates the strong binding of the drug to sheep plasma protein (> 99%).

TABLE 2.5.3

CONCENTRATIONS ($\mu g.ml^{-1}$) OF OXYCLOZANIDE IN PLASMA AFTER ORAL ADMINISTRATION OF "ZANIL" AT A DOSE RATE OF 15 $mg.kg^{-1}$.

Time (days)	Sheep no. 49	77	80	85	88	Mean	± S.E.M.
Base line	0	0	0	0	0		
1	18.0	14.0	27.0	10.0	21.0	18.0	± 3.00
2	10.0	6.0	16.0	15.0	10.0	11.4	± 1.80
3	3.0	2.0	6.0	4.0	3.0	3.6	± 0.70
6	0.2	0.6	0.4	0.4	0.4	0.4	± 0.06
7	0.2	0.4	0.4	0.4	0.4	0.4	± 0.04
8	0.1	0.3	0.2	0.3	0.2	0.2	± 0.04
10	0.1	0.2	0.1	0.3	0.2	0.2	± 0.04
14	0.1	0.2	0.1	0.2	0.2	0.2	± 0.02
17	0	0.2	0	0.1	0.1	0.1	± 0.04
21	0	0.1	0	0	0	0	± 0.01
24	0	0	0	0	0	0	0

TABLE 2.5.4

PHARMACOKINETIC CONSTANTS FOR OXYCLOZANIDE IN FIVE LAMBS AFTER ORAL ADMINISTRATION AT 15 $mg.kg^{-1}$ BODYWEIGHT.

Animal number	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	C $\mu g.ml^{-1}$	α day^{-1}	β day^{-1}	γ day^{-1}	t 1/2 (α) day	t 1/2 (β) day	t 1/2 (γ) day	AUC $\mu g.ml^{-1}.day$
49	120.70	120.40	0.30	2.00	1.30	0.09	0.30	0.60	8.00	38.40
77	76.30	75.6	0.70	2.40	1.30	0.09	0.30	0.50	7.50	33.60
80	123.80	122.70	1.10	2.0	1.00	0.20	0.40	0.70	3.70	60.80
85	170.40	169.00	0.90	3.80	1.50	0.10	0.20	0.50	5.70	78.50
88	108.40	107.80	0.60	2.30	1.20	0.10	0.30	0.60	7.10	46.30
Mean	120.00	119.00	0.70	2.50	1.30	0.10	0.30	0.60	6.40	51.50
+S.E.M.	± 15.30	± 15.10	± 0.10	± 0.30	± 0.08	± 0.02	± 0.03	± 0.04	± 0.80	± 8.20

5.5 Discussion

Following oral administration of oxyclozanide at 15 $mg.kg^{-1}$, absorption appeared to be relatively slow [mean t 1/2 (α) = 7.0 $\pm$ 0.03 h]. This slow rate of absorption from rumen is of value in providing prolonged oxyclozanide concentrations in plasma.

It was unfortunate that the blood sampling schedule was not adequate during the early period after administration to allow better kinetic analysis of the experimental data. The sampling regimen for oxyclozanide had been chosen to provide a direct comparison with the other salicylanilide fasciolicides used earlier in this study.

Oxyclozanide was eliminated rather slowly from the plasma having a mean elimination half life [t 1/2 (γ)] of 6.4 $\pm$ 0.8 days.

It is generally accepted following intravenous administration that the initial rapid decline in plasma concentration can be attributed to rapid distribution of the drug extravascularly into the highly perfused tissues, thereafter the drug will be redistributed into the less perfused tissues and then eliminated from the body whereas after oral administration absorption and distribution occurs simultaneously. Within the blood a fraction of the drug binds reversibly to plasma proteins, usually albumin, and the remainder undergoes simultaneous distribution and elimination (which is referred to as biotransformation and excretion processes).

The intermediate "distribution" phase seen here is rather difficult to explain. For most drugs a "distribution" phase is

seen where drug in plasma equilibrates with tissues and this causes a lowering of plasma concentrations. Since metabolism and excretion are usually relatively slower, the final terminal phase is usually considered to equate with elimination. However from the rafoxanide and closantel studies the concentrations of salicylanilides in tissues are known to be low, also the distribution of drugs to tissues usually occurs much more rapidly i.e. in a few minutes or hours. It is possible that there is strong and weak binding to plasma proteins; that the relatively weakly bound drug is available for metabolism and excretion and this phase accords with "distribution" while the strongly bound drug does not readily dissociate from proteins and gives a long terminal elimination.

Strong binding to plasma proteins often refers to covalent binding but, since oxyclozanide in the terminal phase remains easily extractable with ether (usually one of the criteria for distinguishing ionic and covalent binding) therefore oxyclozanide cannot be too strongly bound. In the absence of more detailed tissue analysis at each sampling time, the correct explanation of the triphasic pattern cannot be proved.

The degree of binding of oxyclozanide to plasma proteins was found to be > 99%. This binding of the drug to plasma proteins is considered to be an important factor limiting penetration of the drug into the animal's tissue and reducing drug toxicity. Although the degree of plasma protein binding of oxyclozanide was found to be similar to that of rafoxanide and closantel, this drug is considerably less active against immature fluke. The

relatively short elimination half life [t 1/2 (γ)], compared to that of rafoxanide and closantel, is presumably the reason for the inactivity of oxyclozanide toward immature flukes.

Broome and Jones (1966) had reported that oxyclozanide is metabolised in the liver to the glucuronide and that its activity against mature flukes is through this metabolite being concentrated in the bile duct where the adult flukes reside. However, they did not show any evidence that the glucuronide is active. It is known that the glucuronides are generally inactive, highly polar metabolites and are rapidly excreted from the body unless they become hydrolysed by the gut flora and enter an enterohepatic circulation (Fingl and Woodbury 1975). Thus it seems unlikely that oxyclozanide is effective through this metabolite.

The activity of oxyclozanide against mature fluke may be related to its high binding to plasma proteins. Young flukes probably ingest mainly liver cells containing less anthelmintic and as they become adult and migrate to the bile ducts they feed mainly on blood rather than on tissues (Jennings et al 1956; Dawes 1961; Pearson 1963; Brown and Neva 1983) and come into contact with the bound oxyclozanide and will be exposed to more toxic concentrations of the drug.

Oxyclozanide tissue residues were not determined in the present study. However, the high binding of the drug to plasma proteins, which acts to reduce the incorporation of the drug to animal tissues, and the small volume of distribution Vd (area) = 345.3 $ml.kg^{-1}$ indicates poor distribution into tissues.

CHAPTER 6

PHARMACOKINETIC STUDIES OF TRICLABENDAZOLE AND FENBENDAZOLE IN SHEEP

6. Pharmacokinetic Studies of Triclabendazole and Fenbendazole in Sheep

6.1 Introduction

6.1.1 Efficacy

Triclabendazole (Fasinex, Ciba-Geigy) : 6-chloro-5 (2-3-dichlorophenoxy)-2-methyl thiobenzimidazole, is a novel benzimidazole derivative recently introduced to the anthelmintic market. The drug has been shown to be a potent fasciolicide efficacious against all stages of F. hepatica in sheep from as early as one day after infection (Boray 1981; Boray et al 1981a, b, 1983; Dorchies et al 1983; Smeal and Hall 1983; Wolff et al 1983; Rapic et al 1984; Turner et al 1984) and against F. gigantica in sheep and goats (Gurlap and Tinar 1984). Efficacy of triclabendazole against F. hepatica infection in cattle was similar to those in sheep (Boray et al 1981a).

Triclabendazole has poor activity against cestodes, nematodes including the important species H. contortus and the trematodes Dicrocoelium dendriticum and Paramphistomum species (Boray et al 1983; Gurlap and Tinar 1984).

Rumen bypass has been shown not to alter the efficacy of triclabendazole and the compound was equally efficient (97.8 - 98.8%) against four-week-old fluke when administered by the oral route or by intraruminal or intraabomasal injection (Boray et al 1983).

Fenbendazole (Panacur, Hoechst) : Methyl-5-(Phenyl-thio)-2-benzimidazole carbamate introduced in 1974 (Baeder et al 1974) has proven to be a well tolerated and broad spectrum

benzimidazole anthelmintic possessing a high degree of activity against many nematode species in sheep and goats (Baeder et al 1974; Duwel et al 1975b; Kirsch and Duwel 1975; Ross 1975; Kelly et al 1975; Duwel 1977), cattle (Baeder et al 1974; Duncan et al 1977; Craig and Bell 1978; Callinan and Cummins 1979) dogs and cats (Roberson and Burke 1980, 1982).

Fenbendazole has also shown a particularly high level of activity against the developing and inhibited larval stages of Ostertagia species in sheep (McBeath et al 1977) and in cattle (Craig and Bell 1978; Duncan et al 1976; Callinan and Cummins 1979).

At recommended dose rates fenbendazole was highly effective against mature and immature stages of lungworm in sheep (Ross 1975; Kelly et al 1975; Corba et al 1979) and cattle (Saad and Rubin 1977; Downey 1980; Oakley 1980). Fenbendazole was also effective against cestodes of sheep and cattle at doses higher than the recommended dose rate (Duwel et al 1975b; Corba et al 1979). The efficacy of fenbendazole against liver fluke infection in sheep has been variable as repeated and high dose rates are required for an effective treatment against fascioliasis (Corba et al 1979; Duwel et al 1975b).

The rumen acts as a reservoir for the orally administered drug releasing it slowly to the omasum and abomasum. It is considered that there is a minimum concentration of benzimidazole necessary for anthelmintic activity (Prichard et al 1978) and also the duration of exposure to this concentration is important. Therefore the introduction of benzimidazole into the rumen will result in prolonging the time for this minimum concentration to

occur essential for activity against parasites.

In ruminants, after the oral administration of aqueous suspension part or all of the administered dose will bypass the rumen and will be available for rapid absorption in the abomasum and duodenum, resulting in lower efficacy against gastrointestinal nematodes. This might explain the low efficacy of fenbendazole against benzimidazole resistant strains of H. contortus and Trichostrongylus colubriformis in sheep when the drug was administered intra-abomasally (Kelly et al 1977).

In a pharmacokinetic study Marriner and Bogan (1980, 1981a, b) found that the kinetics of albendazole, fenbendazole and oxfendazole in sheep were not significantly altered when a proportion of the drug bypassed the rumen (demonstrated by the early appearance of the drug (within 20 minutes) in the abomasum). In cattle, Ngomuo and others (1984) found no significant differences in the area under plasma concentration/time curve for oxfendazole when the drug was given by the oral or intra-ruminal route; neither did albendazole in sheep when it was given as a paste or as a drench suspension (Marriner et al 1981).

6.1.2 Mode of Action

The mode of action of triclabendazole is not clear. The structural similarity of triclabendazole to the general benzimidazole class of drugs would suggest that the compound might act by inhibiting the fumarate reductase system in the parasite as do other benzimidazoles. The lack of activity of triclabendazole against nematodes, including H. contortus leads

to the suggestion that this compound might act similarly to the salicylanilide flukicides by uncoupling of oxidative phosphorylaction, even though, most of the salicylanilide flukicidal drugs show variable activity against H. contortus.

Fasciolicidal drugs generally belong to the salicylanilide and the related substituted nitrophenol chemical group. These drugs have been shown to be highly bound to plasma proteins (Duwel and Metzger 1973, Chapter 2). Therefore they will be delivered to the parasite through ingestion of the drug bound to the plasma proteins (mainly albumin) and this provides the most likely explanation by which these compounds exhibit their activities toward blood sucking parasites (Broome and Jones 1966; Lee 1973; Hall et al 1981; Guerrero et al 1982).

As triclabendazole is also thought to bind strongly to plasma protein (Chapter 6) it is expected that triclabendazole will be consumed similarly by H. contortus and F. hepatica and exert an equivalent activity against both parasites. Therefore the biochemical mode of action of this unusual drug needs further investigation.

Fenbendazole was found to inhibit the fumarate reductase system in the fermentation pathway of nematodes (Prichard 1970; Rehm and Bryant 1977; Rew 1978; Barrowman et al 1984b). Oxfendazole (the principal metabolite of fenbendazole), fenbendazole 2-amino sulfoxide and fenbendazole 2-amino sulfone which are known to be inactive metabolites were also shown to inhibit fumarate reductase system in Ascaris suum in vitro (Barrowman et al 1984b). The drug also appeared to possess a neurotoxic effect in Hymenolepis diminuta (Duwel 1977; Prichard

et al 1978).

Barrowman et al (1984a) demonstrated that fenbendazole and oxfendazole bind to Ascaris suum tubulin thereby inhibiting the polymerisation of tubulin into microtubules.

6.1.3 Safety to Host

In sheep and goat, triclabendazole given at the recommended dose rate of 10 $mg.kg^{-1}$ has been shown to be a well tolerated and safe drug (Wolff et al 1983).

The maximum tolerated dose was found to be 200 $mg.kg^{-1}$, thus having a safety index of 20 when given at 10 $mg.kg^{-1}$ for the treatment of immature flukes and of 40 when given at 5 $mg.kg^{-1}$ for the treatment of adult flukes in sheep (Boray et al 1981a; 1983). Similar observations were found in cattle (Boray et al 1981a,b). No toxicological signs were observed in sheep which received triclabendazole at 150 $mg.kg^{-1}$ concurrently with albendazole, levamisole, oxfendazole and morantel citrate given at dosage three times the recommended dose rate of these compounds (Boray et al 1983).

Fenbendazole has been well documented as a very safe and non-toxic drug. No teratogenic or embryotoxic effects have been reported in animals receiving fenbendazole. Rats, sheep and cattle have tolerated doses of 10,000, 5,000, 2,000 $mg.kg^{-1}$ respectively (Baeder et al 1974; Duwel 1977).

6.1.4 Pharmacokinetics

There is no currently published data on the pharmacokinetics of triclabendazole in sheep while the pharmacokinetics of fenbendazole have already been studied in sheep and cattle.

^{14}C radiolabelled fenbendazole was administered orally to sheep and cattle at 5 $mg.kg^{-1}$. A maximum plasma concentration of 0.40 and 0.74 $\mu g.ml^{-1}$ occurred after 6 - 24 and 30 hours in sheep and cattle respectively after administration (Duwel et al 1975a).

Marriner and Bogan (1981a) measured the plasma concentration of fenbendazole and its principal metabolites, the sulfoxide and the sulfone, separately using an Hplc method. The maximum plasma concentration of fenbendazole, oxfendazole and the sulfone of 0.15, 0.29 and 0.17 $\mu g.ml^{-1}$ occurring at 24, 30 and 48 hours respectively after the oral administration of fenbendazole to sheep at a dose rate of 10 $mg.kg^{-1}$.

It is likely therefore that the high amounts of fenbendazole reported by Duwel et al (1975a) resulted from the failure of the assay method to distinguish between the parent compounds and its metabolites.

6.1.5 Objective

It has been demonstrated that co-administration of two benzimidazoles alters their metabolism, and thus parbendazole has been used to prolong the kinetics and increase the efficacy of oxfendazole (Hennessy 1985). It seemed a high possibility that co-administration of fenbendazole and triclabendazole might alter their pharmacokinetics and thereby possibly increasing or decreasing the efficacy or spectrum of activity of these compounds against liver flukes and gastrointestinal nematodes.

Therefore the pharmacokinetic behaviour of triclabendazole was examined in sheep plasma with or without the co-administration of fenbendazole after the oral administration of triclabendazole separately or in combination with fenbendazole at

a dose rate of 10 $mg.kg^{-1}$ of each compound. The pharmacokinetics of fenbendazole were also examined separately in the same sheep and compared to that when co-administered with triclabendazole.

A special emphasis on the plasma protein binding of triclabendazole and its metabolites was thought likely to be helpful in assessing the unique activity of this benzimidazole compound, among the other members of this chemical group of anthelmintics, against liver fluke infection.

6.2 Determination of Triclabendazole and Fenbendazole in Sheep Plasma

Benzimidazole anthelmintics are compounds poorly soluble in water and partially soluble in organic solvents.

Determination of benzimidazole in body fluids has been carried out by the use of radiolabelled drugs (Van den Bossche et al 1982), but such a technique involves the use of a large quantity of sample and also fails to distinguish the parent compound from their metabolites.

Many other techniques have been developed for the measurement of this group of anthelmintics. A flurometric method was developed by Tocco et al (1965) and Duwel et al (1975a) for the determination of thiabendazole and fenbendazole respectively.

Nerenberg et al (1978) and Michiels et al (1982) developed radioimmunoassay techniques for the determination of oxfendazole and flubendazole respectively in animals.

In addition high performance liquid chromatographic methods have been developed by Karlaganis et al (1979), Alton et al (1979), Bogan and Marriner (1980), Tsina and Matin (1981) and Delatour et al (1983) for the determination of various

benzimidazoles in body fluids and tissue samples of animals and man.

Bogan and Marriner (1980) found that most of the benzimidazole anthelmintics extracted well into di-ethyl ether from alkalinised plasma. The ether was then evaporated and the residue analysed by high performance liquid chromatography. As triclabendazole is a member of the benzimidazole group of anthelmintics it was a logical step to try and extract triclabendazole into di-ethyl ether. Unfortunately, this benzimidazole flukicide was found to be poorly extractable into ether from many different buffers and over a range of pH from acidic to basic.

Subsequently, triclabendazole was determined in the plasma sample in this study by a method slightly modified from that developed by Ciba Geigy, Basle, Switzerland. Fenbendazole was analysed separately by a standardised method by Marriner and Bogan (1981a).

6.2.1 Materials and Methods

6.2.2 Triclabendazole Method of Analysis

6.2.2.1 Reagents and Apparatus

All reagents used were of "Analar" grade, and all the solvents were glass re-distilled except toluene.

Acetone

Hexane

Toluene

Methanol

Absolute alcohol

These solvents were obtained from Rathburn Chemicals Ltd., Walkerburn, Scotland.

Anhydrous sodium sulphate

Ammonium carbonate (0.05 M)

Saturated sodium chloride (brine)

This solution was made up by adding sodium chloride crystals to water with continuous shaking (mechanically) until no more salt was dissolved.

Mechanical shaking device

Griffin and George Ltd., Britain.

Rotary evaporator

Rotavapor-R, Buche, supplied by Orme Scientific Ltd., Middleton, Manchester.

Ultrasonic water bath

Dawe Instruments, U.S.A.

Chromatographic column

1.8 cm i.d. x 20 cm length with teflon stopcock and fritted disc.

Long column

Quick fit 1.8 cm i.d. x 30 cm length.

Alumina Woelm A-Super I Type W 200 acid (Koch-Light Ltd. Colnbrook, Berks., England).

Alumina used in the analysis was of activity Grade V and prepared as follows:

To 500 g alumina 95 ml water was added, the container was stoppered tightly, shaken very well until no lumps could be observed. The alumina was left to stand for two hours before it

was used.

Water

The water used was permanganate distilled to remove any impurities (phenols) which might affect the Hplc column.

6.2.2.2 Hplc Conditions

For the analysis of triclabendazole and its metabolites a high performance liquid chromatographic method was used, while a different Hplc method was used for the analysis of fenbendazole and its metabolites, which will be discussed in Section 6.2.3.

Detector

Cecil CE 2012 variable wave length UV monitor (Cecil Instruments, Cambridge, England).

Pump

Gilson Model 302.

Recorder

Vitatron 10 mV

Absorbance

0.05 a.u.f.s.

Column dimensions

Septum injector 100 mm length x 5 mm i.d. (Shandon Southern Products Ltd., Runcorn, England).

Column packing

Reverse phase ODS. Hypersil, particle size 5 µm (Shandon Southern Products Ltd., Runcorn, England).

Wave length

300 nm.

Mobile phase

Methanol : Ammonium carbonate (0.05 M) 65 : 35.

Flow rate

0.6 ml.minute^{-1}.

Chart speed

1 cm.minute^{-1}.

Injection volume

5 µl using 10 µl microsyringe.

6.2.2.3 Procedure of Analysis

The Hplc method of analysis of triclabendazole and its metabolites involved three steps, an extraction, partition and a column clean up step.

(1) Extraction Step

5 ml of the plasma sample was introduced into a 500 ml round bottom glass flask. 190 ml of acetone was added. Flasks were shaken for 20 minutes on a mechanical shaker.

The suspension was filtered through Whatman filter paper No.1 and 100 ml aliquots of the filtrate were taken for analysis.

(2) Partition Step

100 ml aliquots were transferred into a 1000 ml separatory funnel to which 300 ml of water and 50 ml of brine were added, followed by the addition of 70 ml dichloromethane. The separatory funnel was stoppered and the contents were shaken mechanically for five minutes.

The funnel was left to stand for a few minutes until two clear layers were formed. The bottom dichloromethane layer was taken into a 500 ml glass beaker. A further 70 ml of

dichloromethane was added to the funnel and shaken as above. The dichloromethane layer was then combined with the first extract. A third extraction procedure was carried out and all three extracts were combined.

The combined dichloromethane extract was filtered through a glass column containing a cotton plug and 25 g of anhydrous sodium sulphate. The eluent was collected in a 500 ml round bottom flask and then evaporated to dryness using a rotary evaporator at 40°C.

(3) Column clean up step

In a 100 ml vacuum stable container, a slurry of acidic alumina, activity grade V, in hexane was made up, by adding 20 ml of hexane to 20 g of acidic alumina activity grade V, the slurry was placed in an ultrasonic water bath and de-gassed for five minutes under vacuum.

A chromatographic column was filled with 25 ml of hexane, and slowly, the freshly prepared slurry was added until a constant layer of 8.5 cm height was reached.

The solvent was drained to the level of the top of the column. The drug residue was dissolved in three times 2 ml of toluene and transferred to the top of the column. The toluene was allowed to penetrate the column each time until the last drop touched the top of the column, the next 2 ml of residue in toluene was then transferred to the column.

The flask was rinsed with 50 ml hexane and this was transferred to the column, using a pasteur pipette, slowly and not disturbing the alumina. The eluent was allowed to drain and

then discarded as it contained neither triclabendazole or any of its metabolites.

100 ml of a mixture of hexane : dichloromethane 40 : 60 was used to elute the parent compound, the eluent was collected in a 250 ml round bottom glass stoppered flask (Eluant I).

Triclabendazole metabolites (the sulfoxide and the sulfone) were eluted with a mixture of 120 ml of dichloromethane : methanol 98 : 2 and the eluent was collected in a clean 250 ml round bottom glass stoppered flask (Eluant II).

Solvents of Eluant I and Eluant II were evaporated to dryness using a rotary evaporator (water bath temperature 40°C).

The residues were dissolved in 3 ml of absolute alcohol and transferred by pasteur pipette to 10 ml conical glass tubes. The flask was rinsed twice more with 3 ml of absolute alcohol and the washings transferred each time to the 10 ml conical glass tubes.

The solvent was evaporated to dryness on a dry bath at 50°C under a stream of nitrogen. The walls of the tube were washed with 0.5 - 1.0 ml of absolute alcohol and evaporated to dryness on the dry bath at 50°C under nitrogen.

The residues of Eluant I were dissolved in 100 µl methanol for injection onto the Hplc column. While Eluant II was dissolved in up to 1,000 µl depending on the sulfoxide and sulfone concentrations.

For the analysis of plasma samples for triclabendazole and metabolites, the Hplc system described in Section 6.2.2.2 was used. The retention times for the parent compound and its metabolites (Fig.2.6.1) were:-

Triclabendazole sulfone = 7.8 min.

Triclabendazole sulfoxide = 10.0 min.

Triclabendazole = 36.0 min.

To shorten the retention time for triclabendazole a higher methanol : Ammonium carbonate proportion was used (75 : 25) which resulted in a retention time of 5.80 min. with a flow rate of 1.2 $ml.min.^{-1}$.

The limits of detection of the Hplc method of analysis of triclabendazole and metabolites were 0.05 $\mu g.ml^{-1}$.

Fenbendazole, oxfendazole, and the sulfone were also eluted under the Hplc conditions mentioned in Section 6.2.2.2 (Fig.2.6.1) with a retention time of 12.80 minutes for fenbendazole, while oxfendazole and the sulfone were eluted together to give one peak with a retention time of 3.20 min. Therefore for the analysis of fenbendazole and metabolites in plasma two different Hplc systems were used as described in Section 6.2.3.

Fenbendazole also appeared in eluant II of the triclabendazole method but generally the residue was too dilute for accurate measurement of fenbendazole concentration and, therefore, in all cases, fenbendazole was measured after separate di-ethyl ether extraction (Section 6.2.3).

6.2.3 Fenbendazole Method of Analysis

6.2.3.1 Reagents

All reagents used were of "Analar" grade.

Di-ethyl ether

Glass re-distilled. Washed with 5% aqueous solution of ferrous sulphate $FESO_4.7H_2O$ and then stabilised with pyrogallol as described in Section 1.2.1.1.

Methanol

Re-distilled before use.

Ammonium carbonate

(0.05 M).

Ammonium hydroxide

(0.1 M).

Sodium chloride

6.2.3.2 Hplc conditions

Detector

Reference channel variable wave length UV detector model CE 2012 (Cecil Instruments, Cambridge, England).

Pump

Altex model 110 A solvent monitoring system.

Column dimension

100 mm long x 5 mm i.d. (Shandon), connected to valve injector (Negretti and Zambra Model 190).

Column packing

ODS-Hypersil particle size 5 µm.

Absorbance

0.05 a.u.f.s.

Recorder

Vitatron 10 mV.

Chart speed

1 cm.min.$^{-1}$.

Wave length

292 nm.

Limits of detection

0.01 µg.ml^{-1} for fenbendazole and its metabolites.

6.2.3.3 Procedure of Analysis

A high performance liquid chromatographic method was used for the determination of fenbendazole and its metabolites in the plasma samples.

4 ml aliquots of plasma were transferred into 50 ml glass-stoppered thick-walled tubes using a 4 ml bulb-pipette. 1 ml of 0.1 M NH_4OH and 0.4 g of NaCl crystals were added to each sample.

20 ml of di-ethyl ether was added using an automatic dispenser. The tubes were stoppered and shaken on a rotary mixer for 15 minutes.

15 ml of the upper ether layer were transferred into 50 ml thin-walled glass tubes.

Another extraction was carried out by adding 20 ml of di-ethyl ether to the first tube and the contents were shaken for 15 minutes on a rotary mixer. 20 ml of ether extract was transferred into the thin tubes and combined with the first extract.

The ether extract was evaporated down to a volume of 5-6 ml on a dry bath at 50°C under nitrogen. Then the extracts were transferred into 10 ml conical glass tubes and the walls of the

thin tubes washed three times with 3 ml of ether and combined with the extract in the conical tube.

The extract was evaporated to almost dry on a dry bath at 50°C under nitrogen. The walls of the conical tube were rinsed with approximately 0.5 - 1.0 ml of ether and evaporated to dryness on the dry bath at 50°C.

Residues were dissolved in 100 µl methanol by immersion of the tube in an ultrasonic water bath for 1-2 minutes and injected onto the Hplc column.

Two different Hplc systems were used for the analysis of plasma samples. The first system, Methanol : Ammonium carbonate (0.05 M) 70 : 30 was used for the determination of fenbendazole in sheep plasma. The Hplc conditions are as described in Section 6.2.3.2 with a flow rate of 0.8 $ml.min.^{-1}$ Fenbendazole had a retention time of 5.0 min. while oxfendazole and the sulfone were eluted both together giving one peak in this system. Therefore a second Hplc system was used for the quantitive determination of oxfendazole and the sulfone in sheep plasma.

The Hplc system was Methanol : Water 65 : 35 [Perchloric acid (1.1% w/v) 0.5 $\mu l.ml^{-1}$ solvent]. In this system oxfendazole and the sulfone had a retention time of 4.6 and 2.9 minutes respectively with a flow rate of 0.6 $ml.min.^{-1}$.

6.2.4 Preparation of standard solutions

Stock solutions were prepared from pure drug. 100 mg of each active compound (triclabendazole and its metabolites, fenbendazole and its metabolites) was dissolved in 100 ml methanol to provide a 1000 $\mu g.ml^{-1}$ stock solution. The stock

solution was used to prepare spiked plasma samples.

Triclabendazole and metabolites were gifts from Ciba-Geigy, oxfendazole and the sulfone were gifts from Syntex and fenbendazole was a gift from Hoechst.

6.2.5 Recoveries

Drug-free plasma was fortified with, triclabendazole and its metabolites, and fenbendazole and its metabolites, at varying concentrations. These were then taken through the whole procedure of extraction on each occasion. Table 2.6.1 shows recoveries of triclabendazole and its metabolites on different occasions. Table 2.6.2 shows recoveries of fenbendazole and its metabolites. Calculations of percentage recovery obtained in each case is as described in Chapter 1 Section 1.2.1.4. Concentrations of drug and metabolites were adjusted for recovery using the recovery for the appropriate fortified sample in the same batch, and the same equation used as described in Section 1.2.1.4.

6.2.6 Evaluation of triclabendazole method of analysis

The method of analysis of triclabendazole was evaluated to assess the reproducibility of the assay technique and recoveries from column-clean up step.

20 mg of triclabendazole, triclabendazole sulfoxide and triclabendazole sulfone were dissolved in 100 ml of toluene to make up a stock solution containing 200 $\mu g.ml^{-1}$ of each compound.

A slurry of acidic alumina, activity grade V, in hexane was made up as described in Section 6.2.2.3. The freshly prepared slurry was slowly introduced onto a chromatographic column which contained 25 ml of hexane to form a constant layer of 8.5 cm

height.

The hexane was drained to the top of the column. Three times 2 ml of the stock solution was introduced to the top of the column and the solvent allowed to penetrate the column each time before the next 2 ml was added. 50 ml of hexane was transferred to the column and the elute was discarded.

The parent compound was eluted with a mixture of 100 ml of hexane : dichloromethane 40 : 60 and the eluent was collected as four separate fractions (each fraction of 25 ml) in measuring cylinders.

The metabolites were eluted with a mixture of 120 ml of dichloromethane : methanol 98 : 2 and the eluents were collected as four separate fractions (30 ml each fraction) in measuring cylinders.

Each fraction was transferred to a 50 ml thin-walled glass tubes and evaporated to dryness on a dry bath at 50°C under nitrogen.

Residues were dissolved in 100 µl methanol and injected onto the Hplc column.

Table 2.6.3 shows the percentage recoveries of triclabendazole and its metabolites in toluene.

Aliquots of drug-free plasma were fortified with known amounts of triclabendazole and its metabolites and were taken through the extraction and the partition steps of the method of analysis of triclabendazole. The residues, obtained after evaporation of the solvent in the partition step, were dissolved in 100 µl methanol and injected onto the Hplc column.

The chromatogram showed an extra peak interfering with the sulfone and sulfoxide. Thus the column clean up step was necessary to exclude any substances interfering with the metabolite's peak.

Attempts were made to extract triclabendazole and its metabolites into another solvent i.e. di-ethyl ether. Since triclabendazole is also a benzimidazole it was expected that it would be well extracted into ether. Thus blank plasma and aqueous solution (water) were fortified with triclabendazole and its metabolites in different concentrations (Spikes). They were carried through the extraction procedure for benzimidazoles as described by Bogan and Marriner (1980). The results showed poor recoveries for the three compounds (Mean 16-27%).

Spikes containing triclabendazole and its metabolites were extracted into ether from phosphate buffer with a pH ranged from 2 to 10. The reason for this wide range of pH used was that the pka of triclabendazole at this time is unknown and it might act as a basic drug as are most members of benzimidazole because triclabendazole contains a structure similar to the benzimidazole structure. The benzimidazoles have been shown to extract well into ether from phosphate buffer pH 7.4 (Bogan and Marriner 1980). Alternatively triclabendazole might be an acidic drug as in general most fasciolicidal drugs are and it also has similarities to the chlorinated phenolic flukicides. However recoveries obtained from these extractions were also poor.

Different buffers (phosphate-citrate and borate buffer) were also used to extract triclabendazole and its metabolites using di-ethyl ether and dichloromethane as solvents, but none of them

gave satisfactory recoveries to be suitable for the analysis of plasma samples containing triclabendazole and its metabolites.

Therefore the method described in Section 6.2.2 was chosen for the analysis of triclabendazole and its metabolites. The method was reliable, sensitive to 0.05 $\mu g.ml^{-1}$ for each compound and reproducible (Table 2.6.1) in spite of the fact that the method is quite laborious and a maximum of only four samples could be analysed daily by one person.

TABLE 2.6.1

RECOVERIES OF TRICLABENDAZOLE, TRICLABENDAZOLE SULFOXIDE AND TRICLABENDAZOLE SULFONE OBTAINED ON DIFFERENT OCCASIONS FROM SHEEP PLASMA.

Amount drug added to blank plasma ($\mu g.ml^{-1}$)	Amount drug measured ($\mu g.ml^{-1}$)				Mean	± S.E.M.	Ratio assayed to added
	Triclabendazole						
0.1	0.07	0.09	0.08	0.08	0.08	0.004	0.80
0.5	0.41	0.46	0.42	0.44	0.43	0.010	0.86
1.0	0.80	0.82	0.85	0.88	0.84	0.020	0.84
4.0	4.00	3.52	3.68	3.24	3.61	0.160	0.90
9.0	8.47	7.65	7.47	7.20	7.67	0.250	0.85
	Triclabendazole Sulfoxide						
0.1	0.08	0.09	0.08	0.09	0.09	0.003	0.90
0.5	0.43	0.40	0.41	0.45	0.42	0.010	0.84
1.0	0.85	0.83	0.80	0.97	0.86	0.040	0.86
4.0	3.36	3.44	3.28	3.00	3.27	0.090	0.82
9.0	7.65	8.46	7.29	8.00	7.85	0.250	0.87
	Triclabendazole Sulfone						
0.1	0.09	0.09	0.09	0.08	0.09	0.002	0.90
0.5	0.40	0.41	0.44	0.40	0.41	0.010	0.82
1.0	0.83	0.81	0.91	0.88	0.85	0.030	0.85
4.0	3.32	3.44	3.20	2.96	3.23	0.100	0.80
9.0	7.56	7.74	7.92	7.38	7.65	0.120	0.85

TABLE 2.6.2

RECOVERIES OF FENBENDAZOLE, OXFENDAZOLE AND OXFENDAZOLE SULFONE OBTAINED ON DIFFERENT OCCASIONS FROM SHEEP PLASMA.

Amount drug added to blank plasma ($\mu g.ml^{-1}$)	Amount drug measured ($\mu g.ml^{-1}$)				Mean	$\pm$ S.E.M.	Ratio assayed to added
	Fenbendazole						
0.08	0.06	0.08	0.07	0.07	0.07	0.004	0.88
0.10	0.09	0.07	0.08	0.08	0.08	0.004	0.80
0.20	0.16	0.18	0.17	0.17	0.17	0.004	0.85
0.35	0.31	0.29	0.29	0.28	0.29	0.005	0.83
	Oxfendazole						
0.08	0.06	0.07	0.06	0.07	0.07	0.003	0.88
0.10	0.08	0.07	0.08	0.10	0.08	0.005	0.80
0.20	0.17	0.15	0.14	0.15	0.15	0.005	0.75
0.35	0.26	0.30	0.27	0.26	0.27	0.010	0.77
	Oxfendazole Sulfone						
0.08	0.07	0.07	0.08	0.07	0.07	0.002	0.88
0.10	0.09	0.08	0.09	0.08	0.09	0.003	0.90
0.20	0.16	0.17	0.17	0.18	0.17	0.004	0.85
0.35	0.29	0.31	0.29	0.28	0.29	0.005	0.83

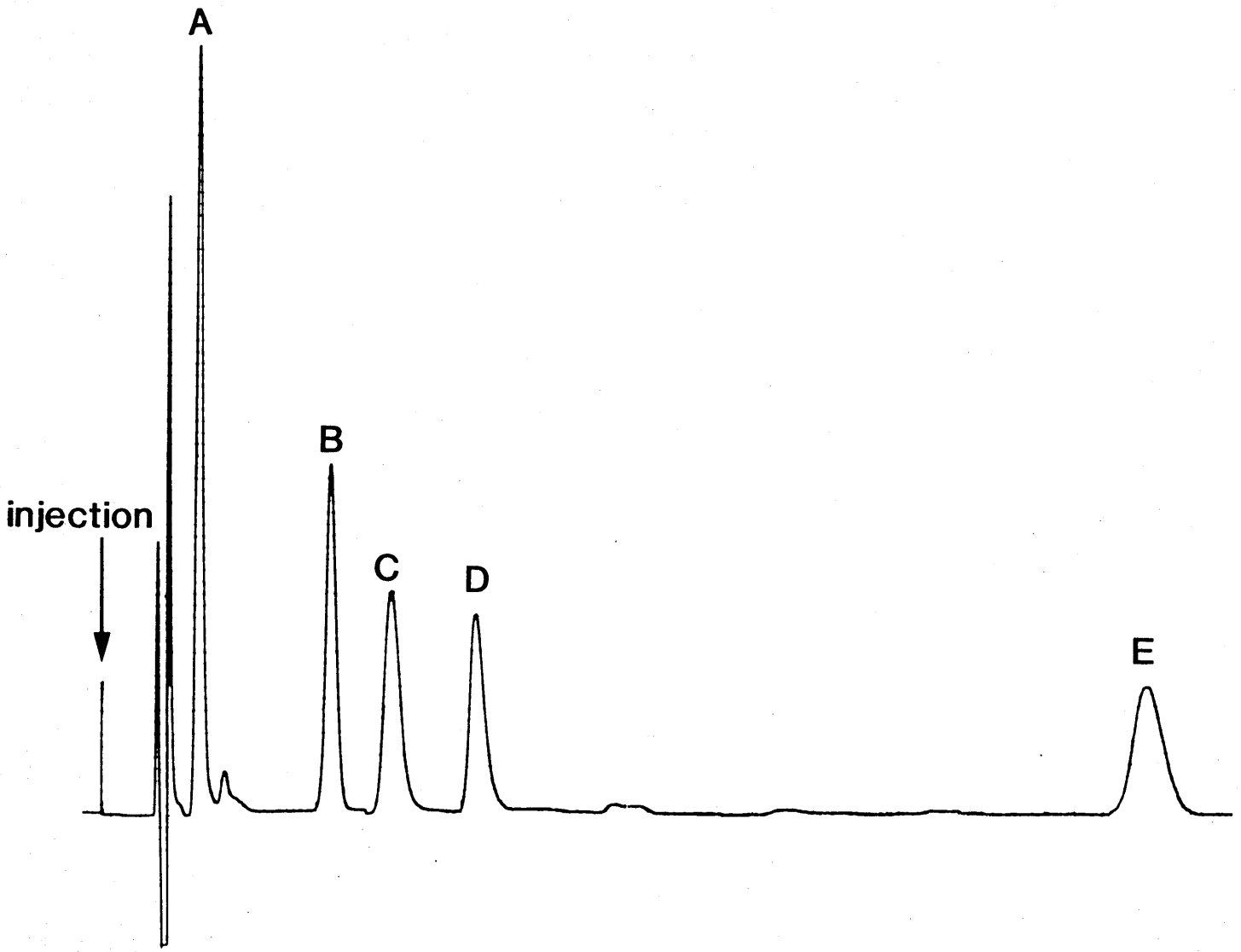
TABLE 2.6.3

PERCENTAGE RECOVERY OF TRICLABENDAZOLE, TRICLABENDAZOLE SULFOXIDE AND TRICLABENDAZOLE SULFONE IN TOLUENE

Elute	% Recovery Fraction I	Fraction II	Fraction III	Fraction IV	Total
I (triclabendazole)	5.40	90.00	4.80	0.34	100.54
II(Triclabendazole Sulfoxide)	0.09	78.70	19.30	0.48	98.57
II (triclabendazole Sulfone)	0.06	78.30	21.30	0.50	100.16

FIGURE 2.6.1

HIGH PERFORMANCE LIQUID CHROMATOGRAMS OF A STANDARD SOLUTION OF OXFENDAZOLE AND THE SULFONE (A), TRICLABENDAZOLE SULFONE (B) TRICLABENDAZOLE SULFOXIDE (C), FENBENDAZOLE (D) AND TRICLABENDAZOLE (E) IN METHANOL.


A
B
C
D
E
injection

6.3 Experiments with triclabendazole and fenbendazole

6.3.1 Materials and methods

6.3.1.1 Animals

Six sheep of different breed and sex, aged between one and five years, weighing 36 - 61 kg. were used in the experiment.

The sheep were divided into three groups (two in each group). Each group was kept separately on straw in a concrete pen and were fed on hay and concentrates diet while water was available ad libitum.

6.3.1.2 Drug administration

Administration of the drug was carried out on three occasions. The sheep were drenched orally using a syringe placed on the back of the tongue and the head of the sheep was held up after the administration to ensure that all the given dose was swallowed by the animal.

The formulations used were in the form of aqueous suspensions; proper shaking was ensured before their administration.

Three formulations were used in this experiment:-

(1) Triclabendazole 5% w/v (Fasinex).

(2)Triclabendazole/Fenbendazole2.5%/2.5%w/v(Fasinex/Panacur).

(3) Fenbendazole 10% w/v (Panacur).

Formulations (1) and (2) were experimental suspensions supplied by Ciba-Geigy. Formulation (3) was purchased.

6.3.1.3 Experimental Design

The animals were weighed one day before the first trial and they were not re-weighed. The dose for each sheep was calculated

on the basis of this weight and remained the same for each of the three trials.

The administration of the formulations were carried out on three occasions. Each group was dosed with one of the formulations on each occasion, at a dose rate of 10 $mg.kg^{-1}$ of bodyweight of each compound.

The interval between each trial ranged from 4-5 weeks. This interval was important to ensure that the sheep had replaced the blood losses due to frequent sampling (as two different analytical procedures were required for triclabendazole and fenbendazole. 20 ml of blood were being taken at each sampling time).

The animal's weight, dose volume and the trial dates are given in Table 2.6.4.

6.3.1.4 Samples for analysis

Blood samples were obtained by venepuncture before and at 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288 and 312 hours after each administration.

Sampling of blood and the treatment of blood samples were as described in Chapter 2 Section 2.2.2.4.

6.3.1.5 Method of analysis

Two different methods of analysis were used for the plasma samples containing triclabendazole and fenbendazole, they were as described in Section 6.2.2 and Section 6.2.3 respectively.

6.4 Binding of triclabendazole and its major metabolites to plasma proteins

Binding of triclabendazole and its major metabolites to plasma proteins in sheep was investigated in vitro. The plasma

sample used in this investigation contained 6, 9, 12, 22 and 30 $\mu g.ml^{-1}$ of each compound.

2 ml of each plasma samples were introduced into Amicon microconcentrators which had a 10,000 molecular weight exclusion (Fig. 2.1.2). The samples were subjected to centrifugation using a fixed rotor MSE centrifuge at 5000 x g for two hours.

Filtrates collected in the reservoir cap were analysed for the concentration of free drug using a high performance liquid chromatographic method as described in Section 6.2.2.

6.5 Biliary excretion of triclabendazole

One sheep weighing 35 kg was given triclabendazole (Fasinex) orally at a dose rate of 10 $mg.kg^{-1}$. Forty-eight hours after administration the sheep was slaughtered and samples of blood and bile were collected for analysis. These were analysed by a high performance liquid chromatography as described in Section 6.2.2.

TABLE 2.6.4

EXPERIMENTAL DESIGN FOR TRICLABENDAZOLE ,

Trial date	Animal no.	Weight (kg)	Volume administered (ml)	Formulation
	11	47.7	9.54	(1)
	91	41.0	8.20	
	90	61.0	24.40	(2)
24.4.84.	23	54.5	21.80	
	0	36.4	3.64	(3)
	12	41.0	4.10	
	11	47.7	19.08	(2)
	91	41.0	16.40	
	90	61.0	6.10	(3)
29.5.84.	23	54.5	5.45	
	0	36.4	7.28	(1)
	12	41.0	8.20	
	11	47.7	4.77	(3)
	91	41.0	4.10	
	90	61.0	12.20	(1)
2.7.84.	23	54.5	10.88	
	0	36.4	14.56	(2)
	12	41.0	16.40	

6.6 Results

6.6.1 Determination of triclabendazole and fenbendazole in sheep plasma

No parent triclabendazole was measured in any plasma samples, at any time. However in a few early samples there was possible trace amounts present at the limit of detection of the method (0.05 $\mu g.ml^{-1}$).

Individual and the mean ± S.E.M. concentration of triclabendazole sulfoxide and triclabendazole sulfone at each sampling time in the plasma of six sheep after the oral administration of triclabendazole alone and in a combined preparation with fenbendazole are shown in Tables 2.6.5.1, 2.6.5.2, 2.6.6.1 and 2.6.6.2 respectively.

The plasma concentration in each sheep and the mean concentration ± S.E.M. of fenbendazole, oxfendazole and oxfendazole sulfone after oral administration of fenbendazole alone or in combination with triclabendazole are presented in Tables 2.6.7.1, 2.6.7.2, 2.6.8.1, 2.6.8.2, 2.6.9.1 and 2.6.9.2 respectively.

Figures 2.6.3 and 2.6.4 respectively show the mean concentrations/time profile for triclabendazole sulfoxide, triclabendazole sulfone and fenbendazole, oxfendazole and oxfendazole sulfone of six sheep after oral administration of Fasinex (F), Fasinex/Panacur (F/P) and Panacur (P).

Appropriate graphical analysis of individual plasma concentration/time curves (Sedman and Wagner, 1976) indicated a triphasic disappearance of triclabendazole sulfoxide and biphasic disappearance of triclabendazole sulfone, fenbendazole,

oxfendazole and oxfendazole sulfone which could be described by the equation ($C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$).

Values of the pharmacokinetic parameters of triclabendazole sulfoxide, triclabendazole sulfone, fenbendazole, oxfendazole and oxfendazole sulfone are shown in Tables 2.6.10, 2.6.11, 2.6.12, 2.6.13 and 2.6.14 respectively.

The maximum mean concentrations of triclabendazole sulfoxides in plasma were 12.84 and 11.04 $\mu g.ml^{-1}$ which occurred 36 hours after administration of F and F/P respectively.

Triclabendazole sulfone reached maximum mean concentrations, in plasma, of 10.90 and 13.00 $\mu g.ml^{-1}$ at 48 hours after administration of F/P and F respectively.

The mean plasma concentrations of triclabendazole sulfoxide and triclabendazole sulfone fell below the limit of detection at 288 and 264 hours respectively after administration of both formulations.

Although there were individual variations in plasma fenbendazole concentrations, the maximum concentration were 0.34 and 0.35 $\mu g.ml^{-1}$ occurring 24 hours after administration of P and F/P respectively.

Oxfendazole attained a maximum concentration in plasma between 24 and 36 hours at 0.45 and 0.41 $\mu g.ml^{-1}$ and at 0.54 and 0.53 $\mu g.ml^{-1}$ after administration of P and F/P respectively.

The plasma concentrations of fenbendazole and oxfendazole were not measurable at 144 and 168 hours respectively after administration of both formulations.

Oxfendazole sulfone attained a maximum concentration in plasma at 48 hours after administration of the single (P) and the combined preparation (F/P). The concentrations of oxfendazole sulfone started to fall below the detection limit between 144 and 168 hours after administration of P and at 192 hours after administration of F/P.

For each of the drug metabolites measured, a two-way analysis of variance was used to compare the variations in the plasma concentration at each sampling time in the six sheep after administration of triclabendazole and fenbendazole alone or in combination (Table 2.6.15).

The results revealed significant differences at a few sample times between the single and combined formulation for triclabendazole sulfoxide, triclabendazole sulfone and fenbendazole. The plasma concentrations at these times were found to be significantly higher ($P > 0.05$) when the combined formulation was administered. At no time, were the concentrations of oxfendazole and oxfendazole sulfone significantly different between formulations. Some significant differences ($P > 0.05$) were found between occasions for all compounds except oxfendazole sulfone with the suggestion that on the second occasion there was greater bio-availability. However, in comparing the formulations there is no clear evidence that an interaction occurs between fenbendazole and triclabendazole such that their pharmacokinetics are affected either advantageously or disadvantageously when each drug was administered alone or co-administered.

6.6.2 Binding of triclabendazole and its major metabolites to plasma proteins

Analysis of plasma ultrafiltrates containing the parent triclabendazole and its metabolites separately showed no detectable concentration of any of these compounds at the limit of detection of the method (0.05 $\mu g.ml^{-1}$).

The filter used had a molecular weight exclusion of 10,000 and since the molecular weight of triclabendazole, triclabendazole sulfoxide and triclabendazole sulfone are 359.6, 375.6 and 391.6 respectively, therefore they should be eluted through the filter.

Since the limit of detection of the method is 0.05 $\mu g.ml^{-1}$ and the highest concentration used is 30 $\mu g.ml^{-1}$ the calculated percentage of binding (according to the equation described in Section 1.4.1) will be:

$$\% \text{ bound} = > 100 - \left(\frac{0.05}{30} \times 100\right)$$

$$= > 100 - 0.17$$

$$= > 99\%$$

The fact that these compounds were not detected in the plasma ultrafiltrate demonstrates that a very high degree of protein binding (> 99%) occurs with triclabendazole and its metabolites.

6.6.3 Biliary excretion of triclabendazole

The parent triclabendazole was excreted in small amounts (0.60 $\mu g.ml^{-1}$) in bile sample obtained at slaughter (48 hours after drug administration) while in the plasma triclabendazole was not detected at the limit of detection of the method

(0.05 $\mu g.ml^{-1}$).

The concentrations of triclabendazole sulfoxide and triclabendazole sulfone in bile were 4.85 and 3.80 $\mu g.ml^{-1}$ and in the plasma they were 7.50 and 10.50 $\mu g.ml^{-1}$ respectively.

TABLE 2.6.5.1

CONCENTRATIONS ($\mu g.ml^{-1}$) OF TRICLABENDAZOLE SULFOXIDE IN PLASMA OF SIX SHEEP AFTER ORAL ADMINISTRATION OF TRICLABENDAZOLE ALONE (FASINEX).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.34	0.21	0.25	0.37	0.32	0.16	0.28	± 0.03
4	1.00	1.72	1.56	3.50	0.98	0.92	1.60	± 0.40
8	6.50	5.46	7.83	8.50	4.11	1.80	5.70	± 1.00
12	6.70	8.00	9.30	11.30	6.57	2.61	7.40	± 1.20
24	12.50	10.00	12.74	19.00	7.20	5.93	11.22	± 1.92
36	10.90	13.30	13.87	20.00	9.46	9.52	12.84	± 1.62
48	7.00	8.60	9.93	15.00	9.07	6.46	9.34	± 1.25
72	4.80	5.80	4.60	7.32	3.75	4.15	5.07	± 0.53
96	2.90	2.20	1.48	2.80	1.33	2.72	2.24	± 0.28
120	1.20	1.00	0.38	1.70	0.69	1.76	1.12	± 0.22
144	0.44	0.47	0.28	1.30	0.58	0.98	0.68	± 0.15
168	0.40	0.20	0.18	1.36	0.49	0.60	0.54	± 0.17
192	0.34	0.18	0.43	0.12	0.40	0.37	0.30	± 0.05
216	0.17	0.16	0.37	0.10	0.25	0.18	0.20	± 0.03
240	0.14	0.05	0.36	0.07	0.08	0.07	0.13	± 0.04
264	0	0	0.12	0	0	0	0.02	± 0.01
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.5.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF TRICLABENDAZOLE SULFOXIDE IN PLASMA OF SIX SHEEP AFTER ORAL ADMINISTRATION OF TRICLABENDAZOLE IN A COMBINED PREPARATION WITH FENBENDAZOLE (FASINEX/PANACUR)

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.53	0.56	0.26	0	0	0.13	0.25	± 0.10
4	1.54	1.05	1.22	0.93	0.45	0.35	0.92	± 0.18
8	4.42	4.40	1.90	4.34	2.27	0.70	3.00	± 0.65
12	5.80	7.48	7.44	9.30	3.88	2.17	6.00	± 1.07
24	10.00	9.10	13.50	7.40	5.87	5.90	8.63	± 1.20
36	9.12	8.10	17.00	9.40	10.38	12.21	11.04	± 1.32
48	6.20	7.75	15.40	9.00	8.17	10.90	9.60	± 1.32
72	2.90	5.10	10.00	4.60	4.25	11.80	6.44	± 1.46
96	1.40	2.74	4.44	1.50	3.44	8.50	3.67	± 1.07
120	0.64	1.60	1.40	1.80	1.68	4.74	1.97	± 0.57
144	0.20	0.57	1.10	0.70	0.91	2.16	0.94	± 0.27
168	0.16	0.37	0.60	0.33	0.33	0.14	0.32	± 0.06
192	0.10	0.23	0.20	0.14	0.29	0.06	0.17	± 0.03
216	0.07	0.16	0.19	0.10	0.15	0	0.11	± 0.02
240	0	0.06	0.10	0.06	0.08	0	0.05	± 0.01
264	0	0	0.05	0	0.07	0	0.09	± 0.01
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.6.1

CONCENTRATIONS ($\mu g.ml^{-1}$) OF TRICLABENDAZOLE SULFONE IN PLASMA OF SIX SHEEP AFTER ORAL ADMINISTRATION OF TRICLABENDAZOLE ALONE (FASINEX).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.09	0.24	0.30	0.23	0.24	0.08	0.19	± 0.03
4	0.30	0.51	0.64	0.83	0.75	0.55	0.60	± 0.07
8	1.50	1.22	2.70	2.12	1.79	1.88	1.87	± 0.21
12	2.50	2.80	4.00	5.00	3.56	3.13	3.50	± 0.37
24	9.80	6.70	9.50	13.00	6.89	9.60	9.25	± 0.94
36	11.30	14.40	15.34	16.20	8.37	9.72	12.55	± 1.31
48	9.50	12.50	14.53	18.00	11.45	11.60	13.00	± 1.21
72	9.00	7.20	10.43	13.00	5.82	6.06	8.60	± 1.41
96	7.10	6.70	5.40	6.30	4.49	2.64	5.44	± 0.68
120	3.70	3.00	3.70	3.40	3.34	1.10	3.00	± 0.40
144	1.23	2.40	1.10	1.20	1.07	0.95	1.33	± 0.21
168	0.80	1.00	0.47	0.42	0.82	0.63	0.70	± 0.09
192	0.43	0.54	0.19	0.26	0.52	0.35	0.38	± 0.05
216	0.20	0.25	0.09	0.13	0.13	0.21	0.17	± 0.02
240	0.09	0.05	0	0.06	0.09	0.07	0.06	± 0.01
264	0.05	0	0	0	0	0	0	± 0.01
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.6.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF TRICLABENDAZOLE SULFONE IN PLASMA OF SIX SHEEP AFTER ORAL ADMINISTRATION OF TRICLABENDAZOLE IN A COMBINED PREPARATION WITH FENBENDAZOLE (FASINEX/PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.10	0.12	0.10	0.18	0	0.5	0.09	± 0.02
4	0.70	0.44	0.24	0.52	0.18	0.28	0.40	± 0.07
8	3.50	2.21	0.31	1.80	0.59	2.44	1.80	± 0.48
12	6.80	3.90	2.10	3.83	1.65	5.00	3.88	± 0.77
24	7.73	7.53	6.00	5.26	3.32	9.64	6.60	± 0.90
36	9.00	7.43	10.00	11.40	8.45	11.24	9.60	± 0.64
48	8.30	9.24	13.00	12.60	8.75	13.30	10.90	± 0.95
72	4.40	6.00	13.80	10.20	8.18	8.10	8.44	± 1.35
96	2.80	4.60	9.20	6.00	7.83	4.34	5.80	± 0.97
120	1.64	2.80	4.14	5.30	3.22	1.83	3.16	± 0.57
144	0.76	2.00	3.70	2.50	2.57	0.78	2.05	± 0.46
168	0.56	1.28	2.00	0.62	1.51	0.73	1.12	± 0.23
192	0.27	0.78	0.80	0.44	0.95	0.45	0.62	± 0.10
216	0.16	0.60	0.40	0.30	0.56	0.20	0.37	± 0.07
240	0.07	0.33	0.18	0.28	0.33	0.8	0.21	± 0.05
264	0.05	0.16	0.10	0.06	0.16	0	0.08	± 0.02
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.7.1

CONCENTRATIONS ($\mu g.ml^{-1}$) OF FENBENDAZOLE IN PLASMA OF SIX SHEEP AFTER ORAL ADMINISTRATION OF FENBENDAZOLE ALONE (PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0.02	0.02	0.04	0	0.01	0
4	0.03	0.09	0.13	0.12	0.15	0.13	0.10	± 0.01
8	0.13	0.20	0.27	0.26	0.26	0.28	0.23	± 0.02
12	0.19	0.23	0.38	0.36	0.28	0.38	0.30	± 0.03
24	0.31	0.30	0.35	0.33	0.27	0.47	0.34	± 0.02
36	0.23	0.22	0.27	0.26	0.21	0.26	0.24	± 0.01
48	0.19	0.17	0.18	0.17	0.15	0.28	0.19	± 0.01
72	0.06	0.04	0.09	0.08	0.12	0.13	0.08	± 0.01
96	0.02	0.03	0.03	0.03	0.06	0.05	0.03	± 0.01
120	0	0	0	0.02	0.05	0	0.01	± 0.01
144	0	0	0	0	0	0	0	0
168	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.7.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF FENBENDAZOLE IN PLASMA OF SIX SHEEP AFTER ADMINISTRATION OF FENBENDAZOLE IN A COMBINED PREPARATION WITH TRICLABENDAZOLE (FASINEX/PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.07	0.08	0	0	0	0.02	0.02	± 0.01
4	0.18	0.22	0.07	0.06	0.04	0.04	0.10	± 0.02
8	0.29	0.30	0.28	0.47	0.13	0.13	0.26	± 0.05
12	0.26	0.28	0.33	0.60	0.15	0.18	0.30	± 0.06
24	0.21	0.27	0.53	0.55	0.25	0.30	0.35	± 0.05
36	0.11	0.16	0.33	0.40	0.20	0.24	0.24	± 0.04
48	0.09	0.15	0.25	0.30	0.25	0.25	0.21	± 0.02
72	0.04	0.07	0.16	0.13	0.15	0.17	0.12	± 0.02
96	0.01	0.02	0.08	0.05	0.08	0.09	0.05	± 0.01
120	0	0.01	0	0	0.03	0.04	0.01	0
144	0	0	0	0	0	0	0	0
168	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.8.1

CONCENTRATIONS ($\mu g.ml^{-1}$) OF OXFENDAZOLE IN PLASMA OF SIX SHEEP AFTER ADMINISTRATION OF FENBENDAZOLE ALONE (PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
4	0	0	0.10	0.10	0.04	0.04	0.04	± 0.01
8	0.04	0.18	0.15	0.16	0.17	0.20	0.15	± 0.02
12	0.10	0.40	0.25	0.24	0.25	0.44	0.28	± 0.05
24	0.29	0.55	0.34	0.34	0.38	0.81	0.45	± 0.07
36	0.26	0.37	0.43	0.42	0.50	0.53	0.41	± 0.03
48	0.23	0.36	0.27	0.30	0.44	0.63	0.37	± 0.05
72	0.11	0.15	0.26	0.26	0.31	0.35	0.24	± 0.03
96	0.05	0.09	0.05	0.06	0.17	0.11	0.08	± 0.01
120	0	0.02	0.07	0.07	0.04	0	0.04	± 0.01
144	0	0	0.05	0.05	0	0	0.01	± 0.01
168	0	0	0.02	0.03	0	0	0	0
192	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.8.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF OXFENDAZOLE IN PLASMA OF SIX SHEEP AFTER ADMINISTRATION OF FENBENDAZOLE IN A COMBINED PREPARATION WITH TRICLABENDAZOLE (FASINEX/PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.08	0	0	0	0	0	0.01	± 0.01
4	0.15	0.08	0.05	0.05	0	0	0.05	± 0.02
8	0.35	0.30	0.18	0.27	0.01	0.10	0.20	± 0.05
12	0.41	0.47	0.50	0.54	0.05	0.17	0.35	± 0.01
24	0.40	0.45	1.10	0.74	0.20	0.36	0.54	± 0.13
36	0.41	0.47	0.98	0.67	0.21	0.47	0.53	± 0.10
48	0.24	0.35	0.98	0.65	0.28	0.45	0.50	± 0.11
72	0.09	0.17	0.58	0.30	0.19	0.30	0.27	± 0.07
96	0.08	0.11	0.26	0.12	0.10	0.24	0.15	± 0.02
120	0.07	0.05	0.04	0	0.03	0.08	0.04	± 0.01
144	0.05	0.04	0	0	0	0	0.01	± 0.01
168	0.04	0.02	0	0	0	0	0.01	0
192	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.9.1

CONCENTRATIONS ($\mu g.ml^{-1}$) OF OXFENDAZOLE SULFONE IN PLASMA OF SIX SHEEP AFTER ORAL ADMINISTRATION OF FENBENDAZOLE ALONE (PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
8	0	0.02	0.01	0.01	0.02	0.02	0.01	0
12	0	0.05	0.03	0.03	0.04	0.05	0.03	0
24	0.08	0.17	0.10	0.10	0.12	0.28	0.14	± 0.02
36	0.12	0.19	0.16	0.16	0.17	0.26	0.18	± 0.01
48	0.13	0.25	0.14	0.16	0.21	0.41	0.21	± 0.04
72	0.11	0.16	0.13	0.13	0.23	0.32	0.18	± 0.03
96	0.07	0.09	0.07	0.07	0.16	0.20	0.11	± 0.02
120	0	0.02	0.05	0.05	0.12	0.11	0.08	± 0.01
144	0	0	0.03	0.04	0.06	0.02	0.03	± 0.01
168	0	0	0.02	0.03	0	0	0.01	0
192	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.9.2

CONCENTRATIONS ($\mu g.ml^{-1}$) OF OXFENDAZOLE SULFONE IN PLASMA OF SIX SHEEP AFTER ADMINISTRATION OF FENBENDAZOLE IN A COMBINED PREPARATION WITH TRICLABENDAZOLE (FASINEX/PANACUR).

Time hours	Sheep no. 10	12	11	91	23	90	Mean	± S.E.M.
Base line	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
8	0.03	0	0	0.04	0	0	0.01	± 0.01
12	0.07	0.04	0.05	0.06	0	0.03	0.04	± 0.01
24	0.11	0.11	0.27	0.19	0.03	0.08	0.13	± 0.03
36	0.12	0.15	0.32	0.28	0.04	0.15	0.17	± 0.04
48	0.11	0.13	0.42	0.32	0.09	0.18	0.21	± 0.05
72	0.07	0.11	0.43	0.23	0.08	0.14	0.17	± 0.05
96	0.06	0.08	0.33	0.15	0.08	0.08	0.13	± 0.04
120	0.04	0.05	0.14	0.05	0.04	0.07	0.06	± 0.01
144	0.03	0.04	0.10	0.04	0.04	0.05	0.05	± 0.01
168	0.02	0.03	0.05	0	0.03	0.02	0.02	0
192	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0
264	0	0	0	0	0	0	0	0
288	0	0	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0

TABLE 2.6.10

PHARMACOKINETIC CONSTANTS OF TRICLABENDAZOLE SULFOXIDE AFTER ADMINISTRATION OF "FASINEX" AND "FASINEX/PANACUR" ORALLY TO SIX SHEEP AT A DOSE RATE OF 10 $mg.kg^{-1}$.

Animal no.	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	C $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	γ h^{-1}	t 1/2 (α) h	t 1/2 (β) h	t 1/2 (γ) h	AUC $\mu g.ml^{-1}.h.$
FASINEX										
0	- 72.20	68.80	3.30	0.06	0.04	0.01	11.80	17.20	52.80	732.00
12	- 39.90	28.70	11.00	0.06	0.04	0.02	11.00	19.30	31.80	675.20
11	- 43.60	42.20	1.40	0.07	0.03	0.01	9.50	20.30	86.50	820.80
91	- 14.20	69.60	55.40	0.30	0.08	0.03	2.70	8.20	24.50	1186.00
23	- 35.70	24.00	11.80	0.06	0.05	0.02	10.80	14.80	35.80	563.00
90	- 3.40	57.40	54.00	0.20	0.04	0.03	3.27	17.00	25.70	598.50
Mean							8.20	16.00	42.80	762.60
$\pm$ S.E.M.							$\pm$ 1.60	$\pm$ 1.70	$\pm$ 9.50	$\pm$ 90.80
Averaged	- 9.80	- 36.70	26.90	0.20	0.10	0.02	3.00	7.30	28.30	758.30
FASINEX/PANACUR										
0	- 31.40	29.60	1.80	0.08	0.04	0.02	8.20	28.60	46.00	540.70
12	4.00	34.00	30.00	0.30	0.07	0.03	2.20	10.40	27.00	678.30
11	- 75.00	50.40	24.60	0.06	0.04	0.02	11.40	18.30	29.40	1133.60
91	- 33.80	29.60	41.20	0.07	0.03	0.02	10.20	21.60	29.30	659.30
23	- 72.60	72.20	0.30	0.05	0.03	0.06	15.00	21.00	44.60	662.40
90	- 65.40	61.70	3.70	0.04	0.02	0.02	18.40	28.70	39.30	1024.60
Mean							11.00	19.80	36.00	783.00
$\pm$ S.E.M.							$\pm$ 2.20	$\pm$ 2.40	$\pm$ 3.40	$\pm$ 94.80
Averaged	- 22.60	83.70	61.00	0.10	0.06	0.03	5.60	11.70	23.00	794.30

TABLE 2.6.11

PHARMACOKINETIC CONSTANTS OF TRICLABENDAZOLE SULFONE FOLLOWING ORAL ADMINISTRATION OF "FASINEX" AND "FASINEX/PANACUR" TO SIX SHEEP AT A DOSE RATE OF 10 $mg.kg^{-1}$.

Animal no.	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	t 1/2 (α) h	t 1/2 (β) h	AUC $\mu g.ml^{-1}.h$
FASINEX							
0	- 81.60	81.50	0.04	0.03	17.20	24.50	858.00
12	- 60.60	60.50	0.05	0.03	14.80	26.00	979.30
11	- 90.30	90.20	0.05	0.03	14.30	21.80	972.80
91	- 98.70	98.60	0.05	0.03	14.50	22.00	1089.00
23	- 43.00	43.60	0.04	0.03	16.60	27.30	675.20
90	- 29.60	29.60	0.06	0.02	11.20	28.40	734.20
Mean	- 67.30	67.30	0.05	0.03	14.70	25.00	884.80
$\pm$ S.E.M.	$\pm$ 10.90	$\pm$ 10.90	$\pm$ 0.01	$\pm$ 0.01	$\pm$ 0.80	$\pm$ 1.00	$\pm$ 63.40
Averaged	- 62.0	62.0	0.05	0.03	15.00	25.00	893.0
FASINEX/PANACUR							
0	- 28.30	28.30	0.06	0.02	11.00	28.20	704.80
12	- 59.80	59.70	0.03	0.02	21.00	31.00	856.20
11	-102.00	102.00	0.04	0.03	19.40	26.50	1041.80
91	- 55.3	55.20	0.04	0.02	17.40	28.30	864.80
23	- 41.50	41.50	0.04	0.02	19.50	33.60	841.80
90	- 43.30	41.30	0.05	0.03	13.40	27.00	844.00
Mean	- 55.00	54.60	0.04	0.02	17.00	29.00	858.90
$\pm$ S.E.M.	$\pm$ 10.20	$\pm$ 10.30	$\pm$ 0.01	$\pm$ 0.01	$\pm$ 1.60	$\pm$ 1.00	$\pm$ 43.00
Averaged	- 50.50	50.50	0.04	0.02	16.50	29.30	934.50

TABLE 2.6.12

PHARMACOKINETIC CONSTANTS OF FENBENDAZOLE AFTER ADMINISTRATION OF "PANACUR" AND "FASINEX/PANACUR" ORALLY TO SIX SHEEP AT A DOSE RATE OF 10 $mg.kg^{-1}$.

Animal no.	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	t 1/2 (α) h	t 1/2 (β) h	AUC $\mu g.ml^{-1}.h$
PANACUR							
0	- 1.60	1.60	0.08	0.05	8.50	14.80	14.30
12	- 0.70	0.70	0.09	0.04	7.40	19.70	12.70
11	- 1.10	1.10	0.10	0.04	6.88	18.57	18.60
91	- 0.70	0.70	0.10	0.03	5.10	21.90	18.00
23	- 0.40	0.40	0.20	0.02	3.34	38.56	19.76
90	- 2.10	2.10	0.07	0.04	9.70	17.40	23.60
Mean	- 1.10	1.10	0.11	0.04	6.80	21.80	17.80
±S.E.M.	± 0.30	± 0.30	± 0.02	± 0.01	± 0.90	± 3.40	± 1.60
Averaged	- 1.70	1.70	0.08	0.04	8.96	16.00	17.30
FASINEX/PANACUR							
0	- 0.50	0.50	0.20	0.04	3.30	17.30	10.80
12	- 0.50	0.50	0.20	0.03	3.50	21.30	13.80
11	- 0.80	0.80	0.10	0.02	6.20	29.20	25.90
91	- 2.20	2.20	0.08	0.04	8.30	17.40	28.60
23	- 0.60	0.60	0.07	0.02	9.80	29.36	17.68
90	- 1.40	1.40	0.05	0.03	13.00	23.00	20.65
Mean	- 1.00	1.00	0.12	0.03	7.40	23.00	19.60
±S.E.M.	± 0.30	± 0.30	± 0.03	± 0.01	± 1.50	± 2.20	± 2.60
Averaged	- 1.20	1.20	0.09	0.04	7.90	18.90	18.30

TABLE 2.6.13

PHARMACOKINETIC CONSTANTS OF OXFENDAZOLE FOLLOWING ORAL ADMINISTRATION OF "PANACUR" AND "FASINEX/PANACUR" TO SIX SHEEP AT A DOSE RATE OF 10 $mg.kg^{-1}$.

Animal no.	A $\mu g.ml^{-1}$	B $\mu g.ml^{-1}$	α h^{-1}	β h^{-1}	t 1/2 (α) h	t 1/2 (β) h	AUC $\mu g.ml^{-1}.h$
PANACUR							
0	- 0.70	0.70	0.08	0.03	8.62	24.40	16.60
12	- 3.10	3.10	0.07	0.04	10.60	16.50	28.80
11	- 1.80	1.80	0.05	0.03	15.20	26.60	28.90
91	- 0.80	0.80	0.07	0.02	9.80	34.50	27.40
23	- 2.40	2.40	0.06	0.03	12.10	21.30	31.84
90	- 3.70	3.70	0.06	0.04	11.80	19.19	38.86
Mean	- 2.00	2.00	0.07	0.03	11.40	23.70	28.70
$\pm$S.E.M.	$\pm$ 0.50	$\pm$ 0.50	$\pm$ 0.04	$\pm$ 0.03	$\pm$ 0.90	$\pm$ 2.60	$\pm$ 2.80
Averaged	- 2.60	2.60	0.06	0.04	11.40	18.50	26.30
FASINEX/PANACUR							
0	- 0.50	0.50	0.20	0.02	3.33	41.80	28.10
12	- 0.90	0.90	0.10	0.02	5.90	30.50	31.60
11	- 4.90	4.90	0.06	0.04	10.70	18.90	57.60
91	- 3.30	3.30	0.07	0.04	10.40	19.70	43.86
23	- 0.70	0.70	0.05	0.02	13.70	29.20	15.30
90	- 1.40	1.40	0.06	0.02	12.4	30.80	36.51
Mean	- 2.00	2.00	0.09	0.03	9.40	29.80	30.70
$\pm$S.E.M.	$\pm$ 0.70	$\pm$ 0.70	$\pm$ 0.02	$\pm$ 0.01	$\pm$ 1.60	$\pm$ 2.80	$\pm$ 3.80
Averaged	- 3.00	3.00	0.06	0.04	11.4	19.00	33.50

TABLE 2.6.14

PHARMACOKINETIC CONSTANTS OF OXFENDAZOLE SULFONE AFTER ADMINISTRATION OF "PANACUR" AND "FASINEX/PANACUR" ORALLY TO SIX SHEEP AT A DOSE RATE OF 10 $mg.kg^{-1}$.

Animal no.	A	B	α	β	t 1/2 (α)	t 1/2 (β)	AUC
	$\mu g.ml^{-1}$	$\mu g.ml^{-1}$	h^{-1}	h^{-1}	h	h	$\mu g.ml^{-1}.h$
PANACUR							
0	- 0.40	0.40	0.05	0.02	14.00	36.80	12.20
12	- 1.40	1.40	0.05	0.04	14.00	20.40	12.40
11	- 0.40	0.40	0.05	0.02	14.00	36.20	14.00
91	- 0.30	0.30	0.06	0.01	10.80	47.60	15.80
23	- 0.80	0.80	0.03	0.02	20.00	38.50	21.70
90	- 1.00	1.00	0.05	0.03	14.70	30.20	23.00
Mean	- 0.70	0.70	0.05	0.02	14.60	35.00	16.50
$\pm$S.E.M.	$\pm$ 0.20	$\pm$ 0.20	$\pm$ 0.01	$\pm$ 0.01	$\pm$ 1.00	$\pm$ 3.60	$\pm$ 1.90
Averaged	- 1.60	1.60	0.04	0.03	16.40	23.50	16.00
FASINEX/PANACUR							
0	- 0.20	0.20	0.08	0.01	9.00	51.60	11.84
12	- 0.30	0.30	0.06	0.01	10.80	50.50	15.40
11	- 1.70	1.70	0.04	0.02	17.30	32.30	37.00
91	- 1.00	1.00	0.05	0.02	14.60	29.30	21.60
23	- 0.20	0.20	0.04	0.01	17.00	62.70	11.40
90	- 0.40	0.40	0.04	0.02	16.00	41.00	14.70
Mean	- 0.60	0.60	0.05	0.02	14.00	44.60	18.70
$\pm$S.E.M.	$\pm$ 0.20	$\pm$ 0.20	$\pm$ 0.01	$\pm$ 0.01	$\pm$ 1.40	$\pm$ 5.00	$\pm$ 3.80
Averaged	- 0.80	0.80	0.04	0.02	16.00	31.80	18.20

TABLE 2.6.15

TWO WAY ANALYSIS OF VARIANCE : TEST FOR SIGNIFICANCE

PLASMA CONCENTRATIONS

Hours	Triclabendazole Sulfoxide Formulation	Triclabendazole Sulfoxide Occasion	Triclabendazole Sulfone Formulation	Triclabendazole Sulfone Occasion	Fenbendazole Formulation	Fenbendazole Occasion	Oxfendazole Formulation	Oxfendazole Occasion	Oxfendazole Sulfone Formulation	Oxfendazole Sulfone Occasion
Pre										
1										
2		*3	*2							
4								*2		
8	*2	*2								
12		*2								
24		*2				*2				
36			*2	*2		*2				
48				*2						
72				*2	*2	*1		*2		
96					*2	*1				
120										
144								*2		
168								*3		
192										
216										
240										
264										
288										
312										

* = Significantly different at the 5% level.
The highest parameter when significance is found is marked.

Formulation 1 = Alone
2 = Combined

Occasion 1, 2 or 3.

FIGURE 2.6.2

MEAN CONCENTRATIONS ($\mu g.ml^{-1}$) OF TRICLABENDAZOLE SULFOXIDE AND TRICLABENDAZOLE SULFONE IN PLASMA OF SHEEP (n=6) FOLLOWING ORAL ADMINISTRATION OF TRICLABENDAZOLE ALONE OR IN COMBINATION WITH FENBENDAZOLE AT A DOSE RATE OF 10 $mg.kg^{-1}$ OF TRICLABENDAZOLE AND OF FENBENDAZOLE.

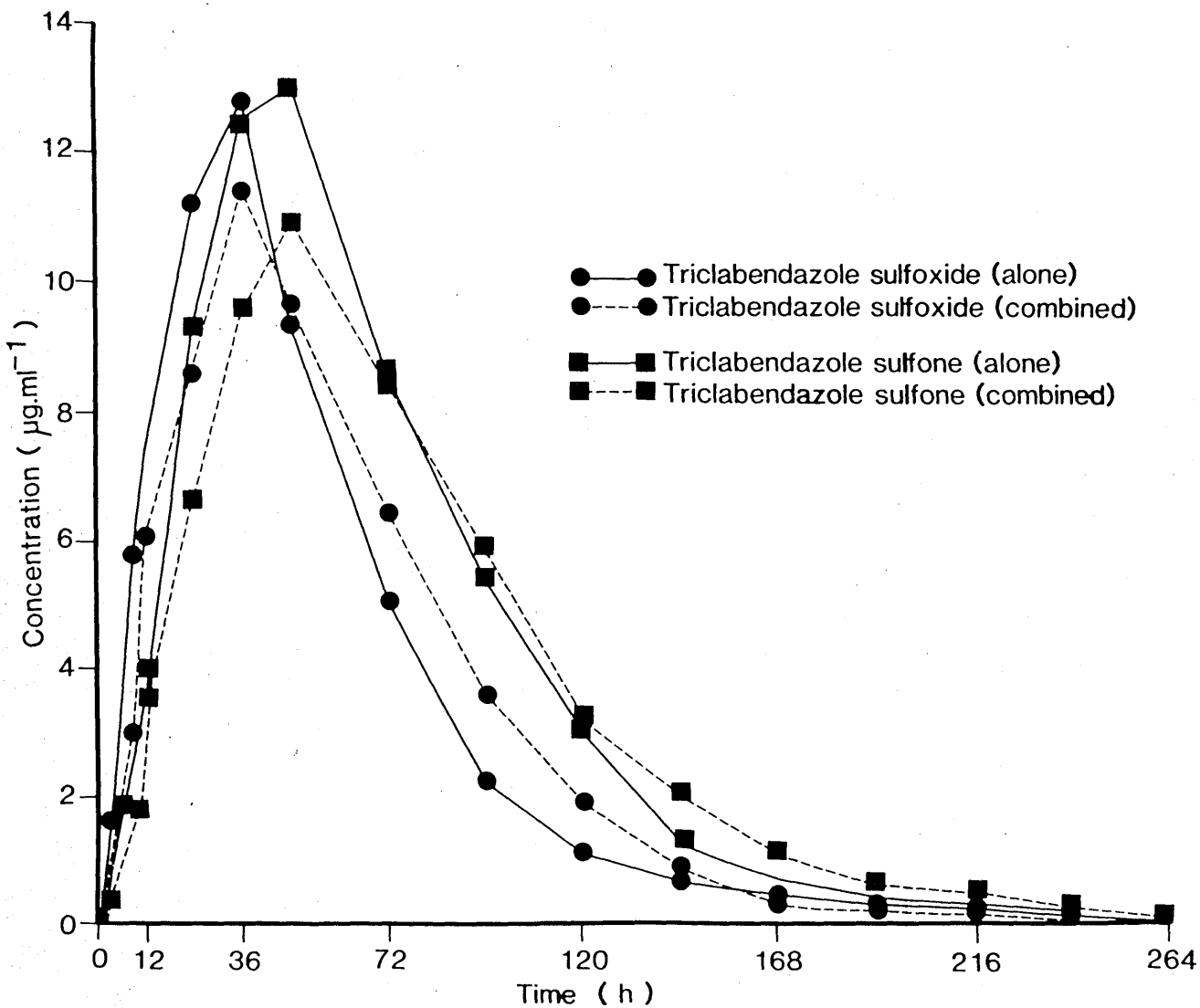
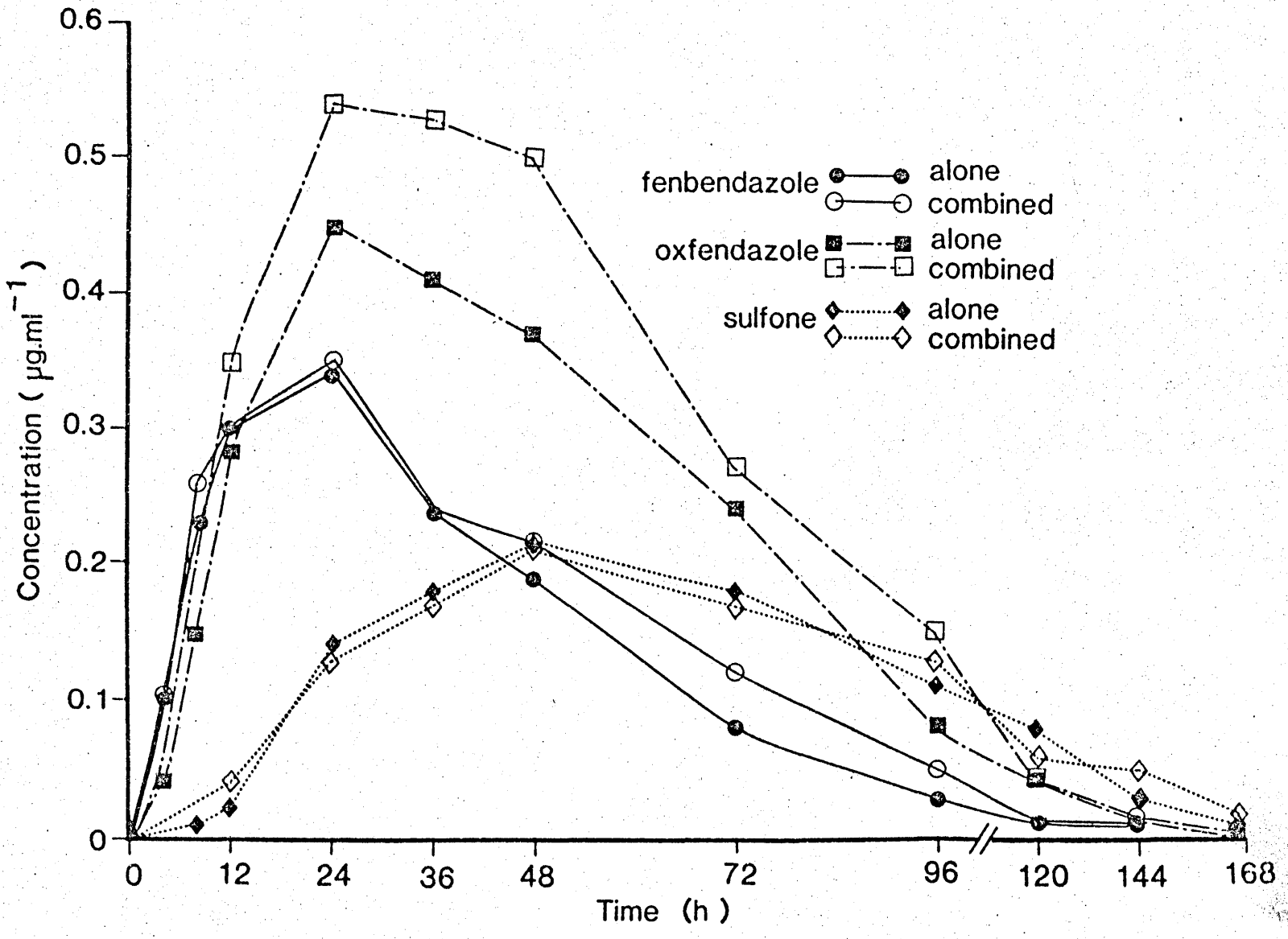

14
12
10
8
6
4
2
0
Concentration (μg.ml⁻¹)
0
12
36
72
120
168
216
264
Time (h)
Triclabendazole sulfoxide (alone)
Triclabendazole sulfoxide (combined)
Triclabendazole sulfone (alone)
Triclabendazole sulfone (combined)

FIGURE 2.6.3

MEAN CONCENTRATIONS ($\mu g.ml^{-1}$) OF FENBENDAZOLE, OXFENDAZOLE AND THE SULFONE IN PLASMA OF SHEEP (n=6) AFTER AN ORAL ADMINISTRATION OF FENBENDAZOLE ALONE OR IN COMBINATION WITH TRICLABENDAZOLE AT A DOSE RATE OF 10 $mg.kg^{-1}$ OF FENBENDAZOLE AND OF TRICLABENDAZOLE.


0.6
0.5
0.4
0.3
0.2
0.1
0
Concentration (µg.ml^{-1})
0
12
24
36
48
72
96
120
144
168
Time (h)
fenbendazole
alone
combined
oxfendazole
alone
combined
sulfone
alone
combined

6.7 Discussion

Immediately after oral administration, triclabendazole was rapidly oxidized to the sulfoxide and subsequently to the sulfone (Fig. 2.6.4). This oxidative pathway is shown to be common for the metabolism of sulphur containing compounds (Meshi et al 1970; Duwel 1977; Marriner and Bogan 1980, 1981a).

The rate of formation of these metabolites was so rapid that the parent compound, triclabendazole, was not detected in the plasma samples and only its metabolites were detected at approximately two hours after administration. A similar finding is reported for another benzimidazole compound, albendazole, in sheep (Marriner and Bogan 1980; Gyurik et al 1981).

Once triclabendazole sulfoxide has entered the blood stream it was reversibly bound to plasma proteins. Based on an inspection of the plasma concentration/time profile (Fig. 2.6.2) it could be concluded that the rapid dissociation and distribution of the free drug, which is immediately replaced by newly dissociated drug, is responsible for the initial decline of the plasma curve of triclabendazole sulfoxide (t 1/2 (β) = 16.0 $\pm$ 1.7h), and the slow decline of the plasma curve (t 1/2 (γ) = 42.8 $\pm$ 9.5h) is thought to be attributed to the slow rate of elimination of the drug since the bound drug is not readily available for distribution, metabolism and elimination.

Following administration of the combined formulation (F/P) the initial decline of the plasma concentration/time profile of triclabendazole sulfoxide (Fig. 2.6.2) was relatively slower (t 1/2 (β) = 19.8 $\pm$ 2.4h) and followed by a relatively faster

elimination rate (t 1/2 (γ) = 36.0 $\pm$ 3.4h) than after administration of triclabendazole alone (F). This might be due to the effect of competition for binding sites and that the subsequent binding of fenbendazole had altered the binding of triclabendazole sulfoxide or might be attributed to alteration in metabolic rate in liver since both triclabendazole and fenbendazole are sulfides and have similar patterns of metabolism via the sulfoxide and the sulfone metabolite.

The decline of the plasma curve of triclabendazole sulfone followed first order kinetics with a mean elimination half life (t 1/2 β) of 25.0 $\pm$ 1.0h and 29.0 $\pm$ 1.0h after administration of F and F/P respectively.

The pharmacokinetic behaviour of triclabendazole in cattle (Bogan et al 1985) appeared to be similar to that in sheep. Following the oral administration, triclabendazole was rapidly metabolised to the sulfoxide and the sulfone. The parent triclabendazole could be detected only at very low concentration in a few early samples ($< 0.05\ \mu g.ml^{-1}$).

The maximum concentration of triclabendazole sulfoxide and triclabendazole sulfone in plasma of cattle after oral administration at a dose rate of 5 $mg.kg^{-1}$ triclabendazole, were 12.5 and 10.1 $\mu g.ml^{-1}$ and occurred at 24-30h and 96-120h respectively. Ignoring the differences in the administered dose of triclabendazole to sheep, compared with cattle, it would appear that the kinetics of triclabendazole sulfoxide in cattle plasma were relatively similar to that in sheep. This was reflected by the similarity in the maximum concentration related to dose rate and the time at which the maximum concentration of

triclabendazole sulfoxide in plasma had occurred in both sheep and cattle. However the situation seems to be different for triclabendazole sulfone. The maximum concentration of triclabendazole sulfone in cattle plasma occurred at a mean time of 96-120h which is later than that in sheep (36-48h). The elimination half life of triclabendazole sulfone in cattle was longer (54.8h) than that in sheep (25.0h).

From this observation it would appear that administration of triclabendazole to cattle at a dose rate of 5 $mg.kg^{-1}$ produces similar plasma concentrations to that in sheep when triclabendazole was given at a dose rate of 10 $mg.kg^{-1}$. The plasma concentrations of triclabendazole sulfoxide, which is probably the anthelmintically active metabolite, achieved in cattle are sufficient for a high activity while triclabendazole sulfone is unlikely to be anthelmintically active. Therefore the commercially recommended dose rate of triclabendazole for cattle which is higher (12 $mg.kg^{-1}$) than for sheep (10 $mg.kg^{-1}$) could be similar to that for sheep.

The pharmacokinetic behaviour of fenbendazole in plasma in sheep, in this study, was in agreement with previous findings (Duwel 1977; Marriner and Bogan 1981a). After absorption of fenbendazole, from the gastrointestinal tract to the peripheral circulation, the drug was metabolised by the hepatic microsomal enzymes to the sulfoxide, oxfendazole and the sulfone (Fig. 2.6.5).

Metabolism of triclabendazole and fenbendazole to the sulfoxide and the sulfone appear to be similar. However the

extent and rate of metabolism of triclabendazole was found to be quicker than that of fenbendazole. Triclabendazole disappeared rapidly from the plasma samples and only its metabolites were detected in much higher concentration in plasma whereas fenbendazole was detected in plasma for longer duration along with its metabolites.

An important factor is to know whether it is the parent compound or one or more of the metabolites which is responsible for the activity of these drugs against helminths. In an efficacy study oxfendazole, which is the major metabolite of fenbendazole, has been shown to be more potent than the parent compound (Averkin et al 1975). Bithionol sulfoxide, which is used as a fasciolicide in ruminants, has been shown to have stronger anthelmintic activity than the sulfide, bithionol (Godfrain et al 1969a, b; Meshi et al 1970; Vishnyauskas and Gudonavitchus 1974; Dagorn 1984).

In a pharmacokinetic study, following the oral administration of albendazole to sheep Marriner and Bogan (1980) reported that the parent compound was virtually absent from the plasma and its major metabolite, albendazole sulfoxide, was found in concentrations much higher than albendazole. They related the anthelmintic activity of this drug to the sulfoxide metabolite. Furthermore administration of albendazole sulfoxide in feed to rats infected with Nippostrongylus brasiliensis was found to be as effective anthelmintically as was albendazole (Marriner 1980).

Subsequent metabolism of the absorbed triclabendazole sulfoxide and oxfendazole to the sulfone is mainly to enhance the process of excretion (Meshi et al 1970; Mulder et al 1982). The

maximum concentration of triclabendazole sulfone and oxfendazole sulfone occurred later and were significantly lower than those of both triclabendazole sulfoxide and oxfendazole respectively. Thereafter the sulfone concentrations tended to be higher than those of the sulfoxide.

One of the main characteristics of flukicidal drugs appears to be that they are extensively bound to blood proteins, mainly albumin, and are therefore excreted slowly from the body (Broome and Jones 1966; Meshi et al 1970; Vishnyauskas and Gudonavitchus 1974). In comparing a number of salicylanilide flukicidal drugs, Lee (1973) found that the flukicidal activity of salicylanilides in sheep was dependent on the extent to which they persist in the plasma. He found that compounds which had half lives of less than 36 h had no detectable fasciolicidal activity whereas compounds with plasma half lives of 48-84 h were markedly more active and those with half lives of 5-6 days were very active. Corba et al (1979) demonstrated the importance of the long exposure of liver flukes to fenbendazole rather than the concentrations of the compound in the body.

The same situation apparently exists for triclabendazole, the strong binding of triclabendazole and its metabolites to plasma proteins (> 99%) would appear to be the most likely route by which this benzimidazole reaches liver flukes. Additional evidence for this suggestion was provided in an experiment in which one sheep received an oral dose of triclabendazole at a dose rate of 10 $mg.kg^{-1}$.

After the oral administration, the bile : plasma ratio of triclabendazole sulfoxide and triclabendazole sulfone was 0.65 and 0.36 respectively. Therefore there is no evidence that flukes are exposed to high concentrations of triclabendazole in bile. Although albendazole has flukicidal activity (Theodorides et al 1975; Knight and Colglazier 1977; Fetterer et al 1982), Morris et al (1985) have also shown that the biliary excretion of albendazole sulfoxide in humans are less than those in plasma.

It has been found that flukes residing in bile ducts feed mainly on blood (Jennings et al 1955, 1956; Pearson 1963; Rowlands 1969) and that each fluke consumes approximately 0.2 ml of blood per day (Jennings et al 1956). This amount of daily blood loss caused by flukes is sufficient to produce marked anaemia which is associated with Fasciola hepatica infections. Thus ingestion of blood bound drug by the flukes is probably responsible for the toxic effect of the drug on Fasciola hepatica.

The pharmacokinetic results presented demonstrate that there was no indication that an interaction between triclabendazole and fenbendazole had occurred, when both compounds were co-administered, and that their pharmacokinetic behaviour was not altered significantly (either potentiated or reduced). This was reflected by the insignificant increase in AUC and their plasma concentration.

It is also clear from this study that triclabendazole functions quite differently from the other benzimidazoles and this may explain the preferential activity of this compound against liver fluke and its lack of activity against nematodes.

Firstly, analytically it cannot be assayed by the same methodology as other benzimidazoles. Therefore chemically it must be functioning in a different way from the others.

Secondly, it is extremely strongly protein-bound (> 99%). Protein-binding of the other benzimidazoles is unknown and for comparison, the binding of albendazole sulfoxide was measured and found to be 49% bound.

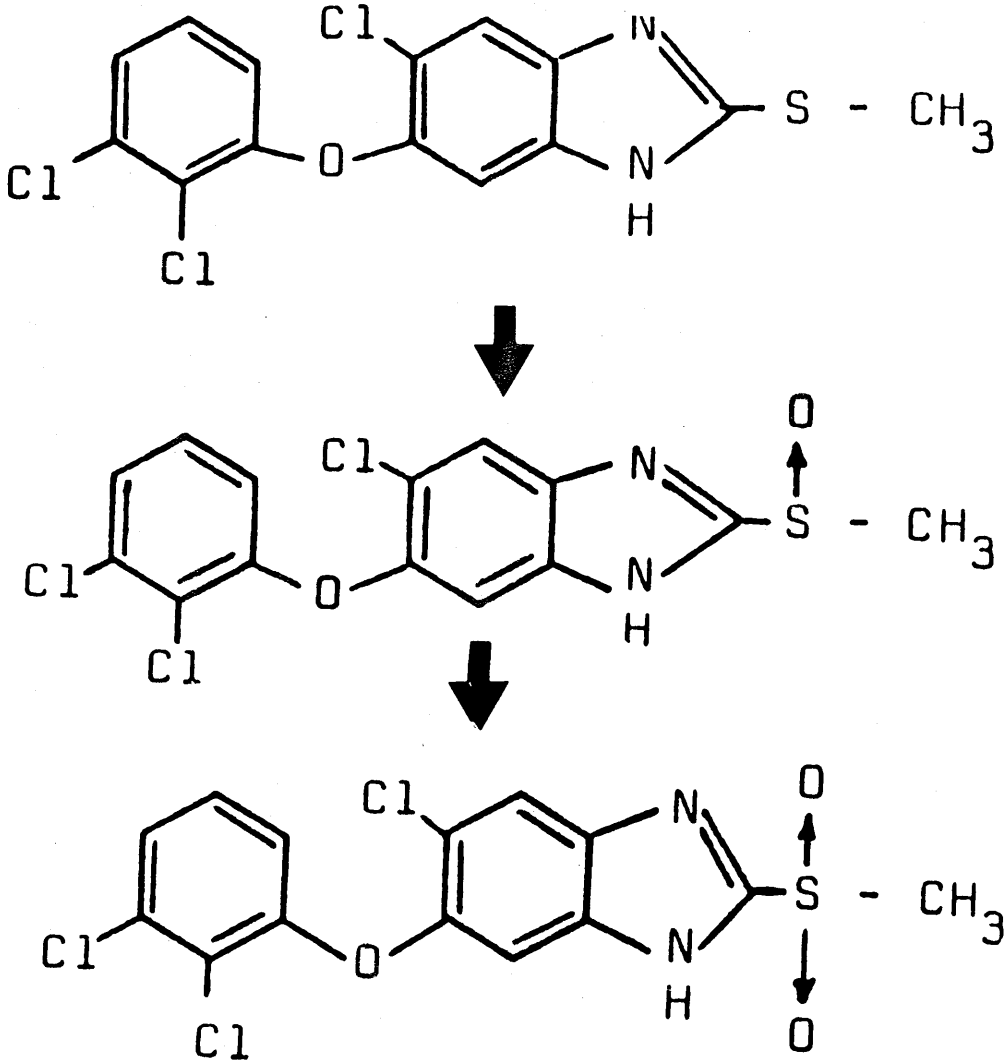
Thirdly, its availability is considerably higher than other benzimidazoles. For example, at dose rates of 10 $mg.kg^{-1}$ the maximum mean concentrations of the parent compound plus metabolites with triclabendazole was 25.84 $\mu g.ml^{-1}$ whereas for parent and metabolites of albendazole, fenbendazole and oxfendazole these were 3.90, 1.00 and 0.96 $\mu g.ml^{-1}$ respectively (Marriner and Bogan 1980, 1981b) (Tables 2.6.7.1, 2.6.8.1 and 2.6.9.1).

Fourthly, triclabendazole produces higher concentrations in cattle than in sheep at the same dose rate in contrast to other benzimidazoles, viz thiabendazole, fenbendazole, oxfendazole and albendazole where the reverse is true (reflected by increased dosage rates in cattle for these compounds).

In conclusion therefore, triclabendazole is a unique benzimidazole and it may be that the other side groups related to salicylanilide may be more important to its action than the benzimidazole nucleus.

FIGURE 2.6.4

METABOLIC PATHWAY OF TRICLABENDAZOLE



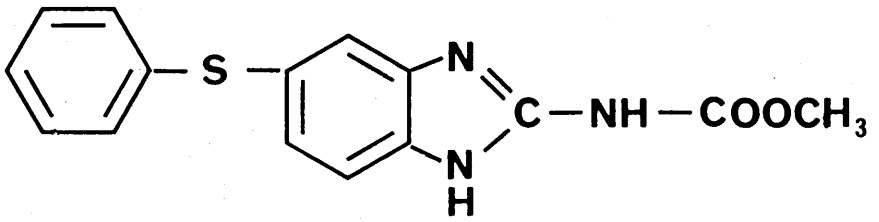
TRICLABENDAZOLE

SULFOXIDE

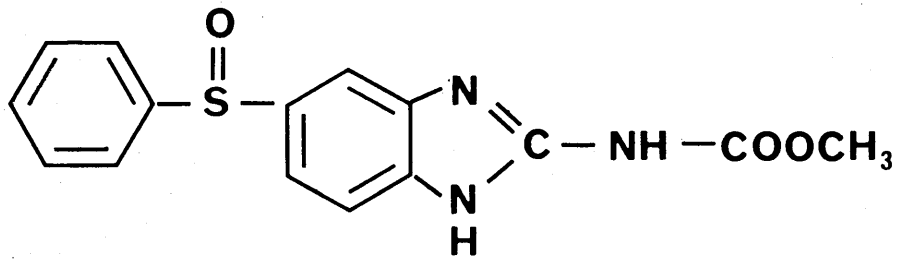
SULFONE

FIGURE 2.6.5

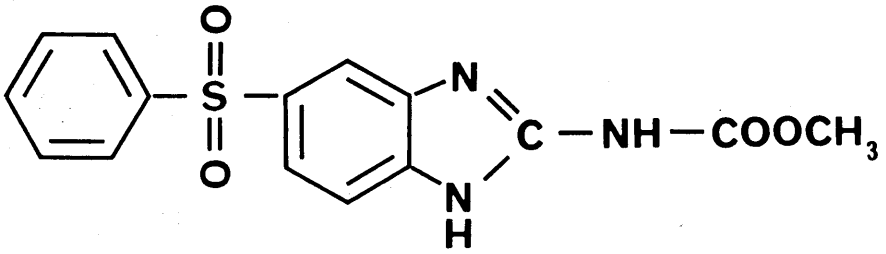
METABOLIC PATHWAY OF FENBENDAZOLE



FENBENDAZOLE



OXFENDAZOLE



SULFONE

GENERAL DISCUSSION

The pharmacokinetic behaviour of the flukicidal drugs studied appears to be very important and relevant to many aspects of their use and efficacy. The importance of the relationship between the long residence time of measurable plasma concentrations and flukicidal activity has been well demonstrated. Two of the salicylanilides studied, rafoxanide and closantel have plasma half-lives much longer than any other commonly used veterinary chemotherapeutic agents.

The differences in efficacy between the three salicylanilide flukicides rafoxanide, closantel and oxyclozanide can be related to the differences in their half-lives especially in the case of oxyclozanide. Thus oxyclozanide which has a relatively short plasma half-life compared with rafoxanide and closantel, has no efficacy claimed against immature fluke.

This study also revealed probable defects in the methods currently used for evaluation of activity against immature flukes and that efficacy claimed against immature fluke may not be truly correct.

It is interesting to consider the practical consequence of the finding that salicylanilides probably do not kill immature flukes but only adults. Normally in the U.K. infection occurs in the autumn and fluke at various immature stages are present in liver during December and January. Lack of activity against immature flukes will therefore fail to prevent the liver damage caused by the migrating fluke, but the extended plasma concentrations will, at least, exert effects against adult flukes

for 15 weeks. The consequence of this is that one, and at most two, treatments given in late autumn/early winter should keep sheep free of chronic infection throughout the year. This would also confirm the results obtained by Armour et al (1973) when they gave rafoxanide twice (at the end of spring and beginning of June) to ewes. The faeces of the ewes remained virtually free of fluke eggs for five weeks after treatment. However if infection is heavy, the possibilities of acute or sub-acute infection are not reduced, and would explain the reason why higher doses (10 and 15 $mg.kg^{-1}$) than 7.5 $mg.kg^{-1}$ were needed for a high efficacy against heavy infection with immature flukes as reported by Campbell et al (1970); Edwards and Parry (1972a).

All the flukicides studied showed a high affinity for plasma-proteins. This property also appears to be important for flukicidal activity especially as flukes ingest blood proteins, particularly albumin, from the time of their invasion of the liver parenchyma.

A common problem with the flukicides is their hazards to the consumer and it seems, from the residue studies with rafoxanide, that the withdrawal period of 28 days may be inadequate.

From the kinetic studies of rafoxanide in plasma and tissue residues, it can be concluded that administration of rafoxanide at low doses and over a period of time would produce similar plasma concentrations adequate for flukicidal activity and also reduces drug toxicity. Thus for example incorporation in feed over a week of the same total dose will result in very similar activity with much reduced possibility of any toxic hazard.

The withdrawal period of closantel recommended by the manufacturers is 14 days. However from the residue experiments it is obvious that consumption of edible tissues especially liver at 14 days continues to be a hazard to consumers.

In the United States, because the residue hazards of salicylanilide fasciolicides are recognised, this group of compounds is not licensed for use in the treatment of liver fluke. With the increase in the problem of fascioliasis especially in cattle, there is an urgent need in that country for a useful flukicide. Albendazole was licensed for use but only as an emergency measure. Recently clorsulon has been licensed for liver fluke treatment in the United States and albendazole has been withdrawn.

With the recent introduction of triclabendazole, it is quite clear that this compound represents a major and different approach to the treatment of fascioliasis. Not only because it possesses better efficacy against all stages of flukes, it would also appear to present much less of residue hazard to the consumer (certainly on a quantitative basis). Although the withdrawal period is also 28 days, it is clear from the kinetic studies that this is adequate and much safer for the consumer.

The pharmacokinetic study of triclabendazole and fenbendazole in sheep given separately or in combination showed that the co-administration of triclabendazole and fenbendazole did not alter the kinetics of either drug. This is important as it is probable that this or a similar combination will be used in cases where mixed nematode and trematode infection are likely to occur.

REFERENCES

Alexander, T. and Collett, R.A. (1974).

Some observations on the pharmacokinetics of trimethroprim in the horse.

British Journal of Pharmacology, 52, 142.

Alexander, T. and Collett, R.A. (1975).

Trimethoprim in the horse.

Equine Veterinary Journal, 7, 203-206.

Alton, K.B.; Patrick, J.E. and McGuire, J.L. (1979).

High performance liquid chromatographic assay for the anthelmintic assay for the anthelmintic agent mebendazole in human plasma.

Journal of Pharmaceutical Sciences, 68, 880-882.

Anderson, E.S. (1967).

Factors of transfer and resistance to antibiotics among enterobacteria.

Annales de / Institut Pasteur (Paris), 112, 547-563.

Anderson, P.H.; Matthews, J.G.; Berrett, S.; Brush, P.J. and Patterson,D.S.P. (1981).

Changes in plasma enzyme activities and other blood components in response to acute and chronic liver damage in cattle.

Research in Veterinary Science, 31, 1-4.

Andreasen, F.; Elsborg, L.; Husted, S. and Thomsen, Q. (1978).
Pharmacokinetics of sulphdiazine and trimethoprim in man.
European Journal of Clinical Pharmacology, 14, 57-67.

Annen, J.M. (1974).
Efficacy and acceptability of rafoxanide and diamphenethide in the treatment of acute and chronic fascioliasis of sheep, with consideration of clinical and biochemical changes.
Helminthological Abstracts, 43A, 827.

Armour, J. (1983).
Modern anthelmintics for farm animals.
In: Pharmacological Basis of Large Animal Medicine, 1st ed., eds. Bogan, J.A., Lees, P. and Yoxall, A.T. p. 185.

Armour, J. and Corba, J. (1970).
The anthelmintic activity of rafoxanide against immature Fasciola hepatica in sheep.
Veterinary Record, 87, 213-214.

Armour, J. and Corba, J. (1972).
The anthelmintic efficiency of diamphenethide against Fasciola hepatica in sheep.
Veterinary Record, 91, 211.

Armour, J.; Corba, J. and Bruce, R.C. (1973).
The prophylaxis of ovine fascioliasis by the strategic use of rafoxanide.
Veterinary Record, 92, 83-88.

Averkin, E.A.; Beard, C.C.; Dvorak, C.A.; Edward, J.A.; Fried, J.H.; Kilian, J.G.; Schiltz, R.A.; Kistner, T.P.; Drudge, J.H.; Lyons, E.T.; Sharp, M.L. and Corwin, R.M. (1975).
Methyl 5(6)-phenyl sulphinyl-2-benzimidazole carbamate, a new potent anthelmintic.
Journal of Medical Chemistry, 18, 1164-1166..

Baeder, C.; Bahr, H.; Christ, O.; Duwel, D.; Kellner, H.M.; Kirsch, R.; Loewe, H.; Schultes, E. and Westen, H. (1974).
Fenbendazole: A new highly effective anthelmintic.
Experientia, 30, 753-754.

Baggot, J.D. (1977).
In: Principles of drug disposition in domestic animals: The basis of veterinary clinical pharmacology, W.B. Saunders & Co., Philadelphia,pp. 155-158.

Baggot, J.D. (1978).
Some aspects of clinical pharmacokinetics in veterinary medicine. II.
Journal of Veterinary Pharmacology and Therapeutics, 2, 111-118.

Barnett, M. and Bushby, S.R.M. (1970).
Trimethoprim and the sulphonamides.
Veterinary Record, 87, 43-51.

Barrowman, M.M.; Marriner, S.E. and Bogan, J.A. (1984a).
The binding and subsequent inhibition of tubulin polymerization in Ascaris suum (in vitro) by benzimidazole anthelmintics.
Biochemical Pharmacology, 33, 3037-3040.

Barrowman, M.M.; Marriner, S.E. and Bogan, J.A. (1984b).
The fumarate reductase system as a site of anthelmintic attack in Ascaris suum.
Bioscience Reports 4, 879-883.

Bassett, D.C.J. (1971).
The sensitivity of Pseudomonas pseudomallei to trimethoprim and sulphamethoxazole in vitro.
Journal of Clinical Pathology, 24, 798-800.

Basson, P.A.; Kellerman, T.S.; Albl P, Von Maltitz, L.J.F.; Miller, E.S. and Welman, W.G. (1975).
Blindness and encephalopathy caused by Helichrysum argyrosphaerum in sheep and cattle.
Onderstepoort Journal of Veterinary Research 42, 135-148.

Batey, R.G. and Smits, A.F. (1976).
The isolation of Bordetella bronchiseptica from an outbreak of canine pneumonia.
Australian Veterinary Journal, 52, 184-186.

Bergan, T. and Brodwall, E.K. (1972).
Human pharmacokinetics of a sulphamethoxazole - trimethoprim combination.
Acta medica Scandinavica, 192, 483-492.

Black, N.M. and Froyd, G. (1972).

The possible influence of liver fluke infestation on milk quality.

Veterinary Record, 90,71-71.

Bogan, J.A. (1983).

Absorption and distribution.

In: Pharmacological Basis of Large Animal Medicine, 1st Ed., eds. Bogan, J.A., Lees, P. and Yoxall, A.T. p13-15.

Bogan, J.A.; Galbraith, A.; Baxter, P.; Mohammed Ali, N.A.K. and Marriner, S.E. (1984).

Effect of feeding on the fate of orally administered phenylbutazone, trimethoprim and sulphadiazine in the horse.

Veterinary Record, 115, 599-600.

Bogan, J.A. and Marriner, S.E. (1980).

Analysis of benzimidazole in body fluids by high performance liquid chromatography.

Journal of Pharmaceutical Sciences, 69, 422-423.

Bogan, J.A.; Marriner, S.E. and Galbraith, E.A. (1981).

Pharmacokinetics of levamisole in sheep.

Research in Veterinary Science, 32, 124-126.

Bogan, J.A.; Mohammed Ali, N.A.K.; Formica G. and Hajdu, P.(1985)
Pharmacokinetic studies with the triclabendazole/fenbendazole combination in sheep and cattle.
11th Conference of the World Association for the Advancement of Veterinary Parasitology, August 1985, Rio de Janeiro, Brazil.

Bohni, E. (1969).
Chemotherapeutic activity of the combination of trimethoprim and sulphamethoxazole in infections of mice.
Postgraduate Medical Journal, Suppl. 45, 18-21.

Bohni, E.; Fust, B.; Rieder, J.; Schaerer, K. and Havas. L.(1969).
Comparative toxicological, chemotherapeutic and pharmacokinetic studies in animals and man.
Chemotherapy, 14, 195-226.

Boisvenue, R.J.; Brandt, M.C.; Galloway, R.B.; Hendrix,J.C.(1983)
In vitro activity of various anthelmintic compounds against *Haemonchus contortus* larvae.
Veterinary Parasitology, 13, 341-347.

Boray, J.C. (1969).
Experimental fascioliasis in Australia.
Advances in Parasitology, 7, 96.

Boray, J.C. (1981).
Control of fascioliasis and paramphistomosis - are there really new concepts and approaches?
Australian Journal of Science, pp 240-242.

Boray, J.C.; Crowfoot, P.D.; Strong, M.B.; Allison, J.R.; Schellenbaum, M.; Von Orelli, M. and Sarasin, G. (1983). Treatment of immature and mature Fasciola hepatica infections in sheep with triclabendazole. Veterinary Record, 113, 315-317.

Boray, J.C. and Happich, F.A. (1968). Standardized chemotherapeutical tests for immature and mature Fasciola hepatica infections in sheep. Australian Veterinary Journal, 44 72-78.

Boray, J.C.; Happich, F.A. and Andrews, J.C. (1967). Comparative chemotherapeutical tests in sheep infected with immature and mature Fasciola hepatica. Veterinary Record, 80, 218-224.

Boray, J.C. and Roseby, F.B. (1969). The effect of the route of administration on the efficacy of clioxanide against immature Fasciola hepatica in sheep. Australian Veterinary Journal, 45, 363-365.

Boray, J.C.; Strong, M.B. and Allison, J. (1981b). Chemoprophylaxis of fascioliasis. Australian Society of Parasitology, Abst. 1981:20.

Boray, J.C.; Strong, M.B.; Schellenbaum, M. and Von Orelli, M. (1981a).
Chemoprophylaxis of fascioliasis in sheep and cattle.
Abstracts of 9th International Conference of the World Association of Advancement of Veterinary Parasitology.
Veterinary Medical Review, 1981:171.

Boyce, C.B.C.; Jones, T.W.T. and Van Tongeren, W.A. (1967).
The molluscicidal activity of N-tritylmorpholine.
Bulletin of the World Helminthology Organization, 37, 1-11.

Bratton, A.C. and Marshall, E.K. (1939).
A new coupling component for sulphanilamide determination.
Journal of Biological Chemistry, 128, 535-550.

Broome, A.W. and Jones, W.G.M. (1966).
A new drug for the treatment of fascioliasis in sheep and cattle.
Nature, 210, 744-745.

Brown, A.R.; Ruben, L.; Hite, M . and Zwickey, R.E. (1972).
Experimental papilloedema in the dog induced by a salicylanilide.
Toxicology and Applied Pharmacology, 21, 532-541.

Brown, H.W. and Neva, F.A. (1983).
In: Basic Clinical Parasitology, Appleton-Century - Crofts/Norwalk, Connecticut, Fifth ed. 1983, p.227.

Bryant, C.; Cornish, R.A. and Rahman, M.S. (1976).
Adenine nucleotides as indicators of anthelmintic efficacy.
In: Biochemistry of Parasites and Host-parasite relationships. ed: Van den Bossche, H. Janssen Research Foundation. pp.559-562, 599-604.

Burchall, J.J. (1973).
Mechanism of action of trimethoprim - sulphamethoxazole.II.
Journal of Infectious Diseases, 128, S437-S441.

Burchall, J.J. and Hitchings, G.H. (1965).
Inhibitor binding analysis of dihydrofolate reductase from various species.
Molecular Pharmacology, 1, 126-136.

Bury, R.W. and Mashford, M.L. (1979).
Analysis of trimethoprim and sulphamethoxazole in human plasma by high-pressure liquid chromatography.
Journal of Chromatography, 163, 114-117.

Bushby, S.R.M. (1980).
Sulphonamide and trimethoprim combinations.
Journal of American Veterinary Medical Association, 176, 1049-1053.

Bushby, S.R.M. and Barnett, M. (1967).

Trimethoprim - sulphonamide : In vitro sensitivity of 348 strains of bacteria.

Proceedings of the 5th International Congress of Chemotherapy, Vienna, 343-344.

Bushby, S.R.M. and Hitchings, S.G.H. (1968).

Trimethoprim: A sulphonamide potentiator.

British Journal of Pharmacology and Chemotherapy, 33: 72-88.

Bye, A. and Brown, M.E. (1977).

An analytical approach to the quantitation of known drugs in human biological samples by H.P.L.C.

Journal of Chromatographic Science, 15, 365-371.

Callinan, A.P.L. and Cummins, L.J. (1979).

Efficacy of anthelmintics against cattle nematodes.

Australian Veterinary Journal, 55, 370-373.

Campbell, N.J. and Brotowidjoyo, M.D. (1975).

Efficacy of clioxanide and rafoxanide against F. hepatica in sheep by different routes of administration.

Australian Veterinary Journal, 51, 500-503.

Campbell, N.J. and Hotson, I.K. (1971).

The anthelmintic efficacy of clioxanide and rafoxanide against Fasciola hepatica and Haemonchus contortus in sheep.

Australian Veterinary Journal, 47, 5-8.

Campbell, N.J. and Narelle, J.R. (1972).

A controlled test of oxyclozanide and rafoxanide against Fasciola hepatica in calves.

Veterinary Record, 91, 647-649.

Campbell, W.C.; Ostlind, D.A. and Yakstis, J.J. (1970).

The efficacy of 3,5-diiodo-3′-chloro-4-(p-chlorophenoxy)-salicylanilide against immature Fasciola hepatica in sheep.

Research in Veterinary Science, 11, 99-100.

Cannon, R.W. (1976).

Clinical evaluation of Tribrissen. New antibacterial agent for dogs and cats.

Veterinary Medicine and Small Animal Clinician, 71, 1090-1091.

Cawdery, M.J.H. (1972).

The effect of regime therapy in Fascioliasis. II. The comparison of four drugs in ewes.

Irish Veterinary Journal, 26 137-143.

Cawdery, M.J.H. (1976).

The effect of fascioliasis on ewe fertility.

British Veterinary Journal, 132, 568-575.

Cawdery, M.J.H. and Donnelly, W.J.C. (1972).

The effect of regime therapy in fascioliasis. III. The comparison of four drugs in wethers and lambs on the basis of mortality and production.

Irish Veterinary Journal, 26 163-170.

Chaia, G.; Chiari, L.; Da Silva, D.C. and Guerrero, J. (1981).
Pilot trials on the treatment of Dermatobia hominis infections in cattle with closantel.
American Journal of Veterinary Research, 42, 1240-1241.

Chevis, R.A.F., Kelly, J.D. and Griffin, D.L. (1980).
The lethal effect of closantel on the metacestodes of Taenia pisiformis in rabbits.
Veterinary Parasitology, 7, 333-337.

Cobb, P.H. and Hill, G.T. (1976).
High performance liquid chromatography of some sulphonamides.
Journal of Chromatography 123, 444-447.

Colegrave, A.J. (1968a).
Fascioliasis: Field trials of nitroxynil in sheep.
Veterinary Record, 82, 343-347.

Colegrave, A.J. (1968b).
Fascioliasis: Field trials of nitroxynil in cattle.
Veterinary Record, 82, 373-376.

Coles, G.C. (1977).
The biochemical mode of action of some modern anthelmintics.
Pesticide Science 8, 536-543.

Corba, J.; Lietava, P.; Duwel, D., and Reisenleiter, R. (1979).
Efficacy of fenbendazole against the most important trematodes and cestodes of ruminants.
British Veterinary Journal, 135, 318-323.

Corbett, J.R. (1974).
The biochemical mode of action of pesticides.
In: London and New York Academic Press.

Corbett, J.R. and Goose, J. (1971a).
The biochemical mode of action of the fasciolicides nitroxynil, hexachlorophane and oxyclozanide.
Biochemical Journal, 121, 41.

Corbett, J.R. and Goose, J. (1971b).
A possible biochemical mode of action of the fasciolicides nitroxynil, hexachlorophane and oxyclozanide.
Pesticide Science, 2, 119-121.

Cornish, R.A. and Bryant, C. (1976).
Changes in energy metabolism due to anthelmintics in *Fasciola hepatica* maintained *in vitro*.
International Journal for Parasitology, 6, 393-398.

Cornish, R.A.; Rehm, C.A.; Butler, R.W. and Bryant, C. (1977).
The *in vivo* effects of rafoxanide on the energy metabolism of *Fasciola hepatica*.
International Journal for Parasitology, 7, 217-220.

Cotteleer, M.C. and Fameree, L.(1971).

Preliminary trials of oxyclozanide in Belgium.

Veterinary Record, 89 114-115.

Craig, G.R. (1972).

The place of potentiated trimethoprim in the therapy of diseases of the skin in dogs and cats.

Journal of Small Animal Practice 13, 65-70.

Craig, T.M. and Bell, R.R. (1978).

Evaluation of fenbendazole as an anthelmintic for gastro-intestinal nematode in cattle.

American Journal of Veterinary Research, 39, 1037-1038.

Craig, G.R. and White, G. (1976).

Studies in dogs and cats dosed with trimethoprim and sulphadiazine.

Veterinary Record, 98, 82-86.

Crossland, N.O., Bennet, M.S. and Hope Cawdery, M.J. (1969).

Preliminary observations on the control of Fasciola hepatica with the molluscicide N-tritylmorpoline.

Veterinary Record, 84, 182-184.

Dagorn, M. (1984).

Plasma kinetics of bithionol sulphoxide and its metabolites in cattle.

Recueil de Medecine Veterinarie, 106, 371-376.

Davis, M.; Lucas, J.M.S.; Rosenbaum, J. and Wright, D.E. (1966).
4-cyano-2-iodo-6-nitrophenol: A new fasciolicide.
Nature, London, 211, 882-883.

Davitiyananda, D. and Rasmussen, F. (1974).
Half-lives of sulphadoxine and trimethoprim after a single intravenous infusion in cows.
Acta Veterinaria Scandinavica, 15, 356-365.

Dawes, B.(1961).
On the early stages of Fasciola hepatica penetrating into the liver of an experimental host, the mouse:
A histological picture.
Journal of Helminthology, R.T. Leiper Supplement 1961, pp 41-52.

Dedek, W.; Grahl, R.; Schwarz, H. and Ludwig, P. (1978).
Metabolism, residues and excretion of the anthelmintic ^{131}I-rafoxanide in blood, milk, meat and urine at the lactating cow.
Archiv fuer Experimentelle Veterinaer-Medizin, Leipzig, 32 951-955.

Dedek, W.; Schwarz, H. and Liebaug, E. (1976).
On degradation and secretion of anthelmintic ^{131}I-rafoxanide on cattle and sheep.
Archiv fuer Experimentelle Veterinaer - Medizin, Leipzig, 30 423-426.

Dedek, W.; Schwarz, H. and Liebaug, E. (1977).
Degradation and Dynamics of residues of the anthelmintic ^{131}I-rafoxanide in blood, milk, meat and fat following different dosages on cattle.
Archiv fuer Experimentelle Veterinaer-Medizin, Leipzig, 31 365-368.

Delatour, P.; Tiberghien, M.P. and Besse, S. (1983).
An H.P.L.C. procedure for the quantification of five metabolites of febantel in sheep serum.
Journal of Veterinary Pharmacology and Therapeutics, 6, 233-235.

De Nollin, S. and Van den Bossche, H. (1973).
Biochemical effects of mebendazole on Trichinella spiralis larvae.
Journal of Parasitology, 59, 970-976.

Divers, T.J.; Byars, T.D.; Murch, O. and Sigel, C. (1981).
Experimental induction of Proteus mirabilis cystitis in the pony and evalution of therapy and trimethoprim-sulphadiazine.
American Journal of Veterinary Research, 42 1203-1205.

Dobbins, S.E. and Wellington, A.C. (1982).

Comparison of the activity of some fasciolicides against immature liver fluke in calves.

Veterinary Record, 111, 177-178.

Dobson, A. (1967).

Physiological pecularities of the ruminant relevant to drug distribution.

Federation Proceedings, 26, 994-1000.

Dorchies, P.; Frang, M.; De Lahitte, J.D. (1983).

Activity of triclabendazole (DCl) on Fasciola hepatica in lambs.

Revue de Medicine Veterinaire, 134, 231-234.

Downey, N.E. (1980).

Effect of treatment with levamisole or fenbendazole on primary experimental Dictyocaulus viviparus infection and on resistance in calves.

Veterinary Record, 107, 271-275.

Druckrey, E. and Metzger, H. (1973).

2,6-Dihydroxybenzoic acid anilides active against liver flukes. A hansch analysis.

Journal of Medical Chemistry, 16 436-439.

Duncan, J.L.; Armour, J.; Bairden, K.; Jennings, F.W. and Urquhart, G.M. (1976).
The successful removal of inhibited fourth stage Ostertagia ostertagi larvae by fenbendazole.
Veterinary Record, 98, 342.

Duncan, J.L.; Armour, J.; Bairden, K.; Jennings, F.W. and Urquhart, G.M. (1977).
The activity of fenbendazole against inhibited 4th stage larvae of Ostertagia ostertagi.
Veterinary Record, 101, 249.

Durr, U.M. (1976).
Clinical experiences with trimethoprim - sulphadiazine in coccidiosis of dogs and cats.
Tierarztliche Umschau, 31 177-178.

Duwel, D. (1977).
Fenbendazole. II. Biological properties and activity.
Pesticide Science, 8, 550-555.

Duwel, D.; Hajdu, P. and Damm, D. (1975a).
The pharmacokinetics of fenbendazole.
Berliner und Munchener Tierarztliche Wochenschrift, 88, 131-134.

Duwel, D.; Kirsch, R. and Reisenleiter, R. (1975b).
The efficacy of fenbendazole in the control of trematodes and cestodes.
Veterinary Record, 97, 371.

Duwel, D. and Metzger, H. (1973).
2,6-Dihydroxybenzoic acid anilides as fasciolicides.
Journal of Medical Chemistry, 16 433-436.

Edwards, C.M. and Parry, T.O. (1972a).
Treatment of experimentally-produced acute fascioliasis in sheep.
Veterinary Record, 90, 523-526.

Edwards, C.M. and Parry, T.O. (1972b).
Experimentally-produced acute fascioliasis in sheep: A further trial with rafoxanide and diamphenethide.
Veterinary Record, 91 483-484.

Egerton, J.R.; Yakstis, J.J. and Campbell, W.C. (1970).
The efficacy of rafoxanide (3,5-di-iodo-3'-4'(p-chlorophenoxy) salicylanilide against Haemonchus contortus in sheep.
Research in Veterinary Science,11, 382-384.

Ellion, G.B.; Singer, S. and Hitchings, G.H. (1954).
Antagonists of nucleic acid derivatives. VIII. Synergism in combinations of biochemically related antimetabolites.
Journal of Biological Chemistry, 208, 477-488.

Falco, E.A.; Hitchings, G.H.; Russell, P.B. and Vanderwerff, H. (1949).
Antimalarials as antagonists of purines and pteroyl-glutamic acid.
Nature, London, 164, 107-108.

Faust, E.C.; Russell, P.F. and Jung, R.C. (1970).
Fasciola hepatica Linnaeus, 1758.
Digenetic trematodes.
In: Craig and Faust's Clinical Parasitology, 8th ed.
eds. Faust, E.C.; Russell, P.F. and Jung, R.C.
Lea and Febiger, Philadelphia 1970, pp 460-465.

Fetterer, R.; Rew, R.S. and Knight, R. (1982).
Comparative efficacy of albendazole against Fasciola hepatica in sheep and calves, relationship to serum drug metabolite levels.
Veterinary Parasitology, 11, 309-316.

Fingl, E. and Woodbury, D.M. (1975).
In: The Pharmacological Basis of Therapeutics.
eds. Goodman, L.S. and Gilman, A. Fifth Edition.
Macmillan Publishing Co. Inc., Bailliere Tindall, London, pages 13-14.

Foreyt, W.J. and Todd, A.C. (1973).
Action of oxyclozanide against adult Fascioloides magna (Bassi, 1875) infections in white-tailed deer.
Journal of Parasitology, 59 208-209.

Froyd, G. (1969).

The efficacy of oxyclozanide in heavy cattle.

Veterinary Record, 85, 705-708.

Gallien, R. (1973).

Antibacterial action of trimethoprim - sulphamethoxazole.

Journal of Infectious Diseases, 128, S486-497.

Gelmo, P. (1908).

Sulphamides of p-aminobenzenesulphonic acid.

Journal of Chemical Research, 77, 369-382.

Genchi, C. and Locatelli, A. (1969).

Sorbitol dehydrogenase and aspartate transaminase in cattle affected with fascioliasis.

Atti della Societa Italiana delle Scienze Veterinarie, 22, 565-567.

Gochin, R.; Kanfer, I. and Haigh, J.M. (1981).

Simultaneous determination of trimethoprim,sulphamethoxazole and N^4-acetylsulphamethoxazole in urine by high-performance liquid chromatography.

Journal of Chromatography, 223, 139-145.

Godfrain, J.C.; Rico, A.G. and Lorgue, C. (1969b).

Study, using radioactive sulphur, of the metabolism of bithionol sulphoxide or BTS in cattle.

Revue de Medecine Veterinarie 120, 441-448.

Godfrain, J.C.; Rico, A.G.; Lorgue, G. and Moris-Varga (1969a).
Metabolism of bithionol sulphoxide in rabbit and ewe.
Revue de Medecine Veterinarie, 120, 145-158.

Gordon, H. McL. (1974).
In: Proceedings of the Third International Congress of Parasitology, Munich, August, 1974.
Facta Publications, Vienna 3, 1392.

Gower, P.E. and Tasker, P.R.W. (1976).
Comparative double-blind study of cephalexin and co-trimoxazole in urinary tract infections.
British Medical Journal, 1, 684-686.

Gruneberg, R.N. and Kolbe, R. (1969).
Trimethoprim in the treatment of urinary infections in hospital.
British Medical Journal, 1, 545-547.

Guerrero, J.; Michael, B.F.; Rohovsky, N.W. and Campbell, B.P. (1983).
The activity of closantel as an equine antiparasitic agent.
Veterinary Parasitology, 12, 71-77.

Guerrero, J.; Page, M.R. and Schad, G.A. (1982).
Anthelmintic activity of closantel against Ancylastoma caninum in dogs.
Journal of Parasitology, 68 616-619.

Guilhon, J.F.; Jolivet, G. and Barnabe, R. (1971).
Study of the acute toxicity, for the sheep, of a new fasciolicide: Rafoxanide.
Bulletin de Academie Veterinaire de France, 44, 33-37.

Guilhon, J.F.; Jolivet, G. and Caillier, R.L. (1970).
Activity of rafoxanide against immature form of Fasciola hepatica.
Bulletin de Academie Veterinaire de France, 63, 419-426.

Guralp, N. and Tinar, R. (1984).
Trematodiasis in Turkey: Comparative efficacy of triclabendazole and niclofolan against natural infections of Fasciola hepatica and Fasciola gigantica in sheep.
Journal of Helminthology,58 113-116.

Gurlap, N. and Weissenburg, H. (1969).
On the treatment and early diagnosis of Fasciola gigantica infection in sheep.
Berliner und Muenchener Tieraeztliche Wochenschrift, 82, 261-263.

Gyurik, R.J.; Chow, A.W.; Zaber, B.; Brunner, E.L.; Miller, J.A; Villani, A.J.; Petka, L.A. and Parish, R.C. (1981).
Metabolism of albendazole in cattle, sheep, rats and mice.
Drug Metabolism and Disposition, 9,503-508.

Hall, G.A.; Kelly, J.D.; Whitlock, H.V. and Ritchie,L. (1981).
Prolonged anthelmintic effect of closantel and disophenol against a thiabendazole selected resistant strain of Haemonchus contortus in sheep.
Research in Veterinary Science, 31 104-106.

Hall, G.A.; McConnel, P.A. and Graham, J.M. (1980).
Anthelmintic activity of closantel against benzimidazole resistant strains of Haemonchus contortus and Trichostrongylus Colubriformis in sheep.
Australian Veterinary Journal, 56, 461-462.

Harfenist, M. (1973).
Diamphenethide - A new fasciolicide active against immature parasites.
Pesticide Science, 4, 871-882.

Harrow, W.T. (1969).
Stilesia infestation in sheep.
Veterinary Record, 84, 564.

Hennessy, D.R. (1985).
Examination and manipulation of the pharmacokinetic behaviour of benzimidazole anthelmintics.
Ph.D Thesis; Macquarie University, Australia.

Hills, N.H.; Bultitude, M.I., and Eykyn, S.(1976).
Co-trimoxazole in prevention of bacteriuria after prostatectomy.
British Medical Journal, 2, 498-499.

Hitchings, G.H. (1973).
Mechanism of action of trimethoprim - sulphamethoxazole I.
Journal of Infectious Diseases, 128, 5433-5436.

Hitchings, G.H. and Burchall, J.J. (1965).

Inhibition of folate biosynthesis and function as a basis for chemotherapy.

Advances in Enzymology, 27, 417-469.

Hitchings, G.H. and Bushby, S.R.M. (1961).

5-Benzyl-2,4-diaminopyrimidines, a new class of systemic antibacterial agents.

5th International Congress of Biochemistry, Moscow, p 165.

Hitchings, G.H.; Elion, G.B.; Vanderwerf, H. and Falco, E.A. (1948).

Pyrimidine derivatives as antagonists of pteryoglutamic acid.

Journal of Biological Chemistry, 174, 765-766.

Hitchings, G.H.; Falco, E.A.; Elion, G.B.; Singer, S.; Waring, G.B.; Hutchison, D.J. and Burchenall, J.H. (1952b).

2,4-Diamino-Pyrimidines as antagonists of folic acid and folinic acid.

Archives of Biochemistry and Biophysics, 40, 479-481.

Hitchings, G.H.; Falco, E.A.; Vanderwerff, H.; Russell, P.B. and Elion, G.B. (1952a).

Antagonists of nucleic acid derivatives. VII. 2,4-Diaminopyrimidines.

Journal of Biological Chemistry, 199, 43-56.

Holenweger, J.A. and Taroco, J.E. (1982).

A new mode of action of closantel.

Gaceta Veterinaria, 44, 420-426.

Holmes, P.H.; Dargie, J.D.; Maclean, J.M. and Mulligan, W. (1968).

Albumin and globulin turnover in chronic ovine fascioliasis.

Veterinary Record, 83, 227-228.

Holmes, P.H.; Maclean, J.M.; Dargie, J.D.; Jennings, F.W. and Mulligan, W. (1967).

The anaemia in fascioliasis, experiments with ^{51}C-labelled red cells.

Veterinary Record, 80, 285-286.

Horak, I.G.; Louw, J.P. and Raymond, S.M. (1971).

Trials with rafoxanide. 3. Efficacy of rafoxanide against the larvae of the sheep nasal bot fly oestrus ovis.

Journal of the South African Veterinary Medical Association, 42, 337-339.

Horak, I.G.; Snijders, A.J. and Louw, J.P. (1972).

Trials with rafoxanide. 5. Efficacy studies against Fasciola hepatica, Fasciola gigantica, Paramphistomum microbothrium and various nematodes in sheep.

Journal of the South African Veterinary Medical Association, 43, 397-403.

Hughes, D.L.; Treacher, R.J. and Harness, E. (1974).

The anthelmintic activity of diamphenethide against immature Fasciola hepatica in goats and the course of experimental infections as demonstrated by plasma enzyme changes.

Research in Veterinary Science, 17, 302-311.

Hughes, D.T.D. (1969).

Treatment of exacerbations of chronic chest infections with combinations of sulphamethoxazole-trimethoprim (including comparison with ampicillin).

Postgraduate Medical Journal, Nov. Suppl. 45, 86-88.

Hughes, W.T.; Feldman, S. and Sanyal, S.K. (1975).

Treatment of Pneumocystis carinii pneumonitis with trimethoprim-sulphamethoxazole.

Canadian Medical Association Journal, 112, 47S-50S.

Ireland, C.M.; Gull, K.; Gutteridge, W.E. and Pogson, C.I. (1979).

The interaction of benzimidazole carbamates with mammalian microtubule protein.

Biochemical Pharmacology, 28, 2680-2682.

Janssen and Sipido (1977).

Janssen pharmaceutica NV: Basic information on closantel (R31520), Beerse 2340, Belgium.

Jennings, F.W.; Mulligan, W. and Urquhart, G.M. (1955).

Some isotopic studies on the blood loss associated with Fasciola hepatica infection in rabbits.

Transactions of the Royal Society of Tropical Medicine and Hygiene, 49, 305.

Jennings, F.W.; Mulligan, W. and Urquhart, G.M. (1956).

Radio-isotope studies on the anaemia produced by infection with Fasciola hepatica.

Experimental Parasitology, 5, 458-468.

Jones, E.H. (1966).

Fascioliasis and oxyclozanide.

Veterinary Record, 79, 716-717.

Kadhim, J.K.; Jabbir, M.H. and Ayoub, A.T. (1973).

Treatment of Fasciola gigantica in sheep.

Tropical Animal Health and Production, 5, 22-26.

Kane, H.J.; Behm, C.A. and Bryant, C. (1980).

The mode of action of closantel, a new flukicide.

In: Programme and Abstracts, 24th conference of the Australian Society for Parasitology, May 1980, 23 Adelaide, South Australia 1980.

Kaplan, S.A.; Weinfeld, R.E.; Cotler, S.; Abruzzo, C.W. and Alexander, K. (1970).

Pharmacokinetic profile of trimethoprim in dog and man.

Journal of Pharmaceutical Sciences, 59, 358-363.

Karlaganis, G.; Munst, G.J. and Bircher, J. (1979).

High pressure liquid chromatographic determination of the anthelmintic drug mebendazole in plasma.

Journal of High Resolution Chromatography and Chromatography Communications, 2, 141-144.

Kelly, J.D.; Hall, C.A.; Whitlock, H.V.; Thompson, H.G.; Campbell, N.J. and Martin, I.A.C. (1977).

The effect of route of administration on the anthelmintic efficacy of benzimidazole anthelmintic in sheep infected with strains of Haemonchus contortus and Trichostrongylus colubriformis resistant or susceptible to thiabendazole.

Research in Veterinary Science, 22, 161-168.

Kelly, J.D.; Whitlock, H.V.; Hogarth-Scott, R.S. and Mears, F.A. (1975).

The anthelmintic efficacy of fenbendazole against mixed nematode infection in sheep.

Research in Veterinary Science, 19, 105-107.

Kelly, W.R. (1974).

Clinical blood chemistry.

In: Veterinary Clinical Diagnosis, 2nd edi. W.R. Kelly, editor, Bailliere Tindall, London, 1974, pp 273-279, 296-297.

Kelsey, F.H. (1966).

Observations on the use of "Zanil" (3,3',5,5',6-pentachloro-2,2'-dihydroxy-benzanilide) against liver fluke disease in Wester Ross.

Veterinary Record, 78, 303.

Kendall, S.B. and Parfitt, J.W. (1962).

The chemotherapy of fascioliasis.

British Veterinary Journal, 118, 1-10.

Kirsch, R. and Duwel, D. (1975).

Laboratory investigations in sheep with a new anthelmintic.

Veterinary Record, 97, 28-31.

Kirchmeier, R.L. and Upton, R.P. (1978).

Simultaneous determination of vancomycin, anisomycin and trimethoprim lactate by high pressure liquid chromatography.

Analytical Chemistry, 50, 349-351.

Knapp, S.E. and Presidente, P.J.A. (1971).

Efficacy of rafoxanide against natural Fasciola hepatica infection in cattle.

American Journal of Veterinary Research, 32, 1289-1291.

Knight, R.A. and Colglazier, M.L. (1977).

Albendazole as a fasciolicide in experimentally infected sheep.

American Journal of Veterinary Research, 38, 807-808.

Kram, T.C. (1972).

Separation of sulfa drugs by high speed liquid chromatography.

Journal of Pharmaceutical Sciences, 61, 254-256.

Kumar, M. and Pachaur, S.P. (1984).

Serum enzyme profile in flukicide treated buffaloes.

Journal of Veterinary Medicine, 4, 13-16.

Lal, S.; Modawal, K.K.; Fowle, A.S.E.; Peach, B. and Popham, R.D. (1970).

Acute brucellosis treated with trimethoprim and sulphamethoxazole.

British Medical Journal, 1, 256-257.

Lee, R. (1973).

The relationship between biological action and structure for some fasciolicidal salicylanilides.

Parasitology, 67, xii.

Leuckart, R. (1882).

Zur Entwicklungs geschi chtet des Leberegels.

Archiv fuer Naturschutz, 1, 80-119.

Lowndes, P. (1984).

Fluke.

Veterinary Practice, Oct. pp 9-10.

Madsen, S.T. and Iversen, P.F. (1964).

Metabolic problems during treatment with long acting sulphonamides.

III International Congress of Chemotherapy, (July 1963), pp 644-648.

Malone, J.B.; Ramsey, R.T. and Loyacano, A.F. (1984).

Efficacy of clorsulon for treatment of mature naturally acquired and 8-week-old experimentally induced Fasciola hepatica infections in cattle.

American Journal of Veterinary Research, 45, 851-854.

Marks, M.I.; Kazemi, Mackay, E. (1973).

In vitro sensitivity of salmonella to ten antimicrobial agents including sulphamethoxazole and trimethoprim, alone and in combination.

Antimicrobial Agents and Chemotherapy, 4, 555-559.

Marriner, S.E. (1980).

The pharmocokinetics of drugs in the ruminant animal.

Ph.D. Thesis, University of Glasgow, 1980.

Marriner, S.E. and Bogan, J.A. (1980).

Pharmacokinetics of albendazole in sheep.

American Journal of Veterinary Research, 41, 1126-1129.

Marriner, S.E. and Bogan, J.A. (1981a).

Pharmacokinetics of fenbandazole in sheep.

American Journal of Veterinary Research, 42, 1146-1148.

Marriner, S.E. and Bogan, J.A. (1981b).

Pharmacokinetics of oxfendazole in sheep.

American Journal of Veterinary Research, 42, 1143-1145.

Marriner, S.E.; Bogan, J.A. and Vandaele, W. (1981).

Comparison of the pharmacokinetics of albendazole and its major metabolites after oral administration of albendazole as a suspension and as a paste formulation to sheep.

Zentralblatt fuer Veterinaer-Medizine Beiheft, 28, 19-26.

Marshall, E.K.Jr.; Emerson, K.Jr. and Cutting, W.C. (1937).

Para-aminobenzenesulfonamide. Absorption and excretion: Method of determination in urine and blood.

Journal of the American Medical Association, 108, 953-957.

Martone, F.; Colella, G.; Palomba, E. and Lovane, G. (1983).

Behaviour of some transferases and lyases in the blood serum of parasitised goats before and after anthelmintic treatment.

Acta. Medica Veterinaria, 29, 129-145.

McBeath, D.J.; Best, M.J. and Preston, N.K. (1977).

Efficacy of fenbendazole against naturally acquired M. expansa infections in lambs.

Veterinary Record, 101, 408-409.

McCaig, J. (1970).

A clinical trial using trimethoprim-sulphadiazine in dogs and cats.

Veterinary Record, 87, 265-266.

McGuinness, B.W. (1969).

A combination of trimethoprim and sulphamethoxazole in upper respiratory infection.

Postrgraduate Medical Journal, Nov. Suppl., 45, 99-101.

Meshi, T.; Yoshikawa, M. and Sato, Y. (1970).

Metabolic fate of bis(3,5-dichloro-2-hydroxy phenyl) - sulphoxide (bithionol sulphoxide).

Biochemical Pharmacology, 19, 1351-1361.

Metzger, H. and Duwel, D. (1973).

Investigations of metabolism in the liver fluke (Fasciola hepatica) as an aid to the development of new anthelmintics.

International Journal of Biochemistry, 4, 133-143.

Michiels, M.; Hendriks, R.; Heykants, S.J. and Van den Bossche, H. (1982).

Pharmacokinetics of mebendazole and flubendazole in animals and man.

Archives Internationales de pharmacodynamie et de Therapie, 256, 180-191.

Miller, E.P.; Gunderson, G.R. and Collier, M.A. (1984).

Clinical field evaluation of a sulphadiazine-trimethoprim combination in horses.

Veterinary Medicine and Small Animal Clinician, 79, 227-231.

Miller, E.P. and Szczech, G.M. (1984).

Safety of an intravenous sulphadiazine-trimethoprim combination in horses.

Veterinary Medicine and Small Animal Clinician, 79, 369-373.

Morris, D.L.; Dykes, P.W.; Marriner, S.E. and Bogan, J.A. (1985).
Albendazole. Objective evidence of response in human hydatid disease.
Journal of the American Medical Association, 253, 2053-2057.

Mrozik, H. (1976).
German offenlegungsschirift 2,556,122
(Chemical Abstracts 85, 94100 n (1976).

Mrozik, H.; Bochis, R.J.; Eskola, P.; Matzuk, A.; Waksmunski, F.S.; Olen, L.E.; Schwartzkopf, G.; Grodski, A.; Linn, B.O.; Lusi, A.; Wu, M.T.; Shunk, C.H.; Peterson, L.H.; Milkowski, J.D.; Hoff, D.R.; Kulsa, P.; Ostlind, D.A.; Campbell, W.C.; Riek, R.F. and Harmon, R.E. (1977).
4-Amino-6-(trichloroethyenyl)-1,3-benzene-disulfonamide, a new potent fasciolicide.
Journal of Medical Chemistry, 20, 1225-1227.

Mrozik, H.; Friedman, J.; Schwartzkopf, G.; Schardt, R.; Patchett, A.A.; Hoff, D.R.; Yakstis, J.J.; Riek, R.F.; Ostlind, D.A., Plishker, G.A.; Butler, R.W.; Cuckler, A.C. and Campbell, W.C. (1969).
A new agent for the treatment of liver fluke infection.
Experientia, 25, 883.

Mulder, G.J.; Temmink, T.J.M. and Koster, H.J. (1982).
The effect of fasting on sulphation and glucuronidation in the rat *in vivo*.
Biochemical Pharmacology, 31, 1941-1944.

Nerenberg, C.; Runkel, R.A. and Matin, S.B. (1978).

Radioimmunoassay of oxfendazole in bovine, equine or canine plasma or serum.

Journal of Pharmaceutical Sciences, 67, 1553-1557.

Ngomuo, A.J.; Marriner, S.E. and Bogan, J.A. (1984).

The pharmacokinetics of fenbendazole and oxfendazole in cattle.

Journal of Veterinary Pharmacology and Therapeutics, 8, 187-193.

Nielsen, P. and Rasmussen, F. (1972).

Elimination of trimethoprim in pigs and goats.

Acta Pharmacologica et toxicologica, 31, suppl. 1, 94.

Nielsen, P. and Rasmussen, F. (1975a).

Elimination of trimethoprim in swine: comparison of results obtained by three analytical methods.

Acta Pharmacologica et toxicologica, 37, 309-316.

Nielsen, P. and Rasmussen, F. (1975b).

Trimethoprim and sulphadoxine in swine. Half-lives, volume of distribution and tissue concentrations.

Zentrablatt fur veterinarmedizin, Reihe A, 22, 564-571.

Nielsen, P. and Rasmussen, F. (1975c).

Half-life and renal excretion of trimethoprim in swine.

Acta pharmacologica et toxicologica, 36, 123-131.

Nielsen, P. and Rasmussen, F. (1976a).

Elimination of trimethoprim, sulphdoxine and their metabolites in goats.

Acta pharmacologica et toxicologica, 38, 104-112.

Nielsen, P. and Rasmussen, F. (1976b).

Influence of age on half-life of trimethoprim and sulphadoxine in goats.

Acta pharmacologica et toxicologica, 38, 113-119.

Nielsen, P. and Rasmussen, F. (1977).

Half-life, apparent volume of distribution and protein binding for some sulphonamides in cows.

Research in Veterinary Science, 22, 205-208.

Nielsen, P.; Romvary, A. and Rasmussen, F. (1978).

Sulphadoxine and trimethoprim in goats and cows: absorption fraction, half-lives and the degradation effect of the ruminal flora.

Journal of Veterinary Pharmacology and Therapeutics, 1, 37-46.

Nolte, H. and Buttner, H. (1973).

Pharmacokinetics of trimethoprim and its combination with sulphamethoxazole in man after a single and chronic oral administration.

Chemotherapy, 18, 274-284.

Oakley, G.A. (1980).

Speed of action of some anthelmintics against Dictyocaulus viviparus infection in cattle.

Veterinary Record, 107, 530-531.

Oakley, G.A.; Cockshott, I.D. and Owen, B. (1984).

Acceptability of levamisole and oxyclozanide for the post parturient ewe and newborn lamb.

Veterinary Record, 114, 381-382.

O'Brien, J.J. (1970).

Toxicological aspects of some modern anthelmintics.

Australian Veterinary Journal, 46, 297-300.

Ostlind, D.A.; Campbell, W.C.; Riek, R.F.; Baylis, P.; Cifelli, S.; Hartman, R.K.; Lang, R.K.; Butler, R.W.; Mrozik, H.; Bochis, R.J.; Eskola, P.; Matzak, A.; Waksmunski, F.S.; Olen, L.E.; Schwartzkopf, G.; Grodski, A.; Linn, B.O.; Lusi, A.; Wu, M.T.; Shunk, C.H.; Peterson, L.H.; Milkowski, J.D.; Hoff, D.R.; Kulsa, P. and Harmon, R.E. (1977).

The efficacy of 4-amino-6-trichloroethenyl-1,3-benzenedisulfonamide against liver fluke in sheep and cattle.

British Veterinary Journal, 133, 211-214.

Pearson, I.G. (1963).

Use of the chromium radioisotope ^{51}Cr to estimate blood loss through ingestion by Fasciola hepatica.

Experimental Parasitology, 13, 186-193.

Penner, M.H. (1975).

Assay of sulphacetamide sodium opthalmic solutions by high-pressure liquid chromatography.

Journal of Pharmaceutical Sciences, 64, 1017-1019.

Pienaar, J.G. (1977).

Nuwere veterinere neuropathologiese toestande in suid-africa.

Journal of the South African Veterinary Medical Association, 48, 13-17.

Piercy, D.W.T. (1978).

Distribution of trimethoprim/sulphadiazine in plasma, tissue and synovial fluids.

Veterinary Record, 102, 523-524.

Pinkiewicz, E. and Madej, E. (1967).

Changes in the peripheral blood of Ca, P, K, Na, Mg and Ap in the course of experimental fascioliasis in sheep.

Acta Parasitologica Polonica, 15, 225-229.

Poet, R.B. and Pu, H.H. (1973).

Analysis of trisulfapyrimidines by high-pressure liquid chromatography.

Journal of Pharmaceutical Sciences, 62, 809-811.

Powers, T.E.; Powers, J.D.; Garg, R.C.; Scialli, V.T. and Hajian, G.H. (1980).

Trimethoprim and sulphadiazine: Experimental infection of Beagles.

American Journal of Veterinary Research, 41, 1117-1122.

Presidente, P.J.A. and Knapp, S.E. (1972).

Anthelmintic effect of rafoxanide against immature Fasciola hepatica in calves.

American Journal of Veterinary Research, 33, 1603-1610.

Prichard, R.K. (1970).

Mode of action of the anthelmintic thiabendazole in Haemonchus contortus.

Nature, London, 228, 684-685.

Prichard, R.K. (1978a)

Sheep Anthelmintics. In: The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia. A.D. Donald, W.H. Southcott and J.K. Dineen (Eds.).

C.S.I. R.O. Melbourne, pp 75-107.

Prichard, R.K. (1978b).

The metabolic profile of adult Fasciola hepatica obtained from rafoxanide-treated sheep.

Parasitology, 76, 277-288.

Prichard, R.K.; Hennessy, D.R. and Steel, J.W. (1978).

Prolonged administration: a new concept for increasing the spectrum and effectiveness of anthelmintics.

Veterinary Parasitology, 4, 309-315.

Prozesky, L. and Pienaar, J.G. (1977).

Amaurosis in sheep resulting from treatment with rafoxanide.

Onderstepoort Journal of Veterinary Research, 44, 257-260.

Pugsley, D.G.; Mwanje, L.; Pearson, C. and Blowers, R. (1969).

Use of trimethoprim and sulphamethoxazole in tropical Africa: Typhoid fever, Salmonella typhi carriage and Staphylococcus aureus sepsis.

Postgraduate Medical Journal, Nov. suppl., 45, 95-99.

Rapic, D.; Dzakula, N.; Caukovic, M. and Stojcevic, D. (1984).

Efficacy of triclabendazole against Fasciola hepatica in naturally infected sheep.

Veterinarski Archiv, 54, 13-18.

Rasmussen, F. (1971).

In vitro antibacterial activity of trimethoprim and sulphonamides on bacteria causing bovine mastitis.

Acta Veterinaria Scandinavica, 12, 131-133.

Rasmussen, F.; Gelsa, H. and Nielsen, P. (1979).

Pharmacokinetics of sulphadoxine and trimethoprim in horses. Half-life and volume of distribution of sulphadoxine and trimethoprim and cumulative excretion of [^{14}C]-trimethoprim.

Journal of Veterinary Pharmacology and Therapeutics, 2, 245-255.

Rasmussen, F. and Svendsen, O. (1976).

Tissue damage and concentration at the injection site after intramuscular injection of chemotherapeutics and vehicles in pigs.

Research in Veterinary Science, 20, 55-60.

Rehberg, P.B. (1943).

A centrifugation method of ultrafiltration using cellophane tubes.

Acta Physiologica Scandinavica, 5, 305-310.

Rehm, M.S. and Bryant, C. (1977).

Studies of regulatory metabolism in Moniezia expansa: Effects of cambendazole and mebendazole.

International Journal for Parasitology, 7, 403-409.

Rehm, W.F.; Thommen, H. and Weiser, H. (1968).

Action of potentiated sulphonamides in avian coccidiosis.

Berliner und Muenchener Tieraeztliche Wochenschrift, 81, 340-343.

Rehm, W.F. and White, G. (1970).

A field trial with trimethoprim and sulphonamide in bacterial diseases of cattle and pigs.

Veterinary Record, 87, 39-40.

Reid, J.F.S.; Armour, J.; Jennings, F.W.; Kirkpatrick, K.S. and Urquhart, G.M. (1967).

The Fascioliasis/Ostertagiasis complex in young cattle. A guide to diagnosis and therapy.

Veterinary Record, 80, 371-374.

Reider, J. (1972).

Quantitative determination of the bacteriostatically active fraction of sulphonamides and the sum of their inactive metabolites in the body fluids.

Chemotherapy, 17, 1-21.

Rew, R.S. (1978).

Mode of action of common anthelmintics.

Journal of Veterinary Pharmacology and Therapeutics, 1, 183-197.

Rew, R.S. and Knight, R.A. (1980).

Efficacy of albendazole for prevention of fascioliasis in sheep.

Journal of the American Veterinary Medical Association, 176, 1353-1354.

Roberson, E.L. (1977).

In: Veterinary Pharmacology and Therapeutics. Editors: Meyer Jones, L.; Booth, N.H. and McDonald, L.E.

Fourth Edition, Iowa University Press, pp 1073-1074.

Roberson, E.L. and Burke, T.M (1980).

Evaluation of granulated fenbendazole (22.2%) against induced and naturally occurring helminth infections in cats.

American Journal of Veterinary Research, 41, 1499-1502.

Roberson, E.L. and Burke, T.M. (1982).

Evaluation of granulated fenbendazole as a treatment for helminth infections in dogs.

Journal of the American Veterinary Medical Association, 180, 53-55.

Robertson, L.; Farrell, I.D. and Hinchliffe, P.M. (1973).

The sensitivity of Brucella abortus to chemotherapeutic agents.

Journal of Medical Microbiology, 6, 549-557.

Romvary, A. and Horvay, M.S. (1976).

Data on the pharmacokinetics of sulphonamide-trimethoprim combinations in suckling pigs.

Zentralblatt fur veterinarmedizin, 23, 781-792.

Roncalli, R.A.; Barbosa, A. and Fernandez, J.F. (1971).

The efficacy of rafoxanide against the larval stages of Oestrus ovis in sheep.

Veterinary Record, 88, 289-290.

Roncalli, R.A.; Fernandez, J.F. and Barbosa, A. (1971).

Field evaluation of Ranizole, a new broad-spectrum anthelmintic in sheep.

Proceedings of 19th World Veterinary Congress, Mexico City, 1971, 2, 615.

Ross, D.B. (1970).

Treatment of experimental Fasciola hepatica infection of sheep with rafoxanide.

Veterinary Record, 87, 110@-111.

Ross, D.B. (1975).

The effect of fenbendazole on nematode parasites in experimentally infected lambs.

Veterinary Record, 96, 357-359.

Ross, J.G. (1970a).

The economics of Fasciola hepatica infection in cattle.

British Veterinary Journal, 126, xiii-xv.

Ross, J.G. and Todd, J.R. (1968).

Epidemiological studies of fascioliasis. A third season of comparative studies with cattle.

Veterinary Record, 83, 695-698.

Ross, J.G.; Todd, J.R. and Dow, C. (1966).

Single experimental infections of calves with liver fluke, Fasciola hepatica (Linnaeus 1758).

Journal of Comparative Pathology, 76, 67-81.

Rowlands, D.ap.T. (1973).

Diamphenethide - A drug offering a fresh approach to the treatment of liver fluke disease in sheep.

Pesticide Science, 4, 883.

Rowlands, D.ap.T. (1974).

Diamphenethide in prophylaxis of ovine fascioliasis.

Veterinary Record, 95, 547-551.

Rowlands, D.ap.T.; Clampitt, R.B. and Macpherson, I.S. (1985).
The ability of diamphenethide to control immature Fasciola hepatica in sheep at a lower than standard dose level.
Veterinary Record, 116, 182-184.

Rowlands, W.T. (1969).
Fascioliasis - the problem.
In: Veterinary Pesticides. S.C.I. Monogr. No. 33., London, Society of Chemical Industry, pp 11.

Saad, M.B.E. and Rubin, R. (1977).
Efficacy of fenbendazole against adult Dictyocaulus viviparus in experimentally infected calves.
American Journal of Veterinary Research, 38, 1427-1428.

Sander, J. and Midtvedt, T. (1970).
The effect of trimethoprim on acute experimental toxoplasmosis in mice.
Acta Pathologica et Microbiologica Scandinavica, 78, 664-668.

Schacht, U.; Duwel, D. and Kirsch, R. (1971).
Uber die Beeinflussung des stoffwechsels von Hymenolepis diminuta durch resorantel.
Zeitschrift fuer parasitenkun De, 37, 278-287.

Schroder, J. (1982).
The safety of injectable rafoxanide in cattle.
Journal of the South African Veterinary Medical Association, 53, 29-31.

Schulman, M.D.; Ostlind, D.A. and Valentino, D. (1982).
Mechanism of action of Mk-401 against Fasciola hepatica inhibition of phosphoglycerate kinase.
Molecular and Biochemical Parasitology, 5, 133-145.

Schulman, M.D. and Valentino, D. (1980).
Fasciola hepatica: effect of 4-amino-6-trichloroethenyl-1,3-benzenedisulfonamide on glycolysis in vitro.
Experimental Parasitology, 49, 206-215.

Schulman, M.D.; Valentino, D.; Cifelli, S.; Lang, R. and Ostlind, D.A. (1979).
A pharmacokinetic basis for the efficacy of 4-amino-6-trichloroethenyl-1,3-benzenedisulfonamide against Fasciola hepatica in the rat.
Journal of Parasitology, 65, 555-561.

Schulman, M.D.; Valentino, D.; Cifelli, S. and Ostlind, D.A. (1982).
Dose-dependent pharmacokinetics and efficacy of MK-401 against old and young-mature infections of Fasciola hepatica in the rat.
Journal of Parasitology, 68, 603-608.

Schwartz, D.E.; Koechlin, B.A. and Weinfeld, R.E. (1969).
Spectrofluorimetric method for the determination of trimethoprim in body fluids.
Chemotherapy, Suppl. ad., 14, 22-29.

Schwartz, D.E. and Reider, J. (1970).
Pharmacokinetics of sulphamethoxazole and trimethoprim in man and their distribution in the rat.
Chemotherapy, 15, 337-355.

Schwartz, D.E. and Ziegler, W.H. (1969).

Assay and pharmacokinetics of trimethoprim in man and animals.

Postgraduate Medical Journal, 45 suppl., 32-37.

Sedman, A.J. and Wagner, J.G. (1976).

CSTRIP, a fortan IV computer program for obtaining initial polyexponential parameter estimates.

Journal of Pharmaceutical Sciences, 65, 1006.

Sewell, M.M.H. (1967).

Serum enzyme activities in acute ovine fascioliasis.

Veterinary Record, 80, 577-578.

Sharma, J.P.; Perkins, E.G. and Bevill, R.F. (1976).

High pressure liquid chromatographic separation, identification and determination of sulfa drugs and their metabolites in urine.

Journal of Pharmaceutical Sciences, 65, 1606-1608.

Sigel, C.W.; Grace, M.E. and Nicol, C.A. (1973).

Metabolism of trimethoprim in man and measurement of a new metabolite: A new fluorescence assay.

Journal of Infectious Diseases, 128, S580-583.

Sigel, C.W.; Ling, G.V.; Bushby, S.R.M.; Woolley Jr., J.L.; De Angelis, D. and Eure, S. (1981).

Pharmacokinetics of trimethoprim and sulphadiazine in the dog: Urine concentrations after oral administration.

American Journal of Veterinary Research, 42, 996-1001.

Simmons, N.A. (1970).

Colistin, Sulphamethoxazole and trimethoprim in synergy against Gram-negative bacteria.

Journal of Clinical Pathology, 23, 757-764.

Sinclair, K.B. and Prichard, R.K. (1975).

The use of disophenol in studies of the pathogenicity of the arrested fourth-stage larvae of Haemonchus contortus in the sheep.

Research in Veterinary Science, 19, 232-234.

Singletary, R.O. and Sancilio, F.D. (1980).

High performance liquid chromatographic analysis of trimethoprim and sulphamethoxazole in dosage forms.

Journal of Pharmaceutical Sciences, 69, 144-146.

Smeal, M.G. and Hall, C.A. (1983).

The activity of triclabendazole against immature and adult Fasciola hepatica infections in sheep.

Australian Veterinary Journal, 60, 329-331.

Smellie, J.M.; Gruneberg, R.N.; Leakey, A. and Atkin, W.S. (1976).

Long term low-dose co-trimoxazle in prophylaxis of childhood urinary tract infections: clinical aspects.

British Medical Journal, 2, 203-206.

Snijders, A.J. and Horak, I.G. (1975).

Trials with rafoxanide. 7. Efficacy against Fasciola hepatica, Haemonchus placei and Bunostomum phlebotomum in cattle.

Journal of the South African Veterinary Medical Association, 46, 265-267.

Snijders, A.J.; Horak, I.G. and Louw, J.P. (1971).

Trials with rafoxanide. 2. Efficacy against Fasciola gigantica in cattle.

Journal of the South African Veterinary Medical Association, 42, 253-257.

Snijders, A.J.; Horak, I.G. and Louw, J.P. (1973).

Trials with rafoxanide. 6. The effect of repeated and single treatments with rafoxanide against Haemonchus contortus and Oestrus ovis.

Journal of the South African Veterinary Medical Association, 44, 251-263.

Snijders, A.J.; Louw, J.P. and Serrano, F.M.H. (1971).

Trials with rafoxanide. 1. Fasciola gigantica in cattle in Angola.

Journal of the South African Veterinary Medical Association, 42, 249-251.

Stephenson, W. (1947).

Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. II. Feeding.

Parasitology, 38, 123-127.

Stuller, T. (1968).

Progress in sulphonamide research.

Progress in Drug Research, 12, 389-457.

Sutherland, I.H. and Batty, A.F. (1971).

Field tolerance of rafoxanide in sheep.

Veterinary Record, 89, 603-605.

Swan, G.E. and Schroder, J. (1981).

A safety trial with rafoxanide in sheep.

Journal of the South African Veterinary Medical Association, 52, 123-125.

Symons, L.E.A. and Roseby, F.B. (1969).

The use of clioxanide as an anthelmintic in sheep infected with Haemonchus contortus.

Australian Veterinary Journal, 45, 385.

Talley, C.; Trenner, N.; Downing, G.V. and Van den Heuvel, W.J. (1971).

Gas chromatographic determination of rafoxanide: [3'-chloro-4'-(4-chloro-phenoxy)-3,5, Di-iodosalicylanilide] in plasma by electron capture determination of its trimethylsily. derivative.

Analytical Chemistry, 43, 1379-1382.

Then, R. and Angehrn, P. (1974).

The biochemical basis of the antimicrobial action of sulphonamides and trimethoprim in vivo. 1. Action of sulphonamides and trimethoprim in blood and urine.

Biochemical Pharmacology, 23, 2977-2982.

Theodorides, V.J.; Gyurik, R.J.; Kingsbury, W.D. and Parish, R.C. (1975).

Anthelmintic activity of albendazole against liver flukes, tape worms, lung and gastrointestinal round worms.

Experientia, 32, 702.

Thomas, A.P.W. (1883).

The life history of the liver fluke (Fasciola hepatica).

Quarterly Journal of Microscopical Science, 23, 99-133.

Thorpe, E. (1968).

Comparative enzyme histochemistry of immature and mature stages of Fasciola hepatica.

Experimental Parasitology, 22, 150-159.

Thorpe, E. and Ford, E.J.H. (1969).

Serum enzyme and hepatic changes in sheep infested with Fasciola hepatica.

Journal of Pathology, 97, 619-629.

Tocco, D.J.; Buhs, R.P.; Brown, H.P.; Matzuk, A.R.; Mertel, H.E.; Harman, R.E. and Trenner, N.R. (1964).

The metabolic fate of thiabendazole in sheep.

Journal of Medical Chemistry, 7, 399-405.

Tocco, D.J.; Egerton, J.R.; Bowers, W.; Christensen, V.W. and Rosenblum, C. (1965).

Absorption, metabolism and elimination of thiabendazole in farm animals and a method for its estimation in biological materials.

Journal of Pharmacology and Experimental Therapeutics, 149, 263-271.

Tsina, I.W. and Matin, S.B. (1981).

Determination of oxfendazole in cow milk by reversed phase high performance liquid chromatography.

Journal of Pharmaceutical Sciences, 70, 858-860.

Turner, K.; Armour, J. and Richards, R.J. (1984).

Anthelmintic efficacy of triclabendazole against Fasciola hepatica in sheep.

Veterinary Record, 14, 41-42.

Udall, V. (1969).

Toxicology of sulphonamide-trimethoprim combination.

Postgraduate Medical Journal, Nov. suppl., 45, 42-45.

Van den Bossche, H. (1972).

In: Comparative Biochemistry of Parasites. Editor Van den Bossche, H. Academic Press, New York and London, pp 455-469.

Van den Bossche, H. (1972a).

In: Comparative Biochemistry of Parasites. Editor Van den Bossche, H. Academic Press, New York and London, pp 139-175.

Van den Bossche, H. (1976).

In: Biochemistry of Parasite and Host-Parasite Relationships. Editor H. Van den Bossche, pp 559-572. Janssen Research Foundation. Elsevier/North-Holland Biochemical Press, Amsterdam.

Van den Bossche, H. and De Nollin, S. (1973).

Effects of mebendazole on the absorption of low molecular weight nutrients by Ascaris suum.

International Journal for Parasitology, 3, 401-407

Van den Bossche, H.; Rochette, F. and Horic, C. (1982).

Mebendazole and related anthelmintics.

Advances in Pharmacology and Chemotherapy, 19, 67-119.

Van den Bossche, H.; Verhoeven, H.; Van Parijs, O.; Lauwers, H. and Thienpont, D. (1979).

Closantel, a new antiparasitic hydrogen ionophore.

Archives Internationales de Physiologie et de Biochemistrie, 87, 851-852.

Van den Heuvel, W.J.A.; Gruber, V.F.; Walker, R.W. and Wolf, F.J. (1977).

Gas liquid chromatographic determination of 4-amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide, a new anthelmintic in biological fluids.

Journal of Agricultural and Food Chemistry, 25, 389-392.

Van Miert, A.S.J. and Groeneveld, H.W. (1969).

Anthelmintics used for the treatment of fascioliasis as uncouplers of oxidative phosphorylation in warm blooded animals.

European Journal of Pharmacology, 8, 385-388.

Vaughan, J.J. (1966).

Preliminary field trials with oxyclozanide, a new fasciolicide.

Veterinary Record, 79, 720-723.

Vishnyauskas, A. and Gudonavitchus, I. (1974).

Studies of metabolism of bithionol-^{35}S, bithionol-^{35}S sulphoxide and bithionol-^{35}S sulphone in sheep's organism and Fasciola.

Proceedings of the 3rd International Congress of Parasitology, Munich (1974).

Vree, T.B.; Hekster, Y.A.; Baars, A.M.; Damsma, J.E. and Van der Kleijn, E. (1978).

Determination of trimethoprim and sulphamethoxazole (Co-trimoxazole) in body fluids of man by means of high performance liquid chromatography.

Journal of Chromatography, 146, 103-112.

Walley, J.K. (1966).

Oxyclozanide (3,3',5,5',6-pentachloro-2,2', dihydroxy-benzanilide) "Zanil" in the treatment of liver fluke Fasciola hepatica in sheep and cattle.

Veterinary Record, 78, 267-275.

Walley, J.K. (1970).

Tetramisole and oxyclozanide in combination in the treatment of gastrointestinal worms, lung worms and liver fluke (Fasciola hepatica) in sheep, goats and cattle.

Veterinary Record, 86, 222-227.

Watson, W.A.; Barnett, K.C. and Terlecki, S. (1972).

Progressive retinal degradation (bright blindness) in sheep: a review.

Veterinary Record, 91, 665-670.

Weinfeld, R.E. and Macasieb, T.C. (1979).

Determination of trimethoprim in biological fluids by high performance liquid chromatography.

Journal of Chromatography, 164, 73-84.

Weir, A.J. and Bogan, J.A. (1985).

Thiabendazole in sheep.

Journal of Veterinary Pharmacology and Therapeutics, in press.

White, G. and Withnell, C.G. (1971).

Experimental salmonellosis in sheep.

Veterinary Record, 88, 371.

Whitelaw, A. and Fawcett, A.R. (1981).

Further studies in the control of ovine fascioliasis by strategic dosing.

Veterinary Record, 109, 118-119.

Wierup, M. (1974).

Sensitivity to sulphamethazole-trimethoprim of Escherichia coli isolated from calves.

Nordisk Veterinaermedicin, 26, 626-633.

Williamson, R.L. and Metcalf, R.L. (1967).

Salicylanilides: a new group of active uncouplers of oxidative phosphorylation.

Sciences, 158, 1694-1695.

Wolff, K.; Echert, J.; Schneiter, G. and Lutz, H. (1983).

Efficacy of triclabendazole against Fasciola hepatica in sheep and goats.

Veterinary Parasitology, 13, 145-150.

Yorke, R.E. and Turton, J.A. (1974).

Effects of fasciolicidal and anticestode agents on the respiration of isolated Hymenolepis dimenuta mitochondria.

Zeitschrift fur Parasitenkunde, 45, 1-10.

Youssef, A.H.S.; El-Gendi, A.Y.I.; El Sayed, M.G.A.; Atef, M. and Abdel Salam, S.A. (1981).

Some pharmacokinetic and biochemical aspects of sulphadiazine and sulphadimidine in ewes.

Journal of Veterinary Pharmacology and Therapeutics, 4, 173-182.