THE DISPOSITION OF METRONIDAZOLE IN GOATS AND ITS RELEVANCE TO THE TREATMENT OF ANAEROBIC INFECTIONS

A THESIS

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ABSTRACT

The recent commercial developments in goat farming in New Zealand, have led to an increase in the value of individual goats and to a growing interest in caprine diseases.

The importance of anaerobic bacteria other than the clostridia, as potential pathogens in humans and animals, has also only recently been recognised, even though anaerobic bacteria have been identified since 1861. Various members of this bacterial group are known to be involved in different conditions of goats, particularly in wound and foot infections.

Metronidazole (Flagyl[1]) is a bactericidal agent which has a specific action against anaerobic micro-organisms. This drug is already widely used in the treatment of selected diseases in dogs, cats and humans, but there was little information available on its use in goats.

The study which forms the basis of this thesis, was to investigate the disposition of metronidazole in eight goats. Both IV and IM routes of administration were studied in the form of a cross-over experiment. Silicone tubing "cages" were implanted subcutaneously, so that the metronidazole concentration versus time profile could be determined, both in serum and in interstitial fluid.

The analysis of serum and tissue cage fluid samples was undertaken using a high pressure liquid chromatography unit, which proved to be reliable over the range of concentrations tested. The system consisted of a Waters Model 6000 A solvent delivery system, a U6K injector, a Z-module radial compression separation system and a Waters programmable automator, Model 710. The mobile phase used was a 75:25 mixture of aqueous potassium hydrogen phosphate and methyl alcohol; this was adjusted to a running speed of 1.5 mls per min. A 450 variable wavelength detector was set at either 0.01 or 0.04 absorbance units, and a constant wavelength of 312 nm.

Given these concentration profiles, a full pharmacokinetic analysis was carried out using standard statistical procedures. The following values were determined:

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[1]. 'Flagyl', May & Baker (NZ) Ltd.

maximum serum and tissue cage fluid concentrations ($C_{max}IV$, $C_{max}IM$, $C_{max}IV_{tc}$), time to maximum serum and tissue cage concentration ($T_{max}IV$, $T_{max}IM$, $C_{max}IM_{tc}$, $T_{max}IM_{tc}$), serum concentration (extrapolated) at zero time (B, B'), half-life ($t_{\frac{1}{2}}$), elimination rate constant (β,β'), volume of distribution ($V_{d(area)}$), area under the concentration curve (AUC), total body clearance (Cl_B), absorption rate constant (k_{ab}), percentage penetration of metronidazole into tissue cage fluid, percentage of drug absorbed into the systemic circulation following IM administration (F), and the total amount of drug which was absorbed into the systemic circulation (in mg/kg).

Following IV administration of a 0.5% w/v solution of metronidazole at a dose rate of 20 mg/kg BWgt, the $t_{\frac{1}{2}}$ at 0.94 ± 0.08 per hour (n=8) was rapid and consistent with a high figure for the elimination rate constant at 0.79 ± 0.09 per hour (n=8). The total body clearance, a more sensitive indicator of the biotransformation and excretion processes than $t_{\frac{1}{2}}$, was also rapid (0.32 ± 0.06 L/kg/hr) which is in keeping with the efficient drug metabolism of the goat. This may account for the low $V_{d(area)}$ which was unexpected for a basic drug of this nature in the ruminant.

Critical parameters for drug concentrations and durations of effect are summarized in Table I.

Metronidazole was rapidly detected in both sera and interstitial fluid (within 0.25 hrs) following the intramuscular administration of a 40% suspension of metronidazole at a dose rate of 20 mg/kg BWgt. The uptake of metronidazole from the injection sites differed markedly between individual goats, resulting in a mean absorption percentage of 42.4% ± 8.8% (n = 8), equivalent to 8.4 mg/kg BWgt. Maximum serum levels were achieved within approximately one hour of IM administration, but the peak was more than ten-fold lower than the corresponding concentration found in serum following IV administration. Peak tissue cage drug concentrations were not achieved until four hours after IM administration.

The maximum drug concentration in tissue cage fluid was greater than the MIC upper threshold for a variety of anaerobic bacteria (12.5 mcg/ml), and this was maintained for 5.5 hrs. The lower limit of the MIC of 3.0 mcg/ml was exceeded for a correspondingly longer period.

TABLE I **Serum and tissue cage fluid drug concentrations and durations of effect, following aministration of metronidazole solutions**

Metronidazole (dose rate : 20 mg/kg BWgt)		C_{max} mcg/ml	T_{max} (hr)	Period that nominated serum conc. was exceeded (hr) 50 mcg/ml	12.5 mcg/ml	3.0 mcg/ml
0.5% w/v solution IV	serum	63.9 ± 12.2 (n=5)	<0.25 (n=5)	0.3 ± 0.05 (n=5)	1.4 ± 0.2 (n=8)	3.1 ± 0.2 (n=8)
	t.c.f	23.67 ± 4.46 (n=8)	1.41 ± 0.58 (n=8)	0	1.5 ± 0.6 (n=6)	7.9 ± 1.0 (n=8)
40% w/v suspension IM	serum	5.5 ± 0.8 (n=8)	1.06 ± 0.6 (n=8)	0	0	4.1 ± 0.7 (n=8)
	t.c.f	13.2 ± 3.9 (n=8)	4.1 ± 0.7 (n=8)	0	5.0 ± 1.5 (n=5)	8.6 ± 1.6 (n=7)

t.c.f Tissue cage fluid

Further pharmacokinetic analysis of the experimental data made it possible to calculate specific medication schedules for the goat. These were established on the basis that serum metronidazole concentrations should be maintained at a level which was bactericidal for the majority of anaerobic bacteria, which included Bacteroides spp., Fusibacterium spp., and Clostridia spp. The recommendation given was that 0.5% w/v metronidazole solution should be administered at a dose rate of 20 mg/kg BWgt and repeated every 4-6 hrs. Using the 40% w/v metronidazole suspension, the dose rate should be 45 mg/kg BWgt and the medication should be repeated every 10-12 hrs. In each case the loading dose was only fractionally greater at 20.3 mg/kg BWgt and 48.5 mg/kg BWgt respectively.

The drug concentration in interstitial fluid (tissue cage fluid), gave some indication of the antimicrobial activity in extravascular tissues, a feature which can not be extrapolated from a profile of serum concentrations.

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CHAPTER 1
GENERAL INTRODUCTION

Goat farming has always been considered an important industry elsewhere in the world. It is a means of providing meat and milk, the mainstay of the diet for a large number of people. The fibre and hides from these animals, are also used for a great variety of purposes. Until recently goats were never very highly regarded in this country, but in the past five years the New Zealand goat industry has undergone rapid expansion. Depending upon the particular breed and geographical area, goats are now being farmed either for dairy, or fibre, or weed control purposes. This development has led to an increase in the value of individual goats and to a growing interest in caprine diseases.

Foot infections, gastrointestinal nematodes, clostridial infections, external parasitism, coccidiosis, mastitis and pneumonia, are among the more important diseases limiting the productivity of this animal species. In particular goats seem very vulnerable to infectious foot conditions. Investigatory work has indicated that the main features are very similar to those found in sheep, in that anaerobic bacteria contribute significantly to the pathogenesis of the disease. Anaerobic bacteria have always been considered important in the diseases of goats and other domestic ruminants, but the involvement of these organisms in a more extended range of disorders of many species is becoming recognized, particularly as specialised isolation and culture methods have been developed for anaerobic bacteria.

The technical problems previously encountered in the culture of anaerobic bacteria meant that their study as potential pathogens has been somewhat neglected. The Clostridia spp. are exceptional, because the syndromes these organisms produce in man, sheep and cattle are usually clinically distinctive. As isolation techniques have improved, clinicians have become aware of the importance of other anaerobic bacteria, with roles both as normal commensal organisms, and as pathogens in a wide variety of pyonecrotic processes. Recent surveys have shown that between 20% (Kimsey and Hirsch, 1978) and 56.3% (Berkhoff, 1978) of samples from clinical infectious processes in different animal species submitted to laboratories, yielded one or more species of anaerobic bacteria.

The antibacterial chemical or drug chosen for therapy in an infectious condition must be active against the causal organism, and in addition be able to penetrate to the site of the infection. The capacity of a drug to penetrate into

extravascular tissue is dependent upon, among other factors; the concentration gradient of the drug from serum to extravascular fluid, the extent of drug binding to protein in sera and tissues, the lipid solubility of the drug, and the degree of ionization in different locations. The binding of the chemical to serum proteins is considered to be one of the most important factors in limiting the penetration of the drug into interstitial fluid, as only the nonionized, unbound form of the drug can pass through the endothelium into the extravascular fluid. Any antibacterial drug should also exhibit an inhibitory effect on the infectious agent, at a concentration that causes minimal toxicity to the host. Ideally, the drug should possess a narrow spectrum of activity and the mechanism of action should be bactericidal. The route of administration should be practical, and result in therapeutic drug concentrations at the site of infection.

Metronidazole (Flagyl)[1] was originally developed for its activity against trichomoniasis, giardia and amoeba, but fortuitously it was found to have a specific bactericidal activity against obligate anaerobic and photosynthetic bacteria. As the understanding of the disorders caused by anaerobic bacteria has increased, so have the therapeutic indications for this drug. Metronidazole is now widely used in the treatment of diseases involving anaerobic bacteria in dogs, cats and humans.

Good pharmacokinetic data are available for the disposition of metronidazole in these species. However, for any ruminant animal, published data are particularly limited. In view of the potential usefulness of metronidazole in controlling anaerobic infections in grazing livestock, a study in goats was undertaken to help define the disposition kinetics of metronidazole in a typical ruminant. From such data it was hoped to propose a rational schedule of treatment, which would achieve and maintain the necessary antibacterial concentrations of the drug in that species.

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1. Flagyl^{R}, May & Baker (NZ) Ltd

CHAPTER II
ANAEROBIC BACTERIA AND ASSOCIATED INFECTIONS : A REVIEW OF THE LITERATURE

CHAPTER II

ANAEROBIC BACTERIA AND ASSOCIATED INFECTIONS : A LITERATURE REVIEW

1. INTRODUCTION

Louis Pasteur in 1861, first proposed the concept that both anaerobic and aerobic micro-organisms existed. Subsequent microbiologists recognized and identified many of the anaerobic bacterial species within a relatively short period, in spite of the primitive equipment and methods of the time (Sonnenwirth, 1976). In the succeeding years, any interest in anaerobic bacteria was largely confined to the pathogenic Clostridia spp. These bacteria produced specific, easily recognizeable conditions of great human importance, such as gangrene, tetanus and botulism.

The potential role of the nonsporing, gram negative, anaerobic bacteria as pathogens in a number of infectious processes has been increasingly investigated over the last decade. Initially, interest was stimulated because of the high number of post-operative infections which occurred in humans, despite antibacterial therapy. The recognition, that anaerobic bacteria can become major aetiological agents in many infections, improves the likelihood of a specific diagnosis. As a result, appropriate therapy can be instituted and a more accurate prognosis can be determined for the patient.

Improvement in the methods of anaerobic culture has aided this developing awareness. The routine use of culture media selective for anaerobes, careful specimen collection, the use of suitable transport media, and the keeping of transport delays to a minimum, have made it feasible for even small clinical laboratories to isolate anaerobic bacteria, and to characterize these isolates into broad groups.

2. ENDOGENOUS ANAEROBIC BACTERIA

The significance of anaerobic bacteria in human disease is no longer in doubt and evidence is being accumulated that indicates a similar situation in other animal species. However, it is important when interpreting microbiological results to be able to differentiate between the commensal bacteria, contaminants cultured from an incorrectly collected swab and potential pathogens. The microbial flora found in most animals consist of aerobic, facultatively anaerobic and obligate anaerobic bacteria, all of which contribute to a dynamic ecosystem. Many of the potentially pathogenic bacteria are found normally as commensals, but most occur in lower numbers than the consistently non-pathogenic bacteria.

Endogenous anaerobic bacteria are predominantly saprophytic and may be found widely distributed around the body, usually in association with mucosal surfaces. The proportion of any particular bacterial species at an anatomical site depends upon the oxygen tension and the nutrients available. Commensal organisms in the mouth and upper gastrointestinal tract utilize simple sugars as a food source. These simple sugars are completely absorbed during intestinal transit, so that organisms resident in the colon must utilize whatever nutrients remain; such as complex polysaccharides. This difference in the food available has led to the induction of specific bacterial enzymes. These and other enzymes manufactured by bacteria, are important in the pathogenesis of infections; some, for example, are able to destroy the protective layer of mucin on the intestinal mucosa, allowing the development of other pathogenic bacteria.

Endogenous and pathogenic bacteria also produce a number of other by-products. Many of these, such as volatile fatty acids, volatile amines and hydroxy acids, have adverse effects on the host. Whereas others, particularly folate, pantothenate, biotin and vitamin K, are beneficial. Anaerobic gram negative bacteria are also involved in fat absorption, bile formation and the regulation of cholesterol metabolism (Finegold et al, 1976). Rolfe (1984) found that a normal microflora stimulated peristalsis, particularly of the small intestine, and assisted the activity of certain enzymes produced by the intestinal epithelium.

3. PATHOGENESIS OF ANAEROBIC INFECTIONS

The majority of infections caused by anaerobic bacteria, occur secondarily to a lowering of local tissue resistance. One of the major defences of the body against anaerobic infection, is the potential of the tissue components to reduce or oxidise compounds by electron transfer (Finegold et al, 1976). If the reduction-oxidation (redox) potential is lowered from its normal Eh of +120 millivolts, the granulocytic, phagocytic bactericidal system fails to function effectively. This allows anaerobic bacteria to multiply, even in tissues such as the mouth or respiratory tract which are normally exposed to air.

Many conditions such as vascular disease, epinephrine injection, cold, shock, oedema, surgery, bruising, tissue necrosis, or the growth of facultative bacteria in a wound, have all been known to adversely affect the redox potential. Anaerobic infections may also occur in tissues adjacent to sites which normally support an abundant endogenous microflora. These sites include: mucous

membranes, the reproductive tract, ear canals and the lower urinary tract (McIntosh, 1985). Haematogenous spread is also possible.

4. DISEASES ASSOCIATED WITH ANAEROBIC BACTERIA

4.1 Prevalence of anaerobic bacterial species

Obligate anaerobic bacteria play an important role in pyonecrotic processes in a wide range of locations. These include wounds, abscesses, uterine infections, gingivitis, otitis externa and pneumonia (McIntosh, 1985).

Until recently, much of the information regarding anaerobic bacteria as commensals or pathogens in animals had been extrapolated from man, as little was known about the identity of anaerobic bacteria in other animals. However, a series of investigations into the type of bacterial species isolated from animal specimens, has shown marked differences. Some authors (Prescott, 1979; Knight et al, 1980; Walker et al, 1983; Hirsch et al, 1985), isolated bacteria in a similar distribution of genera to that found in man, i.e. the majority of bacteria were Bacteroides spp., followed by gram positive cocci, particularly Peptostreptococcus spp. These results differed from those of Berkhoff (1978), who found a 50% incidence of clostridia in anaerobic infections. It was significant that Bacteroides fragilis, an organism which is most commonly isolated from nonspecific infections in man, was much less common in domestic animals.

4.2 Pyonecrotic processes

In general, Bacteroides spp., Fusibacterium spp., Clostridia spp., Actinomyces spp., and Peptococcus spp. constituted the majority of anaerobic bacteria isolated from a number of animal and human infections (Berkhoff, 1978; Prescott, 1979; Carwardine, 1984; Hirsch et al, 1985). The relative proportion of the individual microbial species differed between authors, depending on geographical location and species of animal.

Infectious processes in which anaerobic bacteria are the predominant organisms, often produce foul-smelling discharges, gas in the tissues, and frequently the infection is close to a site which has a rich anaerobic microflora. However, the absence of any of these features does not necessarily rule out the possibility of anaerobic bacteria being present. Microbiologists can now rapidly recognise anaerobic bacteria in purulent material, either by demonstrating characteristic metabolic end products which are produced by the bacteria, or by gas liquid

chromatography, or by their sensitivity to certain antimicrobial compounds (Ferguson, 1979[a]).

5. SITES OF INFECTIONS ASSOCIATED WITH ANAEROBIC BACTERIA

5.1 Oropharynx

The normal oral flora of mammals is essentially anaerobic, consisting of a large number of gram negative organisms such as Bacteroides spp. and Fusibacterium spp., gram positive cocci, i.e. Peptostreptcoccus spp., Peptococcus spp., and the gram positive rods, Actinomyces spp., and Spirochoetes spp,. These commensal anaerobic organisms are found in association with any oral or dental infections, e.g. halitosis, pharyngitis, tonsilitis, feline and canine gingivitis and tooth abscesses. Fusibacterium necrophorum has been implicated as a pathogen in calf diphtheria and necrotic laryngitis.

5.2 Gastrointestinal tract

The normal monogastric stomach and upper duodenum is practically sterile and the number of bacteria progressively increases distally, to reach a maximum in the colon and rectum. Bacteroides spp. account for more than 90% of the intestinal bacterial flora. Bifidobacteria spp., Eubacteria spp., and Propionibacteria spp. are also numerous in normal faeces, but these bacteria are regarded as essentially nonpathogenic. The normal bowel microflora plays an important role as a protective barrier against colonization by potentially pathogenic bacteria such as Vibrio cholerae, Shigella spp., Salmonella spp., Campylobacter spp., and some of the Clostridia spp. (Rolfe, 1984). However, commensal organisms may still become involved in infections secondarily to the development of an anaerobic environment. Clostridia spp. and Bacteroides spp. have been isolated from a number of dogs and cats with chronic diarrhoea (Carwadine, 1984), and Fusibacterium necrophorum has been associated with episodes of diarrhoea in a calf.

5.2.1 Clostridia spp. as important anaerobic bacteria

The most important anaerobic commensals and potential pathogens of animal intestinal tracts, are the clostridial organisms. These bacteria are free-living saprophytes, widely distributed in the soil. Only a small number of species within this genus are responsible for producing disease. The important Clostridia, together with other anaerobic bacteria believed to be involved in goat and other ruminant infections, are mentioned briefly in the following section.

Clostridium chauvoei is a commensal organism of the alimentary tract of animals, responsible for causing blackleg in cattle, sheep and goats. Blackleg was reported in a number of goats, after they had been given a prostaglandin injection to induce abortion (Pauling, 1986). Blackleg vaccine appears to be useful for prophylaxis in this species (Goldberg, 1976).

Clostridium perfringens occurs as a commensal in animal intestinal tracts, but is also found in the environment. C. perfringens Type D produces a toxin which causes severe vascular endothelial damage, resulting in an enterotoxaemia, and brain, heart and lung oedema. Factors which predispose the animal to the effects of this toxin include: a diet rich in soluble and complex carbohydrates, combined with gut stasis. This combination allows for an increase in the permeability of the intestinal mucosa to the epsilon toxin. For prevention, goat kids should be vaccinated with two doses of toxoid, given 3-4 weeks apart, followed by annual re-vaccination.

Clostridium septicum is also ubiquitous in the environment and may cause malignant oedema. This takes the form of a myonecrosis, with swelling, oedema and haemorrhage of the affected tissue. Braxy has not been reported in goats.

Clostridium haemolyticum infection is caused by contact with carrier animals. Bacillary haemoglobinuria is the result of the destruction of erythrocytes and the development of infarcts in the liver, both produced by necrotizing exotoxins.

Clostridium novyi, the causative organism of "black disease", proliferates in areas of the liver which have been damaged by liver fluke. This disease of goats occurs in areas of Australia and New Zealand in which liver fluke is endemic. In New Zealand, the movement of goats out of the fluke-infested areas of the East Coast of the North Island, and the increasingly wide distribution of the intermediate snail hosts, means that black disease may become more prevalent and widespread.

Anaerobic organisms play a major role in liver abscesses (Back et al, 1978).

Clostridium tetani is part of the normal soil bacterial population, and tetanus is a common disease of goats. Susceptibility of goats to the toxin is thought to be comparable to that of man, the horse and the guinea pig (although older goats appear to be more resistent; Pauling, 1986). The bacteria gain entry to

the animal through wounds. Therefore certain management practices may predispose goats to tetanus, e.g. dehorning, disbudding, hoof trimming, tattooing, castration, inoculations and by assistance in cases of dystocia (King, 1980; Pauling, 1986). The treatment advocated is to open up all visible wounds and to administer tetanus antitoxin and penicillin. Prevention is the most effective approach. A toxoid vaccination course consisting of two spaced injections should be started at 3-4 weeks of age, followed by a consolidating dose in 6-10 months and an annual booster dose administered for 2-3 years.

5.3 Respiratory tract

The lower respiratory tract is kept relatively sterile under normal circumstances. Infectious conditions of the lungs, are inevitably associated with the spread of normal upper respiratory tract commensal organisms down the bronchial tree (Phillips, 1979). The predominant genera isolated from lung diseases in cats (Love et al, 1980), cattle (Chirino-Trejo and Prescott, 1983), and horses (Bernard-Strother and Mansmann, 1985; Sweeney et al, 1985), were Bacteroides spp., particularly Bacteroides oralis and B. melaninogenicus, Peptococcus spp., Fusibacterium spp. and Clostridia spp.

5.4 Urogenital tract

The commensal anaerobic microflora of the normal bovine uterus, gradually changes from predominantly gram negative species in the early postpartum period, to gram positive bacteria as the time interval from parturition extends. A mixed bacterial flora was found by Olson et al (1984) and Stephens and Slee (1987) in cows with pyometra, and it was suggested that the gram negative anaerobes may act synergistically with Corynebacterium pyogenes. There was no correlation between the number of anaerobic bacteria isolated from an infected uterus and the severity of the disease process (Messier et al, 1984).

Anaerobic bacteria are also found as commensals of the lower urinary tract, but not of the more proximal parts; such as the bladder, ureters and kidneys. Any focus of infection of the urinary tract may be initiated by the indigenous urethral flora or by bowel microflora. It may also occur secondarily to a bacteraemia, surgical intervention or renal calculi.

Bacteroides fragilis, Peptococcus indolicus, Eubacterium lentium, Eubacterium aerofaciens, Propionibacterium granulosium and an anaerobic Streptococcus sp. have all been isolated from the udders of cows with mastitis (Du Preez et al,

1981; Slee and McOrist, 1985), but no anaerobic bacterial species have been isolated from the udders of healthy lactating dairy cattle.

5.5 Osteomyelitis

Anaerobic bacteria are frequently isolated from cases of osteomyelitis. Bacteroides spp., particularly Bacteroides asaccharolyticus, Fusibacterium necrophorum, and Peptostreptococcos aerobus (Walker et al, 1983), have been the predominant bacteria isolated from clinical cases. Actinomycotic organisms have also been implicated (Nakata and Lewis, 1984). These organisms may gain access to the marrow cavity by the haematogenous route, after direct trauma, as a result of surgical intervention, or by extension of soft tissue infection.

5.6 Myositis

Necrosis of muscular tissue may occur secondarily to faecal and soil contamination of open skin wounds. Intramuscular injections of any irritant substance, with or without an existing generalized bacteraemia, may be important predisposing factors. Clostridial organisms are most frequently implicated. Reactivation and germination of latent spores lodged in the muscle has been suggested as an explanation for certain sporadic infections, when contamination from a local wound seems unlikely.

5.7 Bacteraemia and endocarditis

Attempts to culture organisms from blood is more frequently performed in human patients than in domestic animals. Gram negative non-sporing rods are the predominant isolates, followed by gram positive Clostridia spp., Peptostreptococcus spp., and Eubacterium spp. (Martin et al, 1984).

The source of the bacteraemia has normally been located in the genital, intestinal or respiratory tracts (Werner, 1976), but seeding of bacteria may also occur from sinuses, oropharyngeal infections and infected decubital ulcers or wounds. Certain pre-existing conditions such as: diabetes mellitus, malignancy or corticosteroid therapy, have been shown to favour the development of a bacteraemia in such cases (Ralph et al, 1974).

5.8 Lower limb conditions

Obligate anaerobic bacteria have been associated with a number of lower limb conditions in sheep (West, 1981), horses (Carwadine, 1984), cattle and goats (Skerman, 1987). Interdigital dermatitis, footscald, footrot and foot abscess are

commonly found in goats farmed in New Zealand. The complex aetiology of these conditions is thought to be similar to that demonstrated for sheep (Skerman, 1987). The synergistic action of three principle infective agents: Fusibacterium necrophorum, Bacteroides nodosus, and Corynebacterium pyogenes, combined with other predisposing factors; such as soil moisture and environmental temperature, are important in the aetiology of the ovine conditions. Overgrown hooves, overcrowding, and the introduction of new animals to the flocks present additional risks (West, 1981). B. nodosus, the transmitting agent of ovine footrot and an obligate parasite of certain mammalian hosts, is not found widely distributed in the environment. Various B. nodosus strains differ in their proteolytic activity and hence in their ability to underrun the hoof matrix.

F. necrophorum is ubiquitous, and usually involved in infections as a primary invader following some tissue damage. F.necrophorum produces a number of exo- and endotoxins, including a leucocidal toxin which protects both Corynebacterium pyogenes and itself from phagocytosis. The microflora of foot lesions in goats appears similar to those found in sheep, except that B.nodosus is present in greater numbers (Skerman, 1987). Spirochoeta penortha, previously mentioned in connection with ovine feet, has also been named as a causal organism in goats (Baxendell, 1980).

Footrot may be treated either topically using bactericidal agents following the removal of all undermined horn, or by antibiotic injections (Skerman, 1987).

The commercially available, multivalent ovine footrot vaccine, is not registered for use in goats. However, this product incorporates all the serogroups of Bacteroides nodosus presently identified from caprine footrot in New Zealand, and field observations indicate good prophylactic and therapeutic effectiveness (Skerman, 1987).

F. necrophorum and Corynebacterium pyogenes can be consistently isolated from those suppurative foot conditions which involve the deeper structures of the hoof in ruminants. Local therapy is ineffective once the infection becomes established in the second and third interphalangeal joint. Drainage of the abscess, systemic antibacterial treatment and bandaging are necessary to control infection and minimize the degree of deformity which occurs when the joint ligaments become disrupted (West, 1981).

CHAPTER III
METRONIDAZOLE : A REVIEW OF THE LITERATURE

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CHAPTER III

METRONIDAZOLE : A REVIEW OF THE LITERATURE

1. HISTORY

Metronidazole (1-hydroxyethyl-2-methyl-5-nitroimidazole) was discovered in the late 1950's by the Rhone-Poulenc Company in France, during the search for an agent active against trichomoniasis (Jolles, 1976). The scientists of this company, cultured a streptomyces organism (strain number 6670) from a sample of earth collected from La Réunion Island. Three antibiotics were developed, but only one of them (compound 7080 RP) showed any activity against trichomoniasis. Compound 7080 RP was found to consist of two substances. The first, enteromycin (Fig. 3:1) was active against Escherichia coli, but it possessed only weak activity against Trichomonas vaginalis, and was thermolabile.

The second substance isolated was azomycin (Fig. 3:2). It showed excellent activity against Trichomonas vaginalis and was thermostable.

Azomycin was chosen as the most promising isolate, and further work centered on modifying the properties of this family of nitroimidazoles. This was achieved by the progressive substitution of a nitro group around the imidazole ring. At the same time, the effect of altering the sidechains attached to the nitrogen and carbon atoms was investigated.

Five-nitroimidazole compounds with shortchain substitution were demonstrated to have the most activity against trichomoniasis. These compounds were then tested for safety and 1-hydroxyethyl-2-methyl-5-nitroimidazole, (metronidazole) (Fig. 3:3) was found to have the best therapeutic ratio.

About this time, a related compound 1,2-dimethyl-5-nitroimidazole or dimetridazole[1] (Fig 3:4), was marketed for the prevention, treatment and control of blackhead in turkeys and for the treatment of dysentery in swine.

By the early 1960's, metronidazole was being marketed as Flagyl[2] for therapy against human trichomoniasis (Jolles, 1976). Further research using the same compound revealed substantial activity against both amoeba and giardia.

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1. Emtryl^{R} (Rhone-Poulenc, France)

2. Flagyl^{R} (Rhone-Poulenc, France)

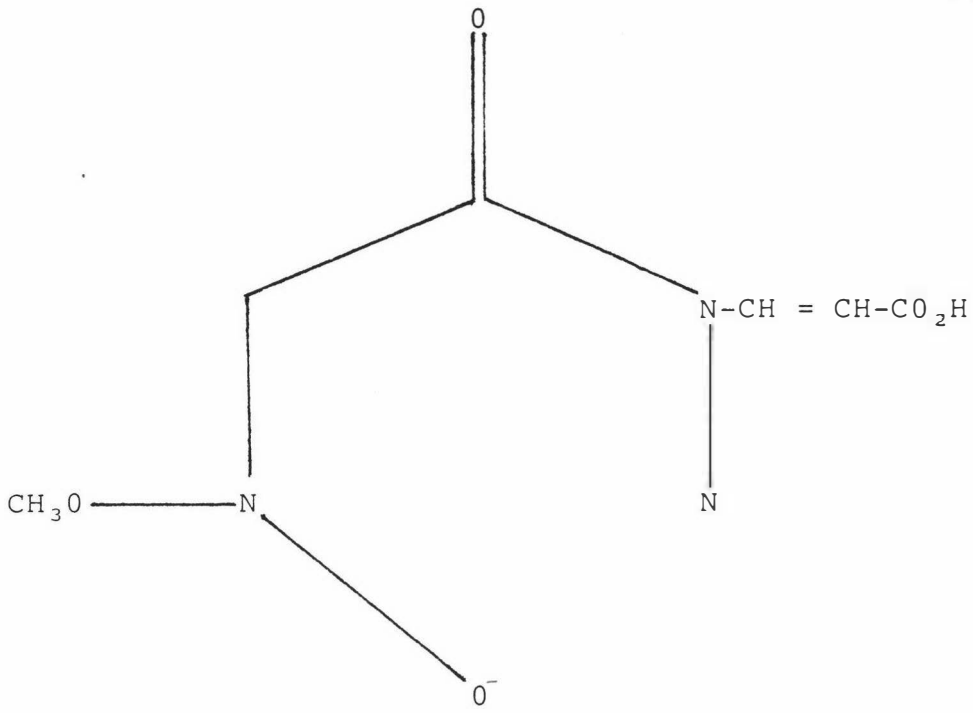


Figure 3:1 Chemical structure of enteromycin

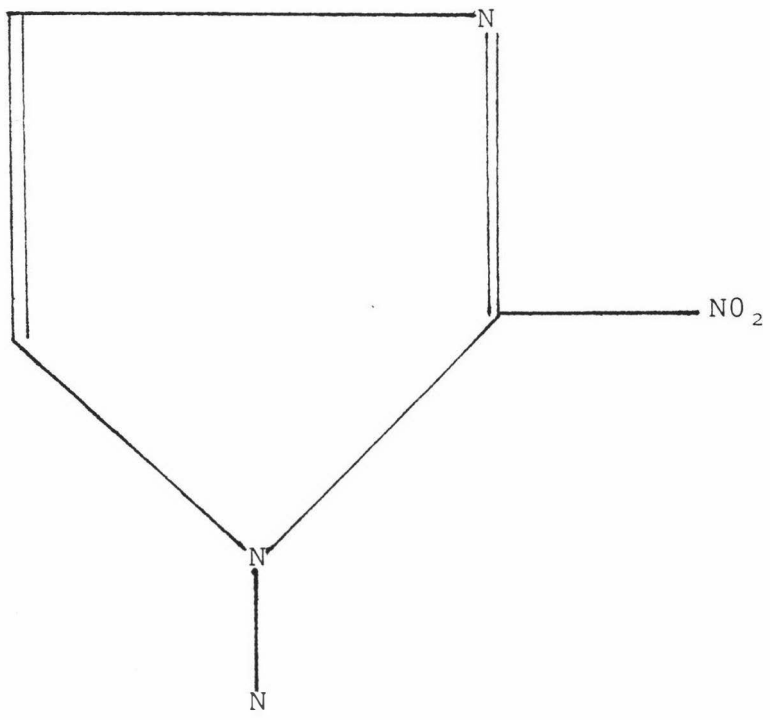


Figure 3:2 Chemical structure of azomycin (2-nitroimidazole)

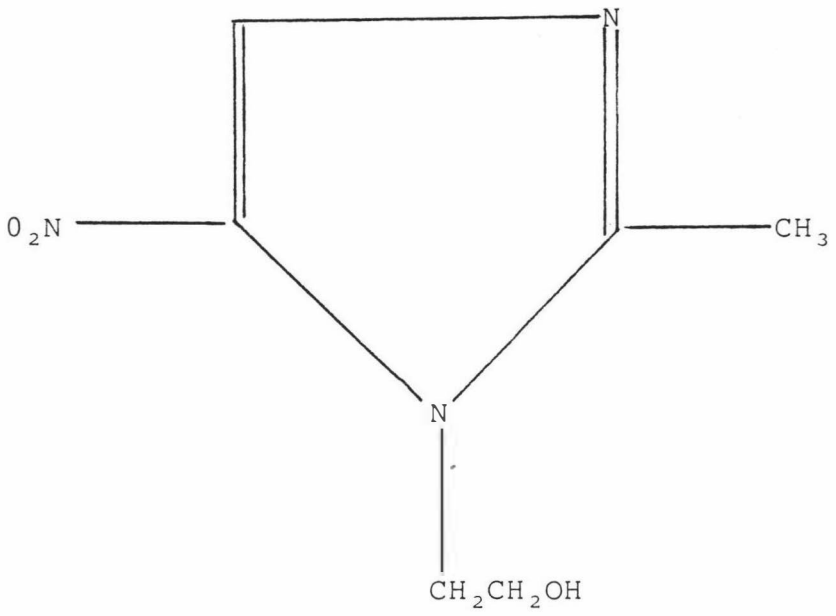


Figure 3:3 Chemical structure of metronidazole (1-hydroxyethyl-2-methyl-5-nitroimidazole)

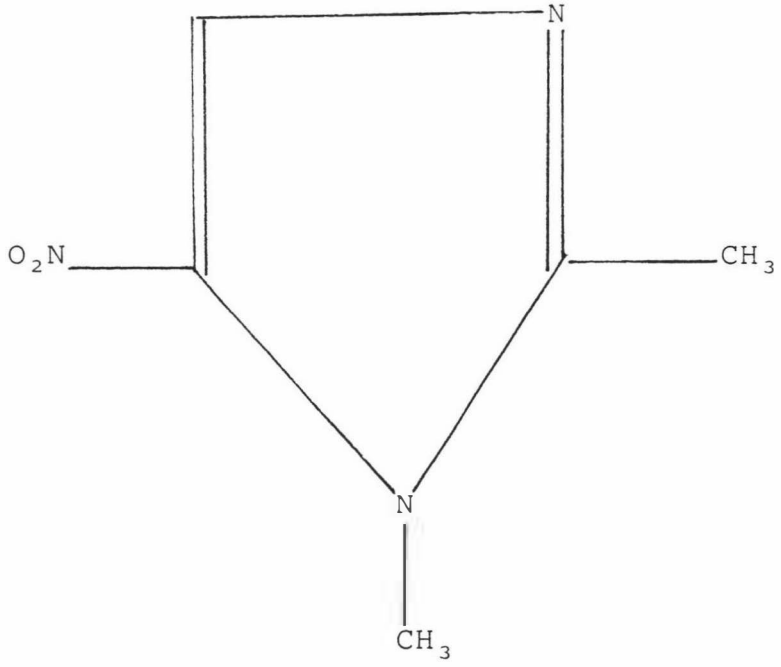


Figure 3:4 Chemical structure of dimetridazole (1,2-dimethyl-5-nitroimidazole)

In the early stages of development, metronidazole had shown a disappointing antimicrobial activity when tested against a variety of aerobic microorganisms. However, a medical practitioner noticed the resolution of Saint Vincent's gingivitis in a patient who was under metronidazole treatment for trichomoniasis (Jolles, 1976). This chance observation stimulated further investigations, using a wider spectrum of organisms. These latter studies revealed an activity by metronidazole against most anaerobic eukaryotic and prokaryotic organisms.

2. MODE OF ACTION

Aerotolerance and carbon dioxide dependence are very important in the context of resistance of an organism to metronidazole. The common property of susceptible microbes, is their anaerobic or photosynthetic nature (Müller et al, 1976). Activity of metronidazole against most aerobic and facultatively anaerobic organisms is virtually non-existent, although Chow et al (1976) have demonstrated some activity against certain microaerophilic bacteria, such as Camphlobacter foetus, Haemophilus vaginalis and Corynebacterium vaginale.

At first it was thought that sensitive organisms selectively accumulated metronidazole, but other authors (Müller et al, 1976; Tally et al, 1978), suggested that the intracellular metronidazole concentration rapidly approaches the extracellular concentration in all tissue cells. This proposal is based on the likelihood, that as a small molecular weight compound (M.Wgt. 171) which is essentially nonionized at physiological pH, metronidazole can readily penetrate cellular membranes. These authors put forward the hypothesis, that the sensitive organisms contain systems which involve either ferredoxin or flavodoxin-type, low redox potential, electron transport proteins. These proteins are thought to be capable of transforming (reducing) the nitro-group of metronidazole, to produce a biologically active compound. The intracellular reduction also has the advantage of promoting a favourable concentration gradient, which increases uptake of the original compound. The reactive metabolite formed, possibly a hydroxylamine, reacts with bacterial DNA. The resultant drug-DNA complex can no longer function as an effective primer for DNA and RNA polymerases, so all nucleic acid synthesis within the bacterial cell ceases. Metronidazole also acts as a non-specific electron trap by reversibly inhibiting many oxidative processes affecting uricase, but not monoamine oxidase. More information on the action of the imidazoles, including metronidazole, is still required.

3. BIOTRANSFORMATION

In his review article, Templeton (1976) states that the majority of experimental studies in animals and man have shown that metronidazole is biotransformed by the oxidation of the aliphatic side chains. The proportions of metabolites formed depends upon the species of animal (Templeton, 1976), although even individuals of the same species may produce different metabolites. Manthei et al (1963) observed that some patients receiving metronidazole therapy passed coloured urine, which was thought to be due to different azoxy compounds being formed by the condensation of two partially reduced molecules of metronidazole or its metabolites. Most of any unchanged parent compound, together with its sulphate and glucuronide metabolites, are excreted in the urine (Ings et al, 1975).

4. DISPOSITION

Early studies on the disposition of metronidazole were based on the analysis of blood samples, and were limited because of difficulties associated with the assay procedure. Distribution studies were performed in rats and mice, using ^{14}C-labelled metronidazole, which was administered either orally or intravenously (Ings et al, 1975; Templeton, 1976).

Orally administered metronidazole is rapidly absorbed in the rat and dog (Neff-Davis et al, 1981). The majority of the compound passes through the mucosa of the stomach and duodenum. Maximum serum concentrations have been obtained at 0.5 to 2.0 hours after administration for the rat (Templeton, 1976), 0.33 to 3.0 hours for man (Houghton et al, 1979[a]), less than 1.0 hour for dogs (Neff-Davis et al, 1981), and 1.0 to 2.0 hours in horses (Sweeney et al, 1986). In man, it was found that food present in the gastrointestinal tract delayed the time to peak concentration and reduced the height of the peak (Ings et al, 1975). An equilibrium is achieved rapidly between plasma and those tissues having a good blood supply such as: the liver, kidney and gastrointestinal tract. Radioactivity in the blood for the first five hours was thought to be associated with the parent compound, which was gradually transformed into more polar metabolites. The clearance of the radio-labelled products from most tissues took 3-4 hours, but the skin and gastrointestinal tract showed a radioactivity which was concentrated and persistent: the clearance values for these tissues was 8 and 14 hours respectively (Templeton, 1976).

In man, 53% of the orally administered dose is excreted in the urine within 24 hours, 24% in the faeces and 6% as carbon dioxide. The carbon dioxide

molecule implies a low level of degradation of the metronidazole. Ings et al (1975) also proposed that there was no significant enterohepatic circulation of metronidazole or its metabolites.

The elimination half-life determined from single intravenous doses of metronidazole was 8.7 hours for man (Selkon, 1979), 2.9 hours for horses (Sweeney et al, 1986), 4.5 hours for dogs (Neff-Davis et al, 1981), and 0.8 hours in sheep (Cooper, pers. comm.). Welling and Munro (1972) investigating multiple dose regimens, found that metronidazole concentrations in plasma plateaued by 3 to 4 days. They concluded that metronidazole probably does not stimulate or inhibit its own elimination after repeated oral administration.

Placidi et al (1970) showed that metronidazole and its metabolites cross the placenta and are evenly distributed throughout the foetus, without any differential concentration in a particular tissue. In man, metronidazole has been detected in vaginal secretions, saliva, milk, cerebrospinal fluid, cerebral and hepatic pus, pulmonary empyaema fluid, bile and seminal fluid. Bamgbose and Bababunmi (1973) found that metronidazole was bound very strongly to human and bovine serum albumin, but a study by Ralph et al (1974) suggested that the amount of drug bound was insignificant at 2-20%.

Pharmacokinetic data following other routes of administration, have been reported for animals and man. Guilland and Carwadine (1987) detected blood concentrations consistently within the therapeutic range for 24 hours following the administration of metronidazole suppositories to an elephant. Sustained blood concentrations in the therapeutic range have also been reported in man following the administration of metronidazole suppositories. However, the serum concentration of metronidazole differed widely between individual patients (Houghton et al, 1979[b]).

A milk withholding period of 24 hours has been advocated for lactating cattle, as a result of pharmacokinetic data obtained following intraperitoneal or intrauterine administration of 0.5% w/v metronidazole solution (Table 3:I). The residue limit was set at 1:10,000,000 (Anon, 1985). The plasma metronidazole concentrations and associated pharmacokinetic values were also determined in sheep following the intraperitoneal administration of 50 mls of 0.5% w/v metronidazole solution (Anon, 1986).

TABLE 3 : I **Disposition values in lactating cattle and sheep following intraperitoneal or intrauterine administration of 0.5% w/u metronidazole solution (expressed as mean ± standard deviation**

	Intrauterine	Intraperitoneal	
	Lactating cows	Sheep	Lactating cows
No. of animals	4	4	4
Quantity of drug solution administered (mg)	150	250	1000
Peak plasma metronidazole concentration (mcg/ml)	0.11 ± 0.06	5 ± 1.08	0.32 ± 0.194
Time to maximum concentration (hours)	4.3 ± 4.6	1.0	2.0 ± 0.82
Area under plasma metronidazole concentration-time curve (mcg.hr/ml)	a	12.8 ± 3.52	a
Elimination half-life (hours)	a	8 ± 0.17	3.4 ± 1.2
Time to last detectable concentration (hours)	7	12	12
Mean milk concentrations measured during first three days (mcg/ml)	< 0.5	< 0.5	a

a = not reported

5. SAFETY ASPECTS

Metronidazole is considered to be a relatively safe drug with few obvious side-effects at normal dose rates, although excessive salivation and nausea may occur if the tablets are chewed or otherwise tasted before swallowing. Gastrointestinal side-effects, sensory peripheral neuropathy and central nervous system signs of ataxia, muscular rigidity and tremors followed by severe prostration, have all been associated with large doses of metronidazole administered for a long time. The clinical signs are usually reversible once the dosage is reduced, or the drug withdrawn (Ingham et al, 1975). Dogs appear to be peculiarly sensitive to the toxic effects of metronidazole (Bost, 1976).

A study of children born to women who had been treated with metronidazole during pregnancy, showed no significant increase in the number of deformities (Roe, 1979). Nevertheless, it was recommended that metronidazole therapy be avoided during the first trimester of pregnancy. Oral administration of metronidazole to pregnant rats failed to produce embryonic, teratogenic or any subsequent change in fertility in either the dam or offspring.

An in vitro organ bath study was undertaken by Essien et al (1985) after reports of myocardial depression following large doses of metronidazole. These chemical and pharmacological studies, showed that metronidazole produces a direct negative inotrophic effect on the isolated heart muscle, by interfering with calcium ions.

Ludwig et al (1983) found that geriatric patients, or those with renal impairment, exhibited a reduced total clearance of the drug and recommended that the standard dose should be reduced by 30-40% for such compromised patients. Patients on renal dialysis did not demonstrate any impairment in the excretion of metronidazole (Gabriel et al, 1979).

6. SPECTRUM OF ACTIVITY

Metronidazole is active against most anaerobic protozoa - trichomonal, giardia, entamoeba and treponema species. However, it is the specific activity against obligate anaerobic bacteria which is of most value in veterinary medicine.

The susceptibility of various anaerobic bacteria to metronidazole (as determined by minimum inhibitory concentration tests - MIC) ranges widely, depending on the bacterial species and the method of determination. Fusibacterium spp.,

Bacteroides spp. and to a lesser extent Clostridia spp. are uniformly sensitive. The majority of these organisms are sensitive to 3.0 mcg metronidazole per ml or less (Sweeney et al, 1986). However, other authors (Freeman et al, 1968; Ingham et al, 1975; Chow et al, 1976; Suter and Finegold, 1976; Warner and Prior, 1976; Kimsey and Hirsch, 1978; Cuchural et al, 1984), suggested that a higher metronidazole concentration was required for more resistant anaerobic organisms. Most authors proposed 12.5 mcg/ml or less.

The following non-sporing gram positive anaerobic bacteria were found to be relatively resistant: Propionbacterium spp., Lactobacillus spp., Eubacterium spp., and Actinomyces spp.

Although there is no apparent activity against aerobic bacteria or the majority of microaerophilic bacteria, Onderdonk et al (1979) found that animals challenged simultaneously with Escherchia coli and Bacteroides fragilis, showed an improvement against both infections when treated with metronidazole. This apparent activity against aerobic bacteria, only occurred in the presence of known susceptible bacteria.

7. PRODUCTS AND ROUTES OF ADMINISTRATION

Metronidazole is a white to pale yellow, crystalline powder. It is colourless, stable in air when in solution, although it darkens upon exposure to light. The powder melts between 159°C to 163°C, and it is only slightly soluble in water and ethanol (Roberson, 1982). The drug is marketed as a tablet formulation for oral use, but in some human patients this route of administration is unsatisfactory because of an individual variability in bioavailability. Neff-Davis et al (1981) found a similar variability in the bioavailability of oral preparations in dogs, ranging between 59% and 100%.

Current investigations involve the incorporation of different base chemicals to improve the bioavailability from oral formulations (Itida and Pilpel, 1986). The oral preparations available are 200 mg and 400 mg white tablets and a buff-coloured suspension (64 mg benzyl metronidazole per ml). An injectable form of metronidazole (Torgyl[3] is marketed as a 0.5% w/v solution for administration intravenously, intraperitoneally and for topical irrigation of superficial wounds. This is a dilute, sterile solution originally designed for intravenous infusion in

[3] Torgyl[R], May & Baker, Port Elizabeth.

human patients over an eight hour period. At the recommended intravenous dose rates, medium to large sized animals require large volumes. Each bottle contains a single dose.

Metronidazole is also presented as a sterile powder to be reconstituted with the given diluent to a 40% w/v suspension. Once prepared, this suspension is stable for one month, and is to be used for deep intramuscular injection. During storage all solutions of metronidazole should be protected from the light.

8. METRONIDAZOLE IN CONJUNCTION WITH OTHER TREATMENTS

Salem et al (1975) investigated the activity of metronidazole in conjunction with other antibacterial agents against Bacteroides fragilis, in vitro. These authors found that concurrently administered spiromycin, rifampicin, clindamycin, tetracyclines or nalidixic acid, reduced the minimum inhibitory concentration of metronidazole. No antagonism was observed between metronidazole and any other agent measured. Ralph and Amtnieks (1980) reported a similar effect with metronidazole and nalidixic acid, spiramycin and rifampicin. The synergism with spiramycin was also apparent against protozoa and spirochaetes. However, the synergism between metronidazole and other anticmicrobial agents may only be true for certain bacteria. Brooke et al (1984) found a synergistic effect produced between metronidazole and gentamycin or penicillin, against strains of Bacteroides melaninogenicus, but not against Bacteroides fragilis.

9. RESISTANCE

The resistance of obligate anaerobic organisms to metronidazole has not been a problem. This is particularly true for clinical isolates in the veterinary field (Chirino-Trejo and Prescott, 1983; Hirsch et al, 1985). The first metronidazole-resistant human strain of Bacteroides fragilis was demonstrated by Talley et al in 1978. The mechanism of resistance is not known, although these authors showed reduced penetration of metronidazole into the bacterial cells.

10. ASSAY PROCEDURES

A number of procedures for assaying metronidazole have been available for some time. Each method has certain advantages, but they also have limitations which may restrict their practical use (Ferguson 1979[b]).

10.1 Polarography

Polarography is a quick, simple and sensitive method of estimating metronidazole without interference from unrelated antibacterial agents. However, polarography measures all of the drug-related material that contains an intact nitroimidazole nucleus and is therefore an estimate which may not necessarily be equivalent to the antibacterial activity.

10.2 Microbiological assay (Levison, 1974 , Ralph et al, 1974)

Bioassay procedures which use modified gel diffusion techniques, have been developed to determine metronidazole levels in biological fluids. The major drawbacks associated with this assay procedure, are that the presence of any other antibacterial agent to which the organism is sensitive, will confuse the interpretation. Any diffusion technique will only have an accuracy of ±10%. Bioassays, although somewhat tedious, do eliminate the problem of having to detect the differing activities of the metabolites, and the results can be directly compared with the therapeutic effect of the drug.

10.3 Thin layer chromatography

The nitroimidazole is extracted from the sample with chloroform, inoculated onto silica gel, the chromatography procedure run, and the plates developed. While thin-layer chromatography can separate metabolites, their recovery from the sample is only about 70%.

10.4 Absorptiometric assay

This procedure lacks sensitivity as azo-compounds of N-substituted 2-nitroimidazoles and their metabolites give a similar colour reaction. The overall recovery from blood and urine is only 70%.

10.5 Direct spectrophotometry

This technique is convenient to use, but there are strong interferences from biological material. The recovery of metronidazole is variable.

10.6 Chromatography

Gas-liquid chromatography is highly sensitive and specific, but the tedious extraction procedures and the high initial cost of apparatus, make it impractical for routine clinical use.

Several methods of high-pressure liquid chromatography (HPLC) seem to be more suitable for assaying biological specimens (Neff-Davis et al, 1981; Sweeney et

al, 1986; Cooper, pers. comm.; Webster, pers. comm.). These methods have been applied to sera, plasma, saliva, and synovial and interstitial fluids. High-pressure liquid chromatography has the advantage of requiring a small sample, it is specific and convenient and it gives reproducible results.

CHAPTER IV

METHODS OF MEASURING THE CONCENTRATION OF DRUGS OUTSIDE THE VASCULAR COMPARTMENT

Among the many factors which determine the success or failure of antibiotic therapy, is the ability of the agent to reach an extravascular site of infection. The concentration of antibiotic that it is hoped will be reached in body tissues, has been extrapolated previously from a measurement of the drug concentration in blood. However, studies using radioisotope-labelled antibiotics have shown that the concentration peak in serum does not necessarily give an indication of the likely peak in the tissues (Calnan et al, 1972: Ziv et al, 1982). Radiolabelled drugs demonstrate the qualitative distribution around the body, but give little indication of the drug concentration obtained in the individual organs. Conventional methods for determining tissue concentrations have been shown to be inaccurate (Ryan and Cars, 1980), because of the uneven distribution of the drug throughout the cellular tissue, blood, lymph, and interstitial fluid.

The implantation of various foreign materials into subcutaneous or other sites, has become a valuable tool for investigating the pharmacokinetics of drugs in laboratory and domestic animals (Table 4:I). Such implants must either absorb or contain an extravascular fluid. The space enclosed by the tissue cage for instance, contains fluid which Guyton (1963) considered to be similar to interstitial fluid. Calnan et al (1972) concurred, although Piercy (1978), suggested that the fluid within the capsule should be more accurately described as an artificially induced transudate. However, most authors agree that the antibiotic concentration in capsular fluid does approximate tissue levels (Guyton, 1963; Calnan et al, 1972; Piercy, 1978; Ziv et al, 1982). Therefore, fluid taken from these artificially introduced containers, can be assayed to provide important information on the pharmacokinetics of the administered drug. In terms of therapy, such data is sometimes more relevant than knowing the drug concentration in blood. Table 4:I lists materials, procedures and anatomical sites which have been developed as a means of determining tissue concentrations.

TABLE 4 : I Materials, procedures and anatomical sites involved in the determination of "interstitial fluid" drug concentrations

Material and procedure	Animal species	Anatomical site	Reference
Cotton threads	rabbits	muscle	Ryan and Cars (1980)
Plastic ring, sides closed with 2 filters. Volume 0.2 ml	rabbits	subcutaneous	Georgopoulos and Schültze (1980)
Plastic ring with millipore filter over site	man	denuded volar skin surface	Tan et al (1972)
Visking[1] tubing chambers containing saline or serum	rabbits	subcutaneous	Larson et al (1983)
Dialysis tubing containing paper discs to absorb molecules less than 10,000 daltons	rabbits	subcutaneous	Landau et al (1981)
Human fibrin to mimic avascular fibrin-rich infectious process	rabbits	subcutaneous	Barza and Weinstein (1974)
Stainless steel mesh cylinders and perforated plastic syringes	rabbits	subcutaneous	Gerding et al (1976)
Polyethylene golf balls	rabbits	intraperitoneal	Gerding et al (1976)
Methacrylate tubes and perforated tennis balls	dogs	subcutaneous	Guyton (1963)

1. Union Carbide Corp., Chicago

Material and procedure	Animal species	Anatomical site	Reference
Polyethylene golf balls ± carrageenin to stimulate inflammation	horses	subcutaneous	Higgins et al (1984; 1987)
Polyethylene golf balls	rabbits	subcutaneous	Tight et al (1975)
Polyethylene golf balls	calves	subcutaneous	Ziv et al (1982)
Polyethylene golf balls attached to a sampling tube	dogs	renal parenchyma	Eikenberg et al (1975)
Perforated silicone tubing	dogs	subcutaneous	Chisholm et al (1973)
Perforated silicone tubing	calves, sheep, dogs	subcutaneous	Piercy (1978)

CHAPTER V

DETERMINATION OF THE DISPOSITION OF METRONIDAZOLE IN GOATS

CHAPTER V
DETERMINATION OF THE DISPOSITION OF METRONIDAZOLE IN GOATS

1. INTRODUCTION

Clostridial infections, foot abscess, scald and footrot, may be very important diseases in a goat herd. Footrot in sheep and goats is essentially due to a synergistic action of the anaerobic bacteria, Bacteroides nodosus and Fusibacterium necrophorum (Skerman, 1987). Metronidazole was considered as a likely therapy for these conditions in goats, as previous surveys (Berkhoff, 1978; Hirsch et al, 1985) had shown the susceptibility of the causative agents to this drug.

There is published information available dealing with the pharmacokinetic properties of metronidazole in mice and rats (Templeton, 1976), human subjects (Houghton et al, 1979[a]), dogs (Neff-Davis et al, 1981), horses (Sweeney et al, 1986), and sheep (Cooper, pers. comm.), but none for goats.

Accordingly, a series of experiments was designed to study the disposition of metronidazole after it had been administered to the goat. From this data, the dose rate and dosage interval were calculated.

2. MATERIALS AND METHODS (see Appendix I)

2.1 Animals

Eight 12-month-old Angora feral crossbred wether goats (Plate 5:1) were individually identified by horn markings, and blood was collected for a routine haemogram prior to the experiment. The goats were housed on straw bedding, and hay and water were given ad libitum.

2.2 Tissue cages

Tissue cages were fashioned out of silastic tubing that was cut into sections, perforated and sealed at both ends (Plates 5:2, 5:3b).

General anaesthesia was induced and maintained in each animal. Six surgical sites (Fig 5:1) on each goat were clipped and prepared for surgery. The sterile tissue cages were inserted subcutaneously and the wounds closed (Plate 5:3). All goats were left untreated for a minimum of two weeks after surgery.

Plate 5:1 Six of the animals used in the experiment.

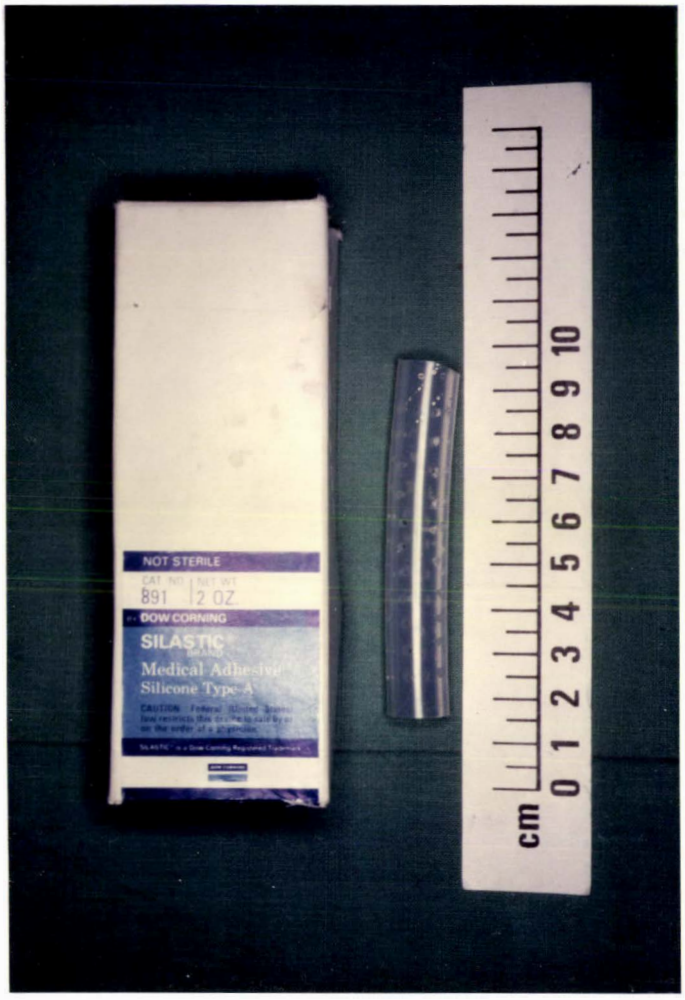
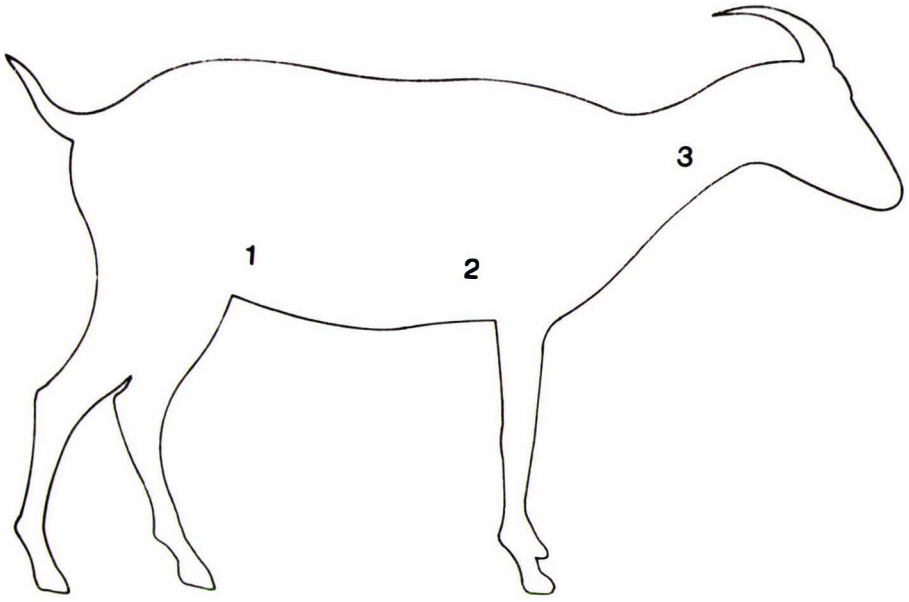
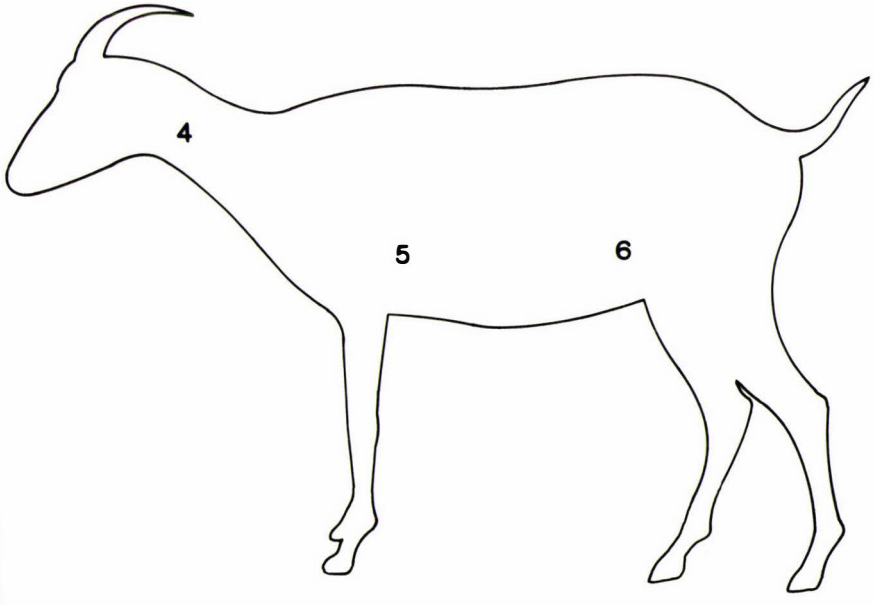


Plate 5:2 Materials used in the manufacture of tissue cages.

Figure 5:1 Diagrams of the right (a) and left (b) sides of a goat, showing the anatomical sites where the tissue cages were implanted subcutaneously. The numbers indicate the random order of tissue cages from which the fluid samples were collected.



(a)



(b)

Plate 5:3 Series of steps involved in the surgical implantation of the tissue cages.

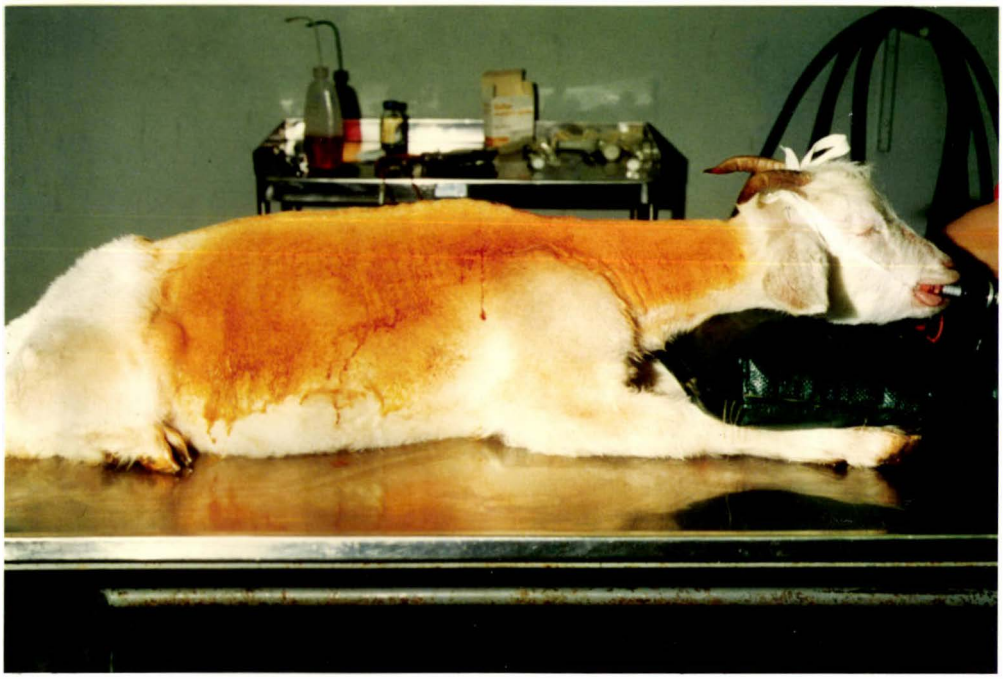
(a) Animal anaesthetised, positioned and prepared for surgery

(b) Positioning of the tissue cage into the subcutaneous space

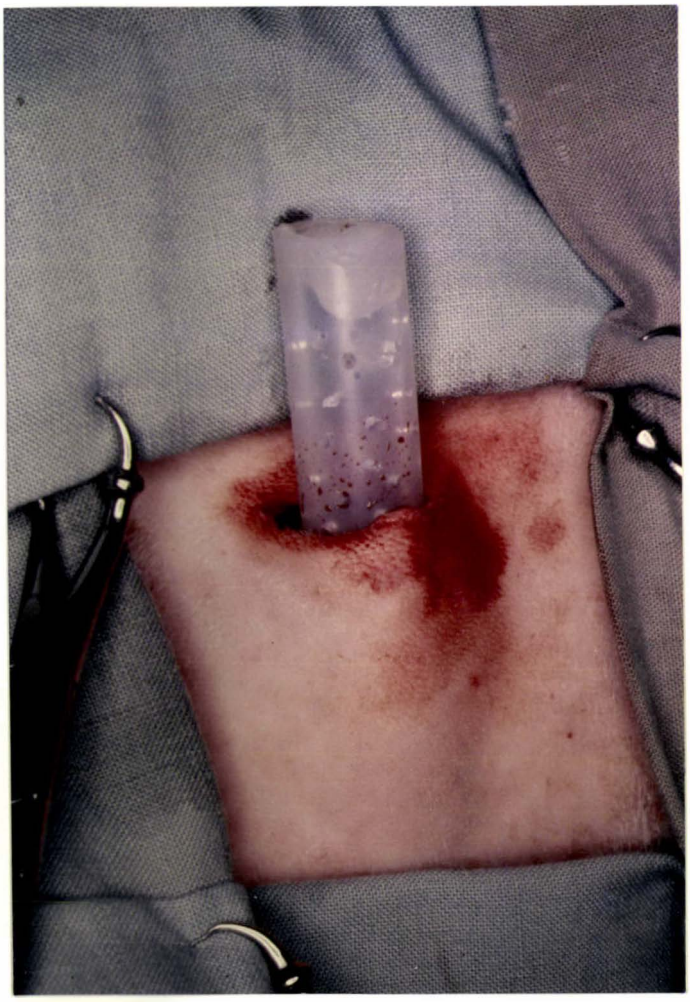
(c) Burial of the cage in the tissue pocket

(d) Tissue cage in place, following wound closure.

(e) Goat after recovery from anaesthesia with all six capsules implanted (3 shown on left side).



(a)



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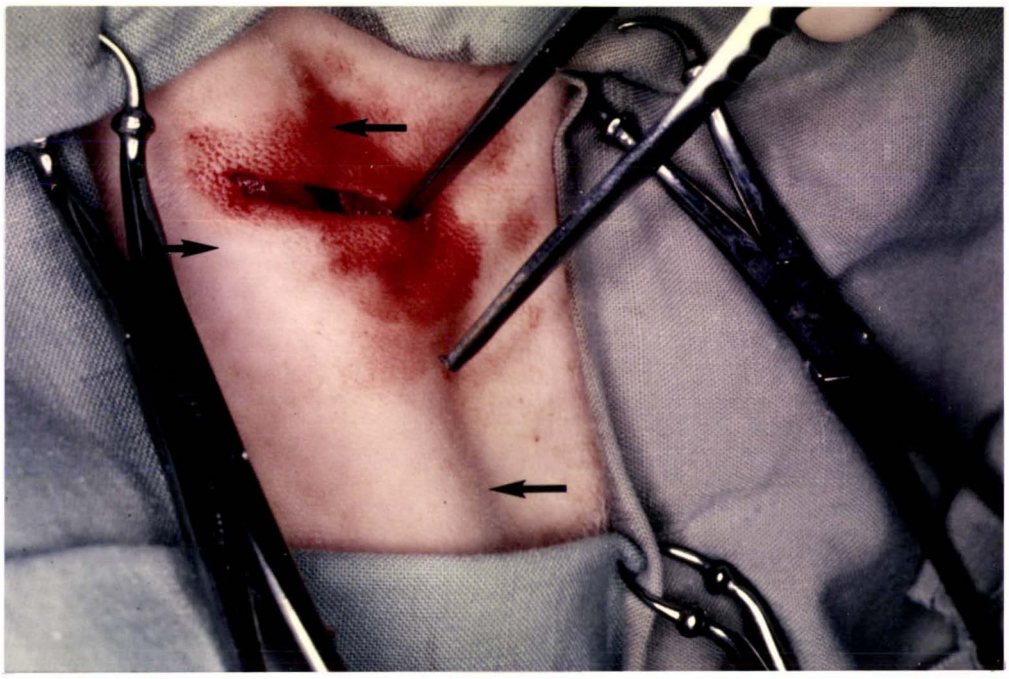

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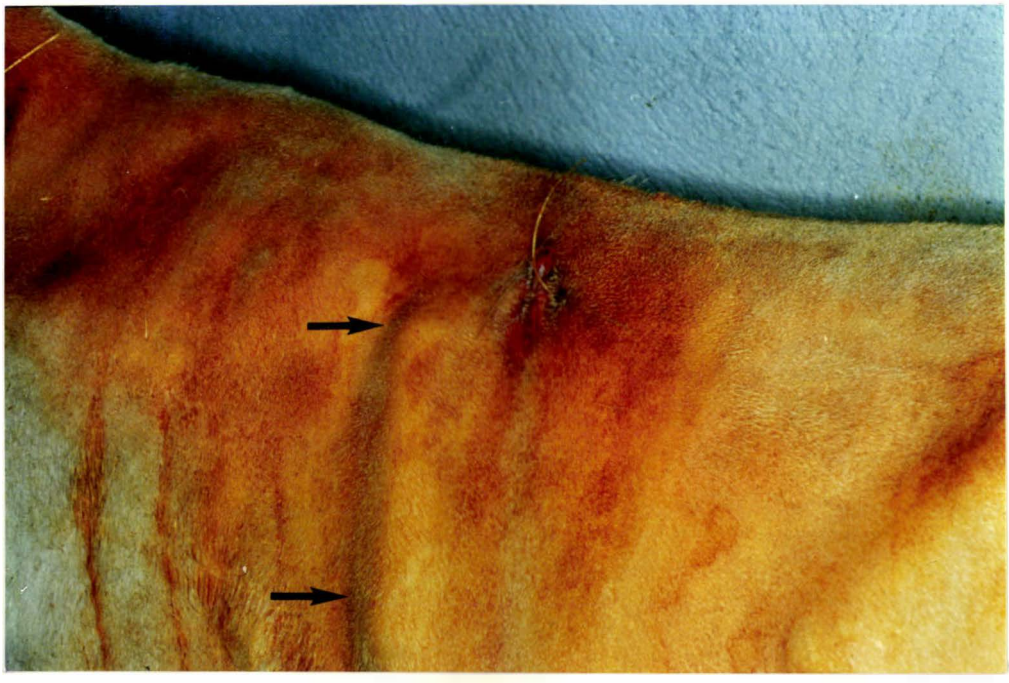

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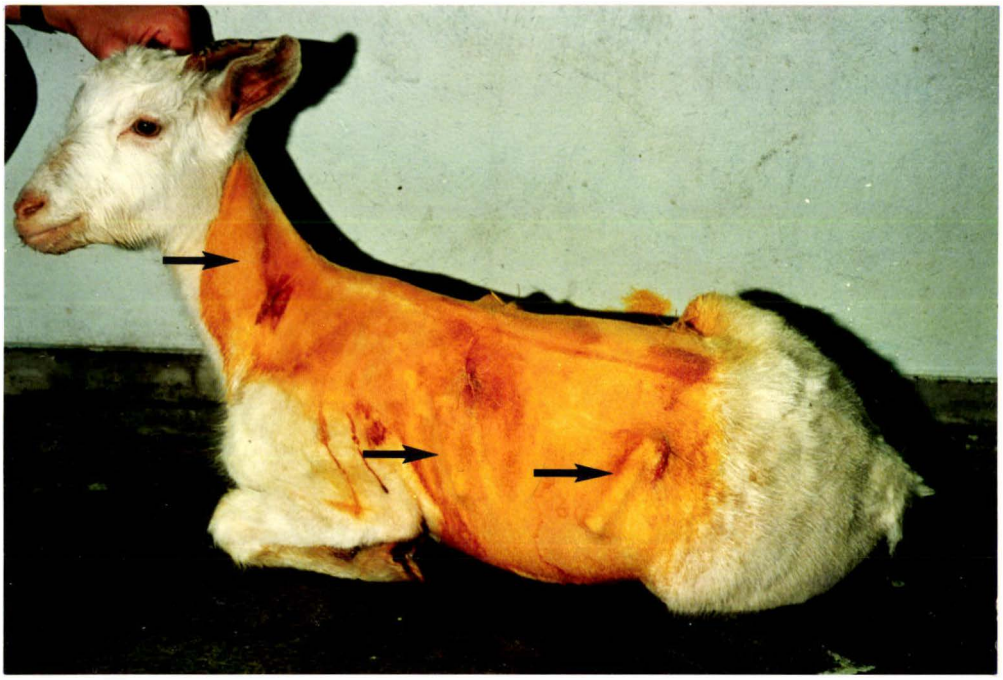

(e)

2.3 Experimental design

The experiment was conducted in a crossover design, with a three-week "wash-out" period between the two alternative treatments.

The metronidazole preparation for intravenous (IV) use was a 0.5% w/v aqueous solution, and for intramuscular (IM) use was a 40% w/v suspension. The dose rate recommended for both routes of administration was 20 mg/kg BWgt.

2.4 Samples of blood and tissue cage fluid

2.4.1 Collection

After preparation of the sites, cages were sampled in a random order (Fig 5:1) at appropriate times after drug administration (Table App. I:I).

2.4.2 Processing

All samples were stored in the dark after collection and during the processing period. After 24 hours the serum was removed from the blood clot, and this together with tissue cage fluids were stored at -20°C.

2.4.3 Extraction procedure

Acetonitrile was added to the thawed samples, and the mixture was centrifuged. The supernatant was taken off, filtered if possible, and then stored in glass vials (Cooper, pers. comm.; Webster, pers. comm.).

2.5 Preparation of the standard curve

A dilution series of pure metronidazole powder in normal goat serum was prepared, ranging from 50 mcg/ml to 0.1 mcg/ml. This was subsequently used as the standard against which unknown concentrations were compared.

2.6 Chromatography

Analysis of the metronidazole concentrations in processed samples was carried out using a high pressure liquid chromatography unit, which incorporated a Waters programmable automator (WHISP^{TM}) Model 710 into the system. The mobile phase consisted of an aqueous potassium hydrogen phosphate ($KH_2 PO_4$) and methyl alcohol mixture.

A 450 variable wavelength detector was set at either 0.01 or 0.04 absorbance

units, and the recording device was either a Varian 5A chart recorder[1] or a Spectra Physics SP4270 chromatography integrator[2].

The height of the peak of the unknown sample was measured and the metronidazole concentration calculated by direct reference to the standard curve.

2.7 Removal of tissue cages

Approximately seven months after the cages had been inserted, blood and tissue cage fluid samples were collected for the last time.

The tissue cage sites were prepared for surgery, prior to the infiltration of local anaesthetic and removal of the tissue cages (Plate 5:4).

2.8 Calculations used in the determination of the disposition kinetics

Following IV or IM administration, the disposition data from individual goats were analysed, according to conventional means (Swintosky et al, 1969; Baggot, 1974, 1977, 1978; Ziv, 1980; Sams, 1984; Teske et al, 1984), and a range of pharmacokinetic values were calculated (Appendix I). The line of best fit for the elimination phase was calculated using a computer program and least squares regression analysis[3].

2.8.3 Therapeutic concentration of metronidazole

A minimum inhibitory concentration (MIC) range of 3.0-12.5 mcg metronidazole/ml serum (Chow et al, 1976; Neff-Davis et al, 1981) was taken to cover the bactericidal concentrations for a majority of susceptible anaerobic bacteria (ref. Chapt. III:6). Because of interfering substances in vivo, it was agreed that the desirable peak serum concentration should exceed the MIC by a factor of four (Koritz, 1984; Powers et al, 1984). Hence, the desirable maximum serum metronidazole concentration C_{pmax} was defined as 50 mcg/ml (4 x 12.5 mcg/ml). The lower serum concentration (C_{pmin}) was defined as 3.0 mcg/ml, that being the MIC for a large majority of anaerobic bacteria. C_{pmax} and C_{pmin} were used to calculate suitable dosage regimens for IV and IM use, according to conventional methods (Appendix I).

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1. Varian, Salmon Smith Biolab Ltd., Auckland, New Zealand
2. Spectra Physics Division, Watson Victor Ltd., Wellington, New Zealand
3. Statistix, NH Analyticals Software, USA.

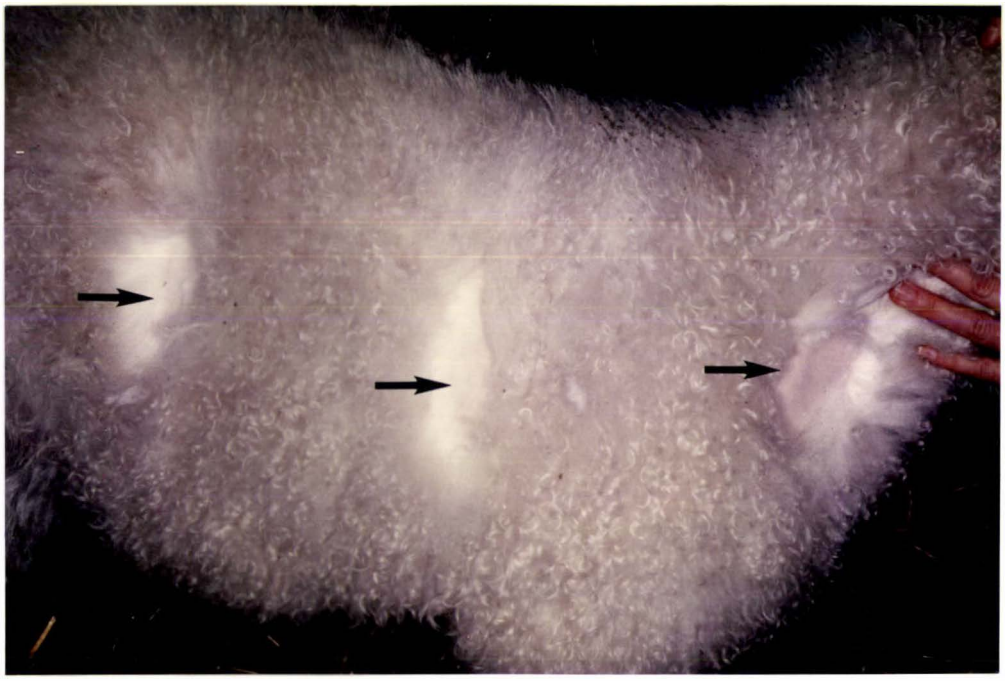
Plate 5:4 Series of steps involved in the removal of the implanted tissue cages.

(a) Three sites, seven months after the tissue cages were implanted.

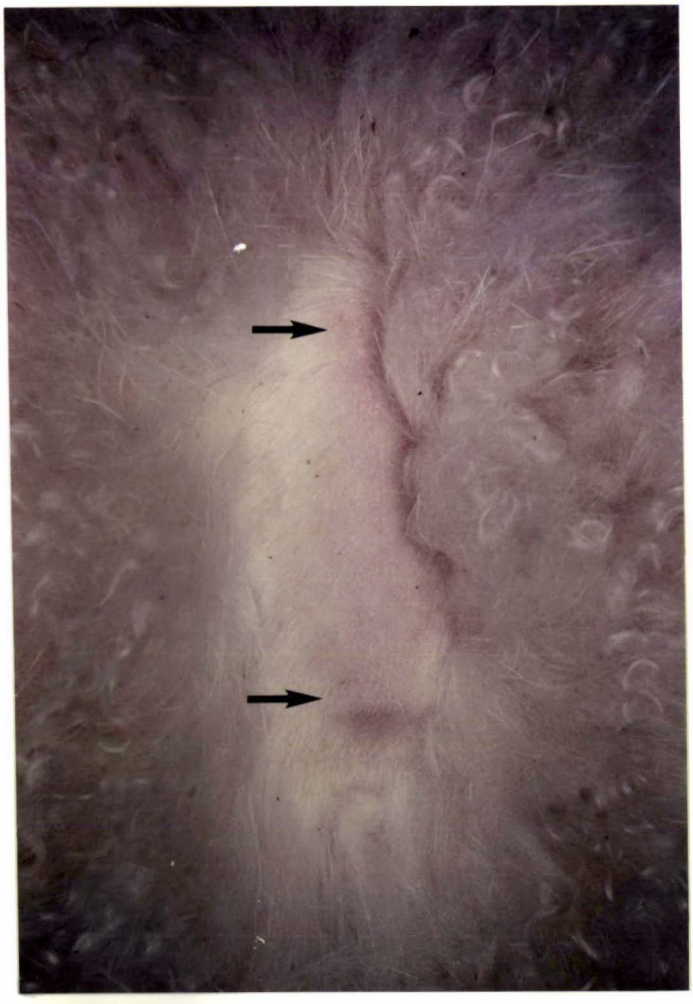
(b) Site prepared for tissue cage removal.

(c) Incision over the most dependent point of the tissue cage, following the administration of a local anaesthetic.

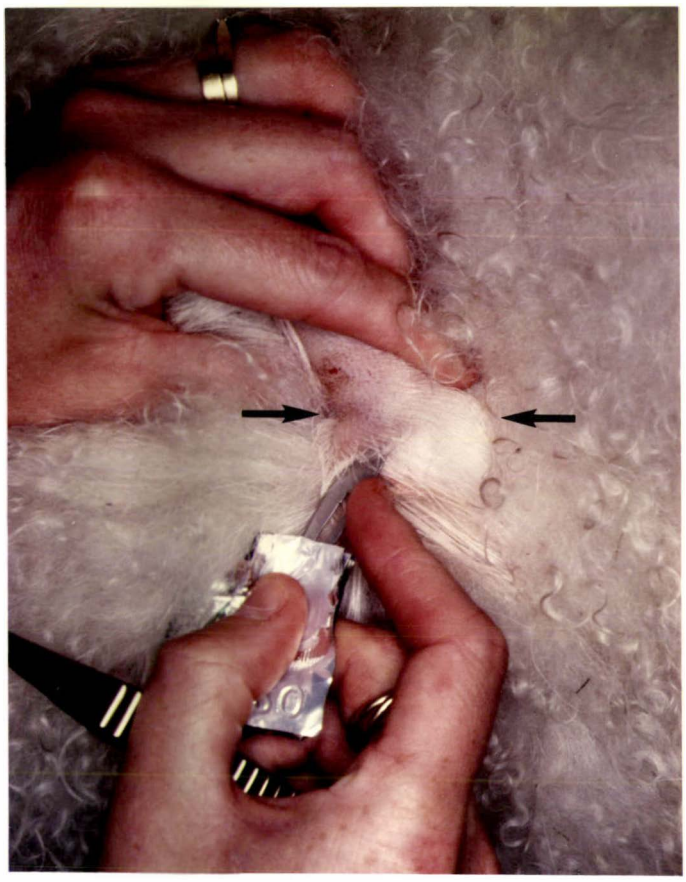
(d & e) Withdrawal of tissue cage from the surrounding capsule.



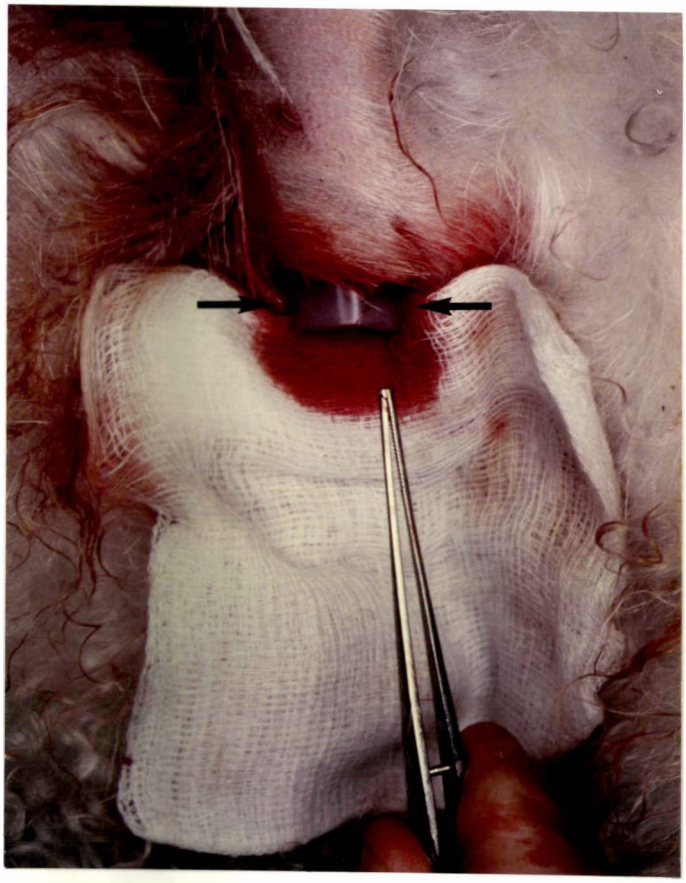
(a)



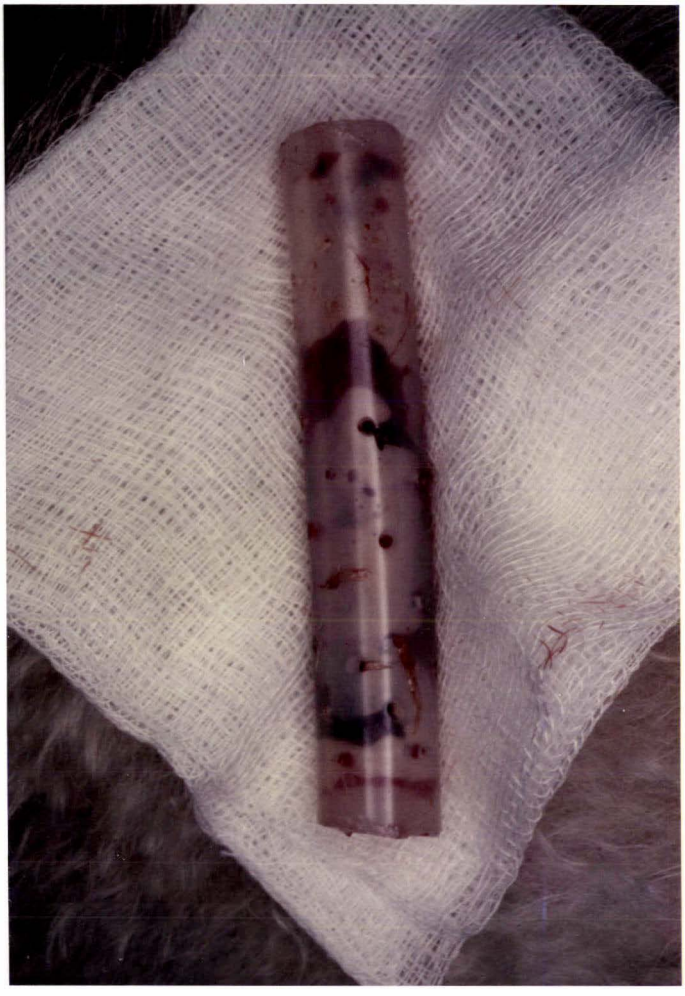
(b)



(c)



(d)



(e)

2.9 Haematology and components of tissue cage fluid

A total and differential white blood cell count was performed on Wright's-stained blood and tissue cage fluid smears. The total protein in the sera and tissue cage fluid samples was measured, and electrophoresis performed to determine the albumin/globulin ratio.

A flow diagram (Fig. 5:2) illustrates the sequence of events involved in the determination of metronidazole disposition.

3. RESULTS

3.1 Tissue cages

Fluid was always available from each implanted tissue cage, even when the cage was being sampled two or three times within a 24-hour period. The volumes obtained ranged from 0.5 to 3.0 ml, and provided it was not contaminated with blood, the fluid was either clear and colourless, or straw-coloured (Plate 5:5). Most samples developed a firm fibrin clot upon standing for more than one hour at room temperature.

The total protein content of the tissue cages was determined approximately seven months after they were implanted. The mean value for 34 tissue cages was 24.53 ± 1.37 gm/dL (Table 5:I). A serum total protein estimation was obtained for each goat, from an average of a pre-experimental and a post-experimental sample. The mean serum total protein was 64.81 ± 0.87 (n=16). The tissue cage protein content was approximately 38% of the total protein in sera, and 54.6% of this tissue cage fluid protein was albumin.

The cellular content of the tissue cages was low, ranging from 0.1 to 2.1 x 10^9 cells/l. The majority of cells were polymorphonuclear leucocytes, lymphocytes and monocytes, in such proportions that indicated the fluid was a sterile inflammatory exudate (Table 5:II).

During the process of extracting the cages, the tough fibrous capsule enveloping the cage was not removed, as it was firmly bound to the surrounding tissue. However, the numerous pillars of fibrous tissue which extended through the perforations in the tissue cage,(into the interior mass of tissue), had to be broken down to permit removal of the cage (Plate 5:6).

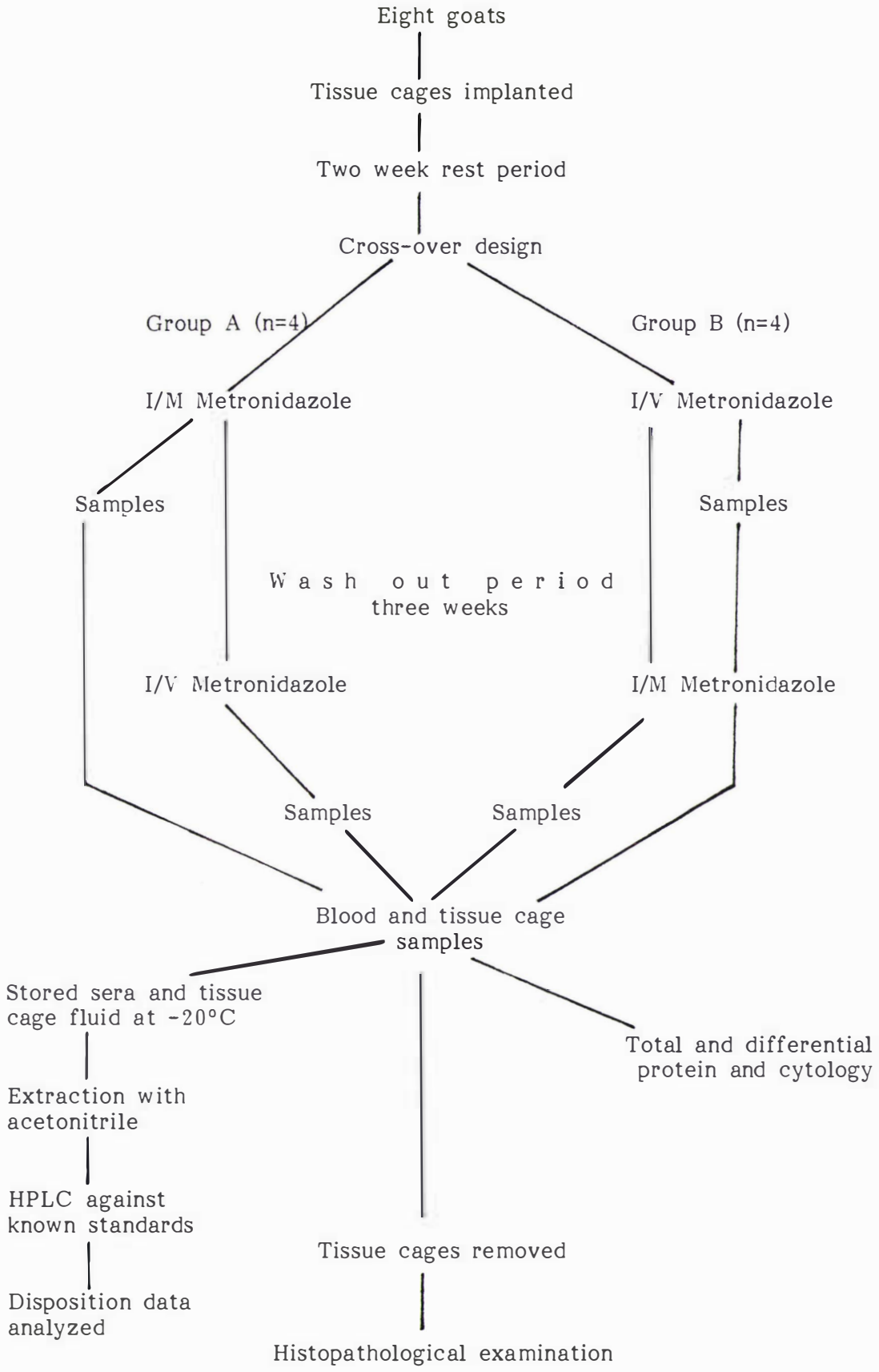


Fig 5 : 2 Sequence of events involved in the determination of metronidazole disposition in goats

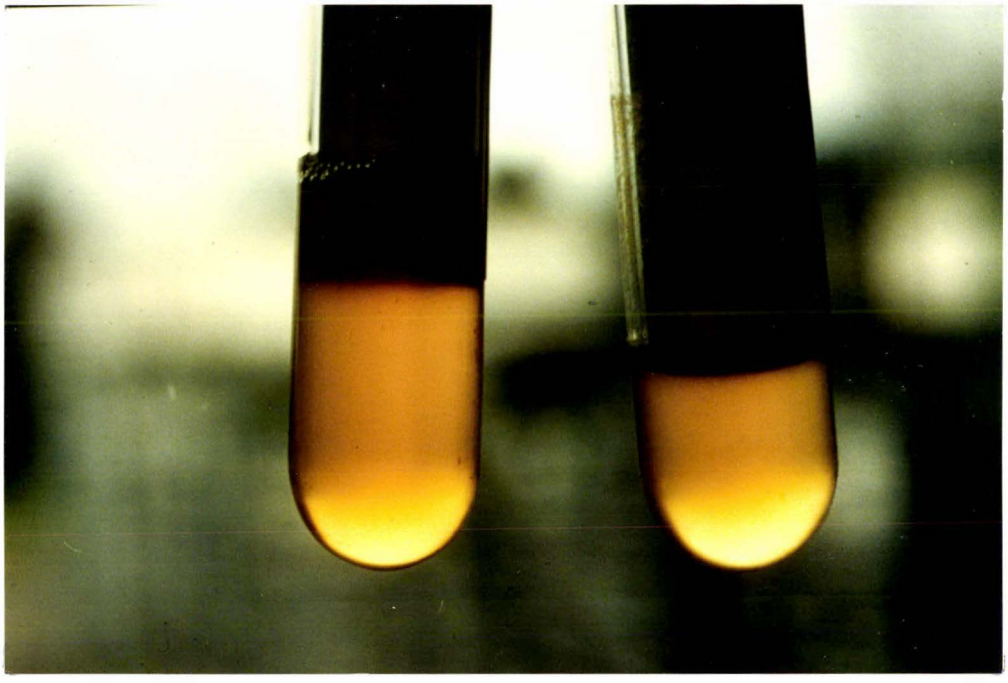


Plate 5:5 Straw-coloured samples of tissue cage fluid.

TABLE 5 : I **Mean total protein in sera and tissue cage fluid**

	ANIMAL								
Protein	**Ba**	**Dc**	**Do**	**Gr**	**Ha**	**Ma**	**Sl**	**Sn**	**Mean**
Mean tissue cage protein (g/dl)	20.33	30.0	25.0	24.6	30.5	31.67	13.5	24.4	24.53
s.e.	0.33	4.0	4.3	2.99	1.5	2.55	1.67	3.14	1.37
n=	3	2	3	5	4	6	6	5	a
Mean serum protein (g/dl)	66.5	66.9	60.5	62.0	66.0	67.5	62.0	65.0	64.81
s.e.	1.5	1.0	0.5	2.0	2.0	1.5	2.0	3.0	0.87
n=	2	2	2	2	2	2	2	2	b
$\frac{\text{TPTC}}{\text{TPS}}$ x 100%	30.5	43.5	41.3	39.7	46.2	47.0	21.8	37.5	37.9

a = total number analysed **34**

b = total number analysed **16**

TPTC = total protein in tissue cage fluid

TPS = total protein in sera

TABLE 5 : II Mean differential cell counts from pooled tissue cage fluid

Cells	Mean ($x10^9$)	s.e.	Proportion of total cells %
Neutrophils	0.4	0.2	44
Eosinophils	0.05	0.004	0.6
Lymphocytes	0.3	0.07	33
Monocytes	0.2	0.07	22
Totals	**0.9**	**0.3**	**100%**

Plate 5:6 Sectioned tissue cage after removal, showing the tissue mass within the cage and fibrous attachments extending through the perforations of the tube.

Microscopic examination of the tissue within the cages showed that it was predominantly regular, dense connective tissue. Fibrocyte nuclei and collagen fibres were aligned parallel to the longitudinal axis of the cylinder, with large blood vessels in the centre of the cylinder. This pattern was interrupted by connective tissue "feeders" that came in through the holes of the cylinder, often carrying a blood vessel. Scattered throughout the tissue were irregularly shaped, clear foreign bodies, which had become walled off by macrophages, giant cells and fibrous connective tissue. Areas where haemorrhage had previously occurred were surrounded by haemosiderin-containing macrophages, accompanied by aggregations of macrophages, lymphocytes, a few plasma cells and occasionally lymphoid cells. Such aggregations tended to occur in the centre of the tissue. The tissue mass within the cage occupied approximately 75% of the lumen.

Fourteen cages of the 48 became infected during the seven month trial period, and regardless of the length of time these cages had been implanted, once infected, they were easy to remove (Plate 5:7). A heavy growth of Staphylococcus aureus was grown from infected material within one of these cages. Microscopic examination of this tissue revealed loose, longitudinally arranged fibrin strands, with large numbers of neutrophils infiltrating from the periphery. Foci of dystrophic calcification were scattered throughout the fibrin strands.

3.2 Intravenous administration of metronidazole

Four animals (Ba, Dc, Ha and Ma) showed initial serum metronidazole concentrations which were greater than the limit of detection of this analytical method. The first metronidazole concentration available for recording in these animals, was two hours following drug administration. The best fitting least squares regression lines for the serum disposition curves (Table 5:III) confirmed that the distribution of metronidazole was rapid and usually complete before any significant elimination occurred (App.I: equation 1). This was true for three goats (Do, Gr and Ha), but for the two remaining goats (Sl and Sn) the metronidazole disposition was biexponential (App.I: equation 3). A representative graph of results from one animal is shown in Fig 5:3; the remaining raw data has been bound in Appendix II.

The total amount of metronidazole which appeared in the serum following IV administration, is calculated as the area under the serum drug concentration

Plate 5:7 Necrotic core within an infected tissue cage.

TABLE 5:III **Least squares linear regression analysis following intravenous administration of 0.5% w/v metronidazole solution (Dose 20 mg/kg BWgt).**

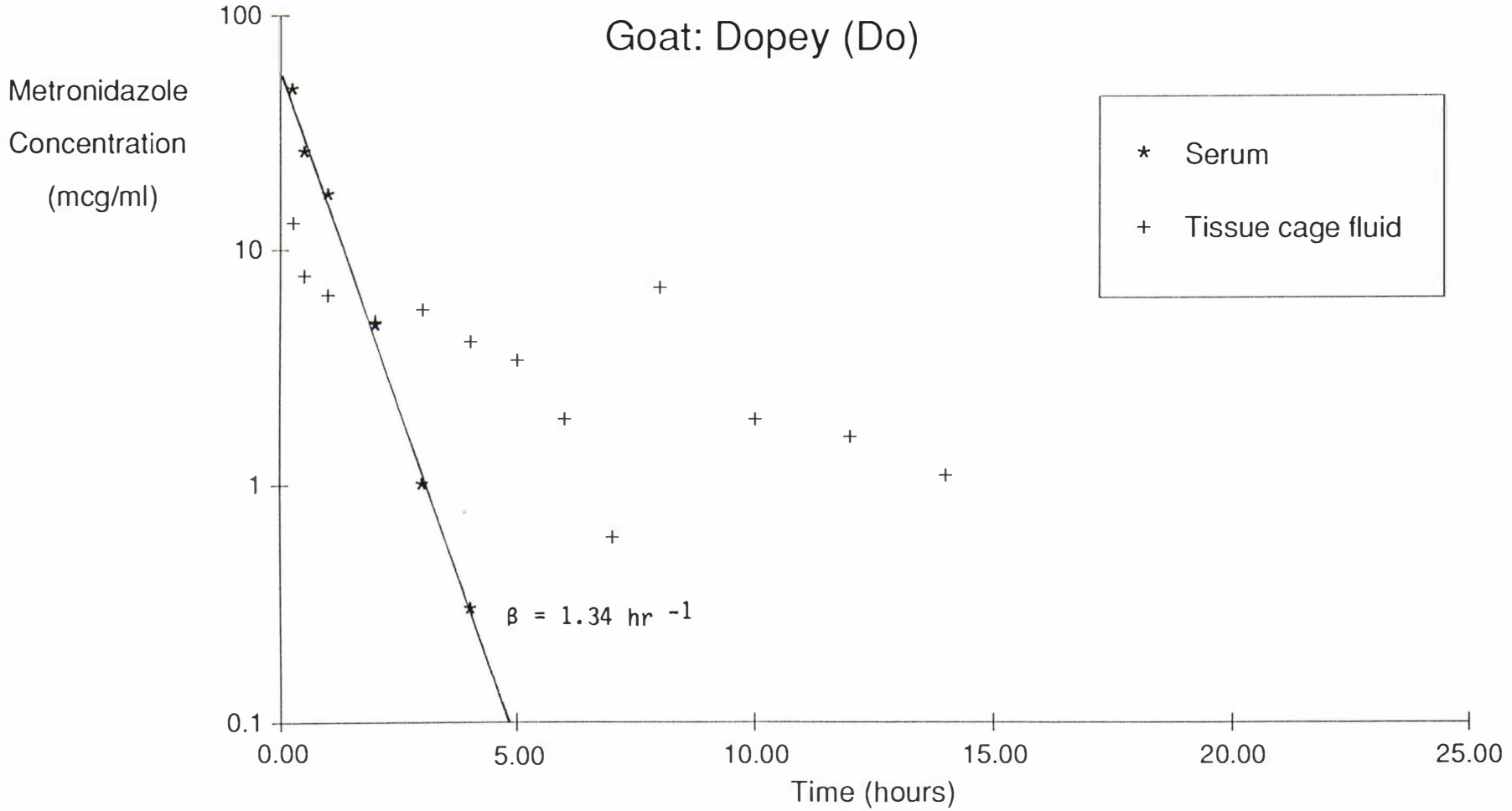
Animal	n	Intercept ± s.e.	Slope ± s.e.	r^2	P
Ba	6	42.36 ± 1.42	0.43 ± 1.09	0.957	<0.0005
Dc	5	36.39 ± 1.15	0.46 ± 1.03	0.995	<0.0001
Do	6	61.52 ± 1.10	0.26 ± 0.14	0.996	<0.0001
Gr	8	47.86 ± 1.09	0.52 ± 1.02	0.995	<0.0001
Ha	5	34.67 ± 1.05	0.42 ± 1.02	0.999	<0.0001
Ma	6	16.60 ±1.51	0.60 ± 1.08	0.910	<0.0022
Sl	6	36.22 ± 1.17	0.50 ± 1.04	0.988	<0.0001
Sn	8	52.97 ± 1.10	0.52 ± 1.02	0.991	<0.0001

n = number of points used in deriving the regression. The zero points and the alpha phase points of Sl and Sn were disregarded.

r^2 = multiple regression coefficient

p = probability of occurrence by chance

Fig 5:3 Metronidazole concentration in serum and tissue cage fluid of goat Dopey (Do), following intravenous administration of 0.5% w/v metronidazole solution (serum metronidazole concentration = 61.52 + 0.26 time +e). $y = a + bx + e$


Goat: Dopey (Do)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
β = 1.34 hr -1
* Serum
+ Tissue cage fluid

versus time curve [AUC] (Fig. App:1.2). For these four animals, this was 68.94 ± 10.72 mcg.hr/ml (n=4).

The concentration of metronidazole in tissue cage fluid reached a peak at 1.41 ± 0.58 hours (n=8) (Table 5:IV). This peak concentration was considerably less than that attained in serum, and occurred later (Table 5:V). Within two hours following the administration of the metronidazole solution, the tissue cage fluid and serum drug concentrations were similar. Metronidazole could be detected longer in tissue cage fluid (20.25 ± 1.83 hours) than in sera (6.38 ± 0.53 hours). The percentage of metronidazole penetration (App.I: equation 10) differed markedly between individuals (Table 5:IV).

All animals achieved peak serum metronidazole concentrations approximately equal to, or greater than, four times the upper value of the minimum inhibitory concentration (4 x MIC), i.e. 50 mcg metronidazole/ml blood. These were maintained for 0.25 to 0.5 hours. Serum concentrations were greater than 12.5 mcg/ml on average for 1.4 ± 0.2 hours (range 1-2, n=8), and greater than 3.0 mcg/ml for 3.1 ± 0.2 hours. No animal achieved a tissue cage fluid metronidazole concentration of 50 mcg/ml, and two animals (Ba and Gr) did not achieve tissue cage fluid concentrations greater than 12.5 mcg/ml at any time. The remaining six animals had tissue cage fluid concentrations approximately equal to or greater than, 12.5 mcg/ml for 1.5 ± 0.6 hours (range 1-4) and all the goats had concentrations greater than 3.0 mcg/ml for 7.9 ± 1.0 hours (range 5-12).

3.3 Intramuscular administration of metronidazole

The peak serum metronidazole concentration obtained following IM injection was well below the corresponding serum peak concentrations following IV administration. The time to the maximum serum concentration was slower following IM injection (Table 5:VI). The concentration of metronidazole achieved in the tissue cage fluid (Table 5:VII), was greater than that in sera, although the period from administration by IM injection to the maximum concentration differed between individual goats. The proportional change of serum metronidazole concentration with time showed a high correlation (Table 5:VIII). A representative graph of one animal is shown in Fig. 5.4. The remaining data has been bound in Appendix III.

TABLE 5 : IV **Pharmacokinetic values for the disposition of metronidazole in goat tissue cage fluid, following the intravenous administration of 0.5% w/v solution (Dose 20 mg/kg BWgt).**

Pharmaco-kinetic values	A N I M A L S								
	Ba	Dc	Do	Gr	Ha	Ma	Sl	Sn	Mean ± s.e.
C_{max} (mg/ml)	4.1	32.4	13.1	9.9	28.6	30.4	33.4	37.5	23.67 ± 4.46 (n=8)
T_{max} (hours)	2.0	0.25	0.25	5.0	2.0	0.5	1.0	0.25	1.41 ± 0.58 (n=8)
Range of detectable levels (hours)	0.25-24.0	0.25-14.0	0.25-14.0	0.25-24.0	0.25-14.0	0.25-24.0	0.25-24.0	0.25-24.0	0.25-20.25 ± 1.83 (n=8)
Percentage penetration	7.2	nd	27.2	23.1	nd	nd	30.1	62	30.7 ±12 (n=5)

nd = not done

TABLE 5 : V **Pharmacokinetic values for the disposition of metronidazole in goat sera, following the intravenous administration of 0.5% w/v solution (Dose 20 mg/kg BWgt).**

Pharmaco-kinetic value	ANIMAL Ba	Dc	Do	Gr	Ha	Ma	Sl	Sn	Mean ± s.e.
C_{max} (mcg/ml)	56.9	atr	48.1	42.8	atr	atr	111.1	60.6	63.9 ±12.2 (n=5)
T_{max} (hours)	<0.25	nd	<0.25	<0.25	nd	nd	<0.25	<0.25	<0.25 (n=5)
B (mcg/ml)	42.36	36.39	61.52	47.86	34.67	16.60	36.22	52.97	41.07 ± 4.8 (n=8)
β (per hour)	0.84	0.77	1.34	0.66	0.86	0.52	0.70	0.65	0.79 ± 0.09 (n=8)
$t_{\frac{1}{2}}$ (hours)	0.82	0.90	0.52	1.06	0.81	1.34	1.0	1.06	0.94 ± 0.08 (n=8)
AUC (mcg.hr/ml)	nd	nd	40.81	65.11	nd	nd	90.45	79.4	68.94 ±10.72 (n=4)
$V_{d(area)}$ (L/kg)	nd	nd	0.37	0.47	nd	nd	0.32	0.39	0.39 ± 0.03 (n=4)
Cl (L/kg/hr)	nd	nd	0.50	0.31	nd	nd	0.22	0.25	0.32 ± 0.06 (n=4)
Range of detectable levels (hours)	0.25-6.0	0.25-6.0	0.25-4.0	0.25-8.0	0.25-5.0	0.25-8.0	0.25-6.0	0.25-8.0	0.25-6.38 ± 0.53 (n=8)

atr = above test range
nd = not done

TABLE 5:VI **Pharmacokinetic values for the disposition of metronidazole in goat sera following the intramuscular administration of 40% w/v metronidazole suspension (Dose 20 mg/kg BWgt).**

Pharmaco-kinetic value	A N I M A L Ba	Dc	Do	Gr	Ha	Ma	Sl	Sn	Mean ± s.e.
C_{max} (mcg/ml)	3.3	4.4	4.4	5.6	3.2	6.8	9.8	6.2	5.5 ± 0.8 (n=8)
T_{max} (hours)	0.5	1.0	1.0	2.0	1.0	2.0	0.5	0.5	1.06 ± 0.6 (n=8)
A' (mcg/ml)	nd	3.8	2.8	8.0	3.6	6.8	nd	nd	5.0 ± 1.01 (n=5)
k_{ab} (per hour)	nd	1.54	0.5	0.55	3.47	2.31	nd	nd	1.67 ± 0.56 (n=5)
$t_{\frac{1}{2}ab}$ (hours)	nd	0.45	1.39	1.25	0.2	0.3	nd	nd	0.72 ± 0.36 (n=5)
B' (mcg/ml)	3.8	6.23	6.17	10.3	3.89	9.55	7.8	6.63	6.71 ± 0.82 (n=8)
$t_{\frac{1}{2}}$ (hours)	3.2	4.2	2.0	3.8	4.8	2.35	2.15	3.0	3.19 ± 0.36 (n=8)
β' (per hour)	0.22	0.17	0.35	0.18	0.14	0.29	0.32	0.23	0.24 ± 0.03 (n=8)
AUC (mcg.hr/ml)	18.37	30.05	15.4	43.75	21.65	28.53	26.1	27.47	26.42 ± 3.07 (n=8)
Range of detect-able levels (hours)	0.5-10	0.5-10	0.5-12	0.5-14	0.5-12	0.5-12	0..5-10	0.5-10	0.5-11.25 ± 0.53 (n=8)
F%	nd	nd	37.7	67.2	nd	nd	28.9	34.6	42.4 ±8.8 (n=4)

nd = not done

TABLE 5:VII **Pharmacokinetic values for the disposition of metronidazole in goat tissue cage fluid following the intramuscular administration of 40% w/v metronidazole suspension (Dose 20 mg/kg BWgt).**

Pharmaco-kinetic value	ANIMAL Ba	Dc	Do	Gr	Ha	Ma	Sl	Sn	Mean ± s.e.
C_{maxTC} (mcg/ml)	11.4	5.7	11.0	29.1	2.2	30.6	12.5	3.0	13.2 ±3.9 (n=8)
T_{maxTC} (hours)	7.0	2.0	4.0	7.0	2.0	2.0	4.0	3.5	4.1 ±0.7 (n=8)
Range of detectable levels (hours)	0.5-24	0.5-24	0.5-24	0.5-24	0.5-14	0.5-24	0.5-24	0.5-12	0.5-21.25 ±1.81 (n=8)
Percent-age penetration	345	259	130	520	68.8	450	127	419	289.9 ±59.8 (n=8)

TABLE 5 : VIII **Least squares linear regression analysis following intramuscular administration of 40% w/v metronidazole solution (Dose 20 mg/kg BWgt).**

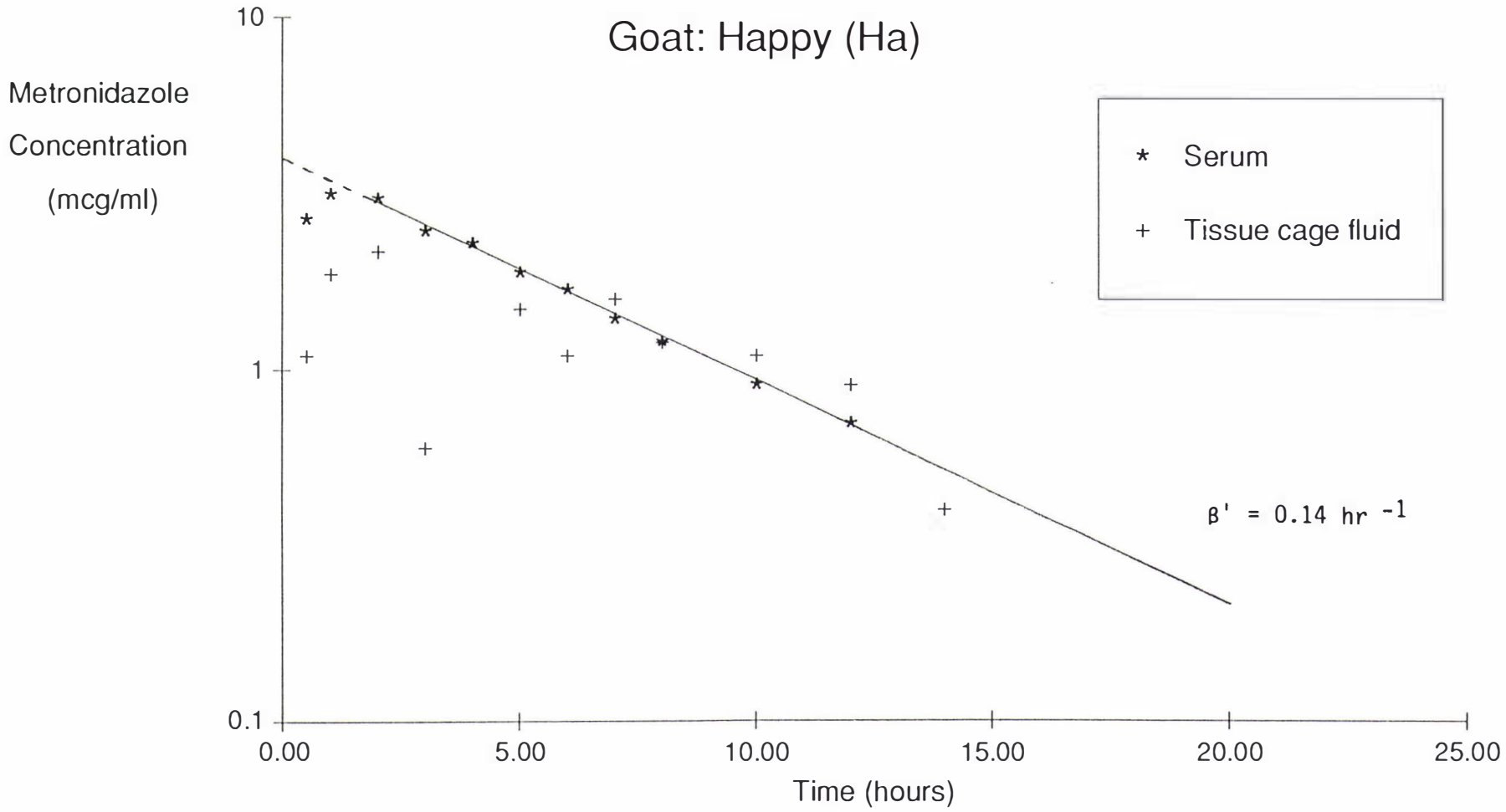
Animal	n	Intercept ± s.e.	Slope ± s.e.	r^2	P
Ba	10	3.77 ±1.08	0.82 ±1.01	0.958	<0.0001
Dc	11	6.23 ±1.06	0.85 ±1.01	0.981	<0.0001
Do	10	6.17 ±1.09	0.70 ±1.01	0.988	<0.0001
Gr	11	10.30 ±1.12	0.83 ±1.01	0.959	<0.0001
Ha	12	3.89 ±1.02	0.86 ±1.00	0.995	<0.0001
Ma	11	9.55 ±1.18	0.74 ±1.02	0.958	<0.0001
Sl	10	7.08 ±1.14	0.73 ±1.03	0.946	<0.0001
Sn	10	6.63 ±1.13	0.77 ±1.02	0.936	<0.0001

n = number of points used in deriving the regression. The zero points and the absorption phase points were disregarded

r^2 = multiple regression coefficient

p = probability of occurrence by chance

Fig. 5:4 Metronidazole concentration in serum and tissue cage fluid for goat Happy (Ha), following intramuscular administration of 40% w/v metronidazole solution (serum metronidazole concentration = 3.89 + 0.06 time + e).


Goat: Happy (Ha)
Metronidazole
Concentration
(mcg/ml)
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.14 hr -1

The ratio of the maximum concentration of metronidazole in tissue cage fluid, to the maximum concentration in sera (percent penetration) for each goat, was very high (289.9% ± 59.8%, n=8).

Metronidazole was present in the sera of four animals 12 hours after administration, and could still be detected in the tissue cage fluid of six animals 24 hours after the administration of the drug.

The systemic availability (App.I: equation 8) of metronidazole following IM injection was 42.4% ± 8.8% (n=4), and therefore the actual quantity of the administered dose that became systemically available was 8.4 mg/kg BWgt. All other serum and tissue cage pharmacokinetic values following IM injection are shown in Tables 5:VI; 5:VII.

None of the animals achieved serum concentrations equal to, or greater than 12.5 mcg/ml (the upper MIC limit). Five animals (Ba, Do, Gr, Ma and Sl) had concentrations equal to or greater than this, in the tissue cage fluid for 5 ± 1.5 hours (n=5, range 2-10). Metronidazole concentrations greater than 3.0 mcg/ml were achieved in the serum for 4.1 ± 0.7 hours (n=8, range 2-8), and in the tissue cage fluid for 8.6 ± 1.6 hours (n=7, range 4 to greater than 14). One animal (Ha) only just achieved concentrations greater than 3.0 mcg/ml in the serum, and did not achieve this concentration in tissue cage fluid.

There was no tissue reaction or pain observed in any goat following IM injection.

3.4 Recommendations for a dosage schedule

Using pharmacokinetic values ($V_{d(area)}$ and F) obtained from the disposition data of the experiment and App. I: equations 11 to 17, dose rates and dosage intervals were calculated for the IV administration of 0.5% w/v metronidazole solution and for IM administration of the 40% w/v suspension of metronidazole (Table 5:IX). The desired maximum serum concentration was set by the upper MIC value multiplied by a safety factor of four, and the minimum serum concentration was set by the lower MIC value.

TABLE 5 : IX **Recommendations for metronidazole administration schedules in the goat**

Product	Route	Loading dose (mg/kg)	Maintenance dose (mg/kg)	Dosage interval (hours)
0.5% w/v metronidazole solution	IV	20.3	19.0	3.6
40% w/v metronidazole suspension	IM	48.3	45.3	9.3

CHAPTER VI
GENERAL DISCUSSION

Many important anaerobic infections of goats and other animals affect devitalised body tissues in which the oxygen tension declines partly because of a markedly reduced blood supply. Typical infections are those affecting the hoof, umbilicus, abdominal wounds and traumatised muscles. Because of the ischaemia, conventional treatment with antibiotics does not necessarily mean that the causative bacteria would become exposed to effective concentrations of the drug. If drug concentrations within the extravascular tissues were known, then more specific recommendations could be made for the use of drugs in those conditions. However, for reasons which will be elaborated below, full use cannot presently be made of that information. Medication schedules are still based solely upon the drug concentrations achieved in serum, although much more knowledge of the distribution of drugs in both vascular and extravascular compartments would provide a more comprehensive guide for the clinical use of antibiotics.

In these experiments, the therapeutic information that could be deduced from determining the metronidazole concentration in serum, was complemented by data describing the drug concentrations in interstitial fluid.

The use of tissue cages as a means of evaluating "tissue levels" of drugs was first reported by Guyton in 1963, and since then there have been many improvements in their design (ref. Chapt. IV). Silicone tubing was selected for use in this experiment, because the material is flexible and inert, it does not support bacterial growth, and the capsule size could be adjusted to suit the goat.

The various sites for cage implantation were chosen because they allowed an easy approach for surgery, would accommodate the extra bulk, were accessible for the collection of samples and would cause no more than minimal inconvenience to the animal. Other authors have used the brisket and sublumbar fossa (Piercy, 1978), and the neck (Higgins et al, 1984).

Previous studies by Calnan et al (1972) and Chisholm et al, (1973), have demonstrated that the fluid which accumulates within the cages is very similar to interstitial fluid. Landau et al (1981), in disputing this, suggested that the large spaces created between cells in these cages is not normal and may inhibit diffusion of antimicrobial agents. These later concerns have not been supported

in dynamic studies using radiolabelled materials (Chisholm, 1978), and furthermore, the distribution of metronidazole into cage fluid in the current experiment was found to be very rapid. Metronidazole was detectable in the tissue cage fluid of all the animals at the first sampling period following administration, i.e. within 0.25 hour.

Piercy (1978) argued that as tissue cage fluid is supplied by recently formed capillaries in the invading granulation tissue, it should be considered to be an artificially induced transudate. Other authors, (Rylander and Norrby, 1983), regarded the tissue cage as an anaerobic "abscess-like compartment". Both of these ideas are consistent with the proposal by Hirsch et al (1985), that the interior wall of an abscess consists of granulation tissue containing permeable immature capillaries and maturing fibrous tissue: features similar to those found in the tissue cages in this experiment.

The ratio of tissue cage fluid protein to serum protein in goats (38%) was comparable to the 30% - 45% found in dogs (Calnan et al, 1972), 33% in rabbits (Gerding and Hall, 1975), and 56% in calves (Piercy, 1978). Albumin was the major constituent of the protein in these goat tissue cages, as it was in the other animal species tested. The concentration of albumin was important, because it is the protein most commonly involved in drug binding. If the protein-drug binding approaches 80% (Baggot, 1980), then the disposition of the drug may be affected by the albumin concentration in tissue cage fluid. This feature is possibly of less significance in the current disposition study, as some authors (Ralph et al, 1974) suggest that metronidazole is not significantly protein bound, at least in humans.

Tissue cage fluid from goats then, is comparable in its protein content to other species and it also has the cellular characteristics of a monocellular exudate, similar to cage fluid taken from rabbits (Tight et al, 1975; Piercy, 1978). Based on these findings, tissue cage fluid from goats can be regarded as "interstitial fluid". As antibiotic concentrations in the cage fluid approximate the "tissue levels" of that antibiotic (Ziv et al, 1982), fluid taken from artificially introduced containers provides vital information on the disposition of the administered drug. The general features of distribution about the body are particularly relevant to an understanding of medication scheduling and to the therapeutic potential of any drug.

The implanted silicone tubes developed a thick fibrous envelope (Plate 6:1), which is similar to that found in other species (Higgins et al, 1984). Subsequent examination of the tissue cages and their contents, showed a connective tissue matrix very similar to the structure described by Piercy (1978) (Plate 5:4^{e}, 5:6). The areas of haemorrhage observed within the tissue mass probably resulted from the repeated aspiration of fluid samples. The clear artifacts noticed under microscopic examination of the tissue, may well have been cores of silicone tubing cut out by hypodermic needles during the sampling procedure.

The relatively high concentration of metronidazole found in tissue cage fluid following either route of administration was not supported by a large value for the apparent volume of distribution. The volume of distribution of basic drugs like metronidazole (Neff-Davis et al, 1981) is usually greater in ruminants than in monogastric animals (Baggot, 1975). This occurs because the majority of the drug is non-ionized in plasma and therefore is likely to diffuse from the systemic circulation into the reticulorumen, where it becomes ion-trapped. The large colon of the horse acts in a somewhat similar way. The small volume of distribution found in goats (0.39 ± 0.03 L/kg) compares unfavourably with the 0.948 ± 0.096 L/kg found in dogs (Neff-Davis et al, 1981), and the 1.7± 0.24 L/kg found in horses (Sweeney et al, 1986). There is no apparent reason for such differences. Possibly the efficiency of biotransformation in the ruminant, and the subsequent uncomplicated excretion of metronidazole metabolites may account for both a low figure for the $V_{d(a)}$ and a short $t_{\frac{1}{2}}$.

The relatively short half-life which was found in goats (0.94 ± 0.08 hr) is similar to that found in sheep (0.82 hour; Cooper, pers. comm.), but considerably shorter than that in dogs (4.28 hours; Neff-Davis et al, 1981), horses (2.9 hours; Sweeney et al, 1986), humans (8.7 hours; Selkon, 1979), calves (approximately four hours), and cattle (approximately ten hours; Anon. 1985). The half-life of any drug which, by definition, is the time taken for blood plasma drug concentrations to reduce by 50%, is influenced by the distribution of the drug into tissues, the biotransformation of the drug, and the excretion of the drug from the body. The short half-life determined for metronidazole in the goat, could have been expected from the obviously rapid distribution of large quantities of the drug into interstitial fluid (tissue cage fluid).

Although the half-life can be used in determining a rational dosage interval for repeated administration, it is not always sensible to apply this method when

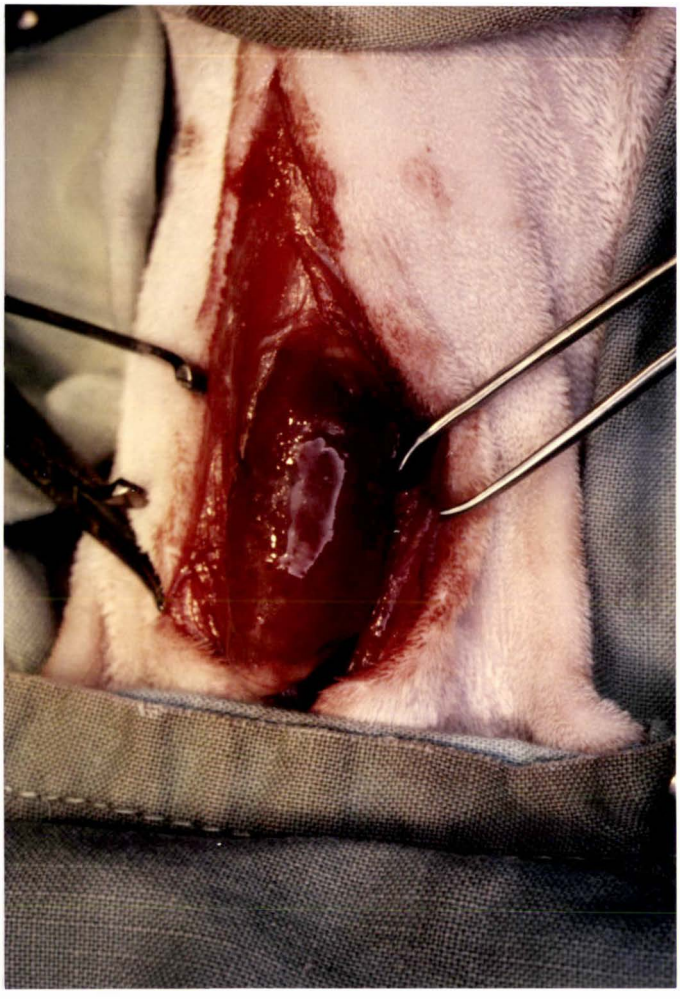


Plate 6:1 Exposure of an implanted tissue cage, to show the thick fibrous capsule.

dealing with antimicrobial agents. Consideration must be given to the spectrum of sensitive bacteria and the range of MIC's for various species. Accordingly, it was elected to calculate the dosage interval for metronidazole on the basis of the MIC for selected bacteria.

The total body clearance parameter differs from the $t_{\frac{1}{2}}$ in being more sensitive to the influence of biotransformation and excretion processes and it is largely unaffected by the distribution process. In keeping with the efficient biotransformation mechanisms of the goat, the clearance of metronidazole from the body was relatively rapid (0.32 ± 0.06 L/kg/hr) and is much the same as for dogs (2.49 ± 0.54 ml/kg/min; Neff-Davis et al, 1981, whose actual figures were 0.15 ± 0.03 L/kg/hr), and horses (0.4 ± 0.05 L/kg/hr; Sweeney et al, 1986).

Various authors (Chow et al, 1976, Neff-Davis, 1981), have reported a range of minimum inhibitory concentrations (MIC) for those anaerobic bacteria sensitive to metronidazole. The range of 3.0 - 12.5 mcg/ml is considered to be the bactericidal concentration in vitro for the majority of susceptible bacteria, (ref. Chapt. III:6), but the practical use of these concentrations in drug scheduling has become a matter for debate. For instance, it has never been conclusively established whether it is better to achieve high concentrations of drugs in the serum rapidly (and thereby achieve high levels in the body tissues) (Prescott and Baggot, 1985), or to have drugs present for a longer period at lower concentrations (Papich, 1987). To obtain bactericidal drug concentrations in tissues, (allowing for membrane barriers and possible interfering substances in vivo), Selkon (1979) and Powers et al (1984) recommend trying to achieve serum levels that are four times the MIC. In the goat experiment this objective was achieved in all animals following IV administration, but not in any animal following IM administration.

Selkon (1979) also suggested that the same therapeutic drug concentration should be maintained for at least 50% of the time between drug doses. Koritz (1984) found that the factors which determined the inhibitory effect on bacteria were a combination of the maximum drug concentration obtained (up to eight times the MIC), and the duration that MIC concentrations were maintained in the serum (particularly up to four hours). However, he found that considerable variation existed among drugs, animal species and bacterial isolates.

Following an IV injection at 20 mg/kg BWgt in the current experiment, the serum metronidazole concentration was greater than 3.0 mcg/ml for

approximately 3.1 hr., i.e. 13% of the experimental time, and for 4.3 hr., i.e. 20% of the experimental time following IM injection. After pharmacokinetic analysis of this information from a single dose study, it was possible to determine suitable dose rates and dosage intervals for multiple dosage schedules by either of the two routes of administration (Table 5:IX).

The closeness of the calculated loading dose to that of the maintenance dose for both IV and IM routes of adminstration (Table 5:IX) is not uncommon in antibiotic usage (Baggot, 1980). Many simply formulated antibiotics have such short elimination half-lives that it is impractical to administer the drug every $t_{\frac{1}{2}}$ period, and accordingly higher doses are used than are necessary on a MIC basis. If slow release preparations such as procaine penicillin are not available, the high dose rate technique is applied, and repeated treatments are given at periods equivalent to several $t_{\frac{1}{2}}$s. Such a schedule is only acceptable because most antimicrobial agents have a wide therapeutic index. By this means of administration, drug accumulation up to steady state does not occur.

Absorption of the drug from the injection site is very dependent on aqueous solubility: as this increases, so does the speed of drug absorption. Metronidazole is poorly soluble in water (Roberson, 1982), and when this drug is injected as a concentrated 40% suspension, some precipitation of the compound probably occurs at the injection site. Uptake from the site is then erratic and delayed, as was found in this experiment. One animal showed very poor absorption following the IM injection and in this individual, serum drug concentrations did not rise above the lowest MIC value. In practice, this individual difference may well result in animals which do not respond to therapy.

In spite of the apparently disappointing "systemic" availability following IM injection, the penetration of metronidazole into the tissue cage fluid was very high: the "interstitial" concentration was maintained above 3.0 mcg/ml for approximately 8.0 hr (n=7). This experiment has confirmed that the measurement of "interstitial" fluid drug concentrations provides invaluable data concerning tissue concentrations, regardless of the serum level. For those infections remote from a substantial blood supply, these tissue cage fluid drug concentrations may be of greater significance in the formulation of drug dosage regimens. It is disappointing, therefore, that the disposition of drugs needs to be calculated from serum data simply because the concentrations determined elsewhere can not be utilised. Mathematical models that can make use of the

interstitial fluid disposition kinetic values in a similar fashion to those used for the analysis of serum disposition data (App. I: equations 9-12), need to be developed. Utilizing the drug concentration at the actual site of action in calculating drug dosage regimens, must improve the therapeutic value of a drug.

SUMMARY

Metronidazole is considered to have a potential use against certain anaerobic infections of the goat and may be administered either intravenously or intramuscularly. Using the disposition kinetics determined experimentally, dosage schedules were calculated for metronidazole given by either route. By adopting such schedules, serum drug levels would be maintained for an appropriate time, at a bactericidal concentration for the majority of anaerobic bacteria, including Bacteroides spp., Fusibacterium spp., and Clostridia spp.

Concurrent determinations of the metronidazole concentration in serum and "interstitial fluid" showed that metronidazole diffused out of the systemic circulation in bactericidal concentrations, and these tissue concentrations were maintained for a longer period than in serum. Drug concentrations in "interstitial fluid" gave some indication of the antimicrobial activity of extravascular tissues; information which can not be extrapolated from a profile of serum concentrations.

REFERENCES

ANON (1985): Plasma and milk concentrations of metronidazole and hydroxymetronidazole (20396 R.P.) following intraperitoneal or intra-uterine administration of metronidazole to lactating cows. May & Baker Ltd., Essex. R. Ph. 62.

ANON (1986): Plasma concentrations of metronidazole and hydroxymetronidazole following intraperitoneal adminstration of a 0.5 % w/v metronidazole solution (50 ml) to sheep. May & Baker Ltd., Essex. AH Tech. 562.

BACK, E.; Hermanson, J.; Wickman, M. (1978): Metronidazole treatment of a liver abscess due to Bacteroides fragilis. Scand. J. infect. Dis. **10:** 152-154.

BAGGOT, J.D. (1974): Principles of drug distribution. Aust. vet. J. **50:** 111-119.

BAGGOT, J.D. (1977): Principles of drug disposition in domestic animals. 1st Ed. W.B. Saunders., London. pp. 238.

BAGGOT, J.D. (1978): Some aspects of clinical pharmacokinetics in veterinary medicine II. J. vet. Pharmacol. Therap. **1:** 111-118.

BAGGOT, J.D. (1980): Distribution of antimicrobial agents in normal and diseased animals. J. Am. vet. med. Assoc. **176:** 1085-1090.

BAMGBOSE, O.H.; Bababunmi, E.A. (1973): Interaction of metronidazole with human and bovine plasma albumin. Biochem. Pharmacol. **22:** 2926-2928.

BAXENDELL, S.A. (1980): Foot problems in goats. **IN** Goats : Proceedings Number 52. University of Sydney, Postgraduate Foundation in Veterinary Science, N.S.W. 351-353.

BERKHOFF, G.A. (1978): Recovery and identification of anaerobes in veterinary medicine : A two-year experience. Vet. Micro. **2:** 237-252.

BERNARD-STROTHER, S. Mansmann, R.A. (1985): Diagnosis and treatment of anaerobic bacterial pleuropneumonia in six horses. Compend. Contin. Educ. Pract. Vet. **7:** S341-S349.

BOST, R.G. (1976): Metronidazole : Toxicology and teratology. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York: 112-118.

BROOKE, I,; Coolbaugh, J.G.; Walker, R.I.; Weiss, E. (1984): Synergism between penicillin, clindamycin or metronidazole, and gentamycin against species of the Bacteroides melaninogenicus and B. fragilis groups. Antimicrob. Agents Chemother. **25:** 71-77.

CALNAN, J.S.; Pflug, J.J; Chisholm, G.D.; Taylor, L.M. (1972): Pathophysiology of tissue fluid. Proc. Roy. Soc. med: **65:** 715-719.

CARWADINE, P.C. (1984): Recent advances in the understanding of anaerobic infections in animals. May and Baker Aust. Pty. Ltd. pp. 25.

CHIRINO TREJO, J.M.; Prescott, J.F. (1983): The identification and antimicrobial susceptibility of anaerobic bacteria from pneumonic cattle lungs. Can. J. comp. Med. **17:** 270-276.

CHISHOLM, G.D. (1978): The tissue cage model in the distribution of antibacterial agents. Scand. J. infect. Dis. Suppl. **14:** 118-124.

CHISHOLM, G.D.; Waterworth, P.M.; Calnan, J.S.; Garrod, L.P. (1973): Concentration of antibacterial agents in interstitial fluid. Br. med. J. **1:** 569-573.

CHOW, A.W.; Bednorz, D.; Guze, L.B. (1976): Susceptibility of obligate anaerobes to metronidazole : An extended study of 1,054 clinical isolates. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York: 368-375.

COOPER, B.S. (1984): pers. comm.

CUCHURAL, G.J.; Tally, F.P.; Jacobus, N.V.; Gorbach, S.L.; Aldrich, K.; Cleary, T.; Finegold, S.M.; Hill, G.; Iannin, P.; O'Keefe, J.P.; Pierson, C. (1984): Antimicrobial susceptibilities of 1,292 isolates of the Bacteroides fragilis group in the United States : A comparison of 1981 with 1982. Antimicrob. Agents Chemother. **26:** 145-148.

DU PREEZ, J.H. et al, (1981): Isolation and significance of anaerobic bacteria isolated from cases of bovine mastitis. Onderstepoort J. vet. Res. **48:** 123-126.

EIKENBERG, H-U.; Waterman, N.G.; Scharfenberger, L. (1975): Concentration of antibacterial agents in renal interstitial fluid : An experimental model. Urol. Res. **3:** 165.

ESSIEN, E.E. et al, (1985): Metronidazole induced myocardial depression : Chemical and pharmacological studies in the role of calcium ions in vitro. J. Pharm. Pharmacol. **37:** 213-216.

(a) FERGUSON, I.R. (1979): The diagnosis of anaerobic infection. **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Edited by Phillips, F.; Collier, J. Roy. Soc. med. London: 13-18.

(b) FERGUSON, I.R. (1979): The assay of metronidazole. **IN** Proceedings of the 2nd International Symposium on anaerobic infections. Geneva. Edited by Phillips, I.; Collier, J. Roy. Soc. med. London: 23-27.

FINEGOLD, S.M ; Rosenblatt, J.E.; Sutter, V.L.; Attebury, H.R. (1976): Anaerobic infections. Upjohn Co. Kalamazoo, Michigan. pp 69.

FREEMAN, W.H.; Fadzean, J.A.; Whelan, J.P.F. (1968): Activity of metronidazole against experimental tetanus and gas gangrene. J. Applied Bacteriology. **31:** 443-447.

GABRIEL, R.; Page, C.M.; Weller, I.V.D.; Collier, J,; Houghton, G.W.; Templeton, R. and Thorne, P.S. (1979): The pharmacokinetics of metronidazole in patients with chronic renal failure. **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Edited by Phillips, I.; Collier, J. Roy. Soc. med. London: 49-54.

GEORGOPOULOS, A.; Schutze, E. (1980): Concentrations of various antibiotics in serum and fluids accumulated in diffusion chambers, implanted in various sites in rabbits. Antimicrob. Agents Chemother. **17:** 779-783.

GERDING. D.N.; Hall, W.H. (1975): The penetration of antibiotics into peritoneal fluid. Bulletin of the New York Academy of Medicine. **57:** 1016-1019.

GERDING, D.N.; Hall, W.H.; Schierl, E.A.; Manion, R.E. (1976): Cephalosporin and aminoglycoside concentrations in peritoneal capsular fluid in rabbits. Antimicrob. Agents Chemother. **10:** 902-911.

GOLDBERG, H.S. (1976): Recent observations on anaerobic infections of animals in metronidazole. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York : 245-258.

GUILLAND, F.M.D.; Carwardine, P.C. (1987): Plasma metronidazole levels in an Indian Elephant (Elephas maximus) after rectal adminstration. Vet. Rec. **120:** 440.

GUYTON, A.C. (1963): A concept of negative interstitial pressure based on pressures in implanted perforated capsules. Circln Res. **12:** 399-414.

Helena Laboratories General Product Catalogue (1984-1985) : 51-53.

HIGGINS, A.J.; Lees, P.; Sedgewick, A.D. (1987): Development of equine models of inflammation. Vet. Rec. **120:** 517-522.

HIGGINS, A.J.; Lees, P.; Wright J.A. (1984): Tissue cage model for the collection of inflammatory exudate in ponies. Res. vet. Sci. **36:** 284-289.

HIRSCH, D.C.; Indiveri, M.C.; Jang, S.S.; Biberstein, E.L. (1985): Changes in prevalence and susceptibility of obligate anaerobes in clinical veterinary practice. J. Am. vet. med. Assoc. **186:** 1086-1089.

(a) HOUGHTON, G.W.; Thorne, P.S.; Smith, J.; Templeton, R. (1979): The pharmacokinetics of intravenous metronidazole (single and multiple dosing). **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Edited by Phillips, I.; Collier, J. Roy Soc. med. London : 35-40.

(b) HOUGHTON, G.W.; Thorne, P.S.; Smith, J.; Templeton, R. (1979): Plasma metronidazole concentrations after suppository administration. **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Edited by Phillips, I.; Collier, J. Roy. Soc. med. London. 41-44.

INGHAM, H.R.; Selkon, J.B.; Hal, J.H. (1975): The antibacterial activity of metronidazole. J. Antimicrob. Chemother. **1:** 355.

INGS, R.M.J.; McFadzean, J.A.; Ormorod, W.E. (1975): The fate of metronidazole and its implications in chemotherapy. Xenobiotica **5:** 223-235.

ITIDA, O.A.; Pilpel, N. (1986): Studies on metronidazole tablet formulations. J. Pharm. Pharmacol. **38:** 81-86.

JOLLES, G.E. (1976): Origin and anti-infective activities of metronidazole. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York. 3-11.

KIMSEY, P.B.; Hirsch, D.C. (1978): Obligate anaerobes in clinical veterinary medicine : Susceptibility to antimicrobial agents. J. vet. Pharmacol. Therap. **1:** 63-68.

KING, N.B. (1980): Clostridial diseases. **IN** Goats: Proceedings Number 52. University of Sydney, Post-Graduate Committee. 217-220.

KNIGHT, H.D.; Hietala, S.K.; Jang, S.S. (1980): Antibacterial treatment of abscesses. J. Am. vet. med. Assoc. **176:** 1095-1098.

KORITZ, G.D. (1984): Relevance of peak and trough concentrations to antimicrobial drug therapy. J. Am. vet. med. Assoc. **185:** 1072-1075.

LANDAU, A.; Halkin, H.; Rubinstein, E. (1981): Interstitial fluid concentrations of cefsulodin, azolocillin and carbenicillin. Scand. J. infect. Dis. **13:** 227-232.

LARSON, T.A.; Gerding, D.N.; Peterson, L.R. (1983); Extravascular penetration of tobramycin and netilmicin in a subcutaneous Visking chamber model in rabbits. Antimicrob. Agents Chemother. **24:** 594-596.

LEVISON, M.E. (1974): Microbiological agar diffusion assay for metronidazole concentrations in serum. Antimicrob. Agents Chemother. **15:** 466-468.

LOVE, D.N.; Bailey, M.; Johnson, R S. (1980): Antimicrobial susceptibility patterns of obligately anaerobic bacteria from subcutaneous abscesses and pyothorax in cats. Austr. vet. Pract. **10:** 168-170.

LUDWIG, E. et al, (1983): Age associated pharmacokinetic changes of metronidazole. Int. J. clin. Pharmacol. Ther. Toxicol. **21:** 87-91.

McINTOSH, M.E. (1985): Importance of anaerobes in veterinary medicine. Irish vet. News. **Nov:** 27-28.

MANTHEI, R.W.; To, W.; Feo, L.G. (1963): Characterization of metabolites of metronidazole (Flagyl). Pharmacologist. **5:** 235.

MARTIN, W.J.; Wilhelm, P.A. Bruckner, D. (1984): Recovery of anaerobic bacteria from vented blood culture bottles. Rev. of infectious Dis. **6:** Supplmt. 1 : 559-561.

MESSIER, S.; Higgins, R.; Couture, Y.; Morin, M. (1984): Comparison of swabbing and biopsy for studying the flora of the bovine uterus. Can. vet. J. **25:** 283-288.

MULLER, M.; Lindmark, D.G.; McLaughlin, J. (1976): Mode of action of metronidazole on anaerobic micro-organisms. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York: 12-19.

NAKATA, M.M.; Lewis, R.P. (1984): Anaerobic bacteria in bone and joint infections. Rev. of infect. Dis. **6:** Supplmt **1:** S165--S170.

NEFF-DAVIS, C.A.; Davis, L.E.; Gillette, E.L. (1981): Metronidazole : A method for its determination in biological fluids and its disposition kinetics in the dog. J. vet. Pharmacol. Therap. **4:** 121-127.

OLSON, J.D. et al, (1984): Aspects of bacteriology and endocrinology of cows with pyometras and retained foetal membranes. Am. J. vet. Res. **45:** 2251-2255.

ONDERDONK, A.B.; Louie, T.J.; Tally, F.F.; Bartlett, J.E. (1979): Activity of metronidazole against E. coli. in experimental intra-abdominal sepsis. J. Antimicrob. Chemother. **5:** 201-210.

PAPICH, M.G. (1987): The -lactam antibiotics : Clinical pharmacology and recent developments. Comp. Contin. Educ. Pract. Vet. **9:** 68-76.

PAULING, B.A. (1986): Clostridial diseases and vaccination of goats. **IN** Proceedings of the 16th Seminar of the Sheep & Beef Cattle Society of the N.Z.V.A. Massey University : 57-72.

PHILLIPS, I. (1979): The spectrum of anaerobic infection. **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Edited by Phillips, I.; Collier, J. Roy. Soc. med. London : 1-6.

PIERCY, D.W.T. (1978): Distribution of trimethoprim/sulphadiazine in plasma, tissue and synovial fluids. Vet. Rec. **102:** 523-524.

PLACIDI, G.F.: Masuoka, D.; Alcaraz, A.; Taylor, J.A.T.; Earle, R. (1970): Distribution and metabolism of ^{14}C-metronidazole in mice. Arch. Int. Pharmacodyn. Ther. **188:** 168-179.

PRESCOTT, J.F. (1979): Identification of some anaerobic bacteria in nonspecific anaerobic infections in animals. Can. J. Comp. Med. **43:** 194-199.

PRESCOTT, J.F.; Baggot, J.D. (1985): Antimicrobial susceptibility testing and antimicrobial drug dosage. J. Am. vet. med. Assoc. **187:** 363-368.

POWERS, T.E.; Varma, K.J.; Powers, J.D. (1984): Selecting therapeutic concentrations : Minimum inhibitory concentrations vs subminimum or supraminimum inhibitory concentrations. J. Am. vet. med. Assoc. **185:** 1062-1067.

RALPH, E.D.; Amtnieks, Y.E. (1980): Potentially synergistic antimicrobial combinations with metronidazole against B. fragilis. Antimicrob. Agents Chemother. **17:** 379-382.

RALPH, E.D.; Clarke, J.T.; Libke, R.D.; Luthy, R.P.; Kirby, W.M.M. (1974): Pharmacokinetics of metronidazole as determined by bioassay. Antimicrob. Agents Chemother. **6:** 691-696.

ROBERSON, E.L. (1982): Antiprotozoan drugs. **IN** Veterinary Pharmacology and Therapeutics Vth Edition. Edited by Booth, N.H.; MacDonald, L.E. Iowan State University Press/Ames : 874-891.

ROE, J.F.C. (1979): A critical appraisal of the toxicology of metronidazole. **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Roy. Soc. med. London : 215-222.

ROLFE, R.D. (1984): Interactions among micro-organisms of the indigenous intestinal flora and their influence on the host. Rev. of infect. Dis. **6.** Supplmt 1 : 573-579.

RYAN, M.; Cars, O. (1980): Antibiotic assays in muscle : are conventional tissue levels misleading as indicators of the antibacterial activity. Scand. J. infect. Dis. **12:** 307-309.

RYLANDER, M.; Norrby, S.R. (1983): Norfloxacin penetration into subcutaneous tissue cage fluid in rabbits and efficacy in vivo. Antimicrob. Agents Chemother. **23:** 352-355.

SALEM, A.R.; Jackson, D.D.; McFadzean, J.A. (1975): An investigation of interactions between metronidazole (Flagyl) and other antimicrobial agents. J. Antimicrob. Chemother. **1:** 387

SAMS, R.A. (1984): Principles of clinical pharmacokinetics of antimicrobial drugs. J. Am. vet. med. Assoc. 1068-1071.

SELKON, J.B. (1979): Choice of chemotherapy for the anaerobe. **IN** Proceedings of the 2nd International Symposium on Anaerobic Infections. Geneva. Edited by Phillips, I.; Collier, J. Roy. Soc. med. London : 29-33.

SKERMAN, T.M. (1987): Footscald and footrot in goats. **IN** Proceedings of the 17th Seminar of the Sheep and Beef Cattle Society of the N.Z.V.A. Hamilton : 65-70.

SLEE, K.J.; McOrist, S. (1985): Mastitis due to a group of pyogenic bacteria. Aust. vet. J. **62:** 63-65.

SONNENWIRTH, A.C. (1976): Anaerobic culture techniques. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York: 236-244.

STEPHENS, L.R.; Slee, K.J. (1987): Metronidazole for the treatment of bovine pyometra. Aust. vet. J. **64:** 343-346.

SUTER, V.L.; Finegold, S.M. (1976): In vitro studies with metronidazole against anaerobic bacteria. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York : 279-284.

SUTTON, R.H. (1982): Clinical pathology : Haematology. Vet. Path. & Public Health Publications No. 13; Massey University : 21.

SWEENEY, C.R.; Divers, T.J.; Benson, C.E. (1985): Anaerobic bacteria in 21 horses with pleuropneumonia. J. Am. vet med. Assoc. **87:** 721-724.

SWEENEY, R.W.; Sweeney, C.R.; Soma, L.R.; Woodward, C.B.; Charlton, C.A. (1986): Pharmacokinetics of metronidazole given to horses by intravenous and oral routes. Am. J. vet. Res. **47:** 1726-1729.

SWINTOSKY, J.V.; Dittert, L.W.; Dolinsio, J.T. (1969): Estimation of drug absorption rates from blood concentration profiles. Am. J. Hosp. Pharm. **26:** 519-22.

TALLY, F.P.; Goldin, B.R.; Sullivan, N.; Johnston, J.; Gorbach, S.L. (1978): Antimicrobial activity of metronidazole in anaerobic bacteria. Antimicrob. Agents Chemother. **13:** 460-465.

TAN, J.S.; Trott, A.; Phair, J.P.; Watanakunakorn, C. (1972): A method for measurement of antibiotics in human interstitial fluid. J. infect. Dis. **126:** 492-497.

TEMPLETON, R. (1976): Metabolism and pharmacokinetics of metronidazole. **IN** Proceedings of the International Metronidazole Conference. Canada. Excerpta Medica, New York: 28-49.

TESKE, R.; Koritz, G.; Riviere, J.E. (1984): The value and limitation of pharmacokinetics in predicting dosage regimens. **IN** Determination of Doses of Veterinary Pharmaceuticals. Edited by Powers, J.D.; Powers, T.E. Ohio State University Press: 86-118.

TIGHT, R.R.; Prior, R.B.; Perkins, R.L.; Rotilie, C.A. (1975): Fluid and penicillin G dynamics in polyethylene chambers implanted subcutaneously in rabbits. Antimicrob. Agents Chemother. **8:** 495-497.

WALKER, R.D.; Richardson, D.C.; Bryant, M.J.; Draper, C.S. (1983): Anaerobic bacteria associated with osteomyelitis in domestic animals. J. Am. vet. med. Assoc. **82:** 814-816.

WARNER, J.F.; Prior, R.B. (1976): Clinical and in vitro studies of the bactericidal activity of metronidazole against anaerobic bacteria. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York: 359-367.

WEBSTER, D. (1984): pers. comm.

WELLING, P.G.; Munro, A.M. (1972): The pharmacokinetics of metronidazole and tinidazole in man. Arzneim Forsch. **22:** 2128-2132.

WERNER, H. (1976): Anaerobes as normal flora. **IN** Proceedings of the International Metronidazole Conference. Canada. Edited by Finegold, S.M. Excerpta Medica, New York: 229-235.

WEST, D.M. (1981): A study of foot abscess in sheep. Thesis, Ph. D., Massey University. pp. 178.

ZIV, G. (1980): Practical pharmacokinetic aspects of mastitis therapy. Vet. Med. Small Anim. Clin. **76:** 277-290.

ZIV, G.; Warner, M.; Nicolet, J. (1982): Distribution of penicillin G, dihydrostreptomycin, oxytetracycline and chloramphenical in serum and subcutaneous chamber fluid. J. vet. Pharmacol. Therap. **5:** 59-69.

APPENDIX I

MATERIALS AND METHODS (ref: Chapt. V)

Animals

The goats were identified by permanent horn markings:- Bashful (Ba), Doc (Dc), Dopey (Do), Grumpy (Gr), Happy (Ha), Max (Ma), Sleepy (Sl) and Sneezy (Sn). Each goat was dosed orally with five ml of 0.08% w/v solution of ivermectin[1] and then shorn. The bodyweights of the animals ranged from 15.0 to 21.5 kg (mean 16.88 ± 0.73 kg) at the beginning of the experimental period and 17.0 to 25.0 kg (mean 20.4 ± 1.07 kg) at the end.

All pharmacokinetic values are reported as the mean ± the standard error, unless otherwise specified.

Tissue cages

Preparation

Silastic Medical-Grade Tubing (internal diameter 9.5 mm by external diameter 14.3 mm), was cut into eight centimetre long cylinders. Holes (0.2 mm diameter) were punched through the walls of the tubing, so that 40-50% of the surface area was perforated. Both ends of the cylinder were then sealed with medical grade silicone Type A glue[2] (Chapt. 5, Plate 5:2).

The tissue cages were thoroughly washed with a mild, non-oily soap and warm water. While still wet, they were pouched in sets of six into plastic envelopes, and autoclaved for 20 minutes at 121°C.

Implantation

After a two week quarantine period, five animals (Ba, Dc, Do, Gr and Ha) were starved for 12 hours but allowed access to water. General anaesthesia was induced in these animals using an intravenous solution of 5% thiopentone sodium[3] administered at a dose rate of 25 mg/kg bodyweight. Anaesthesia was maintained through an endotracheal tube using 1-2% halothane[4], in a circle anaesthetic system (fresh gas flow, 2 litres per minute).

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1. Ivomec; Merk, Sharp & Dohme (NZ) Ltd., Wiri, New Zealand.
2. Silastic^{R} Medical Adhesive A; Dow Corning Corp. (NZ) Ltd.
3. Intraval sodium^{R}: May & Baker (NZ) Ltd., Lower Hutt.
4. Fluothane^{R}; ICI (NZ) Ltd., Wellington

The six surgical sites on each goat were clipped closely and cleansed using aqueous centrimide (1%), then tincture of centrimide (40%), followed by povidone iodine (1%) spray. A four centimetre anterioposterior incision was made in the skin, and a vertical subcutaneous pocket created by blunt dissection. A sterile silicone cage was inserted into each pocket. The wound was closed with two or three vertical mattress sutures using a nonabsorbable nylon suture material[5], and then sprayed with povidone iodine (1%) solution (Chapt. 5, Plate 5:3). The sutures were removed 10 days following the surgery. The remaining three goats (Ma, Sl and Sn) were implanted similarly, four weeks later.

Metronidazole

The metronidazole preparation for intravenous (IV) use was a 0.5% w/v aqueous solution (batch number P 797)[6]. Any metronidazole solution remaining in an opened container at the end of each experiment was discarded.

The concentrated product for intramuscular (IM) use was presented as 20 grams of sterile, powdered metronidazole (T6563, AHSO 2/85)[7], and a suspending agent (T6561, AHSO 2/85)[8]. Immediately before use, 30 ml of the suspending agent was added aseptically to 20 grams of the metronidazole powder, to produce a 40% w/v suspension. Vigorous shaking was necessary to prevent the powder from settling out.

All liquid metronidazole preparations were stored in the dark.

Drug administration

The animals were randomly assigned to one of two groups which received metronidazole, either by IV or IM injection. After a "wash out" period, the administration route for each animal was changed to the alternate option.

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5. Braunamid^{R}; B. Braun, Melsungen A, West Germany

6. Flagyl^{R}; May & Baker (NZ) Ltd., Wellington

7. Metronidazole powder; May & Baker, Dagenham, England

8. Suspending agent for metronidazole powder; May & Baker, Dagenham, England.

Slow IV infusions of appropriate volumes of the 0.5% w/v metronidazole solution were made into the jugular vein through a 1.5 cm long, 20 gauge needle. Either jugular vein was used, but care was taken to collect the first blood sample from the opposite side.

The concentrated 40% w/v metronidazole suspension was administered by deep IM injection using a 1.5 cm long 18 gauge needle into the left cleido-occipital muscle, approximately 10 cm behind the ear. The injection site was then massaged as recommended by the manufacturers. The first blood sample following the injection was always taken by puncture of the right jugular vein.

Samples of blood and tissue cage fluid

Collection

The time intervals for the collection of samples, for each experiment following IV or IM administration of metronidazole are shown (Table App. I:I).

The tissue cage fluid (0.5 to 3.0 ml) was removed by inserting a two cm long, 20 or 22 gauge needle, into the appropriate "cage", and then applying gentle suction to the attached six ml syringe.

The cages were sampled in a random order (Chapt. 5, Fig. 5:1). Initially, if a capsule was missing from the site required, then a sample would be collected from the next cage in the order. For the subsequent experiment, it was decided to sample each chamber only once every seventh sampling interval, to allow some time for equilibration of the cage fluid.

All samples containing metronidazole were protected from the light.

Processing

After 24 hours the blood and fibrin clots were removed, and the samples centrifuged at 1732G for ten minutes. The supernatent was removed immediately and stored in aliquants at -20°C for up to 16 weeks.

Extraction procedure

The frozen samples were thawed slowly, before chilled acetonitrile was added in a ratio of two parts of acetonitrile to one part of the sample. The acetonitrile/sample mixture was vortexed for one minute to ensure maximal mixing of the components, then centrifuged at 1732G for 15 minutes. The

TABLE App. I:I **Time intervals between the taking of blood or tissue cage fluid samples in goats following IV or IM injection of a 0.5% w/v solution or a 40% w/v suspension respectively, of metronidazole.**

Route of administration	Sample	Time of sampling after administration (hours)
IV 0.5% w/v metronidazole	blood	0, 0.25, 0.5 1, 2, 3, 4, 5, 6, 8, 14,20
	tissue cage fluid	0, 0.25, 05, 1, 2, 3,4, 5, 6, 7, 8, 10, 12, 14, 24
IM 40% w/v metronidazole	blood	0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 24
	tissue cage fluid	0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 24

supernatant was removed from the protein precipitate, and if the volume was sufficient, it was filtered through a handheld 0.5μ Millipore filter[9]. Samples thus prepared were stored at +4°C in 1.5 ml glass vials[10], suitable for use in a Waters WISP™ Model 710. The samples were stored in screw capped vials, or analyzed immediately.

Standard curve preparation

Pure metronidazole powder 8823 RP (Ref. LOP 3744)[11], was dissolved in normal goat serum (10 mg in 10 ml) by gentle agitation and warming to 37°C. A dilution series of metronidazole concentrations, ranging from 50 mcg/ml to 0.1 mcg/ml, was prepared. These samples were processed for assay in the same manner used for the unknown samples. A standard curve was derived, from a plot of the magnitude of absorbance against the known metronidazole concentration.

Chromatography

The high pressure liquid chromatography unit consisted of a Waters Model 6000A Solvent Delivery[12] system, a U6K injector and a Z-module radial compression separation system. The Z-module contained a Radial-PAK Cartridge (10μ Bondapak C_{18}, 8 mm x 10 cm), and was set behind an inline filter and a guard column packed with the same material. A Waters programmable automator (WISP™) Model 710 was connected into the system. The injection interval was set at 15 minutes and the injection volume at 50 μl.

The mobile phase consisted of 75% aqueous potassium hydrogen phosphate (KH_2PO_4) and 25% methyl alcohol. The mixture was filtered[13] (0.22μ Millipore filter), and degassed under vacuum. The potassium hydrogen phosphate was made up to a concentration of one gram per litre, without pH adjustment (pH five to six). The running speed of the solvent was 1.5 ml per minute.

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9. Millipore® Corp., Bedford, Massachusetts 01730.

10. Waters Assoc.; Bedford, Massachusetts 01757.

11. May & Baker; Dagenham, England.

12. Waters Assoc.; Bedford, Massachusetts 01757.

13. Millipore® Corp.; Bedford, Massachusetts 01730.

A 450 variable wavelength detector was set at either 0.01 or 0.04 absorbance units. The optimum wavelength of 312 had been determined by a previously performed scan (Cooper, pers. comm.) of the pure material. Initially the recording device was a Varian 5A chart recorder set at a chart speed of 10 cm per hour. This recording device was replaced with a Spectra Physics SP4270 Chromatography integrator, set at a chart speed of 0.2 cm per minute.

Tissue cage removal

Approximately seven months after the cages had been inserted, a sample of fluid was aseptically withdrawn (approximately 0.5 to 3.0 ml), from each cage. The majority of fluid was placed in an individual vacutainer tube, and the remainder was used to make up a 'pool' in an EDTA vacutainer for each animal. Blood samples were collected for routine haemograms.

The tissue cage sites were scrubbed with aqueous centrimide (1%), followed by swabbing with tincture of centrimide (40%). One to two ml of lignocaine hydrochloride (20 mg/ml)[14] was infused about the most dependent point of the cage. After ten minutes, a two centimetre vertical incision was made through the desensitized skin and thick fibrous capsule surrounding the tissue cage. The cage was then grasped with forceps and removed. The wound was sprayed with povidone iodine and left to heal (Chapt. 5, Plate 5:4).

Calculations used for the determination of the disposition kinetics

Intravenous injection

Following intravenous injection of metronidazole, the disposition data from individual goats were analyzed according to a one-compartment open model (Baggot, 1974), which can be described by the monoexponential equation (equation 1). The line of best fit for the data was calculated using a least squares regression analysis computer program[15].

$$Cp = Be^{-\beta t} \qquad (1)$$

Where Cp = the plasma concentration at time **t,** (described in units of mcg/ml)

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[14]. Nopaine, Crown Chem. Comp., Lamberhurst, Kent, England.

[15]. Statistix, NH Analytials Software, USA.

B = plasma drug concentration at zero time, extrapolated back from the elimination phase of the exponential curve.

β = the apparent first order disappearance rate constant, obtained from the slope of the excretion phase of the semilogarithmic plot of serum drug concentration versus time. This constant is described in units of reciprocal time (per min. or per hour).

e = the base of the natural logarithm (ln).

The $t_{\frac{1}{2}}$ is the time taken for the plasma concentration of a drug to be reduced by 50%. An estimate of the $t_{\frac{1}{2}}$ of a drug may be obtained directly from the beta (also termed elimination, or excretion, or terminal) phase, of the semilogarithmic plot of plasma drug concentration versus time, following intravenous (IV) administration of a single dose of a drug (Fig. App. I:1). The $t_{\frac{1}{2}}$ is described in units of either minutes or hours.

The elimination rate constant, β , was calculated by employing equation 2 (Baggot, 1977).

$$t_{\frac{1}{2}} = \frac{\ln 2}{\beta} = \frac{0.693}{\beta} \qquad (2)$$

The zerotime concentration intercept of the elimination phase (B) was obtained by the back extrapolation of the terminal linear segment of the semilogarithmic plot (Fig. App. I:1).

For the raw data of two goats, in which distinct distribution phases indicated consistency with a two-compartment open model, the disposition kinetic values were calculated according to a biexponential expression (Baggot, 1974) (equation 3).

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad (3)$$

Where Cp, B, e, β and t are as defined in equation 1.

A = the plasma drug concentration intercept at zero time of the distribution phase of the disposition curve,

α = the apparent first order disappearance rate constant, obtained from the slope of the distribution phase, of the semilogarithmic plot of plasma drug concentration versus time.

The least squares regression line describing the distribution phase was obtained by the method of residuals (Sams, 1984). The beta phase points, previously determined by extrapolation of the terminal linear segment back to time zero, are subtracted from the actual data points throughout the curve. The calculated differences are called residual concentration-time values, which are then plotted on the semilogarithmic graph of serum metronidazole concentration against time (Baggot, 1977).

The negative slope of this line yields α, and its zero time intercept is A (Baggot, 1977).

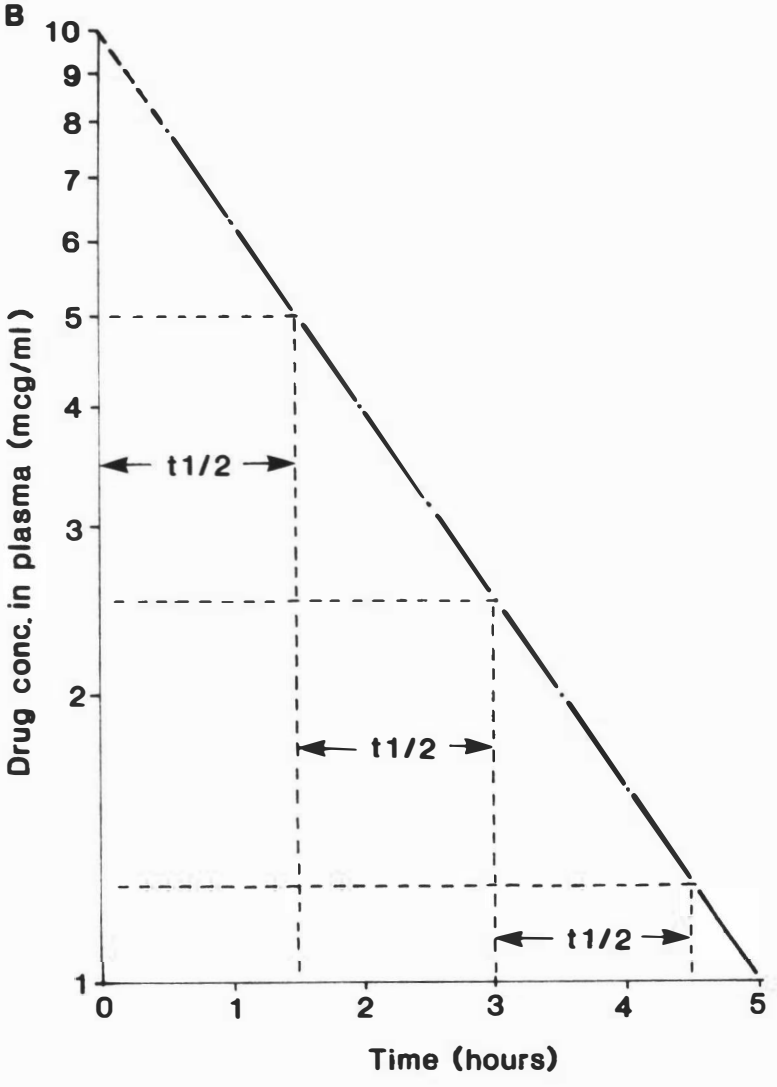
The volume of distribution was calculated by dividing the administered dose (D) by the product of the elimination rate constant (β) and the area under the concentration-time curve (AUC), according to the following (equation 4).

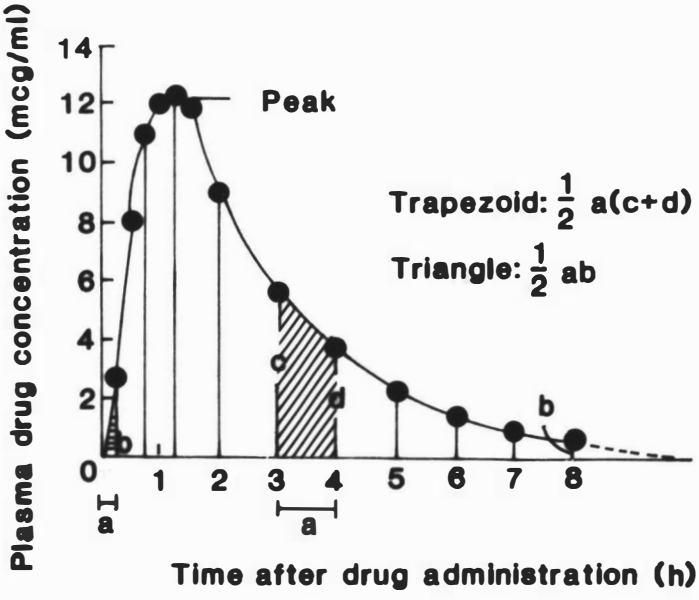
$$V_{d(area)} = \frac{D}{\beta . AUC} \qquad (4)$$

The area under the concentration-time curve (AUC) representing the total amount of drug which appears in the serum after administration, was calculated by the trapezoidal method using values obtained directly from the graph (Baggot, 1978) (Fig. App. I:2).

Fig. App. I:1 Semilogarithmic plot of plasma drug concentration versus time following administration of a single intravenous dose. Graphical technique for estimating the half-life of a drug is illustrated (from Baggot, J.D. [1977]): Principles of Drug Disposition in Domestic Animals. Publ. by Saunders Comp., London.

Fig. App. I:2 Diagrammatic representation of the trapezoidal method used to calculate the area under the plasma concentration time curve (AUC). (From Baggot, J.D. [1978]): J. vet. Pharmacol. Therap. 1:111-118).


B
Drug conc. in plasma (mcg/ml)
10
9
8
7
6
5
4
3
2
1
t1/2
t1/2
t1/2
0
1
2
3
4
5
Time (hours)


Plasma drug concentration (mcg/ml)
14
12
10
8
6
4
2
0
Peak
Trapezoid: 1/2 a(c+d)
Triangle: 1/2 ab
c
d
b
a
a
1
2
3
4
5
6
7
8
Time after drug administration (h)

Vertical lines were drawn from the horizontal (time) axis to the measured serum concentration curve, at each sampling time. The area of each trapezoid (or triangle in the case of the initial and final observations) so constructed, was calculated by dividing the sum of the drug concentrations at adjacent time intervals by 2, and multiplying by the length of the time interval. The AUC, being the sum of all products, was expressed in units of mcg.hr/ml.

The clearance of metronidazole being the sum of all the clearance processes in the body (Cl_B), was calculated according to the expression (equation 5).

$$CL_B = \beta \cdot V_{d(area)} \qquad (5)$$

Intramuscular injection

The disposition data obtained from the semilogarithmic serum concentration versus time curves, following administration of metronidazole by intramuscular injection, were analysed according to equation 6, as a two compartment open model (Baggot, 1977).

$$Cp = A'e^{-k_{ab}.t} + B'e^{-\beta'.t} \qquad (6)$$

Where A' and B' are the zero-time serum drug concentration intercepts of the absorption and elimination phases respectively, of the least squares regression lines of the semilogarithmic plot of serum drug concentration versus time.

k_{ab}, β' = the rate constants of absorption and elimination obtained from the slope of the absorption and beta phases respectively, of the disposition curve.

The absorption phase for the two compartment open model following intramuscular injection was calculated according to the method of Swintosky et al, (1969). This is a method of residuals similar to that used to calculate the distribution phase following intravenous administration. The slope of this line yields the absorption constant, k_{ab}.

The half life for absorption was derived from the following calculation (equation 7).

$$t_{\frac{1}{2}ab} = \frac{\ln 2}{k_{ab}} \qquad (7)$$

The elimination phase of each disposition curve following IM injection was analyzed in a similar manner to that used for calculating the elimination kinetics after IV administration of metronidazole.

The fraction of the dose administered by IM injection, which became available systemically (F), was calculated from a comparison of the AUC following IM or IV use (equation 8).

$$F = \frac{AUC_{IM}}{AUC_{IV}} \qquad (8)$$

This assumes that after IV administration, systemic availability is complete. Using this factor (F), the quantity of the IM administered dose which was absorbed into the systemic circulation was assessed as follows (equation 9) (Ziv, 1980).

$$\text{Absorbed Dose} = F.\ D \qquad (9)$$

The extent of drug penetration into tissue cage fluid was assessed from the ratio of peak cage fluid drug concentration to peak serum drug concentration (equation 10).

$$\text{Percentage penetration} = \frac{\text{Peak tissue cage fluid drug concentration}}{\text{Peak serum drug concentration}} \times 100\% \qquad (10)$$

Dosage regimens for IV and IM administration

The dosage rates and intervals for the administration of 0.5% w/v metronidazole solution were calculated using the following equations. The initial priming dose (D*), which should be given to establish C_{pmax}, was calculated according to equation 11 (Teske et al, 1984).

$$D^* = V_{d(area)} \cdot C_{pmax} \qquad (11)$$

where the maintenance dose (D) to be given, thereafter, was established according to equation 12.

$$D = f_{el}\, V_{d(area)}\, C_{pmax} = f_{el}\, (D^*) \quad (12)$$

where f_{el} = the fraction of the drug which is cleared from the body, during the dosing interval.

The feature, f_{el}, was calculated by equation 13.

$$f_{el} = 1 - e^{-\beta \pi} \quad (13)$$

Where π = the interval of time between maintenance doses, so that serum concentrations would fluctuate between C_{pmax} and C_{pmin} (equation 14).

$$\pi = \frac{(\ln C_{pmax}/C_{pmin})}{\beta} \quad (14)$$

The dosage regimen necessary to establish steady state at a desired concentration, following the IM injection of the 40% metronidazole suspension must take into account the continuing absorption process. The dosage interval $_{IM}$ was calculated using equation 15 (Teske et al, 1984).

$$\pi_{IM} = \frac{\ln (C_{pmax}/C_{pmin})}{\beta'} \quad (15)$$

The priming (D^*_{IM}) and maintenance (D_{IM}) doses for IM administration were calculated according to equations 16 and 17 respectively.

$$D^*_{IM} = \frac{V_{d(area)} \cdot C_{pmax}}{F} \quad (16)$$

$$D_{IM} = \frac{V_{(darea)} \cdot C_{pmax} \cdot f_{el}}{F}$$

$$= f_{el} \cdot D^{*}_{IM} \qquad (17)$$

Haematology and components of tissue cage fluid

The total white blood cell count was determined in both sera and tissue cage fluid using a unopipette[16] and haemocytometer counting chamber according to the method of Sutton (1982). A differential white blood cell count (on 200 cells) was then performed on air-dried blood smears stained with Wright's stain.

As the tissue cage fluid samples were small in volume, they were processed through the Shandon Cytospin, Model SCA 0030[17] at 1000 revs per minute for five minutes. The resulting sediment was smeared on a slide and stained with Wright's stain, and the numbers of different types of cells recorded from a 200 cell sample.

The total protein in the sera and tissue cage fluid samples was measured on a refractometer. Electrophoresis of the total protein was performed by the serum protein method as described by Helena Laboratories, Texas (1984-1985).

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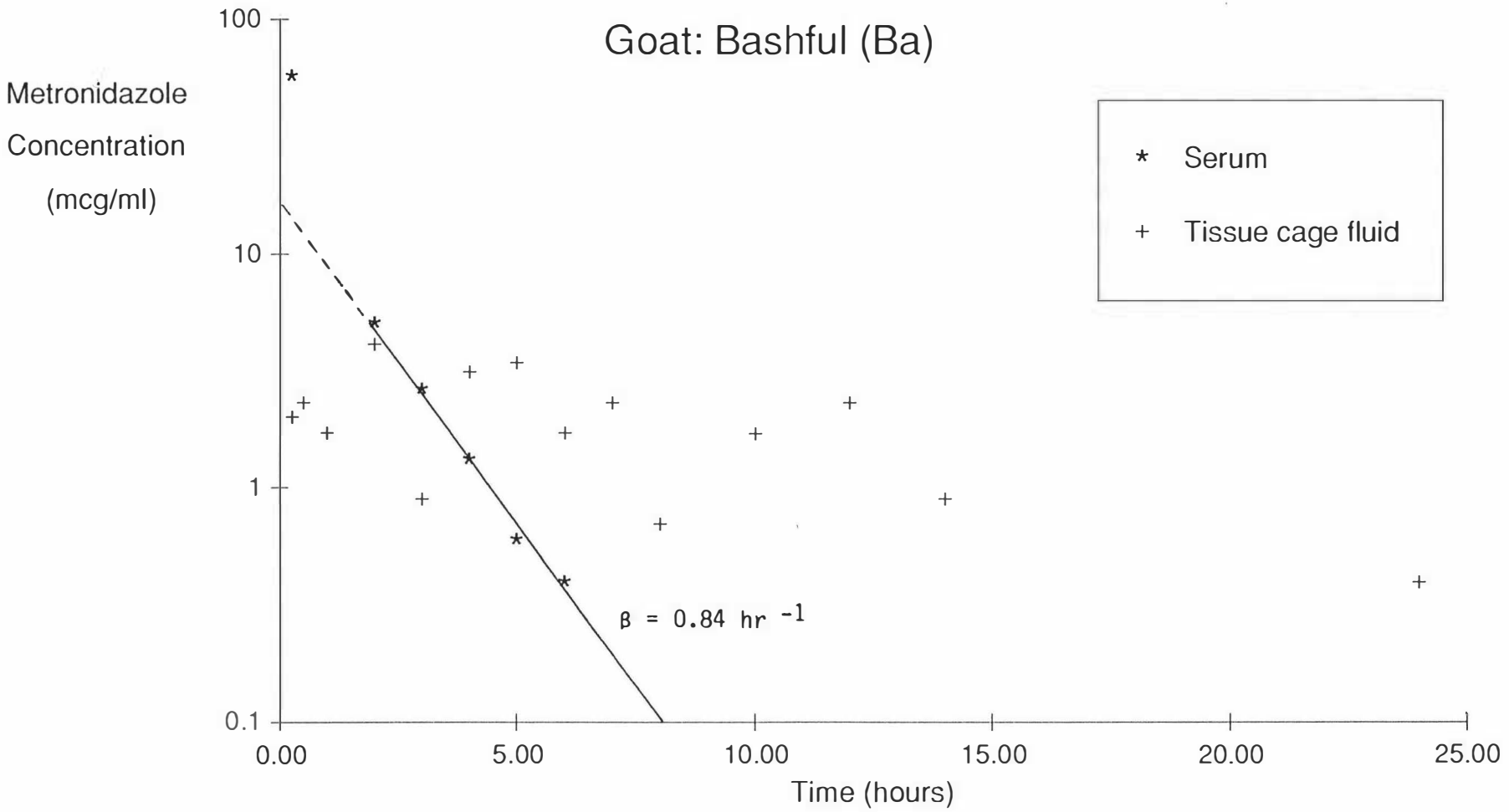
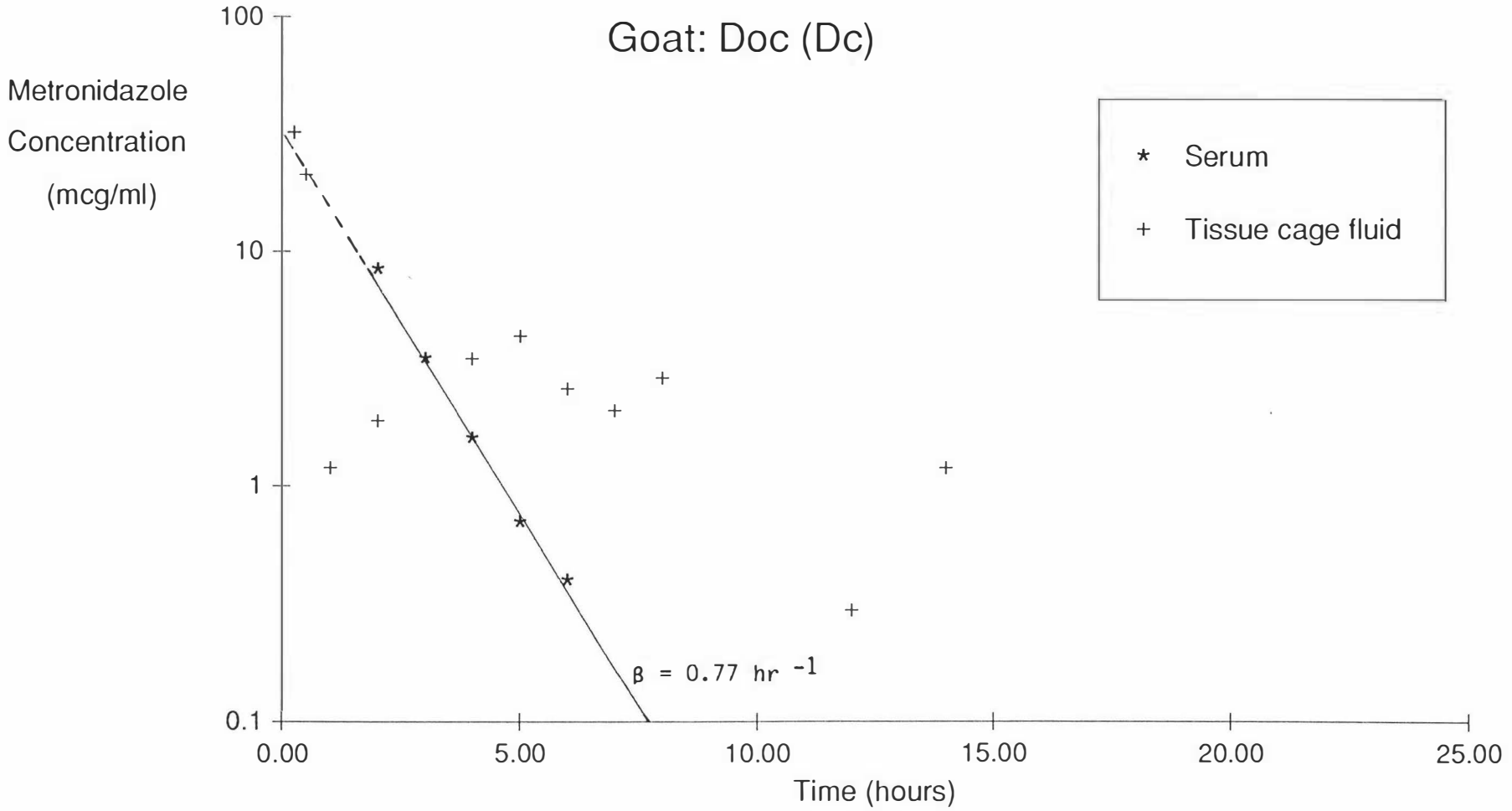
16. Unopipette, Becton-Dickinson & Comp., New Jersey.

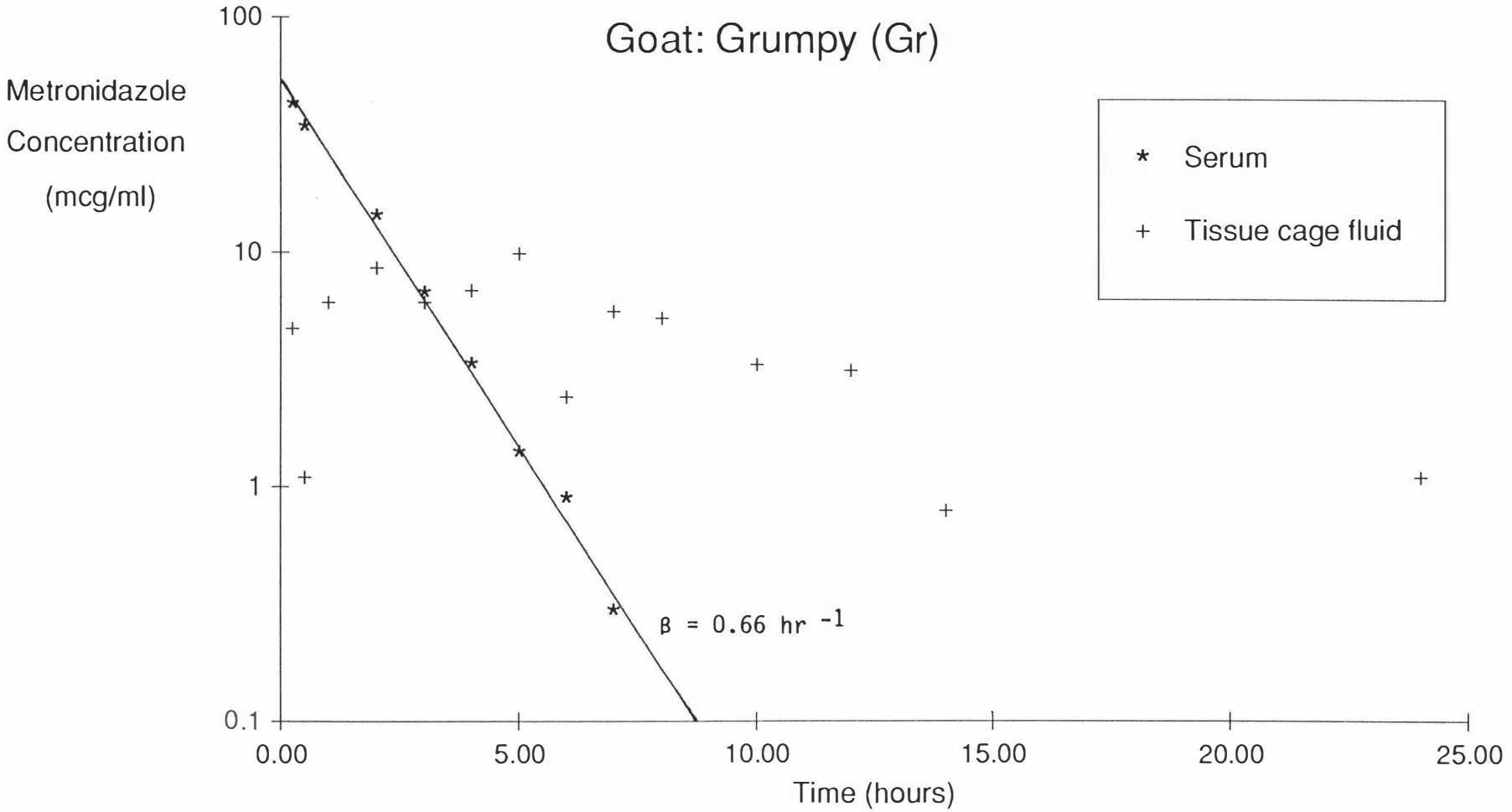
17. Shandon Cytospin, Shandon Southern Products Ltd., England.

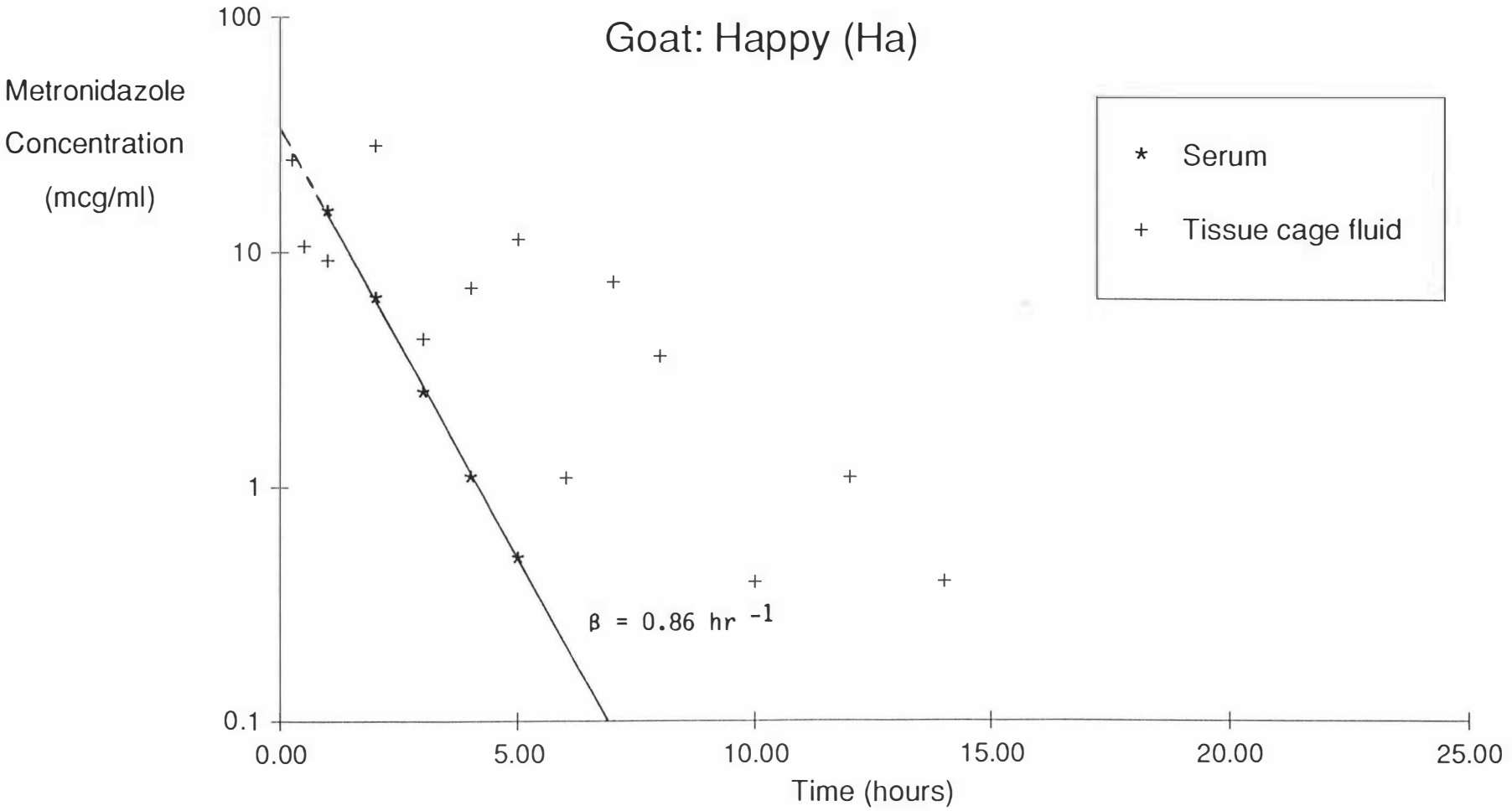
APPENDIX II

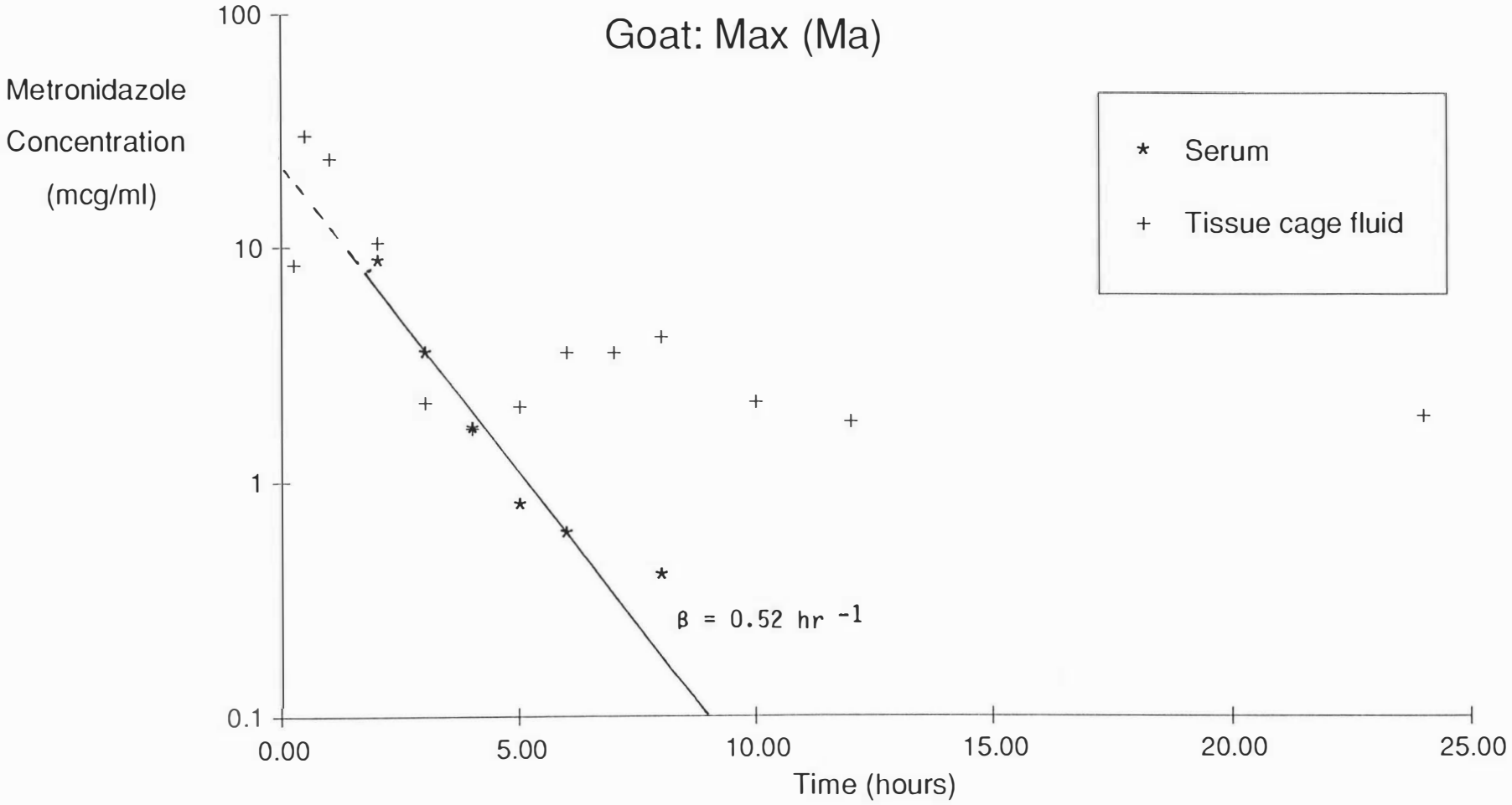
Plots of metronidazole concentration in serum and tissue cage fluid of seven goats, at periods up to 24 hours after IV administration of 0.5% w/v metronidazole solution.

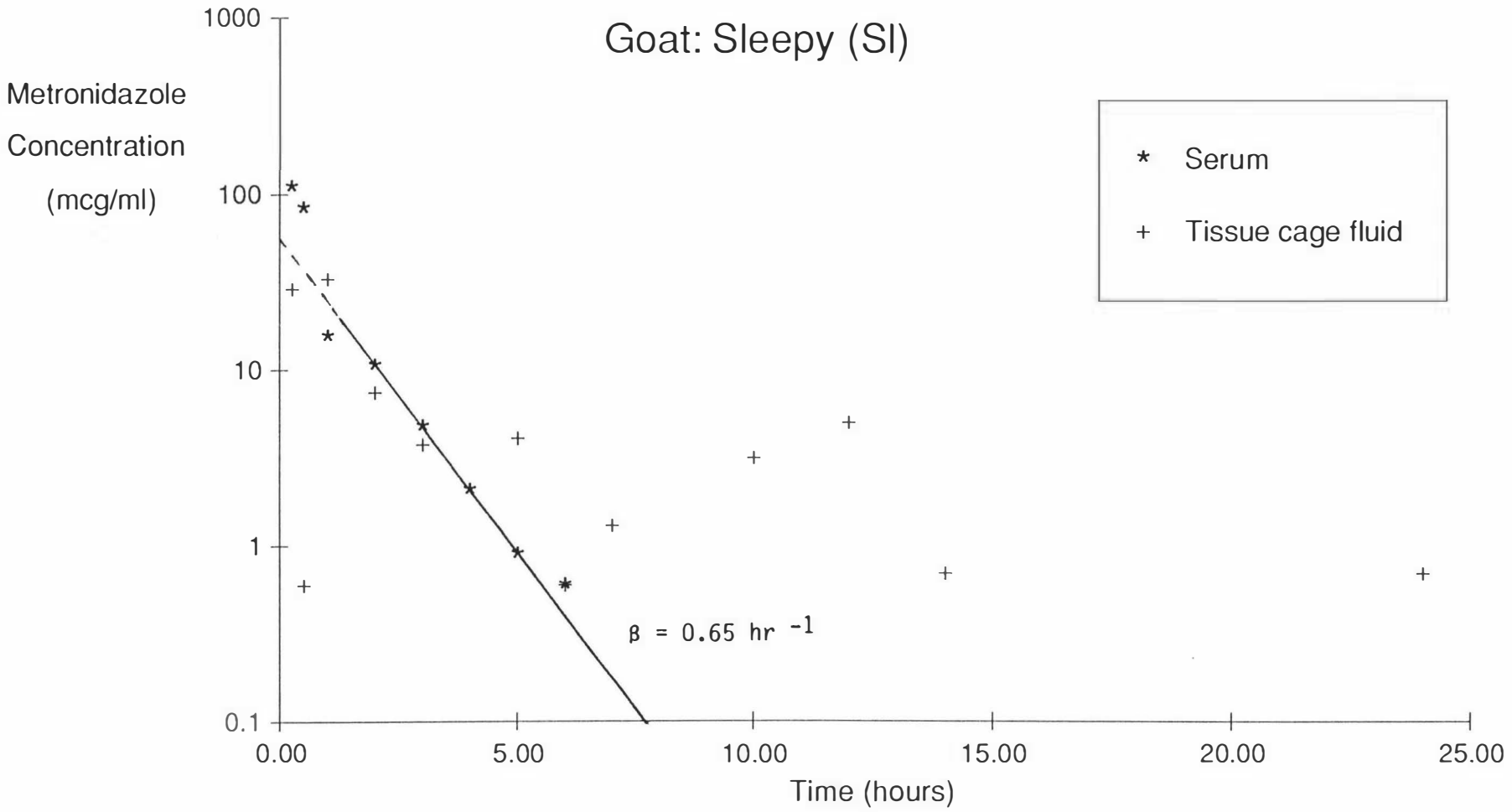

Goat: Bashful (Ba)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.84 hr -1

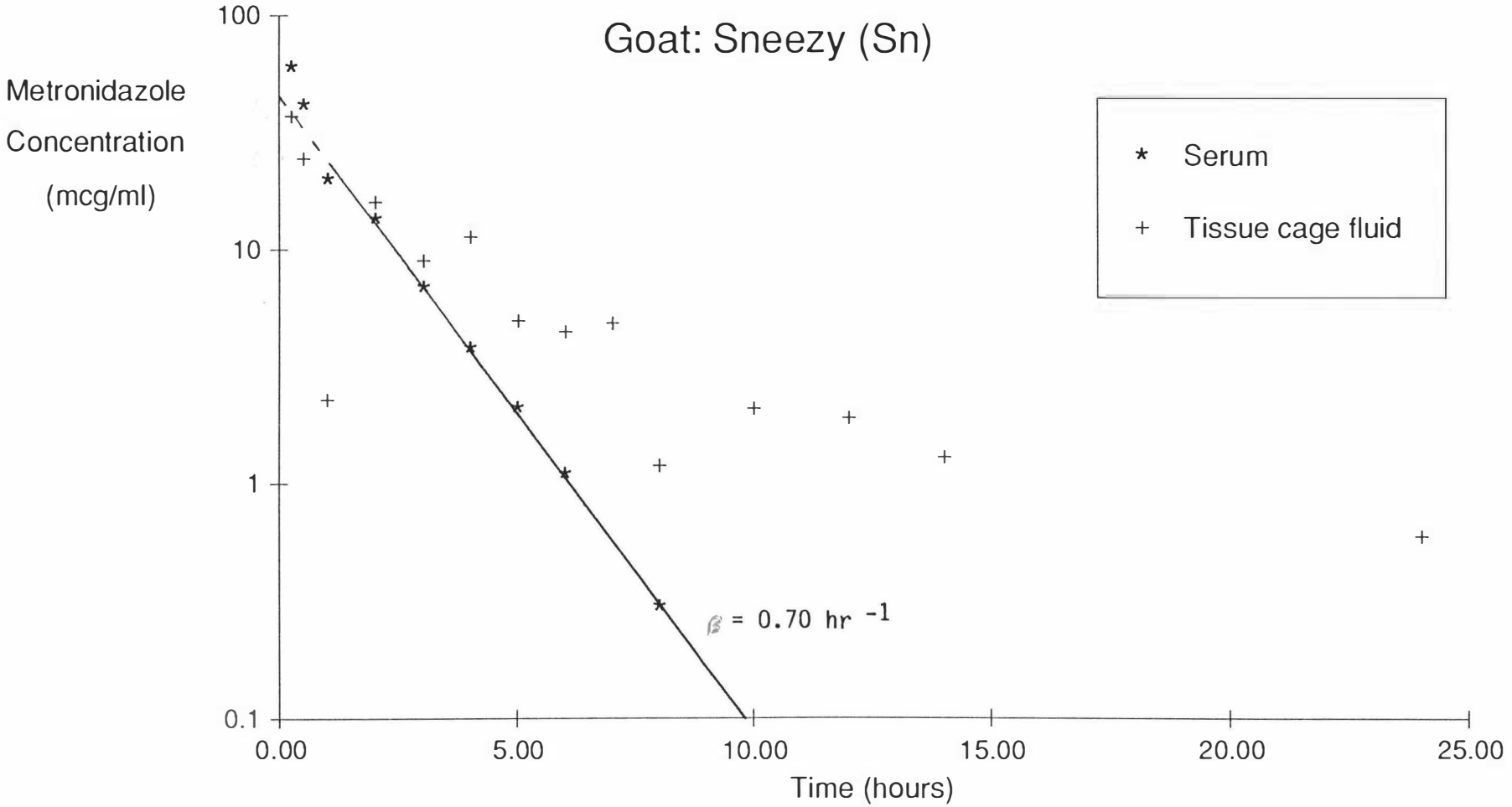

Goat: Doc (Dc)
Metronidazole
Concentration
(mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.77 hr -1


Goat: Grumpy (Gr)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.66 hr -1


Goat: Happy (Ha)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.86 hr -1

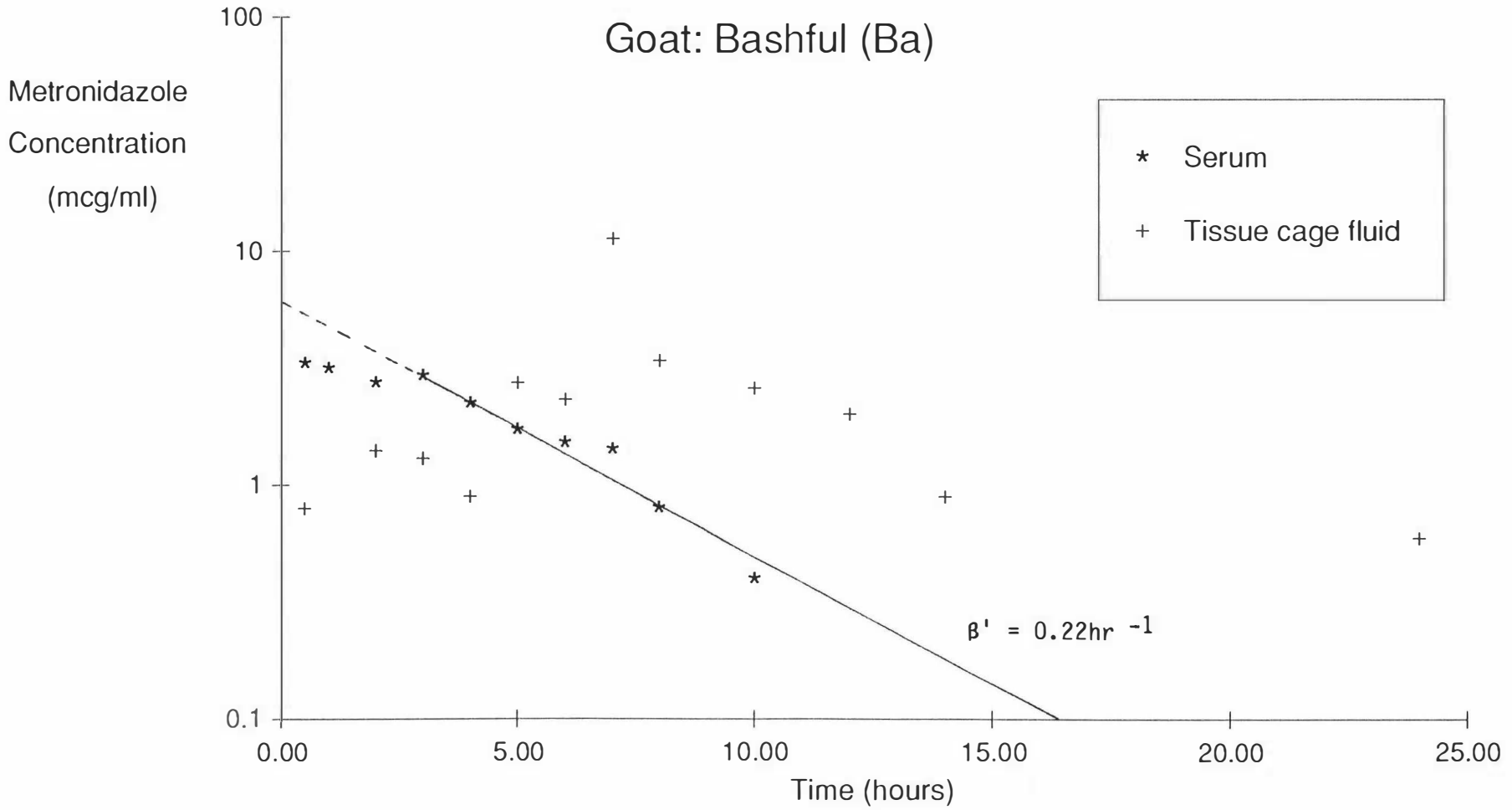

Goat: Max (Ma)
Metronidazole
Concentration
(mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.52 hr -1

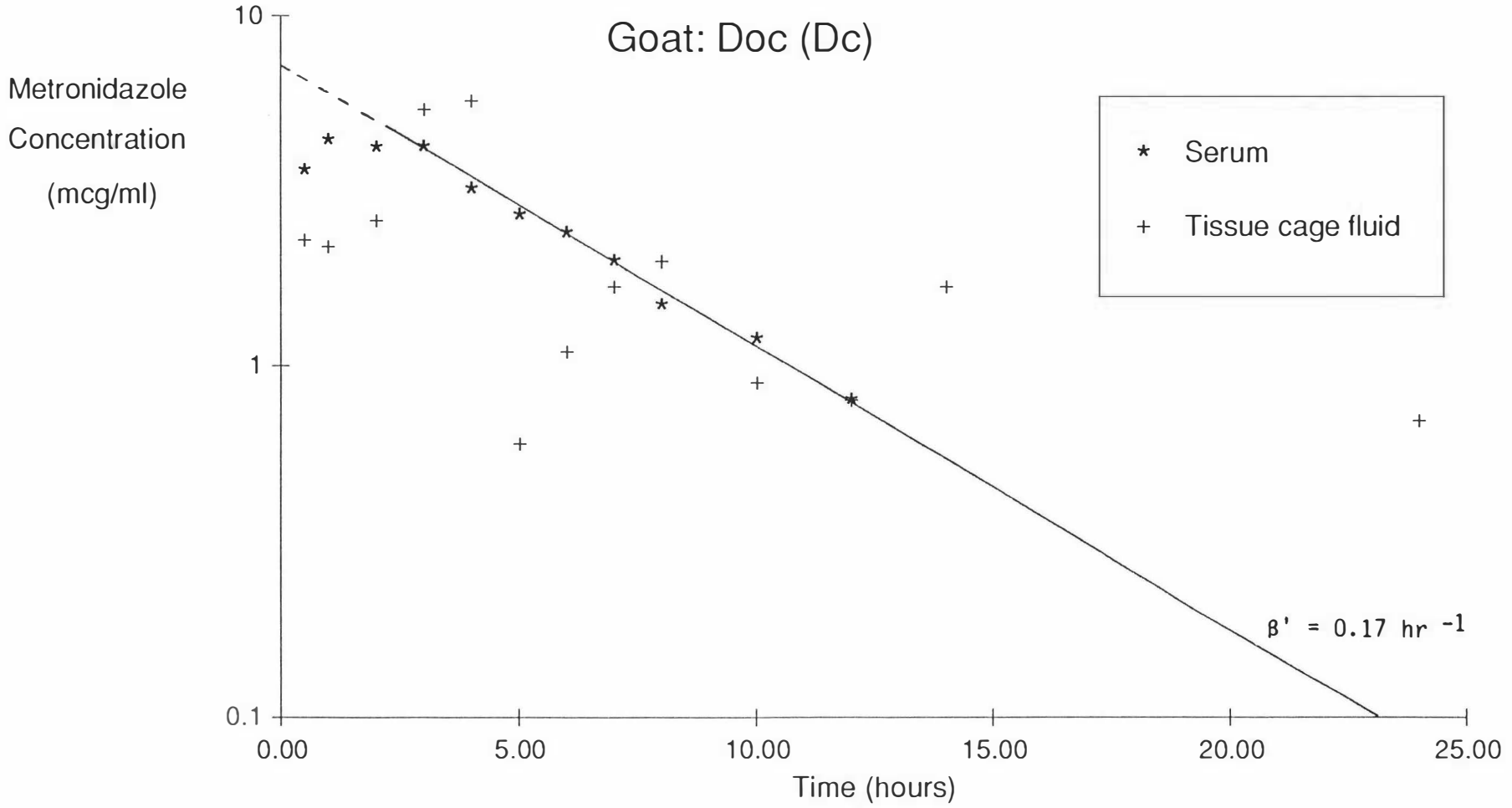

Goat: Sleepy (SI)
Metronidazole Concentration (mcg/ml)
1000
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.65 hr -1

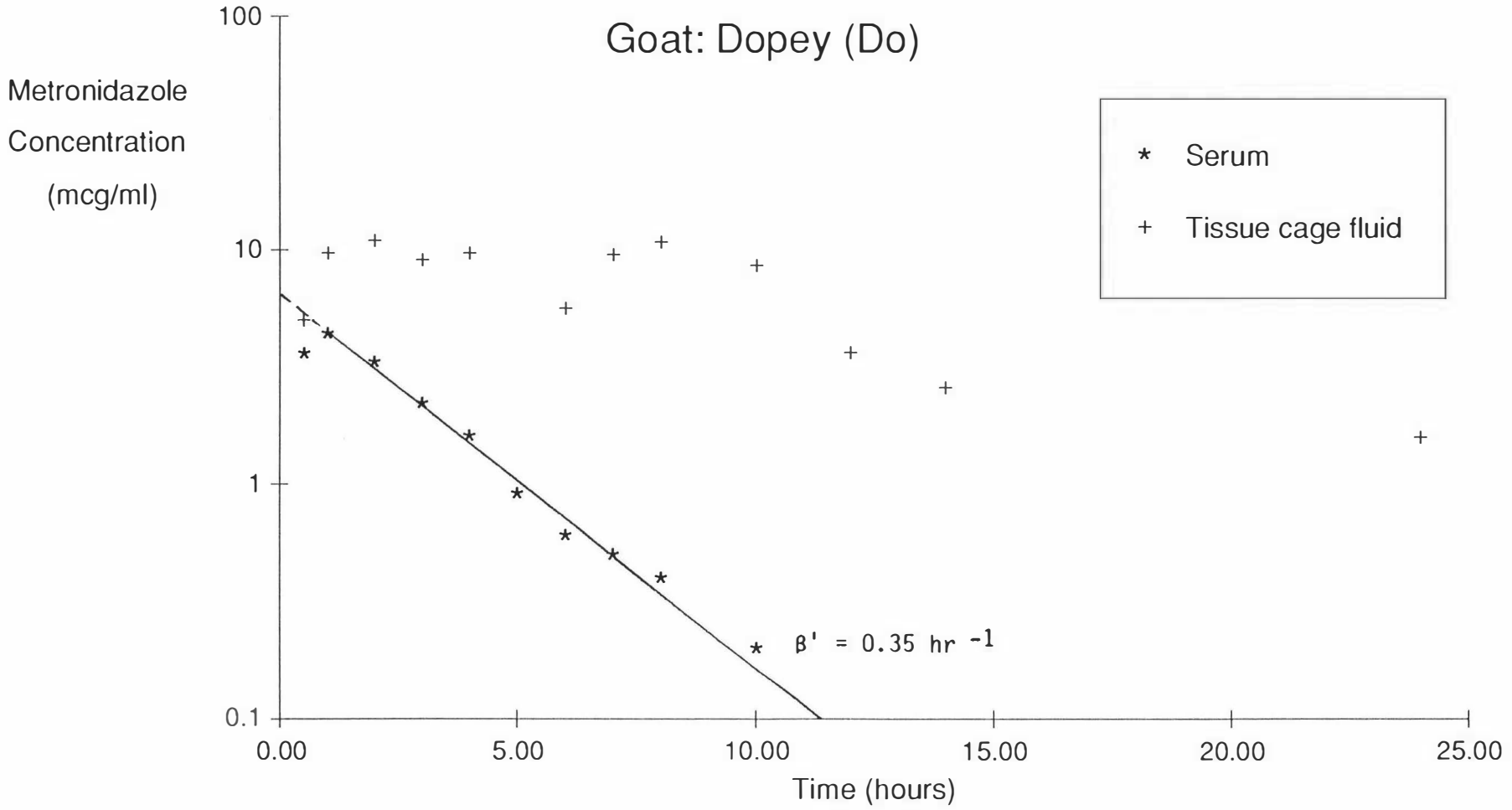

Goat: Sneezy (Sn)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β = 0.70 hr -1

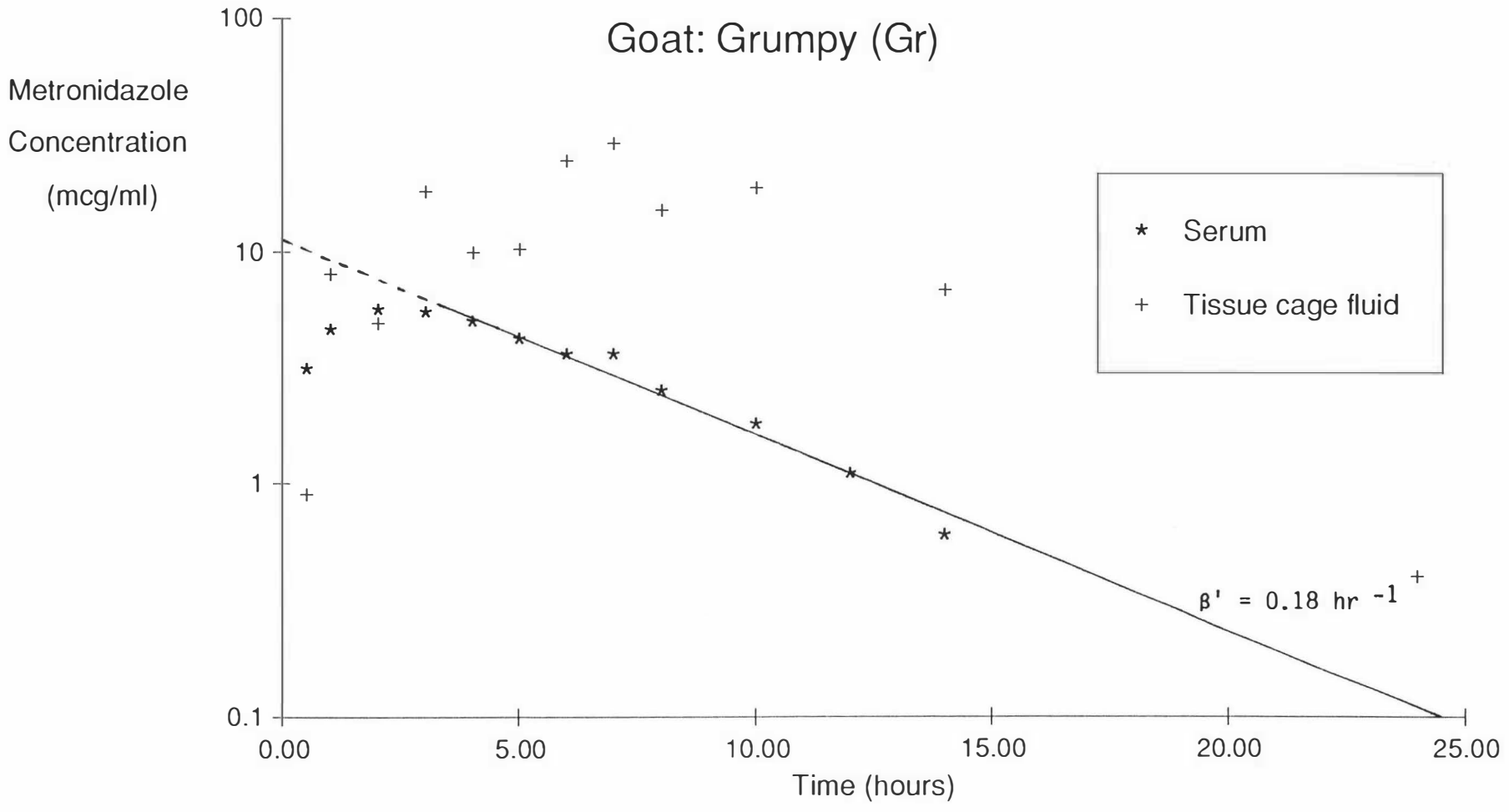
APPENDIX III

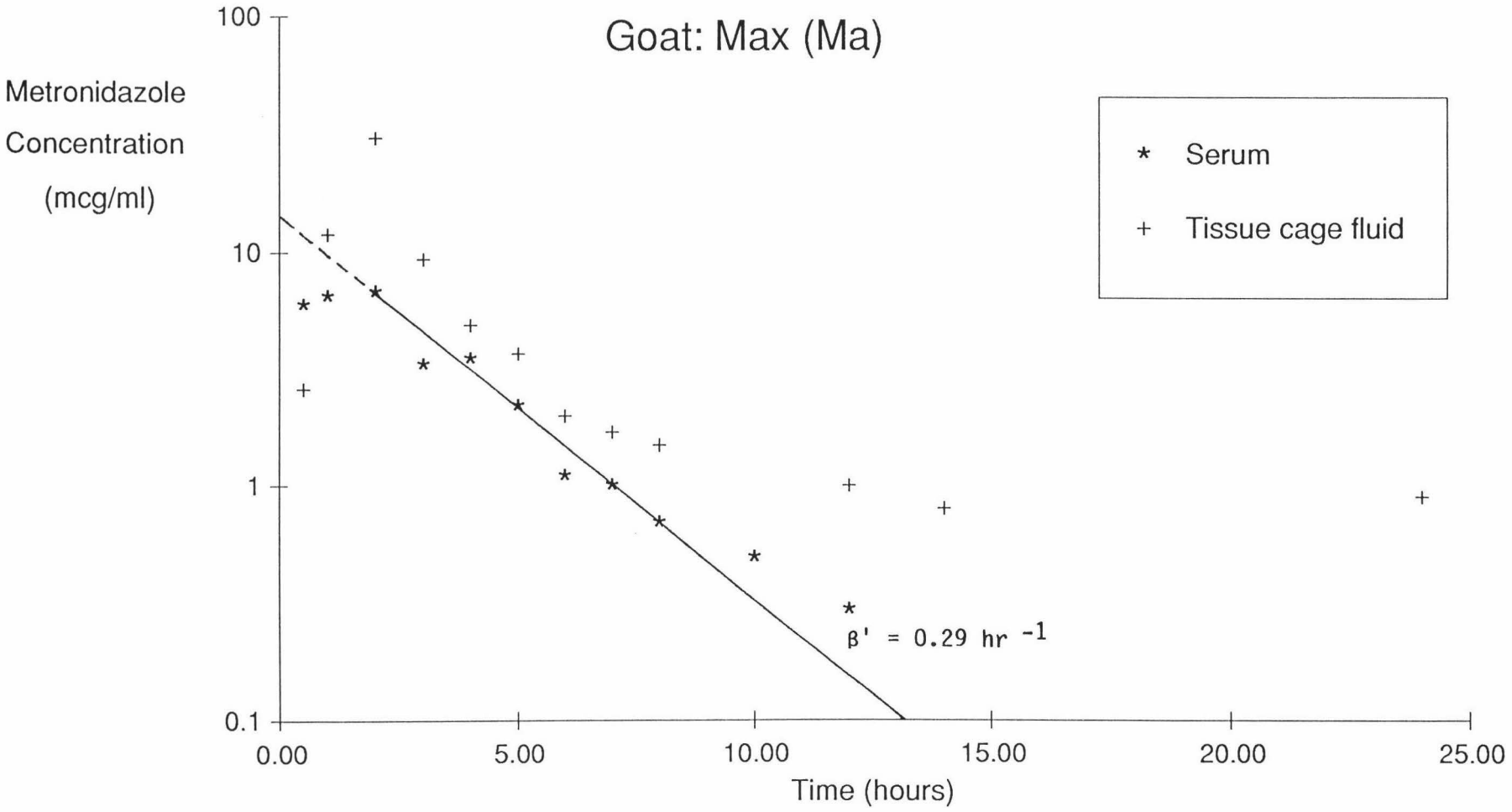
Plots of metronidazole concentration in serum and tissue cage fluid of seven goats, at periods up to 24 hours after IM administration of 40% w/v metronidazole suspension.

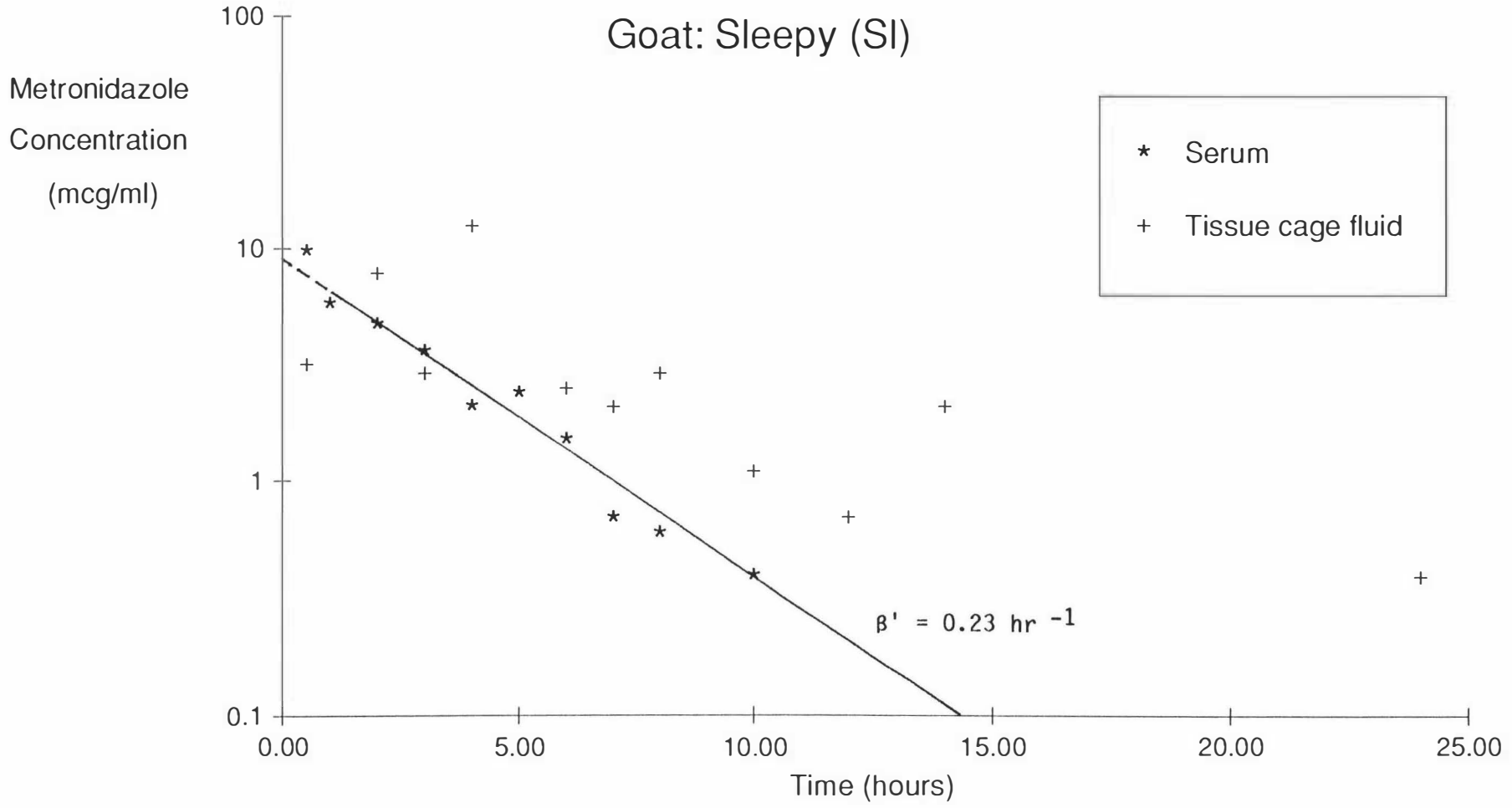

Goat: Bashful (Ba)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.22hr -1

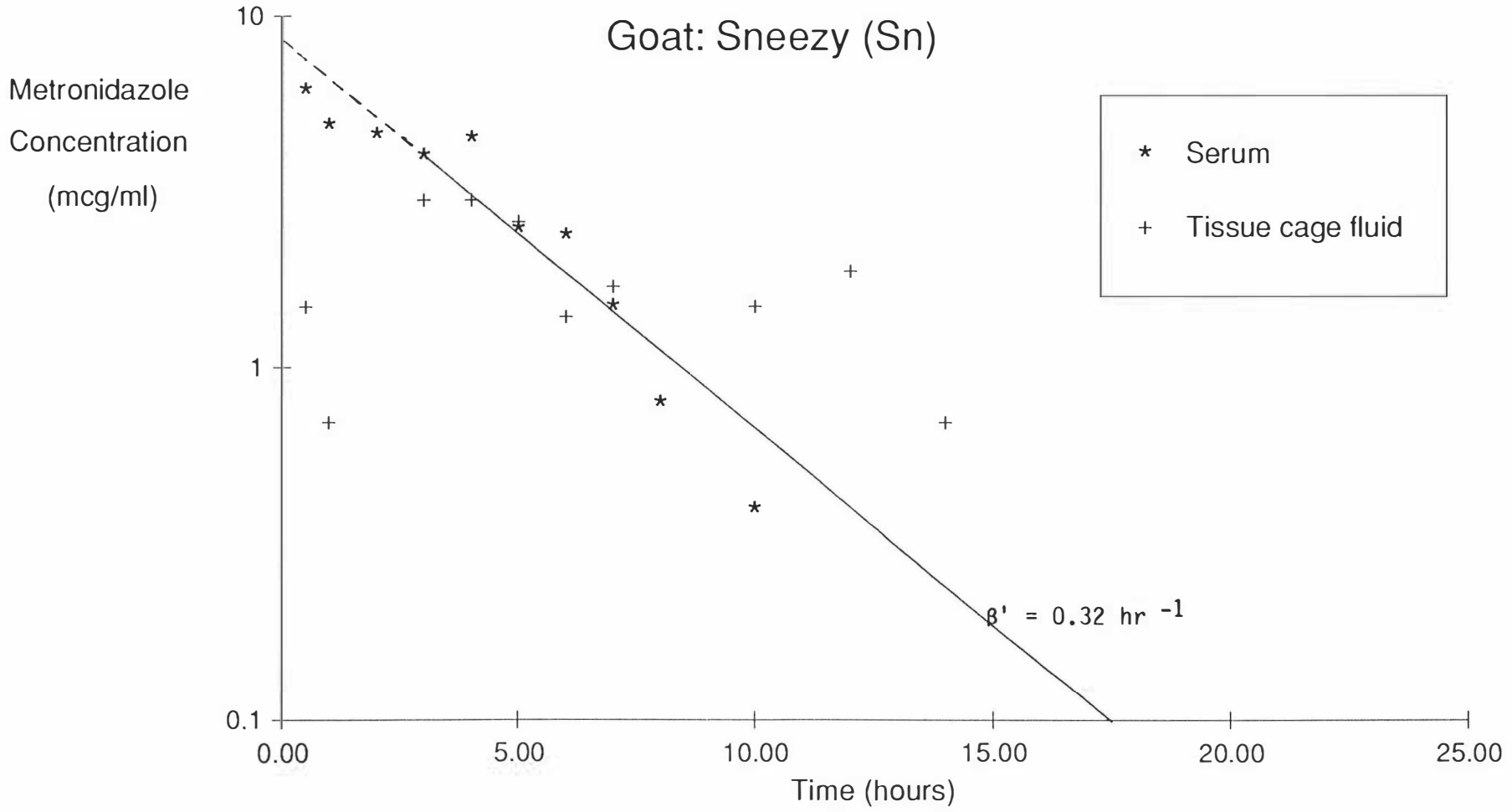

Goat: Doc (Dc)
Metronidazole
Concentration
(mcg/ml)
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.17 hr -1


Goat: Dopey (Do)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.35 hr -1


Goat: Grumpy (Gr)
Metronidazole
Concentration
(mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.18 hr -1


Goat: Max (Ma)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.29 hr -1


Goat: Sleepy (SI)
Metronidazole Concentration (mcg/ml)
100
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.23 hr -1


Goat: Sneezy (Sn)
Metronidazole Concentration (mcg/ml)
10
1
0.1
0.00
5.00
10.00
15.00
20.00
25.00
Time (hours)
* Serum
+ Tissue cage fluid
β' = 0.32 hr -1