PHARMACOKINETIC AND *IN VITRO* SENSITIVITY STUDIES ON AMPICILLIN AND CONGENER PRODRUGS IN *EQUIDAE*

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A thesis submitted for the degree of Doctor of Philosophy

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Contents

List of Tables

Table Page

List of figures

Figure Page

Abbreviations

A. equuli	*Actinobacillus equuli*
&	and
AUFS	absorbance units full scale
bwt	bodyweight
COPD	Chronic obstructive pulmonary disease
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic Acid
e. g.	for example
Eq.	equation
et al.	and others
Fig.	figure
g	grams
g	gravity, 10^{-11}N. m/s^2
h	hours
HPLC	high performance liquid chromatography
i. e.	that is
IV	intravenous
IM	intramuscular
kg	kilogram
l	litre
λ	wavelength
$\log_{10}$	logarithm base 10
LRT	Lower respiratory tract
mg	milligram
MIC	minimum inhibitory concentration
min	minutes
ml	millilitre
mm	millimetres
mV	millivolts
pH	negative logarithm of hydrogen ion concentration
pKa	dissociation constant
ppm	parts per million
Ps.	*Pseudomonas*
rpm	revolutions per minute
s	seconds

S. typhimurium	*Salmonella typhimurium*
S. zooepidemicus	*Streptococcus zooepidemicus*
S. aureus	*Staphylococcus aureus*
SD	standard deviation
SEM	standard error of the mean
spp.	bacterial species
SS	*Salmonella Shigella*
TSI	triple sugar iron agar
U	units
U/L	Units per litre
URT	Upper respiratory tract
u v	ultraviolet
µg	microgram
ºC	degrees centigrade

Pharmacokinetic parameters

A, B	Zero-time plasma drug concentration intercepts (μg/ml) of a biphasic disposition curve where A is based on the distribution slope and B is based on the terminal elimination slope.
α, β	Hybrid rate constants (exponents) (/h) of a biphasic disposition plasma concentration versus time curve related to the slopes of the distribution and elimination phases of a biexponential drug disposition curve, where β is the overall elimination rate constant.
AUC	Total area under the drug concentration versus time or zero moment curve (μg.h/ml) from time 0 to time ∞ after administration of a single dose calculated from $A/\alpha + B/\beta$ or by using the trapezoidal rule (AUC_{obs}).
AUMC obs	Total area under the first moment curve ($\mu g.h^2/ml$) in a non-compartmental model calculated with the trapezoidal rule.
CLb	Total body clearance (ml/h.kg) of a drug calculated from Dose/AUC or Dose/AUC_{obs} for CLb_{obs}.
Cmax	Maximum plasma concentration (μg/ml) following oral drug administration.
Cp0	Initial concentration of the drug in plasma (μg/ml) following an intravenous bolus administration calculated from the sum of the intercepts (A and B) on the y-axis.
Cpss	Plasma concentration (μg/ml) at steady-state during an intravenous infusion.

F	Systemic availability (%) calculated as the fraction of the dose that enters the systemic circulation intact following oral administration and calculated by dividing AUC_{obs} following oral administration by AUC_{obs} following intravenous administration.
k12, k21	First-order transfer rate constants (/h) calculated for drug distribution between the central and peripheral compartments of a two-compartment model.
kel	First-order elimination rate constant (/h) for the disappearance of a drug from the apparent central compartment calculated from Clb/Vc.
MAT	Mean absorption time calculated from the difference between the MRT following a single oral administration and MRT following a single intravenous administration.
MRT	Mean residence time which is a quantitative estimate of the persistence of a drug in the body, or the time for 63.2 % of a drug to be eliminated. calculated from $AUMC_{obs}/AUC_{obs}$.
Ro	Constant (zero-order) intravenous infusion rate (μg/min/ml).
$t_{1/2}\ \alpha$	Distribution half-life (h) following intravenous administration calculated from $0.693/\alpha$.
$t_{1/2}\ \beta$	Elimination (biological) half-life (h) following intravenous administration or time taken for 50% of a drug to be eliminated calculated from $0.693/\beta$.
tmax	Time at which the maximum plasma concentration (Cmax) is reached following oral administration.

Vdarea — Apparent volume of distribution (ml/kg) of the drug, representing the theoretical volume neccessary to contain the amount of drug in the body if distributed uniformly at a concentration achieved in plasma. It is calculated by the area method as Dose/ AUC.β.

Vc — Apparent volume of the central compartment (ml/kg) calculated from Dose/Cp0.

Vdss — Apparent volume of distribution (ml/kg) at steady-state concentration calculated from [(k12+k21)/k21] · Vc or Clb· MRT for $Vdss_{obs}$.

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Declaration

The studies described in this thesis were carried out in the Department of Veterinary Pharmacology at the University of Glasgow Veterinary School.

The author was responsible for all results except where it is stated otherwise.

No part of this thesis has been presented to any university but it has been reproduced in parts in the following scientific papers.

Govan, J. R. W., Sarasola, P., Taylor, D. J., Tatnell, P. J., Russell, N. J. & Gacesa, P. (1992) Isolation of a mucoid alginate-producing *Pseudomonas aeruginosa* strain from the equine guttural pouch. *Journal of Clinical Microbiology*, **30**, 595-599

Horspool, L. J. I., Sarasola, P. & McKellar, Q. A. (1992) Disposition of ampicillin sodium in horses, ponies and donkeys after intravenous administration. Supplement **11**, *Equine Veterinary Journal*, 59-61

Sarasola, P., Horspool, L. J. I. & McKellar, Q. A. (1992) Effect of changes in urine pH on plasma pharmacokinetic variables of ampicillin sodium in horses. *American Journal of Veterinary Research*, **53**, 711-715

Sarasola, P. & McKellar, Q. A. (1992) Effect of probenecid on disposition kinetics of ampicillin in horses. *The Veterinary Record*. **131**, 173-175

Sarasola, P. & McKellar, Q. A. (1993) Pharmacokinetics and applications of ampicillin sodium as an intravenous infusion in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **16**, 63-69

Sarasola, P., Taylor, D. J., Love, S. & McKellar, Q. A. (1992) Secondary bacterial infections following an outbreak of equine influenza. *The Veterinary Record*, **131**, 441-442

Sarasola, P. & McKellar, Q. A. (1993) Ampicillin and its congener prodrugs in the horse. *British Veterinary Journal. In Press.*

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Summary

The present study was conducted to evaluate alternative methods to prolong the persistence of ampicillin in the horse. Artificial alkalinisation (pH > 7.91 ± 0.13) and acidification (pH < 4.24 ± 0.13) of equine urinary pH achieved by intragastric administration of sodium bicarbonate (100, 200, 300, and 400 [x3] mg/kg) over a six day period, and ammonium chloride (200, 300, and 400 [x3] mg/kg) over a five day period respectively, did not produce any significant changes in the plasma disposition of ampicillin when administered as an IV bolus (10 mg/kg) of the sodium salt to horses. These results were attributed to the lack of changes in the extent of reabsorption of ampicillin from the urine into plasma caused by the zwitterion characteristics of the antibiotic. However, the alkalinisation of urine appeared to affect the fraction of the dose of ampicillin recovered in the urine since only a small fraction could be measured, (<15 %) presumably resulting from a degradation of the antibiotic in the basic media.

The plasma disposition of ampicillin was altered when probenecid, a competitor of the active tubular secretion of penicillins, was administered intragastrically to horses at a 75 mg/kg dose rate one hour prior to the administration of ampicillin IV. The presence of probenecid prolonged the elimination of ampicillin significantly ($P< 0.05$), as reflected in an almost twofold increase in the elimination half-life ($t1/2\beta$ = 0.701 h vs $t1/2\ \beta$ = 1.198 h) and mean residence time values (MRT = 48.32 min vs MRT = 95.37 min) of ampicillin when coadministered with probenecid. It was therefore concluded that administration of probenecid was a suitable method for prolonging the persistence of ampicillin in the horse. In addition, the presence of probenecid appeared to restrict the distribution of ampicillin as reflected in a significant reduction ($P< 0.05$) in the volume of distribution of the antibiotic at steady-state ($Vdss_{obs}$ = 207.17 ± 20.89 ml/kg vs $Vdss_{obs}$ = 166.70 ± 11.21 ml/kg).

The short persistence of ampicillin in the horse was also overcome by the administration of the antibiotic as an IV infusion over a prolonged period. This novel method of administration of ampicillin in the horse, enabled accurate control

on the amount of ampicillin delivered per unit of time and was shown to be suitable for those circumstances when a compromised vascular supply would impair the absorption of the antibiotic from the injection site when administered IM. Changes in the infusion rate delivered (Ro= 13.78, 19.34, and 23.48 μg/min/kg) correlated well (R=0.994) with the concentration of the antibiotic at steady-state (Cpss = 4.98, 6.37 and 8.25 μg/ml) and the AUC obtained (AUC = 27.83, 33.89 and 42.10 μg.h/ml) indicating the reliability of this method of administration.

The slower absorption and elimination achieved following oral administration of drugs was investigated by the administration of bacampicillin, an ampicillin prodrug, in horses and ponies. Following intragastric administration of bacampicillin to horses and ponies, more than 40 % was absorbed from the gastrointestinal tract (F = 41.01 %), and the concentrations achieved suggested that oral administration of bacampicillin hydrochloride at dose equimolar to 10 mg/kg of ampicillin sodium every 8 to 12 hours, would be suitable to treat infections caused by sensitive bacteria. In an attempt to slow the hydrolysis of bacampicillin into ampicillin and thus prolong the persistence of the antibiotic within the body, the esterase activity present in plasma and in red blood cells was artificially reduced. The oral administration of dichlorvos, an organophosphate anthelmintic, produced a depression of plasma and erythrocyte esterase activity greater than 95 %, at the time of bacampicillin administration. However, the reduction of esterase activity did not have any influence on the absorption and pharmacokinetics of bacampicillin as no significant differences were found between the pharmacokinetic results obtained following administration of bacampicillin and those following the administration of bacampicillin and dichlorvos. It appeared therefore that the small residual esterase activity present in plasma and red blood cells was sufficient to convert bacampicillin into ampicillin, or that other factors such as the plasma pH and the esterase activity present in other body tissues may have played a role in the results obtainned.

The absorption of bacampicillin from the gastrointestinal tract was impaired when the antibiotic was administered in

conjunction with probenecid, as reflected by significantly ($P<0.05$) lower ampicillin peak plasma concentrations. In addition, the reduced absorption of bacampicillin and consequently higher intestinal concentration of antibiotic, was characterised by the presence of gastointestinal disturbances presumably caused by an alteration of the microbial ecosystem. The transformation of the inactive bacampicillin into ampicillin in the gastrointestinal tract was probably caused by a more alkaline pH in the large intestine and by the production of esterases by enteric bacteria. The presence of probenecid did prolong the fraction of ampicillin present in plasma as reflected in a significantly longer ($P < 0.05$) mean residence time (MRT = 255.5 min vs MRT = 95.82 min) when bacampicillin was administered in conjunction with probenecid.

A three-year survey was conducted to establish the sensitivity patterns of commonly encountered equine bacterial pathogens to a range of antibiotics. Ampicillin appeared to be effective against a number of bacteria including all *Actinobacillus equuli* and *Streptococcus zooepidemicus* tested, however, a large proportion of *Escherichia coli* and *Salmonella typhimurium* isolates were resistant (47.8 % and 81.8 % respectively). In addition all *Pseudomonas* spp. isolates were resistant to ampicillin. This suggests that antimicrobial therapy should be evaluated in every circumstance and indicates the need for routine *in vitro* sensitivity testing particularly on those bacterial strains where resistance is likely to be present. In addition, the survey revealed that other antibiotics, such as the fluoroquinolones, were particularly effective against the bacteria tested and that they might play a rôle in the future of equine antimicrobial therapy, although pharmacokinetic and toxicological studies should be carried out in order to evaluate their therapeutic potential in the equine species.

Chapter 1
General introduction

1.1 Antimicrobial therapy in the horse

1.1.1 General considerations

Antibiotics are probably the most commonly administered drugs in veterinary medicine. They are substances that have the capacity to kill (bactericidal) or to inhibit the growth (bacteriostatic) of bacteria, and are used therapeutically and prophylactically in many conditions.

Many criteria determine which antibiotic and what dosage regimen should be chosen including the organ or tissue infected, the microorganism responsible of the disease, the age of the animal, and others factors which are common to antibacterial treatment in all species. However, there are other considerations which are of particular importance in the horse.

For instance, the enormous variation in size from one animal to another whithin the equine species, requires careful consideration of the dosage to be administered, especially when antibiotics with a narrow therapeutic window such as the aminoglycosides are to be administered. Furthermore, great differences in the value of horses make the costs of treatment another important consideration to be taken into account during equine antibacterial therapy.

Local reactions frequently occur following intramuscular (IM) injection in the neck region of the horse. Thus alternative routes of administration should be considered when prolonged treatments are required or large doses have to be given (Ricketts & Hopes, 1983). The horse's large jugular veins make the IV route suitable for occasions where aggressive antibacterial therapy is needed during acute infections. Nevertheless, this route should be used with care when some antibiotics are administered because of the possibility of phlebitis and subsequent thrombosis (Gabel, 1977). Phlebitis is a very common if not unavoidable sequel to the use of IV route of administration in the neonatal foal.

Other general adverse reactions to antibiotic therapy in the horse include hypersensitivity reactions induced by some drugs, particularly the penicillins (Ingham & Large, 1969) and

enterocolitis frequently attributed to the administration of oxytetracycline (Andersson *et al.*, 1971; Cook, 1973) and lincomycin (Raisbeck *et al.*, 1981).

It is also important to remember that many horses require antimicrobial treatment during training and antibiotics may produce lethargy (Ricketts & Hopes, 1984). In addition, treatment must be withdrawn an adequate time before racing to comply with the Rules of Racing dictated in the U.K by the Jockey Club. A knowledge of the elimination half-life and clearance of the antibiotic being used is therefore necessary, although for most antibiotics the standard period of eight days recommended for the withdrawal of most drugs will be adequate.

Another important consideration to be taken into account during the treatment of equine infections is the emergence of bacterial resistance. This is particularly relevant and has to be taken into account during the local application of antibiotics into the mare's uterus to prevent endometritis and also during the topical use of antibiotics in penile washings in the stallion to prevent the transmission of pathogens to the mare during the coitus. Both procedures can affect the normal commensal bacterial flora leading to superinfection with more resistant microorganisms such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* or overgrowth by yeast and fungi (Asbury, 1986).

In general terms, three main factors are essential to achieve successful antibiotic treatment. The microorganism responsible for the infection has to be susceptible to the antibiotic being used, the drug must be present in sufficient concentrations at the site of infection and the microorganism must be exposed to the antibiotic for a sufficient period of time to be killed or removed (Aronson & Brownie, 1982).

In an ideal situation, before the start of the antibacterial therapy the clinician should identify the microorganism responsible for the infection. Having identified the pathogen, qualitative (disk-diffusion) or if possible quantitative antimicrobial susceptibility testing, minimum inhibitory concentration (MIC), should be performed. Normally the aim of the treatment is to provide plasma concentrations that will exceed the MIC several times (Baggot & Prescott, 1987). Nevertheless for most antibiotics the

extent of such excess has not been well defined. The fact that the MIC values allow tailoring of drug dosage to individual microorganisms, make this data more useful than the qualitative data (Baggot & Prescott, 1987). Having determined which is the most suitable antimicrobial *in vitro*, the treatment should be started. However, in most practical situations the clinician has to initiate the treatment empirically, basing his choice on the probability of the presence of a particular bacteria with likely sensitivity in that specific infection. The successful therapy, will depend on his own clinical experience and on the information available about susceptibility patterns. Several authors have collected such information (Knight & Hietala, 1978; Adamson *et al.*, 1985; Hirsh *et al.*, 1985; Hirsh & Jang, 1987; Snyder *et al.*, 1987; Ensink *et al.*, 1993) which is of considerable value for the equine practitioner. Nevertheless, sensitivity tests and MIC for different antibiotics should be performed in cases were the animal does not seem to respond to the established antibiotic therapy.

In order to succeed during treatment it is also necessary to achieve sufficiently high concentrations of the antibiotic at the site of infection for an adequate period. The achievement of these antibiotic concentrations will depend on several factors including the dose and route of administration and the pharmacokinetic behaviour of the antibiotic in the animals treated.

The route of administration determines the extent and rate of absorption and consequently the achievable plasma and tissue concentrations. The IV route provides rapid and total absorption with the antibiotic reaching high peak plasma and tissue concentrations but also being eliminated rapidly from the body. The direct administration of the drug into the central compartment provides a bioavailability (extent of absorption) of one hundred per cent, and therefore permits very accurate dosage. Nevertheless very high concentrations are attained in plasma and tissues immediately after administration, and consequently this route should be used carefully when antibiotics with low therapeutic indices are administered. In addition, hypersensitivity reactions which sometimes appear following the administration of some antibiotics tend to be much more severe

when the drug has been administered IV (Bogan, 1983). Distribution throughout the body and elimination are very rapid following IV administration, generally making the dose interval shorter compared to alternative routes of administration. For these reasons the IV route of administration is generally restricted to the treatment of acute infections where high plasma and tissue concentrations are required rapidly.

The IM route on the other hand provides lower plasma and tissue concentrations but the antibiotic persists for a longer period in the body. In the horse, the gluteal muscles and the neck muscles (serratus muscle) are the two usual sites for IM administration. It would appear that some antibiotics are better absorbed from the neck region in the horse, probably because there is a greater blood supply and more movement in this area than in the gluteals (Firth *et al.*, 1986). However the local reactions after intramuscular injection seem to be more common in the neck than gluteal region (Keen & Livingston, 1983). The bioavailability following IM injection depends on the formulation of the drug, vascularisation at the site of injection and to a lesser extent on the lipid solubility of the substance (Prescott & Baggot, 1988a).

Despite the fact that the oral route is probably the easiest method of administration, few antibiotics can be administered in this way to the adult horse. This is the result of poor gastrointestinal absorption from the intestinal lumen into the systemic circulation, high intraluminal concentration of the antibiotic and consequently disruption of the intestinal flora commonly leading to colitis (Clark & Becht, 1987). However, some of the antibiotics that do not seem to be absorbed after oral administration in the adult, reach reasonable plasma concentrations when administered orally to foals and do not adversely affect the gut (Brown *et al.*, 1984).

The most common antibiotics administered orally to horses are trimethoprim / sulphonamide combinations although cephalexin, cephadroxil, and chloramphenicol have been administered (Clark & Becht, 1987). Other antibiotics that are effective in the horse when administered orally include metronidazole (Sweeney *et al.*, 1986; Baggot *et al.*, 1988 b) and rifampin (Baggot, 1992) with the former having proven a successful for the treatment of anaerobic

pleuropneumonia (Mair & Yeo, 1987) and rifampin being the treatment of choice when combined with erythromycin for the treatment of pneumonia in foals caused by *Rhodococcus equi* (Hillidge, 1987; Sweeney *et al.*, 1987). Finally, pivampicillin, an ampicillin prodrug for oral administration, has recently been shown to be absorbed to a larger extent than any other penicillin when administered intragastrically to horses and consequently could be a valuable alternative for the treatment of equine infections by the oral route (Ensink *et al.*, 1992).

Intravenous infusion of antibiotics has not been well described in the horse. The aim is to maintain known steady state concentrations of the drug in plasma for a prolonged period of time. Its practical application would be when antibiotics with a narrow therapeutic window have to be administered continuously (intensive care patients) or when defined concentrations of the antibiotic have to be maintained over a specific period of time (prophylaxis during surgery).

The achievement of sufficient concentrations at the site of infection, not only depends on the dose and route of administration of the antimicrobial but also on its pharmacokinetic characteristics. Pharmacokinetic studies provide information about the behaviour of a drug in the body, by showing patterns of plasma and tissue concentrations versus time. Consequently, the pharmacokinetic behaviour of the antibiotic indicates whether the antibiotic will achieve sufficient concentration to kill or inhibit the bacteria and also the period during which this concentration will be maintained. The pharmacokinetic behaviour of any drug is determined by various factors which include the physicochemical characteristics of the drug, the extent of tissue and protein binding, the route of administration and the species to be treated. All these factors, affect the rate of absorption, distribution and elimination of the drug to be administered, and therefore knowledge of pharmacokinetics is required to select the most appropriate antimicrobial and dose to be administered. Finally, other factors which will determine the effective concentration of the drug at the site of infection include the location of the infection and the pathology of the tissue where the infection is occuring.

1.1.2 Treatment failure

When the concentration of the antibiotic achieved is not high enough or when it has not persisted at the site of infection for a sufficient length of time the treatment is likely to fail. Inadequate concentrations may be the result of an incorrect dosage administered and may be accounted for by a number of reasons. Variations between individual animals can be enormous when pharmacokinetic studies are carried out. The recommended dosage rates are based on results from the mean of a group, and variations between individuals, can sometimes explain why some animals treated with the recommended dosage will not respond in the expected way. It is also important to take into account the variation of tissue blood supply within the body, and to consider sites to which antibiotics do not gain access because of physical barriers. Antibiotic concentrations are generally lower in poorly perfused tissues and in sites such as the eye, the brain and the joints where specific barriers such as the blood-brain barrier make the diffusion of drugs more difficult or even impossible depending on the drug. If there is a large amount of tissue debris (pus, fibrin etc) in the infection site the vascular supply will be compromised and the pH altered. These particular conditions at the infection site, will not allow poorly penetrating antibiotics to reach the foci of infection, and may result in treatment failure.

Many antibiotics and especially the bacteriostatic ones, rely on a competent host immunological system, therefore impaired host defence mechanisms will be a possible contributory cause of treatment failure. This fact should be considered when antibiotics are administered concurrently with corticosteroids for a prolonged period of time (Jenkins, 1984) or in the event of any other type of underlying immunodeficency (malignant viral infections, chronic diseases). Conditions where fever, uraemia, dehydration or hypoalbuminaemia exist also alter the pharmacokinetics of drugs and an adjustment of the dose will be necessary when they exist (Baggot, 1989).

The use of antimicrobial combinations is a common practice in veterinary medicine (Brumbaugh, 1986) and can also cause the treatment to fail. Antibacterial combinations are widely used and

can have a synergistic effect when less than half the concentration of each antibiotic is required to inhibit the organism to the same extent as either individual agent alone. If the amount of each antibiotic given in combination is half of the inhibitory concentration required when given alone, the combination is defined as additive and when more than half of the individual concentrations are required when given together then the combination is antagonistic (Rahal, 1978). In general, a synergistic effect is achieved when two bactericidal antimicrobials such as the penicillins and aminoglycosides are combined (Watanakunakorn, 1971). However this is not always the case since it depends on the particular antibiotic used and the bacteria responsible for the infection. Whenever an antagonistic combination is used, the treatment is likely to fail. In general, bacteriostatic agents (tetracyclines, chloramphenicol) when combined with bactericidal ones (penicillins, cephalosporins, aminoglycosides) tend to have an antagonistic effect especially when the bacteriostatic antibiotic is administered first. This has been attributed to the conversion of a growing bacterial population into a static one by the bacteriostatic agent, on which bactericidal antibiotics such as the penicillins cannot exert their effect (Rahal, 1978; Paul, 1987). Also some workers have shown that the presence of a bacteriostatic agent such as chloramphenicol can inhibit the enzymes responsible for bacterial lysis in the presence of penicillins (Rahal, 1978).

Finally, the presence of resistance among bacterial isolates has to be considered as a common cause of treatment failure during antibiotic therapy. Resistance to some antibiotics and variations in the MIC values have appeared more and more frequently in the equine literature. The antibiotics commonly used with prophylactic or therapeutic intention, especially in the field of reproduction, tend to eliminate the susceptible strains of bacteria leaving resistant ones which become the dominant population. The genetic information responsible for the resistance in some strains can also be transmitted, mainly by plasmids, into susceptible bacteria (Linton, 1982). The clinician should be suspicious of the presence of resistant strains in therapeutic failure especially when the dosage being used is based on

relatively old sensitivity and MIC values. In order to limit the potential for development of resistance, antibiotics should not be used indiscriminately.

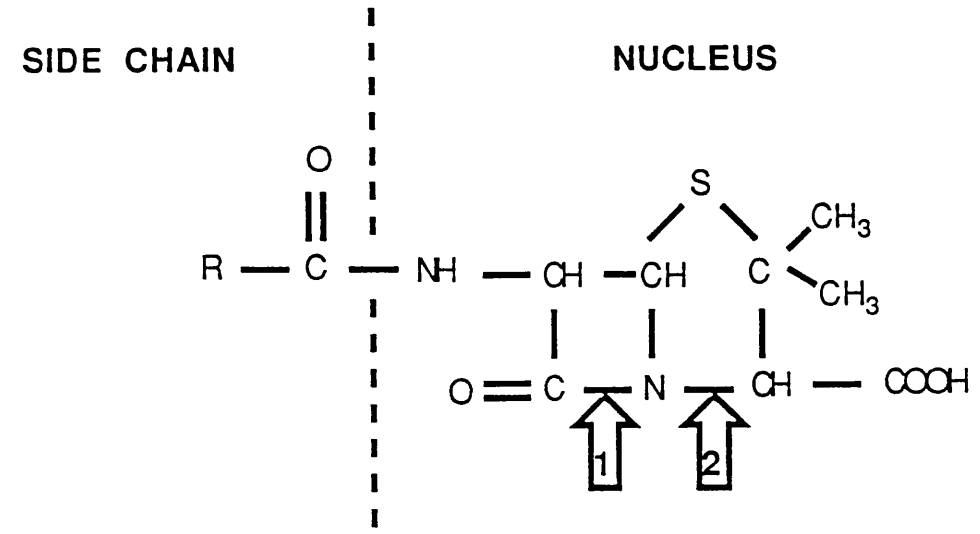
1.2 The penicillins

1.2.1 Chemistry and metabolism

The penicillins constitute a group of antibiotics characterised by a common basic chemical structure consisting of a thiazolidine ring and a beta-lactam ring together with a side chain (Fig 1-1). Penicillin was discovered in 1928 by Alexander Fleming, and subsequently a large number of semisynthetic penicillins were developed.
Variations of the side chain have led to the development of new penicillins which differ in their spectrum of activity, acid stability and pharmacokinetic behaviour. Following administration, the penicillins undergo little metabolism and are excreted mainly as the parent compound .in the urine (Mandell & Sande, 1980).

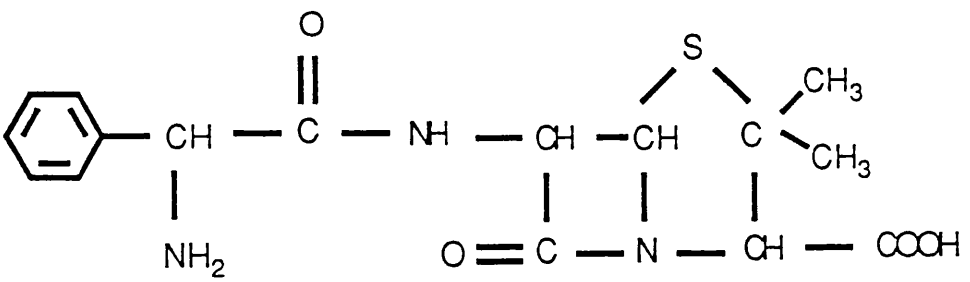
1.2.2 Mechanism of action

The penicillins owe their activity to their effect on the bacterial cell wall. The cytoplasmic membrane of bacteria is surrounded by a robust wall which confers shape on the cell and prevents its osmotic rupture. The cell walls of gram-positive and gram-negative bacteria differ. Both contain peptidoglycan which is formed by two alternating amino sugars (N-acetylglucosamine and N-acetylmuramic acid) with peptide side chains cross-linked by peptide bonds. The peptidoglycan of gram positive bacteria is relatively thick (50 to 100 molecules) and is interspersed and covalently linked to other polymers such as teichoic acid and polysaccharides. Gram-negative bacteria have a thin peptidoglycan layer (1 to 2 molecules) surrounded by an outer membrane consisting of phospholipids, proteins and lipopolysaccharide.



1 Beta-lactam ring

2 Thiazolidine ring



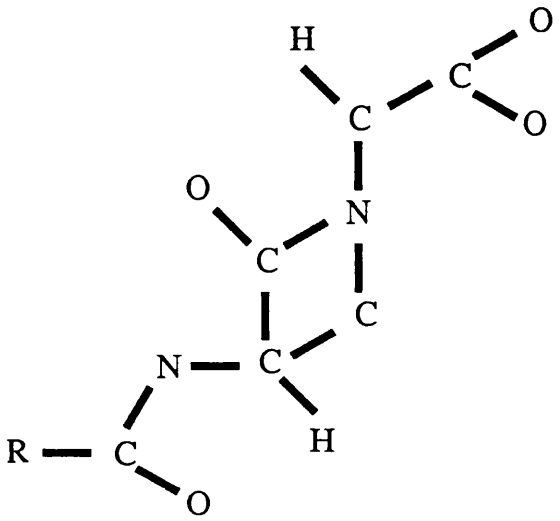
AMPICILLIN

Figure 1-1. Basic molecular structure of penicillin and ampicillin molecules.

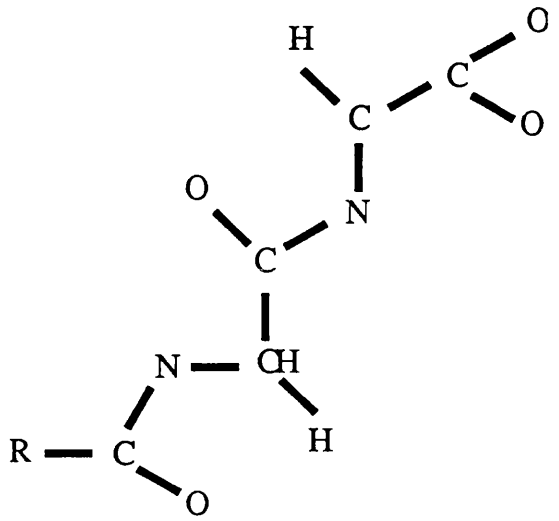
The lack of peptidoglycan in mammalian cells makes this structure an excellent target for antibacterial action since its presence is necessary for bacterial integrity (Brumbaugh, 1987). Peptidoglycan precursors are synthesized in the bacterial cytoplasm and transported across the cytoplasmic membrane on a lipid carrier molecule. The peptidoglycan cross-linking is the final stage in the biosynthesis, and is achieved by a transpeptidation reaction. It is at this final stage of peptidoglycan synthesis that the beta lactam antibiotics work. N-acetyl muramic acid, contains a peptide side chain which cross links to its neighbours. The transpeptidase enzyme interacts with the D-alanyl-D-alanine end of the peptide side chain during the cross linking transpeptidation reaction (Park, 1987). Beta lactam antibiotics are steriochemically similar to D-alanyl-D-alanine (Waxmam *et al.*, 1980) and the transpeptidase enzyme may use the penicillin as a false substrate interaction which inactivates it to a covalently bound penicilloyl-enzyme complex (Fig. 1-2) (Tipper & Strominger, 1965).

Transpeptidase is the main target protein for the beta lactam antibiotics, however many bacteria have been shown to contain other penicillin binding proteins (PBPs) which are enzymes whose interaction may also be detrimental to the organisms (Spratt, 1975). The bactericidal activity of penicillins is the result of lysis of bacterial cells. The lytic action is thought to be associated with cell wall "autolysins" which are normally released in a genetically controlled manner during the separation of daughter cells during bacterial reproduction. It appears that the presence of penicillins with their detrimental effect on the peptidoglycan biosynthesis, cause bacterial death during the phase of autolysin activity. However some workers have suggested that the autolysins are inhibited naturally by amphipathic membrane associated substances within the bacteria and beta lactams may dissociate autolysin inhibitor complexes (Tomasz, 1979; Russell & Chopra, 1990 a).

The penicillins have greater activity against actively growing bacteria than non-growing bacteria. The reason for this is not fully understood although it has been suggested that when PBPs which are not synthesising peptidoglycan are bound by beta



Penicillin



D-alanyl-D-alanine

Figure 1-2. Analogous structure of penicillins and D-alanyl-D-alanine (Tipper & Strominger, 1965).

lactams the interaction does not activate autolysins whereas interaction with active PBPs does activate the autolysins (Russell & Chopra, 1990 a).
Finally, the spectrum of activity of the various penicillins is not related to the affinity of penicillin binding proteins for the antibiotic but rather to the ability of the broad spectrum penicillins to penetrate the outer bacterial membrane of the gram-negative bacteria and thus reach the loci of the target proteins. This penetration of the outer membrane occurs through aqueous channels created by proteins called porins which are present within the lipopolysaccharide and phospholipid of the outer membrane.

1.2.3 Toxicity and adverse reactions

Hypersensitivity, which may present as mild urticaria, fever or angioneurotic oedema or as acute anaphylaxis and collapse, is the most common side effect following the administration of any penicillin and there is cross-sensitization in animals for different members of the group. Penicillins or their break-down products, in particular the penicilloyl moiety (with opened beta lactam ring), become antigenic as haptens upon binding with plasma proteins. Hypersensitivity reactions to penicillins in horses are rare, affecting less than five per cent of treated horses (Ingham & Large, 1969) and where they have been reported it is as a consequence of administration of benzylpenicillin and the associated sodium and potassium salts may have contributed to the reactions observed (English & Roberts, 1983)
The administration of the penicillins by the oral route to adult horses is likely to result in severe gastrointestinal disturbances. This has been reported by Baggot and others (1990) following the administration of penicillin G and penicillin V to adult horses. These disturbances are likely to be the result of a large proportion of the administered penicillin not being absorbed into the systemic circulation and consequently disrupting the microbial ecosystem of the equine large intestine.

Ampicillin achieves high concentrations in the caecum of horses following oral administration and may also be detected in the caecum after intravenous administration although at much lower concentrations. Furthermore the bacterial populations in the caecum change after oral administration of ampicillin with an increase in viable coliforms, streptococci, lactobacilli and clostridia (Horspool, 1992). These changes are similar to those described following administration of oxytetracycline which also caused the development of enterocolitis (Andersson *et al.*, 1971; White & Prior, 1982).

The aetiology of antimicrobial associated enterocolitis is not understood, however it seems likely that a number of factors affect its development. These include the amount of antimicrobial delivered to the caecum and colon and its spectrum of antimicrobial activity, the commensal bacterial population present in the gut, the presence of potential bacterial pathogens in the environment, and other factors which could affect the commensal bacteria such as dietary changes and "stress".

Other forms of toxicity and adverse reactions are extremely rare with penicillin antibiotics although where oil-based preparations are given by the intramuscular route they may cause pain and swelling at the injection site. Penicillin has been associated with haemolytic anaemia and acute hepatic failure although the incidence of this is likely to be very low (Step *et al.*, 1991).

1.3 Penicillins in the horse

1.3.1 Penicillin G and penicillin V

Penicillin G is the basic penicillin molecule and the most active on a molar basis against susceptible gram-positive aerobic bacteria (Sandholm *et al.*, 1990). This antibiotic is widely used in the equine for the treatment of meningitis and polyarthritis caused by *Streptococcus zooepidemicus* in the foal, wound infection, and genitourinary infections (Prescott & Baggot, 1988b). It is also the antibiotic of choice for the treatment of *Streptococcus equi* infections (*ie*, strangles) and is a very useful antibiotic for the

treatment of suppurative respiratory disease in the horse where *S. zooepidemicus* is the most commonly found pathogen (Reif, 1979; Love *et al.*, 1983). It can be administered as the sodium or potassium salt (also called 'soluble' or 'crystalline' penicillin), as the procaine or benzathine salt of penicillin and as the acid resistant phenoxymethyl form for oral administration. These different forms vary in their absorption rates from the site of administration

In the horse there are no phenoxymethyl penicillin (penicillin V) preparations licensed and penicillin G is generally administered IV, IM and as a uterine infusion. When penicillin G was administered orally to horses, only 2.87 % was absorbed from the gastrointestinal tract and severe gastrointestinal disturbances followed (Baggot *et al.*, 1990).

The highly soluble salts (sodium and potassium) are mainly used when rapid high concentrations are required such as the initial stages of acute infections, and they should also be considered the drugs of choice for the treatment of infections caused by beta-haemolytic streptococci which are common in the equine patient (Traver & Riviere, 1981). Nevertheless the high solubility and rapid absorption of these aqueous forms after IM administration and their very short half-life means that, when therapeutic levels are to be maintained, repeated doses (every four to six hours) should be given (Spurlock & Wilcke, 1986). In addition, Dürr (1976) found that 52 to 54 % of penicillin G was bound to plasma proteins and this might limit the tissue concentrations achievable. It should also be borne in mind that the sodium and potassium salts of penicillin G should be used with care in the compromised patient with electrolyte imbalance since the formulations contain 2.3 mEq of sodium and 1.7 mEq of potassium respectively per million iu of penicillin (Brumbaugh, 1987).

To overcome the very short half-life of the sodium and potassium penicillin G salts, a longer acting compound such as procaine penicillin G can be used in later stages of infections caused by gram-positive bacteria. Even with the procaine salt, concentrations greater than MIC values will be achieved very quickly. When used at twenty-four hour dosage intervals the procaine salt is the commonest preparation of penicillin used in

equine medicine (Prescott & Baggot, 1988b). Love and others (1983) recommended that treatment should begin with an IM administration of penicillin G at a 10,000 iu/kg followed by procaine penicillin administered IM at 15,000 iu/kg every 12 h or 30,000 iu/kg every 24 h. However, some workers have suggested that the single administration of procaine penicillin may not be as effective against gram-positive bacteria as the soluble penicillin G salts because lower peak concentrations of the drug are achieved regardless of the amount administered, and for this group of antibiotics peak concentrations may be more important than maintenance concentrations (Sullins *et al.*, 1984). In addition, the possibility of detection of procaine residues two weeks post-treatment and the fact that some adverse reactions to procaine have been recorded in the horse should also be considered before initiating any treatment with this penicillin preparation in the horse (Tobin *et al.*, 1977).

Where very sensitive microorganisms are responsible for infection, benzathine penicillin G, a very long acting form of penicillin G may be used. The very slow absorption of benzathine penicillin G from the site of intramuscular injection allows an interdosing interval of seventy two hours (Prescott & Baggot, 1988b). However, benzathine penicillin is seldom used due to the very low maximum concentrations achievable (Love *et al.*, 1983; Ricketts & Hopes, 1984).

The acid resistant form of penicillin G, phenoxymethyl penicillin (Penicillin V) used for oral administration, has been shown to have a very low bioavailability when administered orally to horses and foals (1.65 % and 16.04 % respectively). In addition, signs of colic and watery diarrhoea were a common feature in the adult horses given this antibiotic (Baggot *et al.*, 1990). Therefore the administration of oral penicillin V is unlikely to prove appropriate for the treatment of penicillin G sensitive pathogens in the horse.

1.3.2 Penicillinase resistant penicillins

Some bacteria such as *Staphylococcus aureus* are capable of destroying the penicillins by breaking down the beta-lactam ring. For successful treatment against infections caused by these microorganisms, penicillinase-resistant penicillins, the isoxazolyl penicillins, have been developed. Oxacillin is the most commonly used in the horse and following IM administration good and rapid absorption is achieved (Stover *et al.*, 1981).
An alternative to the use of a penicillinase resistant penicillin is the use of a combination of a penicillin with an inhibitor of the beta-lactamase enzyme such as clavulanic acid and although this type of treatment is commonly used in other species (dog, cattle) it does not seem to be effective in the horse.

1.3.3 Antipseudomonal penicillins

The carboxypenicillins (carbenicillin and ticarcillin) and the ureidopenicillins (mezlocillin, azlocillin and piperacillin) are in general active against microorganisms resistant to the common broad spectrum penicillins. The carboxypenicillins (carbenicillin and ticarcillin) are very effective against *Pseudomonas aeruginosa* infections, some *Proteus* and enterobacteriaciae although *Klebsiella* spp are resistant (Wright & Wilkowske, 1983). In equine antibiotic therapy they are used for the treatment of bacterial endometritis administered as uterine infusions and also as part of semen extenders (Asbury, 1986). *Klebsiella* spp can be treated with the ureidopenicillins as can infections caused by the anaerobe *Bacteroides fragilis*.
However in general, these new antibiotics are seldom used systemically in equine practice because of their prohibitive price and lack of kinetic data in the horse and their administration should only be recommended in the event of an intransigent penicillin resistant bacterium.

1.3.4 Broad spectrum penicillins

The penicillins described so far, except the antipseudomonal penicillins, have activity mainly against gram-positive bacteria. This makes their use unreliable when the pathogen responsible for the infection is not known or when more than one microorganism is present in the infection. To overcome this problem penicillins with a wider spectrum can be used. Ampicillin and amoxycillin are two broad spectrum penicillins used in equine antibiotic therapy which are characterised by a broader spectrum than penicillin G. They are also destroyed in the presence of beta-lactamase producing bacteria.

It should be emphasised that in the event of an infection caused by susceptible gram-positive bacteria, it is preferable to use penicillin G rather than ampicillin or amoxycillin, since plasma concentrations are likely to exceed MIC values by a greater margin for equivalent doses.

Amoxycillin is widely used in small animals and man. In these species and in calves it has been shown to be absorbed to a large extent from the gastrointestinal tract following oral administration (Zarowny, *et al.*, 1974; Pugh, 1977; Palmer *et al.*, 1983). It has been shown that the systemic bioavailability of amoxycillin trihydrate after oral administration is reasonably high (42.7 %) when administered to neonatal foals and therefore it could be used in systemic infections (Baggot *et al.*, 1988 a). Oral amoxycillin trihydrate achieved concentrations sufficient to treat streptococcal and staphylococcal (non beta-lactamase producing) infections and susceptible *Salmonella typhimurium* and *Actinobacillus equuli* when administered to foals (Love *et al.*, 1981). However, the poor absorption following oral administration of amoxycillin in the adult horse with a bioavailability of 5.3 -10.4 % (Wilson *et al.*, 1988; Ensink *et al.*, 1992), restricts its use to the IM and IV routes of administration in the adult animal.

When sodium amoxycillin was administered IV to mares, the antibiotic was rapidly eliminated from the body as reflected in an elimination half-life of less than forty minutes, and therefore frequent dosages are required to maintain therapeutic

concentrations (Wilson *et al.*, 1988). Another study carried out by Montesissa and others (1988) found that amoxycillin sodium was rapidly distributed and eliminated from the body, the IM bioavailability following administration was 67 % and that 37 % of amoxycillin was bound to plasma proteins. On the other hand, the administration of amoxycillin trihydrate by the IM route was characterised by concentrations greater than 0.5 μg/ml for a period of 24 hours although precipitation at the injection site occurred when the antibiotic was administered as a 25 % formulation (Wilson *et al.*, 1988).
Ampicillin, the other broad spectrum penicillin used in equine practice, was the antibiotic used during our studies and consequently a full description follows.

1.4 Ampicillin in the horse

Ampicillin (Fig. 1-1) is a widely administered broad spectrum antibiotic in equine practice, available as ampicillin sodium for IV and IM administration and ampicillin trihydrate for IM administration. In addition, ampicillin prodrugs, including pivampicillin and bacampicillin, have been developed for oral administration in human medicine. Recently pivampicillin was investigated in the horse as an alternative for the treatment of infectious diseases by the oral route (Ensink *et al.*, 1992).
Ampicillin sodium seems to produce fewer reactions at the site of injection than the trihydrate formulation and better blood concentrations are achieved (Tobin, 1979). However, the soluble sodium salts are unstable and lose their activity a few hours after being reconstituted, the insoluble trihydrate form is much more stable and has a storage lifespan of a few months. The differences in solubility make the sodium preparation more suitable for those situations where higher plasma and tissue concentrations are required. Ampicillin trihydrate on the other hand, produces lower plasma concentrations over longer periods making it very suitable for the treatment of highly sensitive microorganisms. In addition, both formulations can be administered either on their own or in combination with other bactericidal antibiotics such as

the aminoglycosides in order to achieve a synergistic effect or in combination with beta-lactamase inhibitors. The combination of a penicillin with an aminoglycoside has been shown to have a synergistic effect in many infections, particularly when gentamicin is the aminoglycoside used. However, the widely used combination of penicillin with streptomycin did not demonstrate a synergistic effect against many enterococci isolated from urine and wounds (Rahal, 1978).

Ampicillin is used for the treatment of many bacterial infections caused by gram-positive and gram-negative bacteria. Its activity against common equine pathogens, its wide safety margin and relatively low price make this penicillin a popular choice for the treatment of equine infections (Baggot & Prescott, 1987).

Ampicillin has been shown to be effective in the treatment of many equine conditions including respiratory infections, skin infections, skeletal and synovial infections or septicaemia when administered alone or in combination (Keefe *et al.*, 1980; Ricketts & Hopes, 1984; Baggot & Prescott, 1987; Furr, 1989). There are wide variations in the recommended dosage and it may be appropriate to adjust the dose for specific cases depending on the severity of the infection, susceptibility of the pathogens that are likely to be involved and the site of infection. However, a dosage regime of 10 to 20 mg/kg every 6 to 8 hours with ampicillin sodium and every 8 to 12 hours with ampicillin trihydrate has been recommended by different authors as the most suitable for the treatment of sensitive pathogens in the horse (Beech *et al.*, 1979; Prescott & Baggot, 1988b; van Miert, 1988; Horspool *et al.*, 1992).

1.4.1 Chemistry and metabolism

Ampicillin and its ester congeners, bacampicillin (1-ethoxy-carbonyloxyethyl ester) and pivampicillin (pivaloyloxymethyl ester), are semi-synthetic penicillins. The side chain on the beta lactam ring confers greater acid stability, on ampicillin compared to benzylpenicillin, and increases its bioavailability in the host after oral administration and entry into gram-negative bacteria. Both ester congeners are inactive as parent molecules since they

have a side chain on the thiazolidine ring. Following oral administration they are hydrolysed, both in the mucosal cells of the gastrointestinal wall and in plasma, to the active ampicillin molecule. In the horse the ester side chain markedly improves the oral bioavailability of the ampicillin hydrolysate of pivampicillin (30.9 % in fasting horses and 35.9% in fed horses) (Ensink *et al.*, 1992) compared to ampicillin (3.5%) (Horspool, 1992). A small proportion of ampicillin is metabolised, and three metabolites, (5R, 6R)-penicilloate, the (5S, 6R)-epimer and piperazine-2,5-dione, were detected in the urine following administration of ampicillin to human beings (Haginaka & Wakai, 1986). However, like the rest of the penicillins, ampicillin is rapidly eliminated by glomerular filtration and renal tubular secretion mainly in the unchanged form. Experimental studies have shown that ampicillin is partially eliminated in the bile of human beings (Mortimer *et al.*, 1969). Also, a small amount of ampicillin (<4%) may be eliminated via the bile into the gastrointestinal tract following intravenous administration to pigs (Galtier & Alvinerie, 1979) and ponies (Horspool, 1992).
The use of prodrugs could potentially overcome the risk associated with orally administered ampicillin. These drugs are administered in an inactive form which should not affect the enteric microflora, and this rationale has been utilised for the oral administration of pivampicillin (Ensink *et al.*, 1992).

1.4.2 Pharmacokinetics

The efficacy of ampicillin and other antibiotics in the treatment of equine infectious diseases is directly related to the concentration and the duration achieved at the site of infection (Prescott & Baggot, 1988a). These two factors, depend on the physicochemical properties of the antibiotic, the route of administration and the dosage administered.
The pharmacokinetic characteristics of ampicillin have been studied in the horse after intravenous administration at different dosage rates (Dürr, 1976; Bowman *et al.*, 1986; Ensink *et al.*, 1992; Horspool *et al.*, 1992). These studies showed that ampicillin was distributed and eliminated rapidly and had short distribution

and elimination half-lives and high body clearance rates in the horse. For instance, the elimination half-life ranged from a minimum of 0.75 h (Horspool *et al.*, 1992) to a maximum value of 1.72 h (Ensink *et al.*, 1992). These results indicate that frequent dosage is required to maintain therapeutic plasma and tissue concentrations of ampicillin unless the persistence of the antibiotic is prolonged, by formulation characteristics or by competitors of elimination.

The rapid elimination of ampicillin from the body is the result of a very efficient excretory mechanism located in the proximal tubule of the nephron. An active transport mechanism, is responsible for the transfer of ampicillin from the blood stream into the urinary filtrate. In addition, a proportion of the non protein bound fraction of ampicillin is also eliminated into the urine by glomerular filtration. From the urinary filtrate, the transferred substances can passively diffuse back into the plasma from the tubules. This diffusion occurs when the substances present in the urinary filtrate are in their nonionized form which depends on the pH of the filtrate and the pKa of the antibiotic. Alteration of urinary pH has been shown to affect the excretion rate of several drugs and is used therapeutically in human medicine to prolong the effects of some drugs or to increase the elimination rate during intoxication. However, no studies have been carried out to date to evaluate whether changes in urinary pH would affect the pharmacokinetic behaviour of ampicillin in the horse. Potential changes in the plasma disposition of the antibiotic may require the establishment of different therapeutic regimes according to whether the horse had an acidic urine (horses in training) or an alkaline urine (horses grazing).

The concurrent administration of penicillins with probenecid, a competitive inhibitor of the renal tubular secretion of organic anions, decreases significantly the rate of excretion of penicillins and cephalosporins (Juzwiak *et al.*, 1989; Donecker *et al.*, 1986; Levy, 1965; Gibaldi *et al.*, 1968). This could be a practical method of prolonging the excretion of ampicillin in the horse and will be discussed later.

In addition to the characteristic rapid elimination and high clearance rate of ampicillin, determination of other

pharmacokinetic variables in various studies indicates that ampicillin has a relatively poor distribution in the body following administration. This is reflected in the values obtained for the volume of distribution at steady-state ranging from 170.0 ml/kg (Bowman *et al.*, 1986) to 210.8 ml/kg (Horspool *et al.*, 1992). Antibiotic levels in tissue fluids are in equilibrium with the non protein bound fraction of the drug in plasma (Keen, 1989) and ampicillin is bound to equine serum proteins only <10 per cent (Dürr, 1976). It is likely therefore that ampicillin distributes almost exclusively in the extracellular fluid and does not penetrate into the intracellular fluid in significant amounts.

In practice, IM injection is probably the most commonly used route for the administration of ampicillin in the treatment of equine bacterial infections. Several equine studies, have shown that peak plasma concentrations after IM administration of ampicillin sodium are achieved within the first half hour and that the concentrations obtained are sufficient to kill susceptible organisms for several (up to eight) hours (Evans *et al.*, 1971; Knight, 1975; Beech *et al.*, 1979; Keefe *et al.*, 1980; Traver & Riviere, 1982; Firth *et al.*, 1988). On the other hand, when ampicillin trihydrate was administered IM, peak plasma concentrations occurred later (tmax 4-6 hours) and lower plasma concentrations were achieved compared to the ampicillin sodium formulation given at the same dose rate (Cmax 1.48 μg/ml vs 6.0 μg/ml) (Beech *et al.*, 1979; Brown *et al.*, 1982). The differences in absorption could be due to a slower rate of dissolution or precipitation at the injection site. The less soluble trihydrate salts give lower concentrations but can be administered at 12 h intervals (Beech *et al.*, 1979; Prescott & Baggot, 1988b). Both the sodium and trihydrate salts produce plasma concentrations directly related to the dose administered and the absorption of the antibiotic from the site of injection. However, when rapid and high tissue concentrations are required, the intravenous route should be used as tissue levels are directly related to peak plasma concentrations.

The oral administration of most penicillins in the horse is associated with poor absorption and the presence of gastrointestinal disturbances (Baggot *et al.*, 1990). Among the

aminopenicillins, amoxycillin has been administered orally to horses in different experimental studies. In 5-to-10-day-old foals the absorption achieved was 36.2 % (20 mg/kg) and 42.7% (30 mg/kg) (Baggot *et al.*, 1988 a), but only 10.4 per cent (Wilson *et al.*, 1988) and 5.3 per cent (Ensink *et al.*, 1992) of the dose administered was absorbed in studies carried out in adult horses. Ampicillin administered orally achieved peak plasma concentrations of 5 µg/ml in two-day-old foals but only 2.7 µg/ml in 16-to-21-day-old foals. Unfortunately the oral bioavailability was not determined in this study (Brown *et al.*, 1984). In adult animals, the administration of ampicillin orally was characterised by lack of absorption with a bioavailability of 3.5 per cent (Horspool, 1992).

Recently, the experimental oral administration to horses of an ampicillin prodrug, pivampicillin, has shown that high absorption from the gastrointestinal tract can be achieved (Ensink *et al.*, 1992). The rapid transformation of this ester of ampicillin into the active compound is carried out by the intestinal and plasma non-specific esterases. Furthermore, the enhanced absorption of this prodrug reduces the chances of dysbacteriosis that may be caused by the oral administration of the active compound. Once in the plasma the transformation into ampicillin has been shown to be completed in less than ten minutes in man (Neu, 1981). In the horse, the bioavailability of pivampicillin ranged from 30.9 per cent in fasted horses to 35.9 per cent in fed horses (Ensink *et al.*, 1992). The peak plasma concentrations after administration of pivampicillin to fed horses was reached within the first hour and ampicillin was subsequently eliminated in the usual way. The high oral bioavailability obtained with this ampicillin prodrug may allow oral therapy in the horse whereas very few antibiotics, including other penicillins, are suitable for administration by this route.

To evaluate the penetration of ampicillin into equine body cavities, various studies have measured ampicillin in different body fluids. When ampicillin sodium was administered intramuscularly at a 10 mg/kg dosage rate, maximum synovial fluid concentrations were achieved 50 minutes after administration and synovial fluid concentrations greater than 1

μg/ml were maintained for more than eight hours (Firth *et al.*, 1988). Once ampicillin attained equilibrium between the synovial fluid and the plasma, the elimination of the antibiotic from the synovia paralelled the antibiotic decline in plasma (Bowman *et al.*, 1986). In addition, higher concentrations of ampicillin were obtained in the synovial fluid of joints with induced synovial inflammation (Firth *et al.*, 1988). When ampicillin trihydrate was administered to horses by the IM route in two separate studies, mean peak synovial fluid concentrations of 1.65 μg/ml and 2.3 μg/ml were reached at four and six hours respectively, and the antibiotic could still be detected in synovial fluid 48 hours after its administration (Beech *et al.*, 1979; Brown *et al.*, 1982). A mean peak peritoneal fluid concentration of 1.81 μg/ml was also measured 4 hours after the IM administration of ampicillin trihydrate at a dosage of 20 mg/kg bodyweight to adult mares (Brown *et al.*, 1982).

1.4.3 Ampicillin spectrum of activity

Pharmacokinetic data for antimicrobial agents cannot be used to predict dosage regimens without knowledge of the susceptibility of the target organisms. Sensitivities of the bacteria can be obtained *in vitro* by the Kirby-Bauer disc method which gives qualitative information (Bauer *et al.*, 1966) or by the broth dilution method which gives quantitative data (Prescott & Baggot, 1985). Quantitative sensitivity patterns are defined in terms of minimum inhibitory concentrations (MIC), which represent the minimum antibiotic concentration necessary to inhibit visible bacterial growth. Although this *in vitro* measurement of bacterial susceptibility does not take into account many of the effects that occur *in vivo* such as the bactericidal activity of the serum, the enhanced activity of macrophages against antibiotic damaged bacteria and the post-antibiotic effect, MIC data are still the most reliable laboratory method for the determination of bacterial susceptibility. Appropriate dosages based on pharmacokinetics and bacterial sensitivity should ultimately be confirmed in clinical trials (Prescott & Baggot, 1985). In the horse, different workers have investigated the sensitivity of common equine

pathogens to ampicillin and other antibiotics (Knight & Hietala, 1978; Keefe *et al.*, 1980; Adamson *et al.*, 1985; Hirsh & Jang, 1987; Snyder *et al.*, 1987; Moore *et al.*, 1992; Ensink *et al.*, 1993). It has been suggested that an organism is susceptible when the mean plasma concentration obtained during the usual dosing interval is at least twice the MIC value. On this basis, it was suggested that bacteria with MIC values for ampicillin of less than 1 μg/ml should be considered as susceptible to this antibiotic (Adamson *et al.*, 1985). In addition, the National Committee for Clinical and Laboratory Standards (NCCLS), considers bacteria with MIC values for ampicillin of 2 to 16 μg/ml as moderately susceptible and bacteria with MIC greater than 16 μg/ml as resistant (Prescott & Baggot, 1988c). In the United Kingdom, the Veterinary Products Committee (VPC) guidelines recommend that peak plasma concentrations of at least double the MIC value should be achieved and that plasma concentrations greater or equal to the MIC should be maintained for at least half the dosing interval.

Various studies carried out on equine isolates reported that non beta lactamase producing *Staphylococcus aureus, Streptococcus zooepidemicus, Streptococcus equi, Streptococcus pneumoniae, Pasteurella, Corynebacterium pseudotuberculosis, Fusobacterium necrophorum, Bacteroides* species, and most isolates of *Actinobacillus equuli* are sensitive to ampicillin (Keefe *et al.*, 1980; Prescott, 1981; Adamson *et al.*, 1985; Hirsh & Jang, 1987). In addition, many *Escherichia coli, Rhodococcus equi* and *Salmonellae* fall into the moderately susceptible category with *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus* species being resistant to ampicillin. The presence of resistance among the different bacteria such as *Staphylococcus aureus, E. coli, Bacteroides fragilis,* and *E. coli strains,* can be related in general to the production of beta-lactamase enzymes. It is important to point out that the number of bacterial isolates becoming resistant to ampicillin is increasing, particularly among the isolates belonging to the moderately susceptible category *E. coli* and *Salmonella* species (Donahue, 1986; Snyder *et al.*, 1987; Sato *et al.*, 1988).

1.4.4 Resistance

Resistance of bacteria to antimicrobials can be classified as intrinsic such as resistance of some gram-negative organisms to penicillin G or acquired resistance, which is a result of the widespread and often irrational use of antibiotics (Linton, 1982; Ndikuwera and Winstanley, 1987).

Acquired resistance to beta-lactam antibiotics occurs when the permeability of the outer membrane of gram-negative bacteria to the beta-lactam decreases, when beta-lactamases are synthesized or when the penicillin binding proteins are modified (Russell & Chopra, 1990 b).

Reduction in the permeability of the outer membrane may be the result of mutations which lead to the loss of porin proteins. These normally allow the diffusion of the broad spectrum beta lactams through the outer membrane of the gram-negative microorganisms. However, the production of beta-lactmase, an enzyme responsible for the degradation of penicillins into the inactive penicilloic acid is probably the most common mechanism of resistance to beta-lactam antibiotics. The enzyme synthesis can be plasmid or transposon encoded or alternatively some bacteria have the innate genetic ability to generate beta-lacatamase. In these bacteria, beta-lactamase is normally produced at low levels until the bacteria are exposed to a beta lactam antibiotic or a mutation occurs. Whenever this happens, bacteria may be induced to produce beta lactamase in large quantities leading to the appearance of resistance. Finally, mutations can also affect the penicillin binding proteins which become insensitive to the beta lactam antibiotic. This form of resistance has been shown to occur in the treatment of *Streptococcus pneumoniae* with penicillin G in humans (Russell & Chopra, 1990 b).

In veterinary medicine, ampicillin resistant strains of *Proteus* spp., *E. coli* and *Salmonella* spp. (Prescott & Baggot, 1988b; Donahue, 1986; Moore *et al.*, 1992) are a common finding. In addition, a large proportion of *S. aureus* (up to 80% in human infections) produce beta lactamase which diffuses extracellularly due to the absence of the outer cell membrane in gram-positive bacteria (Sande & Mandell, 1985). To overcome the problem

created by the production of beta-lactamases, the penicillins can be combined with beta-lactamase inhibitors. Amoxycillin is currently combined with clavulanic acid and often used in veterinary medicine although not in the horse. Ampicillin has been combined with sulbactam, another beta lactamase inhibitor although this combination has been restricted almost exclusively to human therapy. Both clavulanic acid and sulbactam are irreversible beta-lactamase inhibitors with very little antibacterial activity of their own. Little information is available on their effects in horses, although the administration of clavulanic acid has been shown to produce gastrointestinal disturbances in 10% of treated human patients (Prescott & Baggot, 1988b). Combining the aminopenicillins with beta-lactamase inhibitors would be a useful and rational way of overcoming this type of resistance. Unfortunately the beta lactamase inhibitors are not currently marketed on their own and animal therapy depends on fixed dosage combinations licensed for specific animal species.

The incidence of bacterial resistance is increasing in veterinary medicine and this may be due to the irrational use of antibiotic therapy. For instance errors in the dosage administered such as the administration of small dosages or inadequate frequency of treatment can yield sub-MIC plasma and tissue concentrations which increase the incidence of resistance (Powers *et al.*, 1984). In addition, the choice of the wrong antibiotic can cause the elimination of sensitive organisms, leading to the establishment of a resistant bacterial population while the original aetiological agents are present. In equine therapy, little information is available on the extent of bacterial resistance to antibiotics. However, nosocomial infections are the most common cause of infections with resistant bacteria. These infections are defined as the infections acquired and produced by microorganisms present during hospitalisation. One report on the incidence of nosocomial infections in an equine hospital showed that 3.5% of the horses hospitalised acquired nosocomial infections and of these, 96% were multiresistant to antibiotics. The incidence of ampicillin resistant strains among the nosocomial infections was 100% for *E.coli* and 83% for *Enterobacter* spp compared to an incidence of

57% and 50% for the same bacteria isolated from patients infected oustside the hospital (Koterba *et al.*, 1986). In addition, the incidence of resistant *E. coli* and *Klebsiella* was studied showing that the number of resistant strains isolated from faecal samples increased significantly seven days after hospitalisation (Koterba *et al.*, 1986)

These results show that the use of antimicrobial therapy should be evaluated in every circumstance, taking into acount all the factors that can lead to the appearance of resistance and therefore trying to minimize its incidence especially in those situations where the appearance of resistance is most likely to occur (*ie,* in hospitals).

Chapter 2
General materials and methods

2.1 Animals

Five Thorougbred geldings (numbers 1 - 5) with ages ranging from 7 to 22 years (13.4 ± 2.97, mean ± SEM), two riding mares (numbers 6 and 7) aged 8 and 12 respectively, two Shetland pony mares (numbers 8 and 9) 6 and 18 years of age and two Shetland pony geldings (numbers 10 and 11) aged 13 and 14 years, were used during the experiments.
The weights of each individual animal varied between experiments and the dosage rates were adjusted for each animal according to its weight prior to the experimental procedure.

2.2 Husbandry

All the animals were stabled in boxes during the experimental procedures with free access to water and were fed hay twice a day. A minimum acclimatisation period of four days was allowed for animals that had been kept outdoors before commencing any experiment and a washout period of at least two weeks was allowed between experiments.

2.3 Drug administration and sampling

2.3.1 Intravenous bolus dose administration and sampling

Ampicillin sodium (Penbritin, Beecham Pharmaceuticals) as a 100 mg/ml solution (20 ml sterile, pyrogen free water (Animalcare Ltd, York, UK) per 2 g vial of ampicillin) was administered intravenously, via a Microlance (0.9 mm x 25 mm) needle (Becton, Dickinson and Company, Ireland), at a dose rate of 10 mg/kg bodyweight into the right jugular vein. Heparinised blood samples were collected in lithium heparin syringes (10 ml Monovette, Sarstedt Limited, Leicester, England) by jugular venepuncture from the contralateral vein before drug

administration and at 2, 5, 10, 15, 30 and 45 minutes and 1, 1.5, 2, 4, 8, 12 and 24 hours afterwards. Blood samples were centrifuged at 1700 *g* for 10 minutes, the plasma was removed and stored at 0 - 4 º C together with prepared ampicillin standards in plasma. Plasma samples and standards were subsequently analysed on the following day.

2.3.2 Intravenous infusion administration and sampling

Ampicillin sodium was administered as an intravenous infusion to horses over a four-hour period at various dosage rates.
In order to achieve steady-state levels rapidly, an intravenous bolus dose of 2 mg/kg of ampicillin sodium was administered immediately prior to each infusion. For the administration of ampicillin sodium as an intravenous infusion, 5 ml of local anaesthetic was injected subcutaneously over both jugular veins in the mid-neck region and a 14 gauge, 80 mm IV catheter (Intraflon 2, Trocart catheter IV Teflon, Vygon, France) was placed in each jugular vein connected to a three-way tap (Rocket, England) and sutured to the skin. The catheter was flushed with heparinised saline.
Vials containing 2 g of ampicillin sodium powder (Penbritin, Beecham Pharmaceuticals) were made up with 20 ml of pyrogen free water to give sterile solutions with a concentration of 100 mg/ml. The volume required for infusion was calculated and an equivalent volume removed from a 500 ml sodium chloride fluid replacement bag (Aqupharm, Animalcare Limited, York). This was replaced with the required volume of the 100 mg/ml ampicillin sodium solution, and was connected to an intravenous solution administration set with filter (Travenol Laboratories LDT, Thetford, Norfolk, England). The infusion apparatus was fed through a peristaltic pump (Fig. 2-1) (IVAC 531 Infusion Pump, IVAC House, Bessborough Rd, Harrow) calibrated so that at the established speed a volume of 500 ml was administered over a four hour period (Fig. 2-2).

Fig. 2-1. Peristaltic infusion pump (IVAC 531) used to administer ampicillin IV at a constant rate.

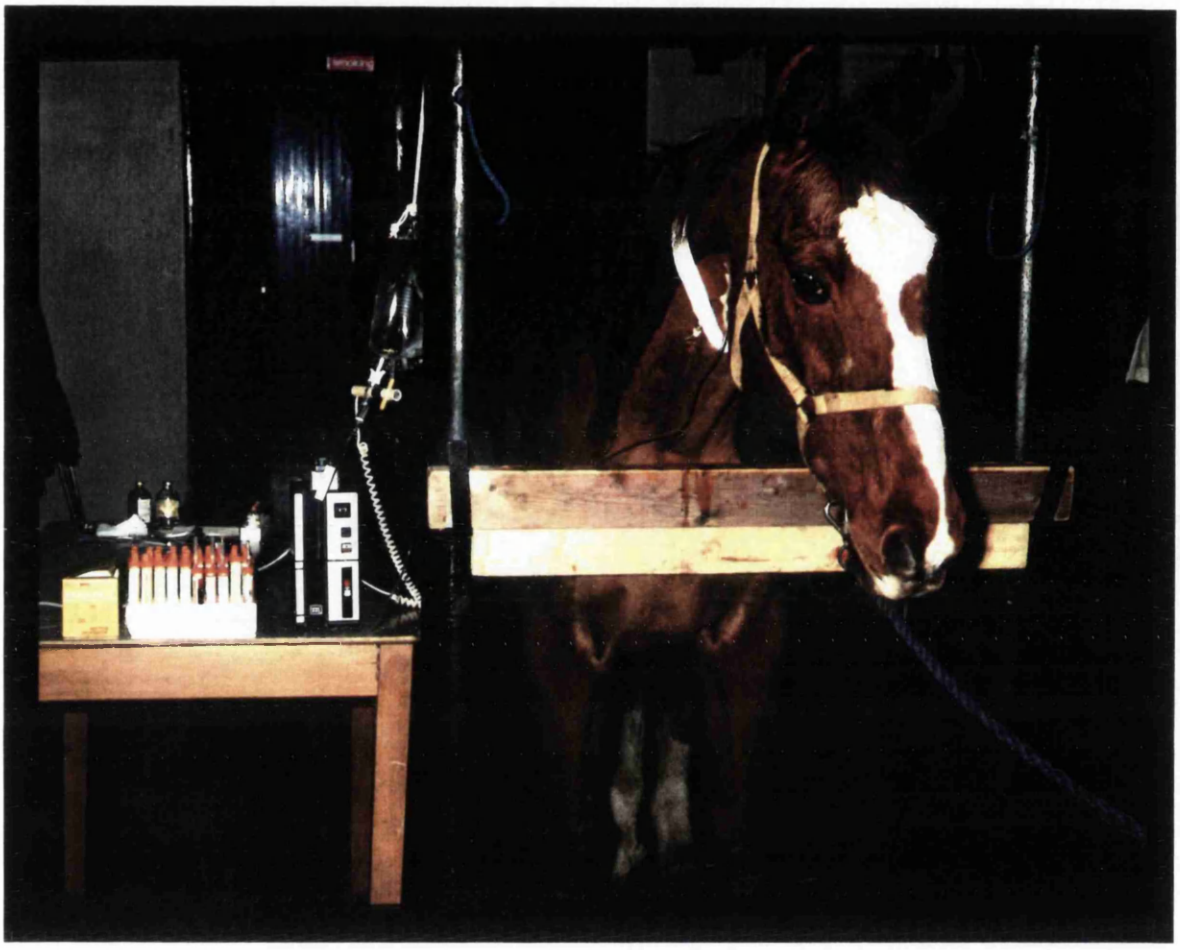


Fig. 2-2. Infusion system during the administration of ampicillin to horse number 4.

Samples were taken in lithium heparin tubes from the contralateral jugular vein at 2 , 5 , 10, 15 , 30 and 45 minutes and at 1 , 1.25 ,1.5 , 1.75 , 2 , 2.25 , 2.5 , 2.75 , 3 , 3.25 , 3.5 , 3.75 and 4 h during the infusion period. Immediately after the 4 h sample, the infusion pump was disconnected and further samples were taken at 2, 5, 10,15, 30 minutes and 1, 2, 4, 8, 12, and 24 hours thereafter. The samples were centrifuged at 1700 *g* for ten minutes and stored as described previously.

2.3.3 Intragastric drug administration and sampling

Drugs administered intragastrically were suspended in water (500 ml for horses and 250 ml for ponies) and administered via stomach tube (18 mm x 3.5 m for horses; 15 mm x 3 m for ponies, Vet Drug Company plc, Falkirk, U.K). A volume of water equivalent to the volume of the suspension was used to flush the stomach tube immediately after drug administration. When probenecid was the drug administered intragastrically, blood samples were collected in lithium heparin syringes from the left jugular vein before the administration and at 15, 30 and 45 minutes and 1, 1.5, 2, 4, 6, 8, 12 and 24 hours afterwards. The samples were subsequently centrifuged as described previously and stored at - 20 º C until analysis during the following two weeks.

2.4 Ampicillin analysis

2.4.1 Microbiological analysis

Ampicillin sodium concentrations in plasma were assayed using the agar diffusion microbiological assay described by Bennett and others (1966). Antibiotic concentrations were calculated by comparing the zones of inhibition of bacterial growth produced by the samples on an agar plate seeded with bacteria, to the zones of inhibition produced by known standard antibiotic concentrations made up in blank equine plasma.

2.4.2 Preparation of ampicillin standards

The range of ampicillin standard concentrations for each assay was prepared according to the concentrations expected in the animals after administration of each dosage regimen.
When ampicillin was administered as a 10 mg/kg intravenous bolus, ampicillin standards were prepared in plasma at 0, 0.25, 1, 5, 10, 20 and 50 μg/ml. Ampicillin (Sigma Chemical Co Ltd., Dorset, UK) was used to fortify blank equine plasma. To carry out the preparation of standards in plasma, an initial stock standard solution of 500 μg/ml of ampicillin was prepared in 0.05 M sterile phosphate buffer at pH=7.0. From the initial stock, serial dilutions were carried out in buffer and were subsequently used to prepare the plasma standards. A maximum volume of buffer stock solution equivalent to one tenth of the total volume of the plasma standard was used.
When the experiments carried out were expected to produce lower levels of ampicillin, such as the intravenous infusion and the oral administration of bacampicillin hydrochloride studies, ampicillin plasma standards of 0, 0.25, 0.5, 2, 5, 10, and 20 μg/ml were used to inoculate the plates.

2.4.3 Plating

Glass plates (30 x 30 cm) were attached to an aluminium frame by eight clips. The plate, was levelled on a triangular levelling stand, cleaned with alcohol and flamed with a Bunsen flame to eliminate contamination. Aluminium covers were available for the plates (Fig. 2-3).

2.4.4 Agar and spore preparation

Antibiotic medium 2 (Difco Laboratories, Detroit, USA) was used to prepare the agar. Fifty one grams of the medium were added to two litres of warm tap water and then placed in a steamer until the powder was completely dissolved (approximately 1 h).

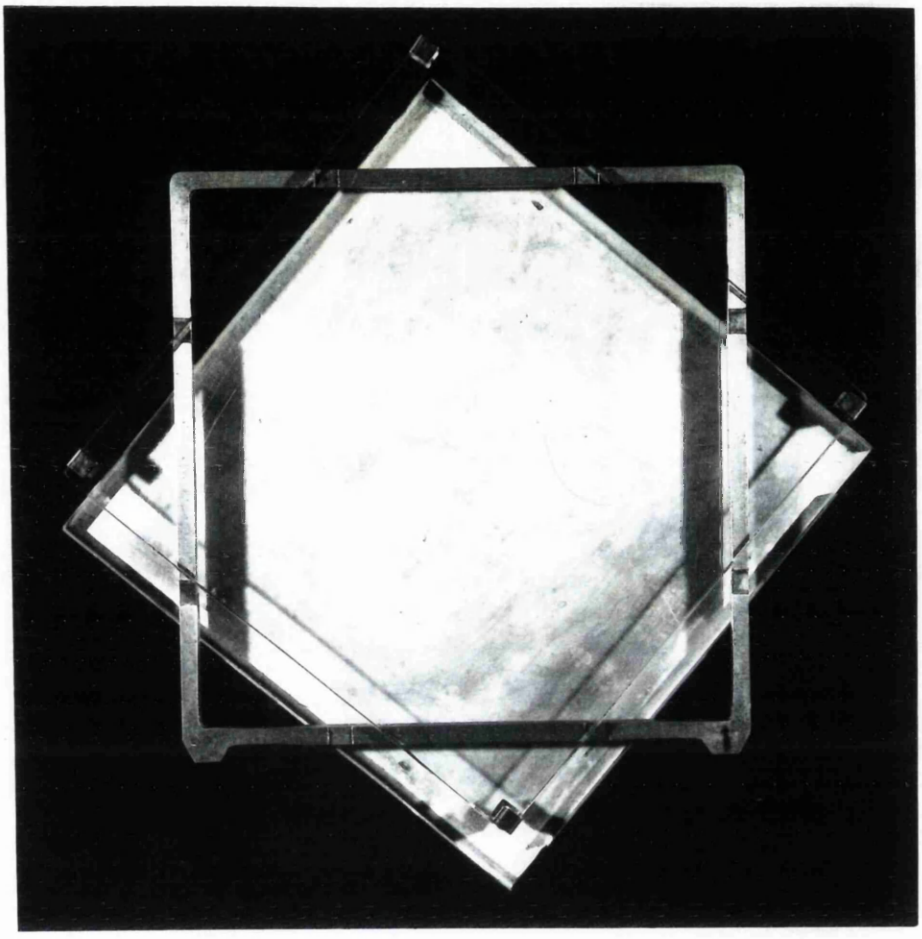


Fig. 2-3. Glass plate, metal frame and aluminium cover used for the determination of ampicillin by microbiological assay.

The two litres were then divided into 310 ml volumes, which were autoclaved and then stored at 0 - 4 º C until the day of assay. On the day of assay, the autoclaved bottles containing the agar were liquefied in the steamer and placed in a 54 º C bath for a minimum period of one hour. Each bottle was then inoculated with one millilitre of a 1 in 20 sterile distilled water dilution of *Bacillus subtilis* spore suspension (Subtilis Spore Suspension, Difco Laboratories, Detroit, USA). The agar inoculation was gently swirled to prevent bubble formation while mixing the spore suspension. Immediately after inoculation and thorough mixing, the agar was poured in a circular motion onto the plate covering equally all the parts of the plate. The plated agar was then left to cool for half an hour on the levelling stand and subsequently placed over a template. Sixty-four holes were punched in an even matrix pattern with a hand-held agar-cutting tool of nine millimetres external diameter and the contents of the wells were removed by suction.

The 64 wells were then inoculated with seven plasma standards and ten samples. Each sample and five of the plasma standards were inoculated in four different wells with the remaining two standards inoculated in two wells each. All the samples and standards were distributed throughout the plate in a Quasi-Latin square design (Sutherland & Rolinson, 1978). Following inoculation the plates were incubated for 18 hours at 31º C.

2.4.5 Reading the plates

The zones of inhibited bacterial growth (Fig. 2-4) were measured using an electronic digital calliper (Electronic digital calliper Max-Cal 0-6", CP Instruments, Bishop Stortford, England) and recorded on a printer/calculator (QC-Printer, CP Instruments, Bishop Stortford, England) connected to the calliper (Fig. 2-5). This recorder calculated the mean of the measurement of the four wells corresponding to each standard or sample.

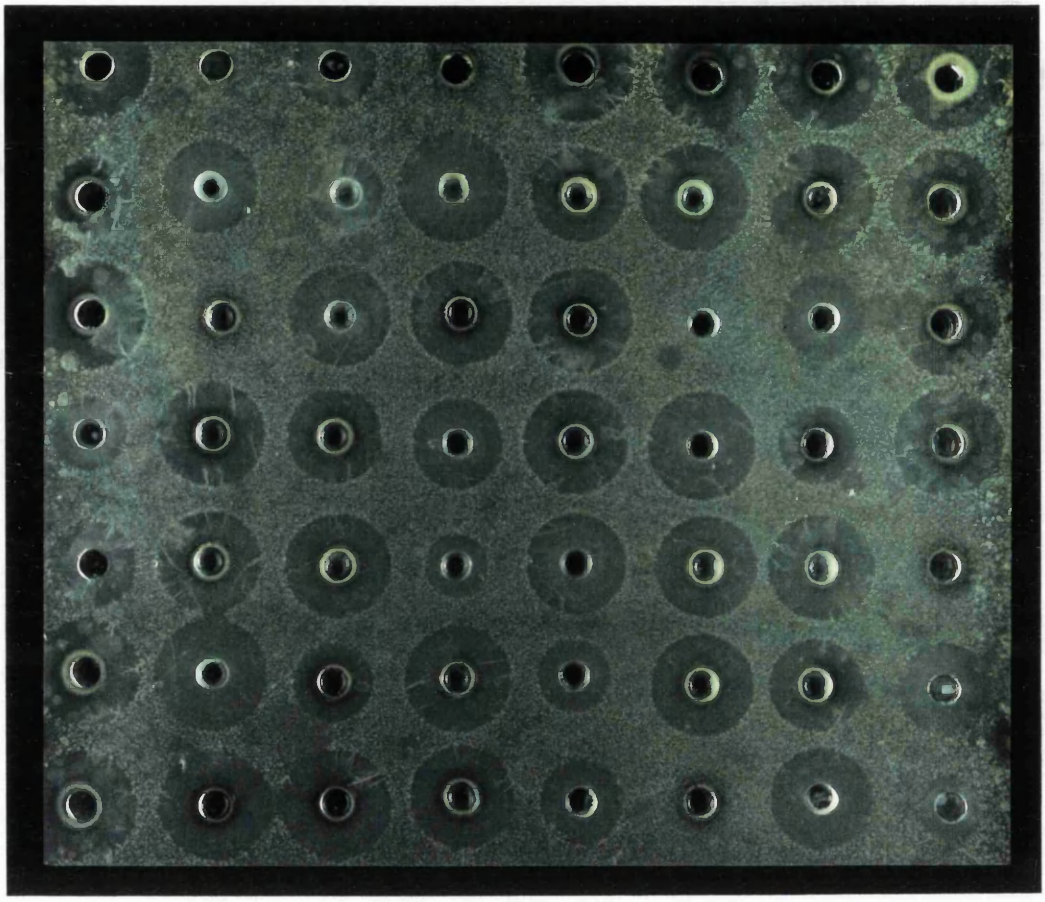


Fig. 2-4. Antibiotic agar number 2 after 24 hour incubation period showing zones of bacterial growth inhibition corresponding to different antibiotic concentrations inoculated.

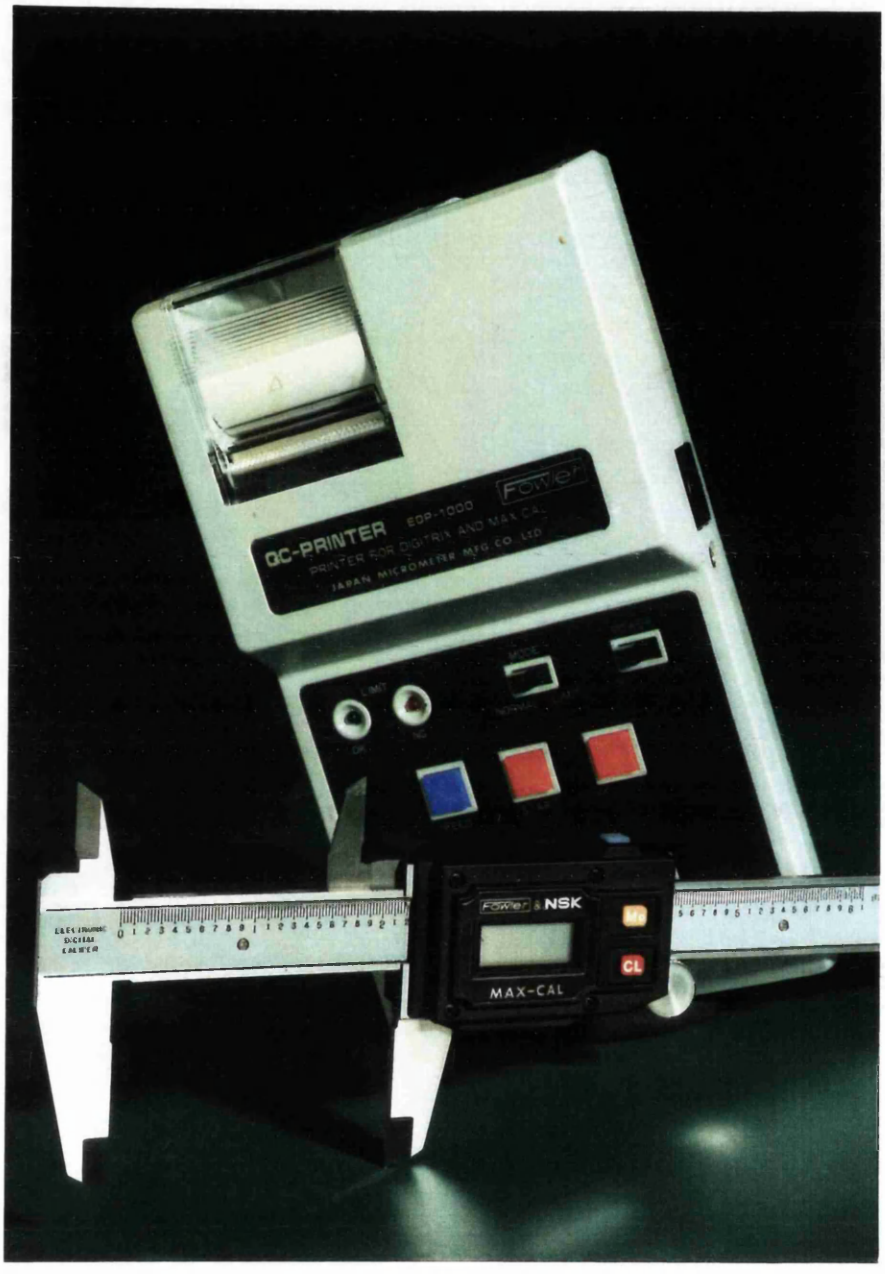


Fig. 2-5. Digital calliper and recorder for the measurement of zones of bacterial growth inhibition.

When all the measurements were taken, the diameters of the inhibition zones around the ampicillin standards allowed preparation of a standard curve from which unknown sample concentrations could be measured and allowed the calculation of a correlation coefficient for the accuracy of each assay.

2.4.6 Sensitivity and reproducibility of the assay

To evaluate the sensitivity of the assay the bacterial growth inhibition zone diameters from 12 replicates (4 measurements per sample in each replicate) of a range of ampicillin standards were plotted against the $\log_{10}$ of the concentrations. The best fit line for ampicillin was described by the equation $y= 10.448 \log_{10}(x) +28.334$ where y is the zone of inhibition diameter in mm and x is the concentration (μg/ml). In addition, a good linear correlation coefficient (r) of 0.996 was obtained for ampicillin in plasma standards. Considering that the minimum zone of inhibition that could be measured accurately in this assay was 12 mm, a limit of detection of 0.03 μg/ml was found after replacing y=12 in the equation.

To assess the accuracy and reproducibility of the microbiological assay a range of standards was inoculated on four different plates on the same day and on eight different plates on separate days. The readings allowed the calculation of the coefficient of variation by dividing the standard deviation by the mean of the zone diameter for a particular concentration of ampicillin. The results from the samples analysed the same day and on separate days showed little variation and good reproducibility as shown by the coefficients of variation of less than 5% (Table 2-1).

2.5 Probenecid analysis

2.5.1 Standard preparation and extraction

Probenecid was analysed by high performance liquid chromatography (HPLC).

Conc. (μg/ml)	Zone diameter (mm) (mean ± SD)	Coefficient of Variation (%)	
		Within-day	Between-day
0	0.00 ±0.00 (n=12)	0.00 (n=4)	0.00 (n=8)
0.25	21.18 ±0.77 (n=12)	4.36 (n=4)	3.27 (n=8)
0.5	25.41 ±0.77 (n=12)	2.12 (n=4)	3.43 (n=8)
2	32.48 ±1.51 (n=12)	4.00 (n=4)	3.40 (n=8)
5	36.17 ±1.26 (n=12)	1.55 (n=4)	3.45 (n=8)
10	38.68 ±1.39 (n=12)	1.36 (n=4)	3.79 (n=8)
20	41.14 ±1.52 (n=12)	1.04 (n=4)	3.83 (n=8)

Table 2-1. Zone diameters (mean ± SD) and coefficients of variation of ampicillin in equine plasma.

An initial probenecid (Sigma Chemical Co Ltd., Dorset, UK) stock solution was prepared in methanol (Methanol HPLC grade, Rathburn Chemicals Limited, Walkerburn, Scotland) at a 20 mg/ml concentration. Subsequently, serial dilutions of the initial stock solution were prepared at 2 mg/ml, 500 μg/ml, 100 μg/ml and 20 μg/ml. A range of standards in blank equine plasma was then prepared at 500 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml, 10 μg/ml, 2 μg/ml, and 0 μg/ml.
The extraction method was carried out by adding 1 ml of 0.1 N HCl (Convol ®, The British House Chemicals Limited) and 5 ml of dichloromethane (DCM, Rathburn Chemicals Limited, Walkerburn, Scotland) to 1 ml of standard in plasma. The mixture was gently mixed in a rotor for ten minutes at 30 rpm and then centrifuged for ten minutes at 1700 *g*. After centrifugation, the aqueous layer was removed by aspiration and 3 ml of the remaining organic phase were transferred into clean glass tubes and evaporated under air at 50 ° C. The residues were then reconstituted in methanol and injected into the HPLC system.

2.5.2 Injection

A Gilson pump (Model 302, Scotlab, Coatbridge, UK) equipped with a Gilson manometric module (Model 802, Scotlab, Coatbridge, UK) was used to pump the mobile phase through an ODS hypersil column (5 μ, 100 x 5 mm) (Shandon Scientific Ltd, Cheshire, England, UK). A variable λ uv/vis detector (Model SP8450, Spectra Physics, Burke Electronics, Glasgow, UK) was connected to the column and the results were recorded on a variable chart recorder set at 10mV with a 5 mm/min speed (Vitatron, M. S. E. Scientific, Crawley, UK). The mobile phase was a mixed solution of methanol (Methanol HPLC Grade, Rathburn Chemicals Limited, Walkerburn, Scotland, UK), potassium permanganate distilled water (60:40) and perchloric acid (1.2 ml per 500ml of mobile phase). The perchloric acid used was a one in fifty-five water dilution of a 70% perchloric acid solution with specific gravity of 1.7 (Analar, BDH Chemicals Limited, Poole, England, UK).

Before pumping the mobile phase through the HPLC system, it was degassed for fifteen to thirty minutes using a sonic bath and a vacuum pump. When the mixture was free of air bubbles, it was pumped at 1 ml/min with the detector set at a wavelength of 254 nm and the absorbance at 0.02 absorbance unit full scale (AUFS). Once the baseline became stable, normally 30 minutes after the start of pumping, the plasma drug standards and the samples were injected into the system. After every two samples, a probenecid standard made up in methanol at a 20 parts per million concentration, was injected. The values obtained from the probenecid standards were used to evaluate the recoveries of the plasma standards and work out the standard curve for the calculation of plasma probenecid concentrations. A retention time (time from injection of the sample until appearance of its corresponding peak on the chromatogram) of 2.5 minutes was observed for probenecid (Fig. 2-6).

2.5.3 Sensitivity and reproducibility of the assay

To evaluate the reproducibility of the analysis, results from probenecid plasma standards ranging from 2 to 500 µg/ml were compared by means of a coefficient of variation test. The coefficient of variation was calculated by dividing the standard deviation of the recovery by the mean recovery for a particular probenecid concentration. Three sets of standards analysed during the same day showed little variation as shown by a maximum coefficient of variation of 5.65 %. When the standards were analysed on separate days, the reproducibility was also good as reflected in a maximum coefficient of variation of 6.01 % (Table 2-2). The mean recovery of probenecid plasma standards (range 2 - 500 µg/ml) was 100.23 %.

To evaluate the sensitivity and linearity of the assay the probenecid plasma peak concentrations were plotted versus the peak heights obtained. A good linearity (r=1.00) was obtained between the plasma probenecid concentration and the peak heights.

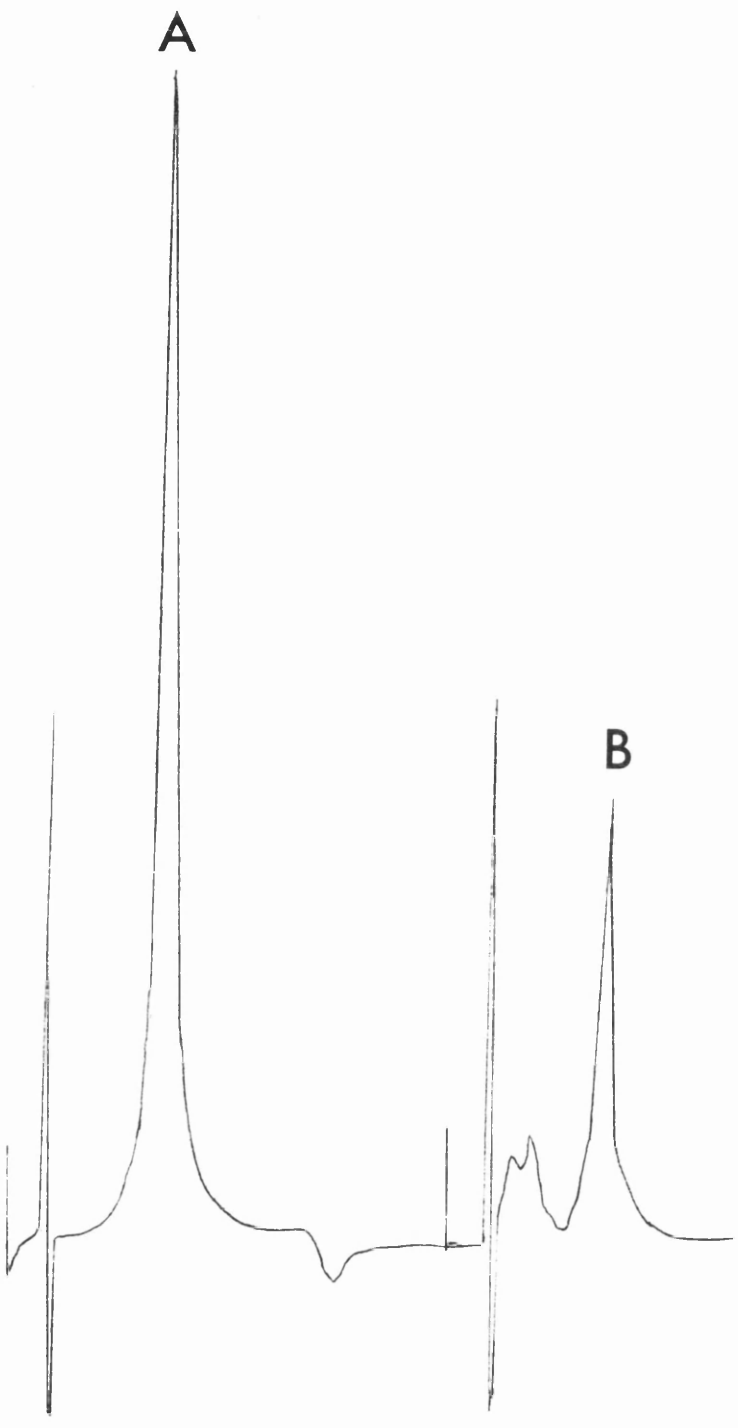


Fig. 2-6. Typical chromatographic trace of probenecid.
A : Probenecid in buffer. B: Probenecid in equine plasma

Conc. (μg/ml)	Recovery (%) (mean)	Coefficient of Variation (%) Within-day	Between-day
2	99.57 (n=6)	NS	5.34 (n=6)
10	96.70 (n=9)	3.63 (n=3)	4.63 (n=6)
25	99.71 (n=9)	1.07 (n=3)	6.01 (n=6)
50	98.67 (n=9)	5.65 (n=3)	3.85 (n=6)
100	104.32 (n=9)	2.50 (n=3)	2.47 (n=6)
500	102.43 (n=9)	1.57 (n=3)	4.58 (n=6)
Mean	100.23 (n=51)	2.88 (n=15)	4.48 (n=36)

Table 2-2. Mean recoveries and coefficients of variation of probenecid in plasma samples.

The best fitting line equation obtained (y= 2.22x + 0.662) was used to calculate the limit of detection of 0.15 μg/ml for probenecid in plasma taking one unit as the minimum height peak that could be read accurately.

2.5.4 Effects of probenecid on the microbiological assay

To ensure that the presence of probenecid in the plasma samples did not affect the bioassay results, three sets of plasma standards containing ampicillin, ampicillin in combination with probenecid and probenecid on its own, were measured by microbiological assay.

The results showed that the presence of probenecid did not enhance nor reduce the activity of ampicillin as shown by the coefficients of variation of less than 1 % when the zone diameter of plasma containing ampicillin was compared to the zones obtained for the ampicillin and probenecid combination (Table 2-3). In addition, no bacterial growth inhibition was observed when the plasma standards inoculated contained only probenecid.

2.6 Pharmacokinetic analysis

Plasma concentration versus time data were analysed by compartmental model analysis and noncompartmental model analysis described in the literature (Baggot, 1977a; Gibaldi & Perrier, 1975; Kinabo & McKellar, 1989; Riegelman & Collier, 1980; Sams, 1987).

2.6.1 Compartmental model analysis

Plasma concentrations versus time from each animal were analysed using a curve stripping computer programme CSTRIP (Sedman & Wagner, 1976).

Concentration (μg/ml)	1- Ampicillin (mm) (mean ±SD)	2- Ampicillin & Probenecid (mm) (mean ±SD)	Coefficient of Variation (%)
0	0 ± 0.00	0 ± 0.00	(n=8) 0.00
2	31.13 ± 0.11	31.26 ± 0.21	(n=8) 0.64
5	34.48 ± 0.09	34.75 ± 0.19	(n=8) 0.63
10	37.48 ± 0.21	37.67 ± 0.18	(n=8) 0.64
20	39.59 ± 0.33	39.64 ± 0.18	(n=8) 0.64

Table 2-3. Zone diameters (mean ± SD) and coefficients of variation of plasma standards containing ampicillin and ampicillin with probenecid.
The concentrations (μg/ml) are both for ampicillin and probenecid.

The observed plasma concentrations were fitted with mono-, bi- and triexponential equations in the form of $Cp= Be^{-\beta t}$, $Cp=Ae^{-\alpha t} + Be^{-\beta t}$ and $Cp= Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ respectively. Cp represents the plasma concentration, P and A are the intercepts of the distribution slopes and B is the intercept of the elimination slope on the y-axis while π and α the distribution constants and β the overall elimination rate constant. The number of coefficients and exponents of the equations best describing the data were confirmed using Akaike's information criterion (Yamaoka *et al.*, 1978). Plasma distribution half-lives ($t1/2\pi$ and $t1/2\alpha$) were estimated as $0.693/\pi$ and $0.693/\alpha$ and the elimination half-life ($t1/2\beta$) was calculated as $0.693/\beta$. The elimination half-life represents the time taken for the plasma drug concentration to decrease by half its value.; where β is the hybrid rate constant for the terminal phase of drug concentration decline in plasma. The initial plasma concentration at time 0 for an intravenous bolus administration, Cp0 (μg/ml) was calculated by adding the y-axis intercepts P, A and B or A and B for a biexponential curve. The area under the zero moment curve (AUC), in μg.h/ml, was calculated by adding the y- axis intercepts and exponents ratios i.e. $A/\alpha + B/\beta$ for a biexponential curve. The apparent volume of the central compartment Vc (ml/kg) was calculated as the dose, D in the appropriate units, divided by Cp0. Whenever the drug fitted a two-compartmental model, the apparent volume of the distribution, Vd(area) in ml/kg was calculated by the area method, where $Vd(area)= D/AUC\cdot\beta$. The apparent volume of distribution at steady-state, Vdss in ml/kg, was calculated with the equation $Vdss= [(k_{12} + k_{21})/k_{21}]\cdot Vc$ in a two-compartment system and $Vdss= [(k_{21}k_{31} + k_{12}k_{31} + k_{21}k_{13}) /k_{21}k_{31}]\cdot Vc$ in a three-compartment system were k_{12}, k_{21}, k_{13}, and k_{31} represent the first-order transfer rate constants between the central and the peripheral compartments. Finally, the total body clearance Clb (ml/h·kg) was calculated as Clb = D/AUC and the rate of elimination from the central compartment, k_{el} (/h), was calculated as $k_{el}= Clb/Vc$.

2.6.2 Non-compartmental model analysis

Various parameters were calculated by non-compartmental analysis based on the statistical moments theory. The area under the plasma concentration versus time curve for observed values (AUC_{obs}) from 0 to ∞ in μg.h/ml was calculated with the linear trapezoidal equation where $AUC_{obs} = [(C_n + C_{n-1})/2].\Delta t$ with C representing the plasma concentrations, and $\Delta t = t_n - t_{n-1}$ where $n-1$ and n are adjacent data point times. When the drug was administered IV, the AUC_{obs} for the first trapezoid, $AUC_{first-obs}$ was calculated by substituting C_{n-1} by the initial plasma concentration at time 0, *ie.* Cp0. The area under the last triangle, $AUC_{last-obs}$ was calculated by dividing the last detectable concentration C_{last} by the elimination rate constant β, when the drug was administered IV. When the drug was administered orally, C_{last} was divided by the slope of the best fitting line, λ_z, obtained after plotting three or more points in a semilogarithmic graph to a monoexponential equation (Riegelman & Collier, 1980). The area under the first moment curve $AUMC_{obs}$ from 0 to ∞ in $\mu g.h^2/ml$ was calculated with the linear trapezoidal equation $AUMC_{obs} = [(t_n C_n + t_{n-1}C_{n-1})/2].\Delta t$. The $AUMC_{first-obs}$ was calculated as $[(t_{n-1}C_{n-1})/2]\cdot\Delta t$ since tn was 0. For the last triangle, $AUMC_{last-obs}$ was calculated with the extrapolation equation where $AUMC_{last-obs} = (t_{last}\cdot C_{last})/\beta + C_{last}/(\beta)^2$ when the drug was administered IV. When the drug was administered orally, the β value was substituted by λ_z.

The mean residence time, MRT, was calculated as $MRT = AUMC_{obs}/AUC_{obs}$ and expressed in hours. The MRT is defined as the average time that a molecule spends in the body between the time it is administered and the moment when it is eliminated or as the time for 63.2% of the administered dose to be eliminated by all processes. In addition, the total body clearance observed Clb_{obs} (ml/h·kg) was calculated as the dose administered IV, D (mg/kg), divided by the AUC_{obs} (μg.h/ml). The volume of distribution at steady-state was subsequently calculated as $Vdss_{obs} = Clb_{obs}\cdot MRT$.

Whenever the drug was administered orally, the mean absorption time MAT in hours, was calculated as the difference between MRT following oral administration and MRT following intravenous administration. In addition, the maximum plasma concentration after oral administration C_{max} and the time at which this concentration was achieved, t_{max}, were noted.

Finally, the oral bioavailability, F (%), which represents the extent of drug absorption into the systemic circulation following oral administration, was calculated by dividing the AUC_{obs} following oral administration by the AUC_{obs} following IV administration.

Chapter 3

Studies on the effects of urinary pH on plasma pharmacokinetics of ampicillin in the horse

3.1 Introduction

Renal excretion is probably the most common route for the elimination of polar, water-soluble drugs from the body and an alteration in the rate of renal excretion should affect the plasma disposition of drugs excreted by this pathway. However, this is not a feature common to all drugs and changes in the renal excretion of phenylbutazone, amphetamine and procaine did not affect their overall plasma disposition in the horse (Piperno *et al.*, 1968; Houston *et al.*, 1985; Baggot *et al.*, 1972; Evans & Lambert, 1974). It has been claimed that urinary pH is an important determinant for plasma concentrations of penicillin and cephalothin (Whelton *et al.*, 1967), although most of the studies undertaken when the urinary pH was altered have measured exclusively the urine concentrations of the drugs and not the plasma concentrations. Plasma pharmacokinetic studies on penicillins where the urinary pH was artificially altered have not been described in the horse.

In the present study, a two-part experiment was undertaken to evaluate the effects of artifitial alteration of urinary pH on the plasma disposition of ampicillin sodium in the horse. In the first part, sodium bicarbonate a urine alkalinizer, was administered orally to horses until the urine became alkaline, ampicillin sodium was then administered intravenously and the plasma disposition of the drug determined. The same model was used when the urine was acidified by oral administration of ammonium chloride. In addition, urinary concentrations of the antibiotic, volume of urine excreted and urinary pH were measured during the forty-eight hours following the administration of ampicillin sodium.

3.2 Materials and methods

3.2.1 Animals

Three Thoroughbred geldings and a riding mare (numbers 1, 2, 3 and 6) and weighing 621, 587, 510, and 494 kg, respectively, were used.

3.2.2 Alkalinisation of urine

Urinary pH was monitored for two days before administration of the alkaliniser (days 0 and 1). Sodium bicarbonate (Analar, BDH Chemicals, Merck Ltd, Dorset, England) was suspended in 500 ml of water and then administered via stomach tube during six consecutive days (days 2, 3, 4, 5, 6, and 7) at the dose rates of 100, 200, 300, 400, 400 and 400 mg/kg respectively until the urine became consistently alkaline (pH > 7.91 ± 0.13). Ampicillin sodium was administered IV on day 6 at a 10 mg/kg dosage rate. A washout period of sixty days was allowed before starting the second part of the study.

3.2.3 Acidification of urine

Acidification of urine was carried out by administering ammonium chloride (Analar, BDH Chemicals, Merck Ltd, Dorset, England) via stomach tube. The urinary pH values during the two days prior to the start of ammonium chloride administration (days 0 and 1) were used as baseline values. To achieve urinary acidification, ammonium chloride was diluted in 500 ml of water and administered during five consecutive days (days 2, 3, 4, 5 and 6) at the doses rates of 200, 300, 400, 400 and 400 mg/kg respectively until the urine became consistently acidic (pH < 4.24 ± 0.13). Ampicillin sodium was administered IV on day 5 at a 10 mg/kg dosage rate.
Blood sampling, storage and measurement of ampicillin were carried out as described previously (See General Materials and Methods).

3.2.4 Urine collection

The urine was collected in plastic urine bags positioned under the geldings' penis (Fig. 3-1). These were emptied and their position checked regularly to ensure that all the urine was collected. A fresh urinary catheter was used once daily to collect the urine from the mare (animal number 6).

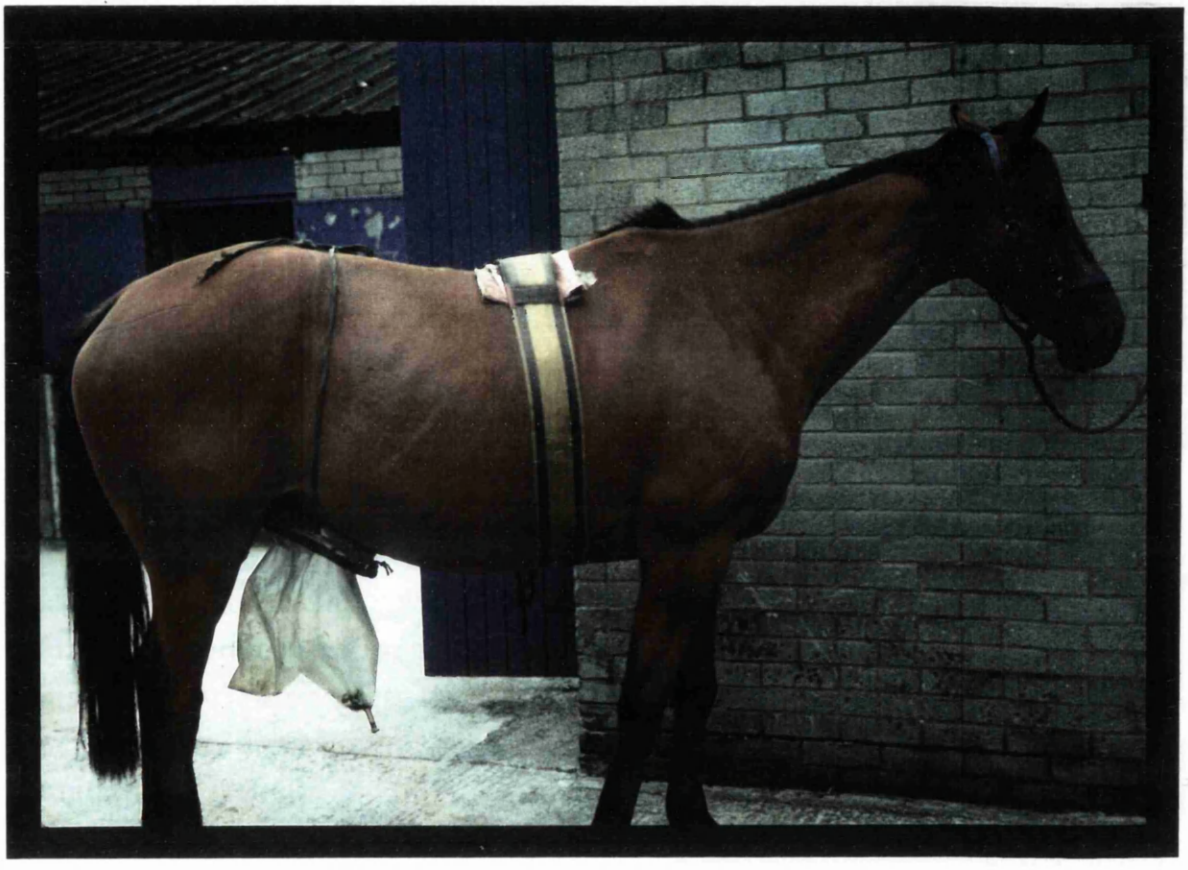


Fig. 3-1. Urine collection bag in animal number 2.

Following administration of ampicillin sodium in both parts of the study, all the urine was collected for 48 hours. During this period an indwelling Foley catheter (Argyle, Sherwood Medical) was placed in the mare's urethra and connected to a urine bag (Fig. 3-2). The volume and pH of each sample collected during the 48 h period were measured. From each sample 10 ml of the urine was stored at 0 - 4 ºC for retrospective measurement of ampicillin concentrations.

3.2.5 Urine pH measurement

The pH values were determined with a portable pH meter (Whatman®, pHμ-Sensor) which was calibrated before the measurement of each urine sample collected, using standard pH buffers (Buffer solutions, BDH Chemicals, Merck Ltd, Dorset, England). The time of collection and the pH measured were systematically recorded.

3.2.6 Urine ampicillin measurement

A similar bioassay was utilised for urine measurement of ampicillin concentrations as the one used for plasma ampicillin measurement. However the samples were diluted in blank urine in order to bring them within the range of the standard curve. Standards of 0, 2, 5, 10, 20, 40 and 80 μg/ml were used when urine concentrations were measured.

3.2.7 Statistical analysis

Statistical evaluation was carried out using a paired Student's t-test to compare plasma ampicillin concentrations and urine pH values. A Mann Whitney-U test for nonparametric data was used to compare pharmacokinetic parameters following urinary acidification and alkalinisation (Powers, 1990). A value of $P < 0.05$ was considered to be statistically significant, and $P < 0.01$ was considered to be highly significant.

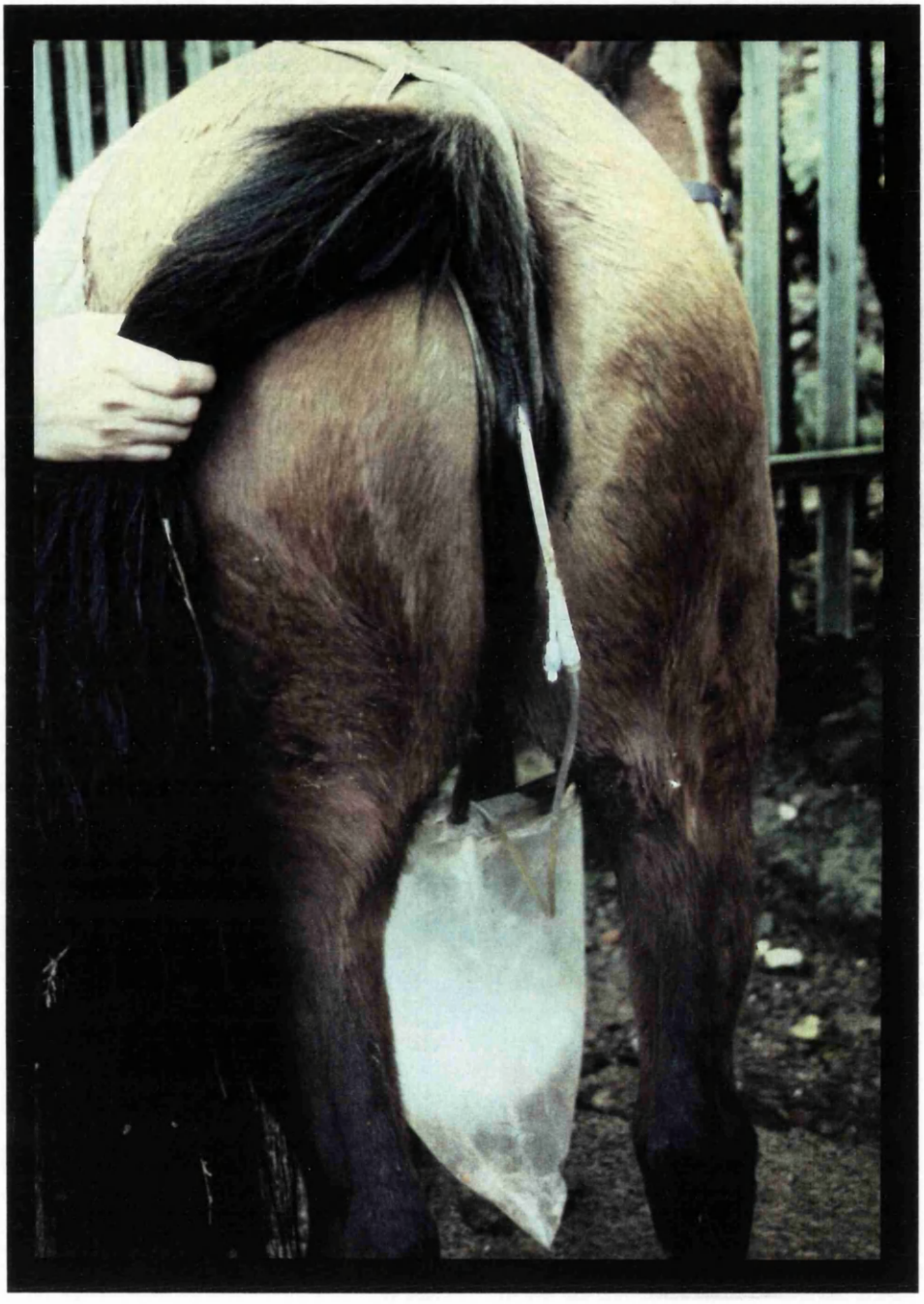


Fig. 3-2. Foley catheter in position in the mare's urethra and connected to a urine collection bag.

3.2.8 *In vitro* degradation of ampicillin

To evaluate the effects of alkalinisation on urine samples containing ampicillin an *in vitro* study was carried out. Blank urine (pH = 7.2) was collected and ampicillin urine standards of 5, 10, 20, 40 and 80 µg/ml were prepared as described previously. In addition, part of the blank urine was alkalinized by adding sodium bicarbonate until the pH became consistently alkaline (pH = 8.5). The same ampicillin urine standards were then prepared in the basic urine. Both sets of urine standards, were kept at 0 - 4 º C and measured 1, 4, 7 and 10 days thereafter. Each day new freshly made urine standards, were measured together with the original sets. This permitted observation of the degree of degradation of ampicillin in normal and alkaline urine.

3.3 Results

3.3.1 Alkalinisation of urine

3.3.1.1 Urinary pH values

The administration of sodium bicarbonate orally resulted in a rise in the urinary pH from the 4th day of administration (day 5) onwards. The pH became gradually more alkaline as the dose of sodium bicarbonate was increased. A dose of 300 mg/kg was needed to increase the pH substantially and with a dose of 400 mg/kg the pH stabilised at around a value of pH = 8 (Fig. 3-3). On day 4 of administration of sodium bicarbonate (day 5) the urinary pH was significantly different ($P < 0.05$) from the normal values and on days 6, 7 and 8 the urinary pH values became highly significantly different ($P < 0.01$) from the normal values (Table 3-1).

3.3.1.2 Urine volume.

The urine volumes collected during the 48 hours following the administration of ampicillin sodium are shown in Table 3-2.

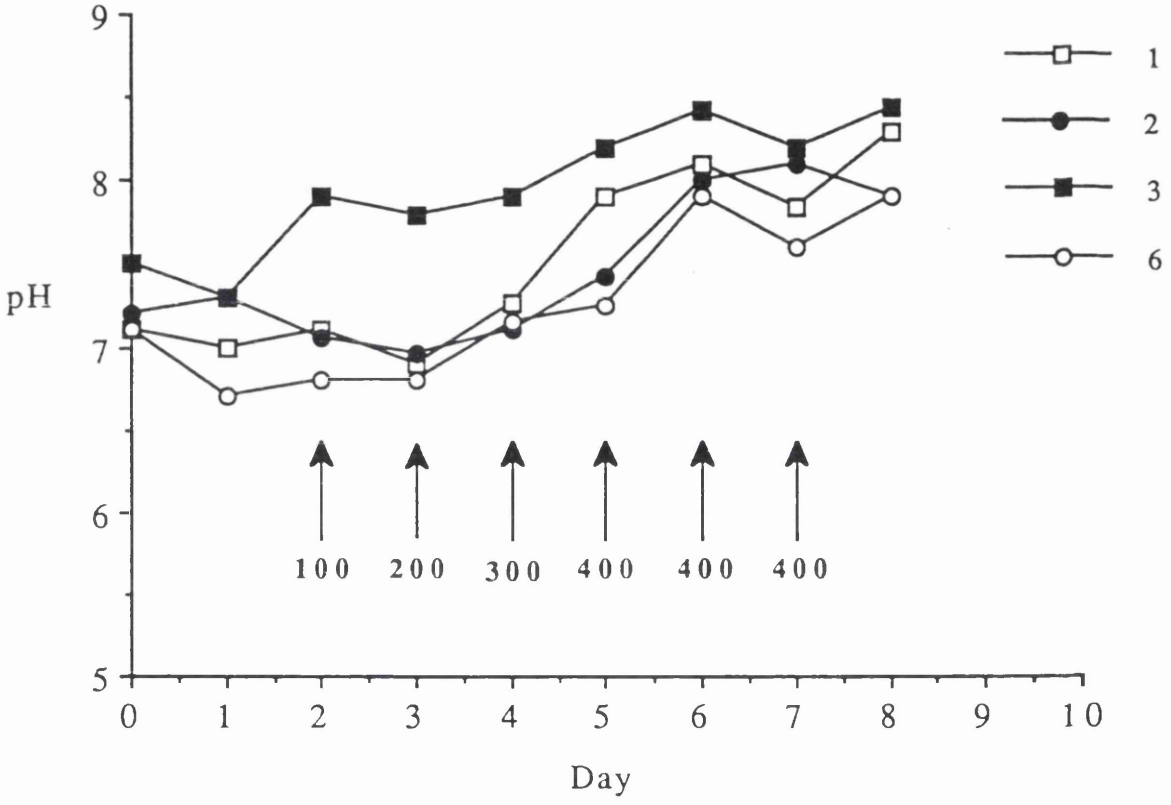


Fig. 3-3. Urine pH values in 4 horses during administration of sodium bicarbonate. Arrows, indicate daily administration of sodium bicarbonate (100, 200, 300, 400 [x 3] mg/kg). Ampicillin sodium was administered at a dose rate of 10 mg/kg on day 6.

		Animal number			
Day	Dose	1	2	3	6
0	0	7.10	7.20	7.10	7.50
1	0	6.70	7.30	7.00	7.30
2	100	6.80 ± 0.20	7.05 ± 0.15	7.03 ± 0.09	7.90
3	200	6.80 ± 0.10	6.97 ± 0.12	6.90 ± 0.21	7.80
4	300	7.15 ± 0.15	7.10	7.27± 0.26	7.90
5*	400	7.25 ± 0.25	7.43 ± 0.09	7.90 ± 0.04	8.20
6**	400	7.82 ± 0.08	8.10 ± 0.13	8.10 ± 0.05	8.40 ± 0.03
7**	400	7.60 ± 0.05	8.10 ± 0.26	7.80 ± 0.09	8.16 ± 0.16
8**	0	7.90 ± 0.25	7.90	8.30 ± 0.07	8.43 ± 0.03

Table 3-1. Mean ± S.E.M. urinary pH values in four horses after administration of sodium bicarbonate. Dose in mg/kg.

* Significantly different ($P<0.05$)

** Highly significantly different ($P<0.01$)

	Animal number				
	1	2	3	6	Mean ± SEM
Urine volume (l)	14.48	8.89	12.38	9.12	11.22 ± 1.34
Animal weight (kg)	621	587	510	494	553 ± 30.42
Daily urinary excretion (ml/kg)	11.66	7.58	12.14	9.23	10.15 ± 1.06

Table 3-2. Urine volume and excretion during 48 hours after administration of ampicillin sodium to horses with alkaline urine.

A mean ± S.E.M value of 11.22 ± 1.34 litres was collected from the animals. The mean daily excretion of urine per animal was calculated to be 10.15 ± 1.06 ml/kg of bodyweight.

3.3.1.3 Plasma ampicillin concentration

Ampicillin sodium was administered when the urinary pH was 8.1 ± 0.12. The plasma ampicillin concentration (Table 3-3; Appendix A Tables A-1 and A-2) versus time data fitted a bi-exponential decay curve (Fig. 3-4). None of the pharmacokinetics values obtained (Tables 3-4 and 3-5; Appendix A Tables A-6 and A-7) were statistically different from the values obtained for horses with normal urinary pH (Appendix A Tables A-4 and A-5) or from the results obtained during the acidification of urine (Appendix A Tables A-8 and A-9).

3.3.1.4 Urine ampicillin amount (fraction of dose)

The results from the urine concentrations of ampicillin sodium show that only a small percentage of the ampicillin sodium administered intravenously was recovered in the urine. Only 14.20 ± 1.92 % of the total dose administered was recovered in the alkaline urine. Of the total ampicillin recovered in urine, more than 90% was excreted during the first six hours. However after 30 hours some ampicillin sodium could still be detected in urine from all four animals and some ampicillin could be measured in one of the animals after 36 hours (Table 3-6; Fig. 3-5).

3.3.2 Acidification of urine

3.3.2.1 Urinary pH values

A highly significant reduction ($P < 0.01$) of the urinary pH (pH of 4.24 ± 0.13) was achieved in all animals when ammonium chloride was administered at a 400 mg/kg dosage on the fourth day of administration (day 5). Urinary pH levels ranging between 3 and 5 were maintained during day 6 and 7 of the study. (Fig. 3-6; Table 3-7).

Time (h)	Normal (n=4) * Mean ± SEM	Alkaline (n=4) Mean ± SEM	Acidic (n=4) Mean ± SEM
0.0333	57.22 ± 2.13	60.33 ± 4.18	66.45 ± 2.37
0.0833	47.38 ± 2.95	50.24 ± 2.43	61.02 ± 4.14
0.25	35.28 ± 1.74	36.34 ± 3.76	38.44 ± 1.22
0.50	24.59 ± 3.07	23.24 ± 1.65	24.59 ± 1.16
0.75	16.44 ± 2.38	14.15 ± 0.27	16.28 ± 0.56
1	10.85 ± 1.97	11.61 ± 0.55	11.13 ± 0.59
1.5	6.11 ± 0.98	7.88 ± 0.94	5.76 ± 0.45
2	3.27 ± 0.37	4.03 ± 0.38	3.52 ± 0.33
4	0.63 ± 0.21	0.92 ± 0.25	0.61 ± 0.10
8	0.04 ± 0.03	0.02 ± 0.01	0.05 ± 0.02
12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3-3. Plasma concentrations of ampicillin in horses with normal, alkaline and acidic urine.

* Three of the animals from Horspool *et al.*, 1992.

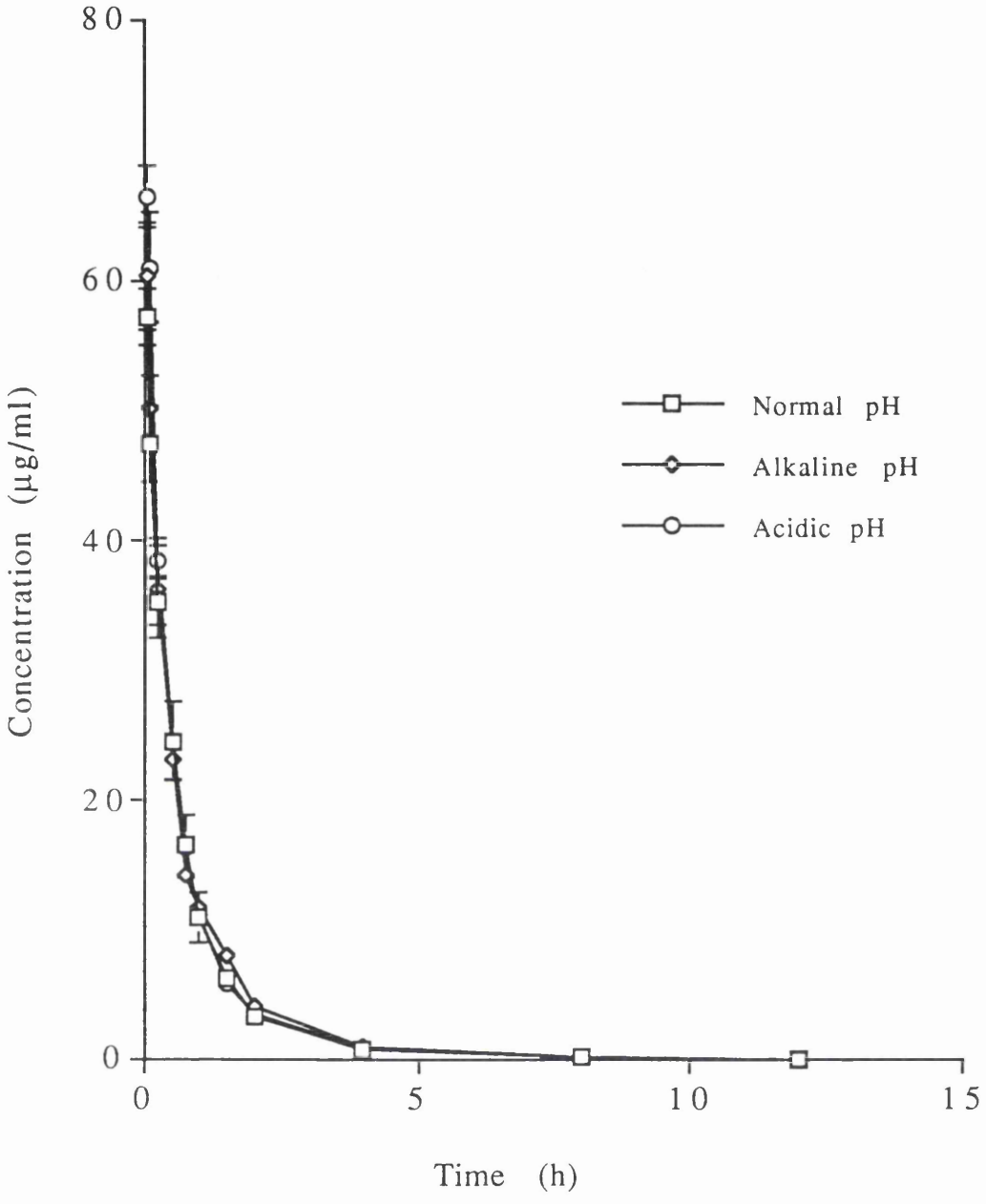


Fig. 3-4. Mean ± SEM (µg/ml) concentration of ampicillin in plasma of horses with normal, alkaline and acidic urine after IV administration of ampicillin sodium at a dose rate of 10 mg/kg.

Variable	Normal urine * (n=4)	Alkaline urine (n=4)	Acidic urine (n=4)
α (/h)	3.72 ± 1.14	6.64 ± 2.85	3.93 ± 0.46
β (/h)	0.88 ± 0.13	0.90± 0.07	0.85 ± 0.12
t1/2 α (h)	0.186	0.105	0.176
t1/2 β (h)	0.787	0.772	0.820
Cp0 (μg/ml)	60.16 ± 3.01	71.31 ± 8.72	73.91 ± 4.24
AUC (μg.h/ml)	39.01 ± 3.98	40.53 ± 1.57	41.61 ± 1.62
Vc (ml/kg)	167.47 ± 8.35	145.92 ± 15.67	136.65 ± 7.87
Vd_{area}(ml/kg)	316.40 ± 40.30	281.37 ± 23.55	300.22 ± 37.80
Vdss (ml/kg)	230.55 ± 18.16	235.47 ± 20.79	220.35 ± 15.95
Clb (ml/h.kg)	265.30 ± 29.63	248.30 ± 9.84	241.35 ± 9.28
k_{21} (/h)	2.22 ± 0.93	3.14 ± 1.08	1.90 ± 0.38
k_{el} (/h)	1.59 ± 0.18	1.78 ± 0.24	1.78 ± 0.08
k_{12} (/h)	0.78 ± 0.29	2.62 ± 1.59	1.104 ± 0.16

Table 3-4. Pharmacokinetic values obtained after compartmental analysis in horses with normal, alkaline and acidic urine.
* Three of the animals from Horspool *et al.*, 1992.

Variable	Normal urine * (n=4)	Alkaline urine (n=4)	Acidic urine (n=4)
AUC_{obs} (μg.h/ml)	39.17 ± 4.10	41.75 ± 1.31	41.89 ± 1.66
$AUMC_{obs}$ ($\mu g.h^2/ml$)	33.57 ± 6.44	38.61 ± 5.07	34.73 ± 3.24
MRT (min)	50.32 ± 4.65	55.09 ± 6.13	49.57 ± 3.63
$Vdss_{obs}$ (ml/kg)	217.17 ± 16.27	220.17 ± 21.17	197.47 ± 13.92
Clb_{obs} (ml/h.kg)	264.32 ± 29.04	240.26 ± 7.79	239.81 ± 13.92

Table 3-5 Pharmacokinetic values obtained after non-compartmental analysis in horses with normal, alkaline and acidic urine. * Three of the animals from Horspool *et al.*, 1992.

	Animal number				
Time (h)	1	2	3	6	Mean ± SEM
6	17.17	15.82	8.88	11.67	13.39 ± 1.90
12	1.16	0.18	0.78	0.45	0.64 ± 0.21
18	0.08	0.06	0.05	0.21	0.10 ± 0.03
24	0.04	0.04	0.06	0.00	0.03 ± 0.01
30	0.02	0.03	0.01	0.03	0.02 ± 0.00
36	0.00	0.00	0.05	0.00	0.01 ± 0.01
48	0.00	0.00	0.00	0.00	0.00 ± 0.00
Total	18.47	16.13	9.83	12.36	14.20 ± 1.92

Table 3-6. Percentages of ampicillin sodium recovered in alkaline urine after IV administration of ampicillin sodium at a 10 mg/kg dosage rate.

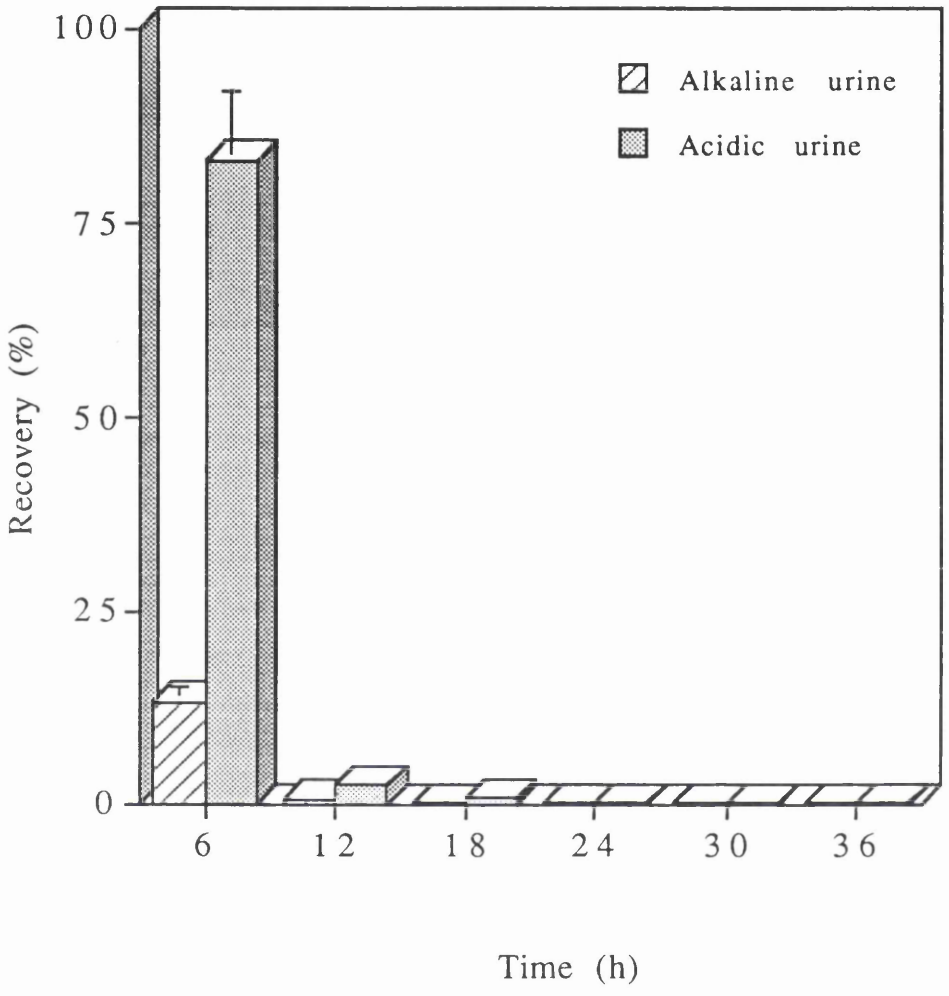


Fig. 3-5. Urinary recoveries of ampicillin following administration of ampicillin sodium at a 10 mg/kg dose rate to horses with alkaline and acidic urine.

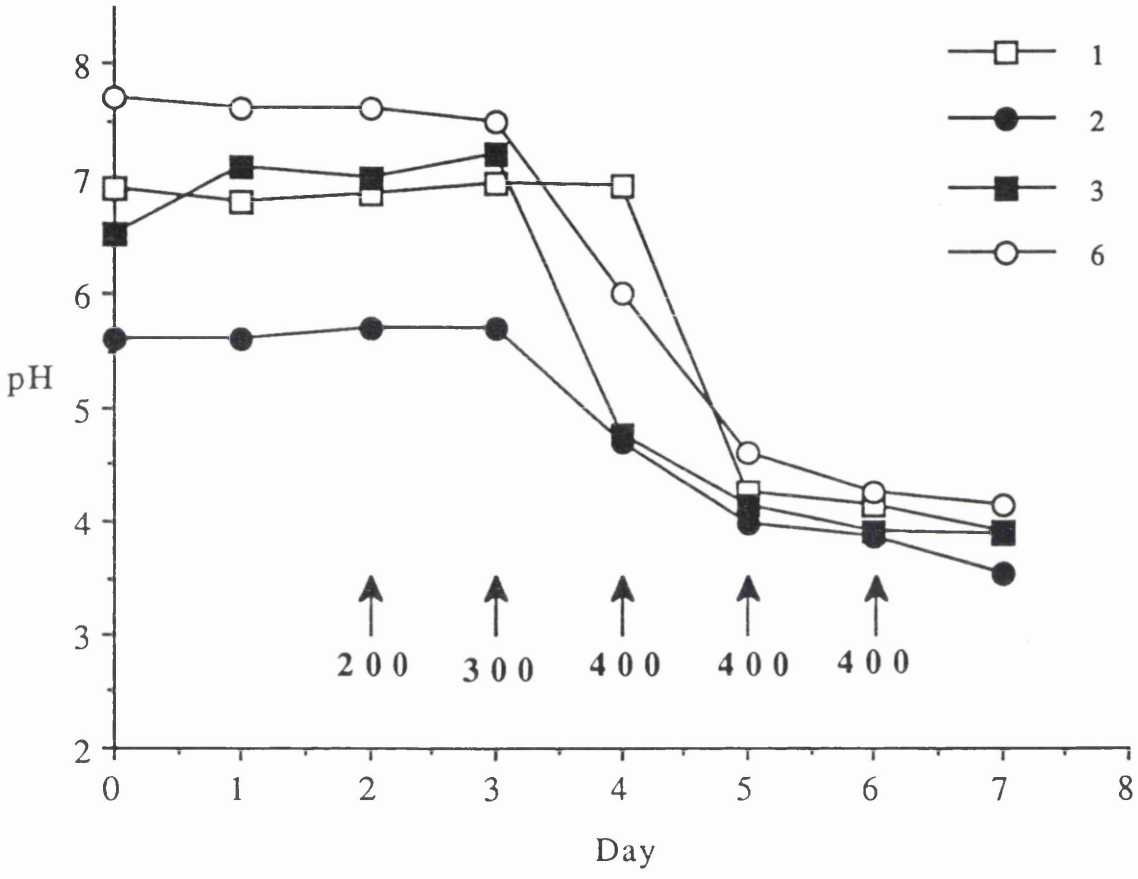


Fig. 3-6. Urine pH values in 4 horses during administration of ammonium chloride. Arrows, indicate daily administration of ammonium chloride (200, 300, 400 [x 3] mg/kg). Ampicillin sodium was administered at a dose rate of 10 mg/kg on day 5.

		Animal number			
Day	Dose	1	2	3	6
0	0	6.90	5.60	6.50	7.70
1	0	6.80	5.60	7.10	7.60
2	200	6.85 ± 0.05	5.70	7.00 ± 0.10	7.60
3	300	6.95 ± 0.15	5.70	7.20 ± 0.10	7.50
4	400	6.93 ± 0.38	4.70 ± 0.10	4.76± 0.09	6.00
5**	400	4.25 ± 0.21	3.97 ± 0.17	4.13 ± 0.14	4.60 ± 0.31
6**	400	4.13 ± 0.10	3.86 ± 0.06	3.91 ± 0.10	4.26 ± 0.21
7**	0	3.90	3.52 ± 0.06	3.88 ± 0.06	4.14 ± 0.03

Table 3-7. Mean ± S.E.M. urinary pH values in four horses after administration of ammonium chloride. Dose in mg/kg.
Single values are given when only one urine sample was collected.

** Statistically highly significant ($P<0.01$)

3.3.2.2 Urine volume

A mean urine volume of 31.14 ± 5.64 litres was excreted by three of the animals during the 48 hours after the administration of ampicillin sodium. This is equivalent to a mean daily urinary excretion of 27.4 ± 4.84 ml/kg bodyweight by the horses (Table 3-8). Not all the urine could be collected from the mare (animal number 6) and therefore it was excluded from the calculations.

3.3.2.3 Plasma ampicillin concentrations

Mean ± S.E.M plasma ampicillin concentrations are shown in Figure 3-4 , Table 3-3 and Appendix A table A-3, and pharmacokinetic values are given in Tables 3-4 and 3-5 and Appendix A Tables A-8 and A-9. There were no statistically significant differences in the pharmacokinetic parameters obtained in normal horses compared with horses following urinary acidification. There were also no significant differences in pharmacokinetic values of ampicillin when comparing horses with acidic or alkaline urine. The concentration of ampicillin at 45 minutes was significantly ($P<0.05$) lower after alkalinisation than after acidification, but was significantly ($P<0.05$) higher at 90 minutes. Significant differences were not observed on any other occasion.

A Mann Whitney-U test did not reveal any significant differences between pharmacokinetic data from an experiment in which three of the horses were administered an IV bolus of ampicillin sodium at the same dose rate without urinary alterations (Horspool *et al.*, 1992) compared to the ampicillin pharmacokinetic values obtained after alkalinisation and acidification of the urine.

3.3.2.4 Urine ampicillin amount (fraction of dose)

The mean recovery of ampicillin in acidic urine was 86.64 ± 8.68 % of the total dose administered. Animal number 6 was excluded in this calculation as the total urine volume could not be collected.

	Animal number			
	1	2	3	Mean ± SEM
Urine volume (l)	24.86	42.41	26.17	31.14 ± 5.64
Animal weight (kg)	621	587	510	572.7 ± 32.8
Daily urinary excretion (ml/kg)	20.02	36.52	25.66	27.4 ± 4.84

Table 3-8. Urine volume and excretion during 48 hours after administration of ampicillin sodium to horses with acidic urine.

More than 90% of the ampicillin recovered was excreted in the first 6 hours after administration of the antibiotic. Ampicillin could still be detected in the acidic urine 30 hours after the ampicillin sodium administration in one animal and 36 hours in the two other horses (Table 3-9; Fig. 3-5).

3.3.3 *In vitro* degradation of ampicillin

High concentrations of the antibiotic suffered less degradation (%) than the smaller concentrations (Table 3-10). However, this might be due to the logarithmic relationship between the zone of bacterial inhibition and the concentration of the standard. This was observed in normal and basic urine standards during each measurement. When comparing the degradation of ampicillin in normal and alkaline urine, it appears that the drug disappeared more quickly in the basic urine with only a maximum of 27.5 % left on day 4 and 7.5 % on day 10 compared to 77.7 % on day four in normal urine and 63 % on day 10.

3.4 Discussion

Most pharmacokinetic studies carried out in the horse are undertaken in resting animals. This could be misleading as it has been observed that horses under hard training conditions produce more acidic urine (Evans & Lambert, 1974). This is probably the result of an increase in lactic acid production (Wood *et al.*, 1990) or an increase in protein in the diet of those animals (Baggot, 1977b). Care must therefore be taken when predicting the excretion of drugs in animals where their urinary pH could be altered by natural physiological mechanisms (Moss, 1976).
Following absorption most antimicrobials are metabolised by the liver and then excreted by different routes including the kidneys (English & Roberts, 1979). Penicillins however, are excreted in the urine mainly unchanged (Baggot, 1977b).

	Animal number			
Time (h)	1	2	3	Mean ± SEM
6	75.24	100.53	73.54	83.10 ± 8.73
12	3.84	1.59	1.86	2.43 ± 0.70
18	1.24	1.07	0.18	0.83 ± 0.32
24	0.06	0.57	0.14	0.26 ± 0.15
30	0.02	0.03	0.00	0.02 ± 0.00
36	0.01	0.01	0.00	0.01 ± 0.00
48	0.00	0.00	0.00	0.00 ± 0.00
Total	80.41	103.80	75.80	86.64 ± 8.68

Table 3-9. Percentages of ampicillin sodium recovered in acidic urine after IV administration of ampicillin sodium at a 10 mg/kg dosage rate.

Conc.	Day 1	Day 4		Day 7		Day 10	
(μg/ml)	A	N	A	N	A	N	A
5	28.0	42.0	7.6	24.0	3.2	13.9	1.6
10	40.9	46.4	10.6	24.5	4.0	16.7	2.2
20	60.2	65.0	17.6	41.5	5.9	28.5	3.7
40	73.2	67.5	21.4	62.6	9.4	49.9	5.4
80	79.6	77.7	27.5	75.1	13.1	63.0	7.5
Mean %	56.38	59.72	16.94	45.54	7.12	34.40	4.08

Table 3-10. *In vitro* degradation of ampicillin in alkaline urine expressed as percentages. A: alkaline urine. N: normal urine.

Although detectable amounts of penicillin are found in faeces after biliary excretion (Mandell & Sande, 1980), it is considered that the kidney is the main pathway of excretion for the penicillins. More precisely, it has been shown in human patients that between 75 and 100% of ampicillin is excreted unchanged in urine (Rang & Dale, 1987a).

Renal excretion of drugs may occur by glomerular filtration and tubular secretion. Glomerular filtration itself accounts for a small proportion of the excreted drug in the urine because only 10% of the blood reaching the kidney is filtered at this level and in addition, only the unbound fraction of the drug is capable of crossing this physiological barrier.

The drug fraction which is not excreted at the glomerulus passes into the peritubular capillaries, and may get into the renal tubule by non specific carrier mechanisms which principally exert their effect in the proximal tubule. There are thought to be two major types of carrier mechanisms; one for acidic substances and the other for basic ones. The first is responsible for the transfer of ionized ampicillin from the peritubular capillaries into the proximal tubule lumen and has been shown to account for the excretion of 80% of penicillin in the equine urine (Knudsen, 1960). In human beings it has been shown that approximately 10 % of penicillin is excreted in the urine by glomerular filtration, the rest being excreted by the carrier system (Rang & Dale, 1987b).

Once the drug is inside the renal tubule, it may diffuse back into the blood across the epithelium of the distal tubule. The extent to which the drug will diffuse back through the tubular epithelium into the peritubular capillaries depends on different factors such as the concentration gradient across the membrane, the thickness of the membrane, the surface over which transfer occurs and the diffusion constant of the drug. This last factor is determined by the molecular weight of the compound, its solubility in the membrane and its steric configuration. In addition, the diffusion of weak organic compounds (*ie* most drugs) across biological membranes is largely determined by their degree of ionisation. since only the nonionised fraction of the drug is capable of crossing physiological barriers. The degree of ionisation of a

particular compound is dependent on its dissociation constant (pKa), which is the pH of the aqueous solution in which the compound is 50 % ionised, and the pH of the media, and it can be calculated when pH and pKa are known by use of the Henderson-Hasselbalch equation (Curry, 1980). This phenomenon is of major importance when considering the excretion of drugs, since the ionised form of the drug is not capable of diffusing across the phospholipid membranes of the distal tubule back into the blood stream and will therefore be excreted rapidly in the urine. On the other hand when the drug present in the urinary filtrate is largely in the nonionised form, the overall renal elimination will be reduced since the drug will diffuse back into the blood.
When the pH of the urine increases (alkalinisation) the fraction of a weakly acidic drug in the nonionised form is also increased (Equation 3-1).

$$pH - pKa = \log \frac{[\text{concentration of ionised acid}]}{[\text{concentration of nonionised acid}]} = \log \frac{[A^-]}{[AH]}$$

Eq. 3-1. Henderson-Hasselbalch equation for weak acids.

Weakly acidic compounds like the penicillins, will therefore tend to be ionised in an alkaline urinary filtrate and consequently eliminated rapidly, a phenomenon described as the ion-trapping effect. On the contrary, when the urine is acidic the weakly organic acids will be mainly unionised and thus will be reabsorbed into the bloodstream and therefore less rapidly eliminated. The property of ion-trapping can be used to alter the excretion pattern of many drugs by artificially changing the urinary pH and therefore the diffusion of the drug across physiologic membranes. From a practical point of view the urinary pH can be changed with the intention of prolonging the effects of some drugs by retaining them in the plasma and tissues or to obtain a more rapid elimination in the event of drug intoxication. In addition, artificial changes in the urinary pH can be induced when doping tests are to be carried out (Wood *et al.*,

1990) or when high concentrations are required for therapeutic effect within the urinary tract.

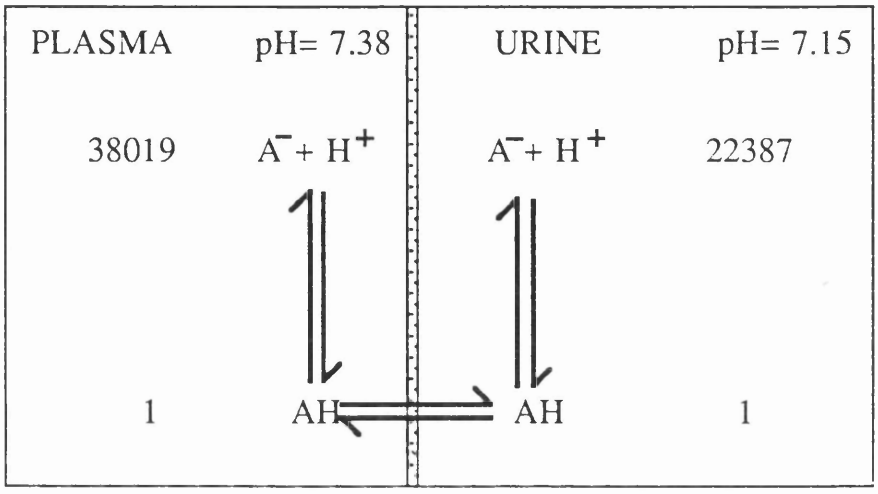
The urine pH values obtained during this two-phase study permitted the calculation of theoretical changes in the extent of ionisation both in plasma and urine for ampicillin. These changes calculated on the basis of ampicillin with a pKa of 2.8, reflect a 8.5 decrease in the number of molecules on the plasma side of the tubule when the urine is alkalinised to pH = 8.10, compared with normal urinary pH values of pH = 7.15, suggesting that the plasma concentration of the antibiotic should decrease more rapidly when the urine is alkaline (Fig. 3-7). On the other hand the number of molecules in plasma should theoretically increase 2300 fold when the urine is acidified to pH = 4.24, leading to a prolonged elimination half-life and a lower body clearance of the antibiotic (Fig. 3-8).

It is important to point out that only a qualitative prediction of the effects of changes in urine pH on the plasma disposition of ampicillin can be made since a quantitative estimation of changes on plasma disposition would require knowledge of additional factors such as renal blood flow.

Theoretical calculations indicate that urinary pH changes should have a direct effect on ampicillin plasma disposition. However, the results obtained showed that no substantial change occurred in either part of the study. None of the pharmacokinetic variables calculated were significantly different or were different from pharmacokinetic values obtained in clinically normal horses (Horspool *et al.*, 1992). Thus changes in urine pH did not affect the behaviour of ampicillin in the horse.

Several studies carried out in the horse have demonstrated the effects of urinary pH on the pharmacokinetics and rate of excretion of certain drugs. For instance, phenylbutazone a widely used nonsteroidal antiinflammatory drug in the horse was shown to persist longer in the body when administered to animals with acid urine (Moss & Haywood, 1973). Although the elimination in the urine of phenylbutazone, which is a weak organic acid (pKa = 4.7) was slower when the urine was acidic, other workers demonstrated that this did not affect the plasma kinetic values.

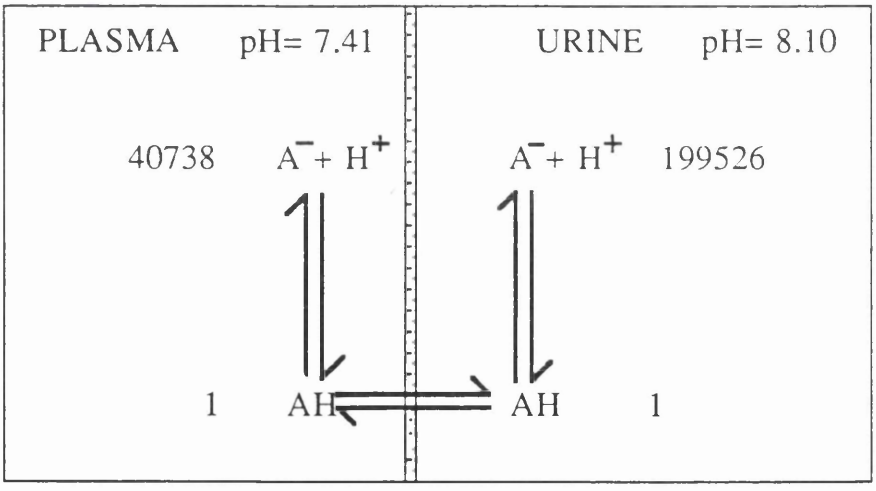
Before alkalinisation of urine



Total = 38020 Total = 22388

For each molecule in urine there are 1.7 molecules in plasma.

After alkalinisation of the urine.

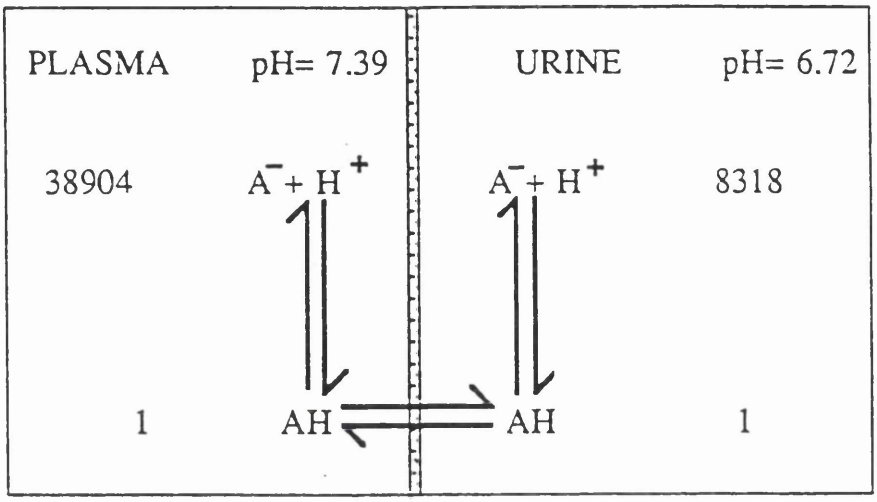


Total 40739 Total 199527

For each molecule in urine there are 0.2 molecules in plasma.

Fig. 3-7. Theoretical effect of urinary alkalinisation on ampicillin molecules ratio across the tubular epithelium.

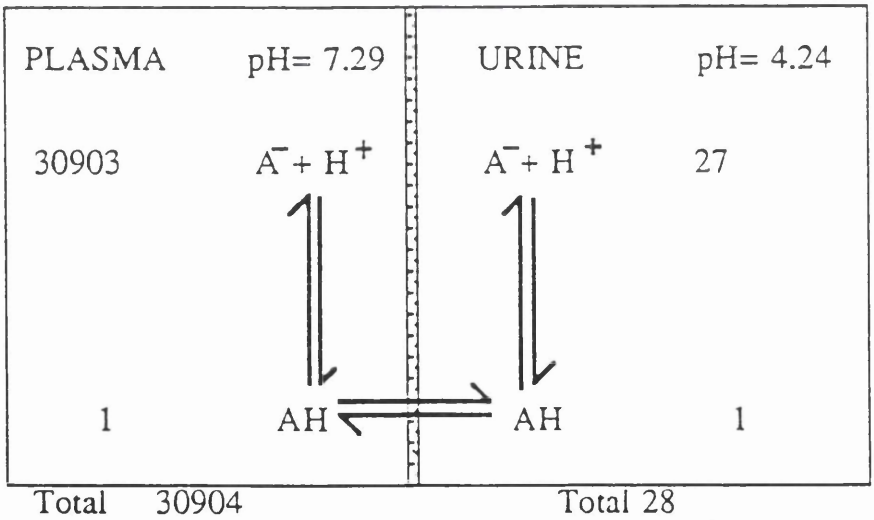
Before acidification of urine



Total 38905 Total 8319

Therefore for each molecule in urine there are 4.8 molecules in plasma.

After acidification of urine



Total 30904 Total 28

For each molecule in urine there are 11034 molecules in plasma.

Fig. 3-8. Theoretical effect of urinary acidification on ampicillin molecules ratio across the tubular epithelium.

In fact, plasma concentrations of phenylbutazone were independent of the urinary pH values (Piperno *et al.*, 1968; Houston *et al.*, 1985). Similarly urinary pH has been shown to affect the rate of excretion of amphetamine in the horse. This weak organic base (pKa = 9.9) was excreted much faster in acidic urine, although the plasma disposition of the drug was not apparently altered (Baggot *et al.*, 1972). Finally, Evans and Lambert (1974) observed that after administering procaine, another weak organic base (pKa = 8.95), to horses with acidic urine, the concentrations in urine increased. However this change did not alter significantly the plasma pharmacokinetics of the drug.

The reason that changes in the excretion of phenylbutazone, amphetamine and procaine in the horse's urine did not affect their plasma disposition, may be attributable to the fact that these drugs are excreted unchanged in the urine to a very limited degree. Only 3.7% of the administered phenylbutazone, 2% of the amphetamine and less than 1% of procaine were recovered unchanged in the urine. Therefore although the urine concentrations can vary widely when the pH is altered, the amount of unchanged drug lost by this route is not sufficiently large to have a major influence on the plasma half-life of these drugs. It seems more likely that the plasma disposition of these drugs is determined by their metabolism rather than by their renal excretion (Tobin & Woods, 1979).

From a clinical point of view, the alteration of the urinary pH may only affect the plasma disposition of drugs that are mainly excreted unchanged in urine (Baggot, 1977b) and for these drugs a significant difference in urinary excretion will produce a major difference in the overall removal of the compound from the body (Curry, 1980).

It was anticipated that a change in urinary pH would affect the plasma kinetics of ampicillin sodium since the drug is thought to be principally excreted by the kidney unchanged. According to theoretical calculations the changes of pH obtained should have had an effect on the excretion and plasma pharmacokinetics of ampicillin. However all plasma concentration values obtained after alkalinisation or acidification of urine were not significantly

different from the normal values (except the 45 minute sample in horses with alkaline urine). The 45 and 90 minute values during urinary alkalinisation were statistically different when compared to horses with acidified urine. Thus the changes in plasma concentration were insignificant when the pH was altered and the alteration of the pH does not seem to play a major role in the overall plasma disposition of ampicillin sodium when administered intravenously to horses.

It has been reported that following increased diuresis some drugs have altered plasma disposition. It was observed that following administration of furosemide the plasma concentrations of codeine declined more rapidly than in normal animals. Morphine, a metabolite of codeine biotransformation, reached higher levels rapidly in urine when diuresis was established (Stevenson *et al.*, 1990). The normal urinary excretion of the horse is 3 to 18 ml/kg per day (Gans & Mercer, 1984) therefore the excretion of urine following sodium bicarbonate in the present study can be considered normal as the average urine excreted following the administration of ampicillin sodium was 10.15 ± 1.06 ml/kg of bodyweight (Table 3-2). However, a marked diuresis with a mean urinary output of 27.4 ± 4.84 ml/kg of bodyweight was observed following acidification of urine with ammonium chloride (Table 3-8). This diuresis during the acidification experiment, could explain why ampicillin plasma disposition was not altered when the urine was acidified. Theoretically, at a lower urine pH, higher ampicillin plasma concentrations of the drug should have been observed. However, excessive diuresis would have resulted in dilution of the ampicillin in the tubules and thus reduction in the concentration gradient with a consequent increase in the excretion of ampicillin in urine. However when the urine was alkalinised, no diuresis was observed, yet the plasma pharmacokinetics did not change. On this occasion, lower plasma concentrations would have been expected, and it seems likely that there may be another explanation for the results observed. These results correlate with those of Stevenson *et al.* (1990) since they found that codeine plasma concentrations were reduced when a diuresis was established, however plasma disposition was

unaltered for theophylline, phenylbutazone, pentazocine, guaifenesin and flunixin.

Penicillins have been claimed to persist in individuals with acidic urine, however the fact that they are very water-soluble may explain why they were not dramatically affected by the pH of the urine in the present study. The solubility of a drug in a biological membrane will affect its diffusion, in the present case this represents a lipid-to-water partition coefficient (*ie.*, lipid solubility) since the renal tubular epithelium is a phospholipid bilayer. It may be that because penicillins are very water-soluble in both the ionised form and the unionised form they will be excreted rapidly even in acidic urine and therefore the pH should not play a major role (Katzung, 1984). Ampicillin sodium is very water-soluble and poorly lipid-soluble, and may be excreted in the urine regardless of the urinary pH especially after IV administration where the bioavailability is total and the drug reaches the kidney immediately.

However, the presence of an amino (NH_2) group in addition to the carboxyl (COOH) group within the ampicillin molecule has been shown to confer zwitterion characteristics to ampicillin. This would appear to be the most likely reason of the results obtained in this study since the presence of a carboxyl group with a pKa of 2.8 and of an amino group with pKa of 7.24, means that ampicillin will have one of its groups or even both in the ionised form at any pH (Hou & Poole, 1969). Consequently, it seems logical to think that the artificial changes of urinary pH carried out during this study, would not affect the extent of reabsorption of the antibiotic from the urinary filtrate across the distal tubule, since no ampicillin free of ionised groups was likely to be found.

In the present study very small amounts (14.20 ± 1.92 % of the dose) of ampicillin were detected in the alkaline urine. It is most likely that because the samples were stored at 0 - 4 º C for nine days prior to analysis, the drug was degraded or inactivated in the basic media. It has been reported that in alkaline urine, two penicillanic acid sodium salts, mezlocillin and azlocillin, suffered a degradation of 70% after 8 hours of incubation at pH = 9 (Gundert-Remy & Weber, 1981).

The *in vitro* experiment showed that ampicillin was degraded faster in alkaline urine as reflected in a maximum of 7.5 % of the original ampicillin remaining on day 10 when tested in alkaline urine compared to 63 % on day 10 in normal urine. It seems therefore that the length of time that the urine samples were kept before being analysed and their high pH ranging from 8 to 9 could explain the poor recoveries obtained when the urine concentrations were measured. However, it is impossible to extrapolate the results obtained *in vitro* and calculate what concentrations should have been attained in urine during the *in vivo* experiment.

Artificial acidification of urine after administration of ammonium chloride has been reported to enhance the activity of some antibiotics such as the tetracyclines and penicillins in humans (Rang & Dale, 1987c). Many studies have shown the relationship between the alteration of the pH of the growth medium and the antibacterial activity of penicillin (Foster & Woodruff, 1943; Abraham & Duthie, 1946; Eagle *et al.*, 1952; Mou, 1962). All these studies found that penicillin was more active when the medium was acidified possibly because penicillins are less ionised when the pH is lowered and they are thus more lipid-soluble, and can penetrate more readily into the bacterial cell. As most antibiotics have to penetrate the bacterial cells to act, a change in the penetrating ability (changes in ionisation) affects directly their activity. It is unlikely that changes in the degree of ionisation would increase the activity of penicillin in acidic medium since penicillins act on the cell wall and therefore do not have to penetrate the bacteria to exert their activity. It is more likely that the penicillins suffer some type of degradation in basic media and that is the reason for the greater activity in an acidic environment. Galbraith, (1984) demonstrated by fluorimetry, that ampicillin suffered a degradation of greater than 20% in plasma after 14 days storage at 2ºC but was not degraded after the same period of time when the samples were kept in an acidic environment. It seems likely that the penicillins are less effective in the basic media mainly because of an increased rate of degradation. The urine samples collected during the acidification experiment were analysed after two days and the recoveries

obtained (86.64 ± 8.68 %) were similar to the normal values that would be expected (75 to 100%) in humans (Rang & Dale, 1987a). Following both acidification and alkalinisation of urine, more than 90% of the total ampicillin sodium excreted in urine was recovered in the first 6 hours, however low concentrations of antibiotic could still be detected in the urine after 30 hours.

3.5 Conclusion

In the horse the excretion of many drugs may be affected by the urinary pH, however, possibly because of its high water solubility and the presence of two ionisable groups within its molecule, ampicillin sodium excretion does not appear to be greatly affected by the urinary pH when administered by the intravenous route. Although great variations in pH have been observed in the horse population, ranging from 4.5 to 9.0 (Houston *et al.*, 1985), it appears that the plasma disposition of ampicillin sodium will not be affected by the urinary pH. It is unlikely that special considerations, such as the status of training or type of diet require to be taken into account when treating horses with ampicillin sodium.

It appeared that ampicillin was rapidly degraded in alkaline urine, something that should be considered when animals with alkaline urines (e.g. grazing) are to be treated for cystitis. In this event, the normal dosage interval should be shortened as the ampicillin is degraded and therefore loses its activity, however further studies in the horse's urine should determine the extent of such degradation.

Chapter 4

Studies on the effects of probenecid on ampicillin pharmacokinetics in the horse

4.1 Introduction

One of the major drawbacks in the clinical use of penicillins is their rapid elimination from the body and consequently, frequent dosage regimes have to be used to maintain adequate plasma and tissue levels of the antibiotic over prolonged periods of time. Active secretion in the proximal convoluted tubule of the nephron is the main mechanism responsible for the effective excretion of penicillins into the urinary filtrate (Knudsen, 1960) and this is reflected in an elimination half-life of less than two hours for ampicillin sodium in the horse (Dürr, 1976; Bowman *et al.*, 1986; Baggot & Prescott, 1987; Horspool *et al.*, 1992; Ensink *et al.*, 1992).

As already discussed (Chapter 3), ampicillin has an acidic and a basic group within its molecule, and these physicochemical properties restrict its ability to diffuse from the urinary filtrate into the peritubular capillaries at any pH. It appears then, that the elimination of ampicillin from the body and its plasma disposition, cannot be altered by changing the pH of the urinary filtrate. On the other hand, the presence of a nonspecific carrier mechanism responsible for the transfer of penicillins from plasma into the urine, has led to the use of substances that can compete for this carrier and therefore delay the rapid excretion of penicillins and cephalosporins.

Probenecid (Fig. 4-1) is a competitive inhibitor of the renal tubular secretion of organic anions and has been shown to prolong the elimination of beta-lactam antibiotics (Levy, 1965; Gibaldi & Schwartz, 1968; Gibaldi *et al.*, 1970; Kampmann *et al.*, 1972 ; Galtier & Alvinerie, 1979; Ziv *et al.*, 1979; Ziv & Horsey, 1979; Juzwiak *et al.*, 1989). In addition, studies carried out in the horse have shown that the bioavailability of probenecid after oral administration was greater than ninety per cent and that maximum plasma concentrations were achieved approximately one hour after administration (Donecker *et al.*, 1986). The present study, comprised a two phase experiment, was carried out to compare the effects of an oral dose of probenecid on the plasma disposition of ampicillin sodium in the horse with the disposition of ampicillin when administered alone.

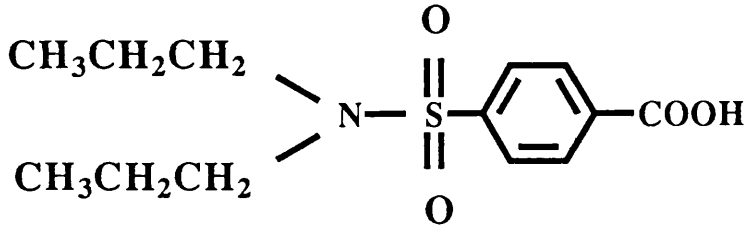


Figure 4-1. Structural formula of probenecid.

4.2 Materials and methods

4.2.1 Animals

Three Thoroughbred geldings (animals 1, 2, 5) and a mare (animal 7) weighing 582, 568, 603 and 507 kg respectively were used during both phases of the experiment.

4.2.2 Ampicillin sodium and probenecid administration

Ampicillin sodium was administered at a dose rate of 10 mg/kg into the right jugular vein in both phases of the study. Sample collection, storage and analysis was carried out as described previously. During the second phase of the experiment, probenecid (Benemid, Merck Sharp & Dohme Limited) was suspended in 500 ml of water and administered via a nasogastric tube at a dose rate of 75 mg/kg one hour before the administration of ampicillin sodium. Collection of samples, storage and probenecid analysis were carried out as described previously

4.2.3 Pharmacokinetic Analysis

Ampicillin plasma concentrations were analysed by compartmental and noncompartmental pharmacokinetic analysis as described previously. Probenecid plasma concentrations were analysed by noncompartmental analysis and Cmax and tmax were also determined.

4.2.4 Statistical analysis

A Mann Whitney-U test for nonparametric statistical analysis was used to compare the pharmacokinetic values for ampicillin obtained after administration of ampicillin and probenecid with values obtained from animals where ampicillin was given on its own. A paired Student's t-test was carried out to compare plasma ampicillin concentrations obtained on both occasions.
A value of $P < 0.05$ was considered to be statistically significant and $P < 0.01$ was considered to be highly significant.

4.3 Results

When probenecid was administered intragastrically, a peak plasma concentration (Cmax) of 188.60 ± 19.27 μg/ml was achieved at 120.00 ± 21.21 min (Table 4-1; Appendix B Table B-1). Thereafter, the plasma concentration declined; however relatively high values (17.69 ± 7.38 μg/ml) were still found after 25 hours (Table 4-2; Appendix B Table B-2; Fig. 4-2). The non-compartmental analysis of the probenecid plasma concentrations indicated an area under the plasma concentration versus time curve for observed values (AUC_{obs}) of 2028.00 ± 346.00 μg.h/ml, an area under the first moment curve ($AUMC_{obs}$) of 21339.00 ± 6505 $\mu g.h^2/ml$ and a mean residence time (MRT) of 9.70 ± 1.62 hours (Table 4-1; Appendix B Table B-1). No adverse effects were observed in any of the animals following administration of probenecid.

When analysed by compartmental analysis, plasma disposition of ampicillin sodium was best described by a biexponential decline curve on both occasions. When ampicillin was administered on its own, it was detected up to four hours after its administration (Table 4-3; Appendix B Table B-3; Fig. 4-3). Plasma concentrations were still detected in all four animals at 12 hours when antibiotic was administered after probenecid (Fig. 4-3; Table 4-3, Appendix B Table B-4). When plasma concentrations of ampicillin in horses coadministered with probenecid were analysed statistically, all were highly significantly different ($P<0.01$) from the control values except at the 2 min and the 90 min samples which were significantly different ($P<0.05$) (Table 4-3). The compartmental pharmacokinetic variables obtained in both phases of the study for ampicillin were calculated (Table 4-4, Appendix B Tables B-5 and B-6). The statistical analysis showed a significant increase ($P<0.05$) in the elimination half-life of ampicillin when probenecid was administered (t1/2ß = 1.198 h versus 0.701 h; harmonic mean). The prolonged antibiotic elimination was reflected in a significantly ($P<0.05$) greater AUC (AUC = 92.33 ± 5.10 μg.h/ml versus 36.55 ± 3.42 μg.h/ml), and reduced ($P<0.05$) body clearance Clb (Clb = 109.40 ± 6.71 ml/h.kg versus 280.87 ± 26.19 ml/h.kg).

Variable		Mean ± SEM (n=4)
Cmax	(μg/ml)	188.6 ± 19.27
tmax	(min)	120.00 ± 21.21
AUC_{obs}	(μg.h/ml)	2028.00 ± 346.00
$AUMC_{obs}$	($\mu g.h^2/ml$)	21339.00 ± 6505.00
MRT	(h)	9.70 ± 1.62

Table 4-1. Probenecid pharmacokinetic variables (mean ± SEM) following intragastric administration of probenecid at a 75 mg/kg dosage rate.

Time (h)	Probenecid (n=4) Mean ± SEM
0.25	63.08 ± 5.55
0.5	109.78 ± 8.16
1	146.73 ± 21.57
1.50	175.7 ± 26.36
2	167.54 ± 19.02
3	161.93 ± 9.36
5	135.60 ± 17.49
9	81.55 ± 18.66
13	50.47 ± 9.61
25	17.69 ± 7.38

Table 4-2. Probenecid plasma concentrations (mean ± SEM) following intragastric administration of probenecid at a 75 mg/kg dosage rate.

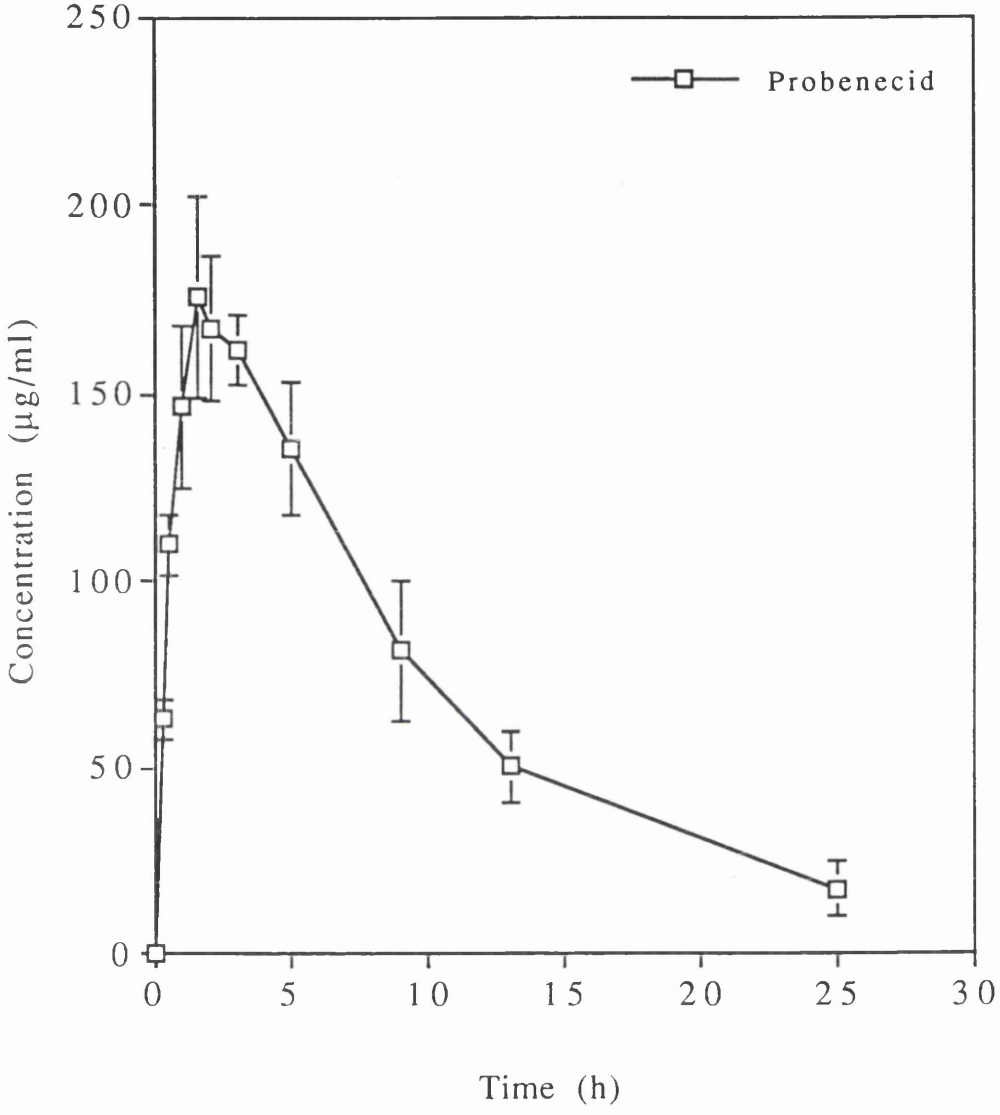


Fig. 4-2. Mean plasma concentrations (± SEM) of probenecid in four horses after intragastric administration at a dose rate of 75 mg/kg.

Time (h)	Ampicillin (n=4) Mean ± SEM	Ampicillin and Probenecid (n=4) Mean ± SEM
0.0333	58.07 ± 2.86	83.84 ± 4.01 *
0.0833	44.27 ± 1.62	67.95 ± 1.85 **
0.25	32.98 ± 1.12	53.99 ± 3.60 **
0.50	21.91 ± 2.89	39.35 ± 2.71 **
0.75	15.03 ± 2.03	31.92 ± 2.20 **
1	10.43 ± 1.96	27.19 ± 1.96 **
1.5	5.98 ± 1.13	20.08 ± 1.02 *
2	3.46 ± 0.64	14.46 ± 1.23 **
4	0.59 ± 0.12	4.65 ± 0.43 **
8	0.00 ± 0.00	0.61 ± 0.08 **
12	0.00 ± 0.00	0.05 ± 0.00 **
24	0.00 ± 0.00	0.00 ± 0.00

Table 4-3. Mean ± SEM plasma concentrations of ampicillin in horses after the administration of an IV bolus dose (10 mg/kg) of ampicillin sodium with or without the previous administration of an oral dose of probenecid at 75 mg/kg.

* Significantly different ($P<0.05$).

** Highly significantly different ($P<0.01$).

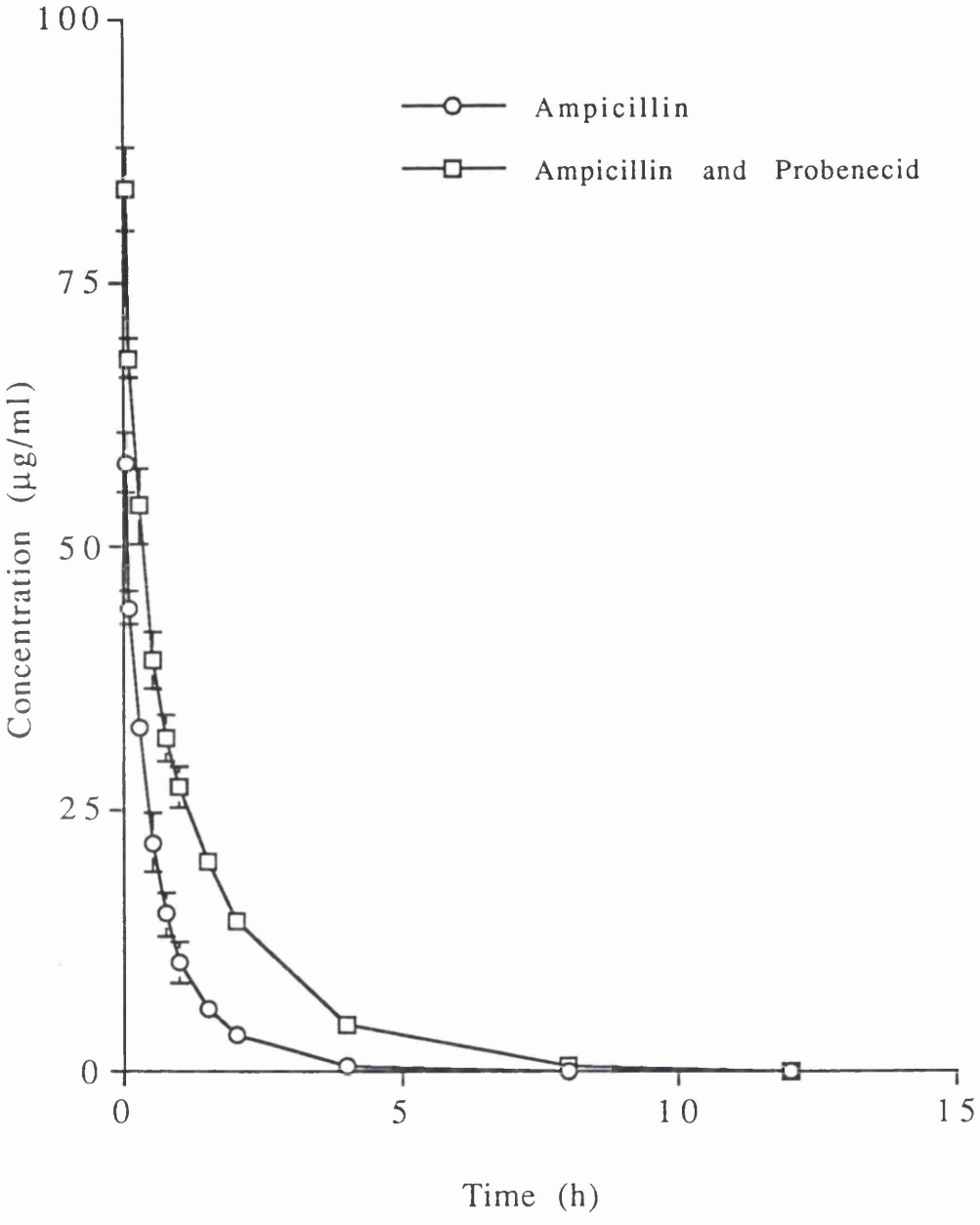


Fig. 4-3. Mean (± SEM) plasma concentrations of ampicillin in four horses after IV administration of a single bolus dose (10 mg/kg) with or without probenecid.

Variable		Ampicillin (n=4)	Ampicillin and Probenecid (n=4)
α	(/h)	11.70 ± 6.74	11.87 ± 5.16
β	(/h)	0.99 ± 0.02	0.58 ± 0.02
t1/2 α	(h)	0.059	0.058
t1/2 β	(h)	0.701	1.198 *
Cp0	(μg/ml)	70.70 ± 9.01	102.14 ± 10.46
AUC	(μg.h/ml)	36.55 ± 3.42	92.33 ± 5.10 *
Vc	(ml/kg)	148.31 ± 18.11	101.10 ± 10.45
Vd_{area}	(ml/kg)	285.67 ± 30.87	191.27 ± 18.79
Vdss	(ml/kg)	235.67 ± 13.72	177.45 ± 14.04
Clb	(ml/h.kg)	280.87 ± 26.19	109.40 ± 6.71 *
k_{21}	(/h)	5.66 ± 3.15	6.02 ± 2.49
k_{el}	(/h)	1.94 ± 0.16	1.10 ± 0.07
k_{12}	(/h)	5.09 ± 3.53	5.33 ± 2.62

Table 4-4. Ampicillin pharmacokinetic values obtained after compartmental analysis in horses following administration of ampicillin sodium and ampicillin with probenecid.
* Significantly different ($P<0.05$).

In addition, the values obtained for the volume of the central compartment, Vc, the apparent volume of distribution at pseudodistribution equilibrium, Vdarea, and the apparent volume of distribution at steady state, Vdss, were smaller when probenecid was administered concomitantly although not significantly different.

When the results were analysed by noncompartmental analysis (Table 4-5, Appendix B Tables B-7 and B-8) significant differences ($P < 0.05$) were found in all parameters. When ampicillin was administered after an intragastric dosage of probenecid, the AUC_{obs} of ampicillin increased from 36.89 ± 3.60 µg.h/ml to 96.50 ± 5.70µg.h/ml. The $AUMC_{obs}$ was also increased from 30.17 ± 4.79 µg.h^2/ml to 153.39 ± 9.52 µg.h^2/ml. A marked reduction in the $Vdss_{obs}$ was observed when ampicillin was administered after probenecid (166.70 ± 11.21 ml/kg versus 207.17 ± 20.89 ml/kg). The presence of probenecid in the body almost doubled the MRT of ampicillin, (from 48.32 ± 2.77 minutes to 95.37 ± 1.64 minutes) and reduced the total body clearance observed CLb_{obs} from 278.58 ± 25.83 ml/h.kg to 104.86 ± 6.95 ml/h.kg.

4.4 Discussion

Studies carried out in horses to evaluate the effects of probenecid on para-aminohippuric acid (PAHA) clearance revealed that plasma concentrations greater than 55 µg/ml were neccessary to reduce the renal elimination rate of PAHA (Gronwall & Brown, 1988). The clearance of PAHA was reduced by 50% when oral doses of probenecid were administered at a dose rate of 75 mg/kg. Since the excretion of ß-lactam antibiotics from the body is very similar to that of PAHA (partly by glomerular filtration but mainly by active transport in the proximal renal tubule) it was considered reasonable to extrapolate the results obtained by Gronwall and Brown (1988) with PAHA, and a dosage of 75 mg/kg was chosen to be administered during the present study.

Variable		Ampicillin (n=4)	Ampicillin and Probenecid (n=4)
AUC_{obs}	($\mu g.h/ml$)	36.89 ± 3.60	96.50 ± 5.70 *
$AUMC_{obs}$	($\mu g.h^2/ml$)	30.17 ± 4.79	153.39 ± 9.52 *
MRT	(min)	48.32 ± 2.77	95.37 ± 1.64 *
$Vdss_{obs}$	(ml/kg)	207.17 ± 20.89	166.70 ± 11.21 *
Clb_{obs}	(ml/h.kg)	278.58 ± 25.83	104.86 ± 6.95 *

Table 4-5. Ampicillin pharmacokinetic values obtained after non-compartmental analysis in horses following administration of ampicillin sodium and ampicillin with probenecid.
* Significantly different ($P<0.05$).

The oral route of administration of probenecid was chosen as it has been shown that probenecid has an oral bioavailability in the horse greater than 90% (Donecker *et al.*, 1986; Gronwall & Brown, 1988; Juzwiak *et al.*, 1989), reaching a Cmax at approximately one hour after its administration (Donecker *et al.*, 1986). Other routes of administration such as IV have been used in sheep (Guerrini *et al.*, 1985) but have been claimed to cause irritation in the horse (Donecker *et al.*, 1986). On this basis, a 75 mg/kg oral dose of probenecid was administered in the present study and ampicillin sodium was administered at the predicted time of maximum plasma concentration of probenecid (one hour post-probenecid).

The probenecid plasma concentration profile showed a peak plasma concentration, Cmax, two hours after its administration, representing slower absorption than in the previous reports. This, however, did not appear to be a problem because plasma probenecid concentrations greater than 55 μg/ml were present from 15 minutes after administration and persisted in plasma for a period of at least 9 hours (Table 4-2). The results obtained after noncompartmental analysis of the probenecid plasma concentrations showed that when the drug was administered orally it persisted for a long time in the body as reflected by an MRT of almost 10 hours (Table 4-1). It should, however, be emphasized that a proper evaluation of the probenecid behaviour in the equine body after oral administration cannot be described from this study since these results were obtained when ampicillin was present in the animals, and this drug acts as a competitor of probencid's renal excretion.

Ampicillin plasma concentrations were significantly higher in animals treated with probenecid. These results are in agreement with those found after administration of probenecid followed by ampicillin sodium in pigs (Galtier & Alvinerie, 1979), calves (Ziv & Horsey, 1979) and man (Levy, 1965). The pharmacokinetic parameters obtained after compartmental analysis, showed that the volume of the central compartment, Vc, and the volumes of distribution, Vd_{area} and Vd_{ss} although not significantly reduced were smaller when the animals received ampicillin concomitantly with probenecid (Table 4-4). The volume of distribution of ampicillin obtained after noncompartmental analysis, $Vdss_{obs}$,

showed a statistically significant reduction when probenecid was co-administered (Table 4-5). These alterations of the distribution parameters of beta-lactam antibiotics have already been described in man (Gibaldi & Schwartz, 1968) and animals (Galtier & Alvinerie, 1979; Juzwiak *et al.*, 1989, Villa *et al.*, 1991). In addition, the administration of probenecid in combination with phenylbutazone to mares, reduced significantly the Vd_{ss} value of the non-steroidal antiinflammatory drug (Zertuche *et al.*, 1992). It appears that probenecid might limit the access of penicillins and other drugs to some parts of the body, either by competing with active transport mechanisms or by competitive tissue binding. This would reduce the concentrations of the antimicrobial in some body organs and thereby increase the plasma concentrations (Gibaldi & Schwartz, 1968). This was shown in rats where probenecid administered concurrently with radiolabelled benzylpenicillin limited the penetration of the antibiotic into liver and kidney parenchyma (Bergholz *et al.*, 1980). In the present study, the alteration of ampicillin plasma disposition when administered in conjunction with probenecid, was mostly due to a decrease in its elimination rate from the body. This was reflected in a longer elimination half-life (t1/2 ß) and a reduced total body clearance (Clb) when compared to animals receiving ampicillin alone (Table 4-4). The prolongation of the elimination half-life and the reduction of the total body clearance produced an increase in the area under the plasma concentration versus time curve when the animals were administered probenecid. The noncompartmental model analysis showed similarly a significant reduction in the observed body clearance, Clb_{obs}, and an increase in the MRT as a consequence of substantial increases in the AUC_{obs} and the $AUMC_{obs}$ when probenecid was administered (Table 4-5).

Since the penicillins are almost entirely excreted into the urine (Rang & Dale, 1987b), it appears that the decreased body clearance of ampicillin is due to a reduction of the excretory capacity of the kidney in the presence of probenecid. This has been previously demonstrated and is the result of competition for the active transport of organic anions in the proximal tubule of the nephron (Beyer *et al.*, 1951; Levy, 1965). When the horses

were given ampicillin and probenecid, plasma concentrations of ampicillin sodium were almost 1 μg/ml 8 h after antibiotic administration and were still detected after 12 h. Bacteria with minimum inhibitory concentration (MIC) of less than 1 μg/ml are considered susceptible to ampicillin (Baggot & Prescott, 1987). These include many common pathogens of the horse such as *Streptococcus zooepidemicus*, *Streptococcus equi*, non beta-lactamase producing *Staphylococci*, *Actinobacillus equuli*, *Corynebacterium pseudotuberculosis*, *Fusobacterium necrophorum* and a large percentage of *Pasteurella* species (Knight and Hietala, 1978; Keefe *et al.*, 1980; Adamson *et al.*, 1985; Hirsh & Jang, 1987). Since bacterial growth does not resume until a few hours after plasma concentrations fall below the MIC (post-antibiotic effect) (Koritz, 1984), dosage every 12 h would be sufficient when the antibiotic is administered together with probenecid. This represents a two-fold increase in the dosing interval recommended for ampicillin in the horse (Prescott & Baggot, 1988b; Horspool *et al.*, 1992). However, it should be pointed out that probenecid might limit the penetration of penicillins into certain body "compartments" as the reduction in volumes of distribution suggests. This phenomenon could lead to lower ampicillin concentrations in some tissues. It is impossible to determine to what extent this would occur in different tissues without measuring specific ampicillin concentrations in the target tissue following concurrent administration with probenecid.

4.5 Conclusion

The concurrent administration of an intragastric dose of probenecid and an intravenous dose of ampicillin sodium prolonged the persistence of the antibiotic in horses. Probenecid was well tolerated and was an appropriate agent to prolong the ampicillin dosing interval as reflected in the reduction in the total body clearance of the antibiotic when the combination was given. The noncompartmental analysis of the ampicillin plasma concentrations in both phases of the study, revealed a statistical decrease in the $Vdss_{obs}$ for ampicillin administered in conjunction

with probenecid. These results suggest that probenecid might limit the penetration of ampicillin to some areas of the body.

Chapter 5

Intravenous infusions of ampicillin in horses

5.1 Introduction

Ampicillin sodium is administered mainly for the treatment of bacterial infections, however it is also commonly administered as a prophylactic for the prevention of post-operative infections.
During surgical procedures, it is important to administer antibiotics at the correct dose and the right time in order to have sufficient concentration at the site and at the time of bacterial contamination since it has been shown that these are crucial factors for the prevention of post-surgical infections (Hirschmann & Inui, 1980; Gorbach, 1983; van den Bogaard & Weidema, 1985; Brown, 1986; Kirk, 1986). In practice, many different regimens are used depending on the type of surgery to be performed and the likelihood of bacterial contamination during the surgical procedure and these involve different types of antibiotic, time of administration and dosage (Guglielmo *et al.*, 1983; Bartlett & Burton, 1983). Some authors have suggested that the optimal regimen for administration of antibiotic for the prevention of such infections is immediately prior to the operation when administered intravenously or at least half an hour before if given intramuscularly and one hour before when administered orally (Clark, 1980). Several investigators have also concluded that in most circumstances, that antibiotics should only be administered during the surgical procedure (Van Scoy & Wilkowske, 1983; Hirschmann & Inui, 1980).
Among the routes of administration of antibiotics during surgical procedures, continuous intravenous infusion have been evaluated (Alexander & Alexander, 1976). Infusion administration is extensively used in human medicine for prophylactic antibiotic therapy and for drugs with narrow therapeutic indices. It allows control over the amount of drug delivered to the (body) systemic compartment at each moment and permits maintainance of desired concentrations over prolonged periods of time.
In the present study, an experimental model for prolonged administration of ampicillin sodium to horses was developed. The objectives of the study were to achieve steady-state plasma concentrations when infusing the drug and to determine the dose rate required to obtain plasma concentrations between 5 and 10

μg/ml. In addition, the method was evaluated as an alternative way of administering prophylactic antibiotics during equine surgical procedures.

5.2 Materials and methods

The experiment involved IV infusion of ampicillin at a constant rate with a peristaltic pump over a 4-hour period after an initial intravenous bolus dose of 2 mg/kg.

5.2.1 Animals

Three geldings 7, 21 and 22 years old (animals 1, 3. and 4) and weighing 600, 540, and 580 kg respectively were used in this experiment. The animals were healthy and had water and hay available *ad libitum* during the experiments. Each animal was restrained in stocks, with an intravenous catheter placed in each jugular vein.

5.2.2 Intravenous bolus and infusion administration

An IV bolus dose (2 mg/kg) of ampicillin sodium was administered prior to infusion in order to achieve steady-state plasma concentrations rapidly. The constant infusion pump was activated and infusion was carried out as described previously.

5.2.3 Calculation of the intravenous infusion dose rate

The amount of ampicillin required for the infusion was calculated according to the equation 5-1:

$$Cpss = Ro / Vdss.\beta \quad \text{(Eq. 5-1)}$$

with: Cpss = plateau (steady-state) concentration of drug in the plasma during constant rate (zero-order) infusion.

Ro = rate of intravenous infusion (constant zero-order)

Vdss = steady-state volume of distribution.

β = overall elimination rate constant of drug from the body.

The pharmacokinetic parameters used to calculate the rate of infusion during the present study were determined previously following single dose IV administration of ampicillin to horses (Horspool *et al.*, 1992). A Vdss of 210.8 ±16 ml/kg and β of 0.0154 min^{-1} were used. The Cpss required during the infusion period was estimated to be 5 - 10 μg/ml. It was therefore calculated that an infusion rate of Ro = 20.0 μg/min/kg would be adequate to achieve the desired concentration and this was administered to three animals in the first phase of the experiment.

In addition, in a second phase of the experiment, one animal (animal 3) received two further intravenous infusions at different dose rates (13.78; and 23.48 μg/min/kg) to assess if variations in the dose administered would correlate directly with the plasma concentrations obtained. The amount of ampicillin sodium required for each animal was calculated according to the weight of the animal. In each case, the IV infusion was continued for 4 hours.

5.2.4 Sampling regime and drug analysis

During the infusion period samples were collected from the contralateral jugular vein at 2, 5, 10, 15, 30 and 45 minutes and at 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75 and 4 h. Immediately after collection of the 4 h sample, the infusion pump was disconnected and further samples were taken at 2, 5, 10, 15, 30 minutes and 1, 2, 4, 8, 12, and 24 hours thereafter. Sample storage and analysis was carried out as described previously (See General materials and methods).

5.3 Results

5.3.1 First Phase

At the first blood sampling time (2 min after intravenous injection of the bolus dose) the mean ± SEM concentration of ampicillin in plasma was 16.75 ± 0.59 μg/ml and declined

progressively during the first hour until it reached a steady state level at approximately 1.25 h (Table 5-1; Appendix C Table C-1; Fig 5-1).

When the peristaltic pump was disconnected after the four-hour infusion period the concentration of the antibiotic declined in an exponential manner and the last sample in which ampicillin could be detected was four hours after the end of the infusion period (Fig. 5-1). The plasma concentration values obtained during this period allowed calculation of the actual values of the elimination rate constant, β and elimination half-life ($t_{1/2}\ \beta$) shown in Table 5-2. The area under the plasma concentration versus time curve for observed values, AUC_{obs} = 4.34 ± 0.45 μg.h/ml and the area under the first moment curve for observed values, $AUMC_{obs}$ = 3.83 ± 0.66 $\mu g.h^2/ml$ enabled calculation of an MRT value of 0.87 ± 0.07 h for ampicillin after disconnection of the pump (Table 5-2; Appendix C Table C-2).

During the infusion, the dose (infusion) rate (Ro) varied from 19.07 to 19.34 μg/min/kg in the three animals with a mean of 19.19 ± 0.08 μg/min/kg (Table 5-3; Appendix C Table C-3). The mean plasma concentration during the steady-state period (1.25 h - 4 h) was $Cpss_{(actual)}$ = 5.95 ± 0.10 μg/ml with a maximum steady-state ampicillin plasma concentration Cpmax of 6.73 ± 0.50 μg/ml and a minimum steady-state ampicillin plasma concentration Cpmin of 5.42 ± 0.33 μg/ml.

When substituting the actual values obtained, Ro, Cpss and β in equation (5-1), a $Vdss_{(actual)}$ of 204.3 ± 13.9 ml/kg was obtained. Also a total body clearance $Clb_{(actual)}$ of 194.7 ± 10.6 ml/h.kg was obtained after dividing the infusion rate Ro by the ampicillin plasma concentration at steady-state $Cpss_{(actual)}$ (Table 5-3; Appendix C Table C-3).

In addition, the total area under the curve ($AUC_{obs-total}$) for the infusion and post-infusion period, was calculated and a mean ± SEM value of 31.78 ± 2.19 μg. h/ml was obtained (Table 5-3).

Infusion period	
Time (h)	Mean ± SEM (n=3)
0.033	16.75 ± 0.59
0.083	14.47 ± 0.74
0.166	12.12 ± 1.23
0.25	10.30 ± 0.91
0.50	8.77 ± 0.89
0.75	7.46 ± 0.61
1.00	7.01 ± 0.63
1.25	6.62 ± 0.60
1.50	6.17 ± 0.50
1.75	6.51 ± 0.37
2.00	5.96 ± 0.29
2.25	5.96 ± 0.32
2.50	6.00 ± 0.23
2.75	5.77 ± 0.38
3.00	5.69 ± 0.22
3.25	5.98 ± 0.31
3.50	5.49 ± 0.31
3.75	5.66 ± 0.26
4.00	5.56 ± 0.38

Post-infusion period	
Time (h)	Mean ± SEM (n=3)
0.033	5.50 ± 0.20
0.083	5.09 ± 0.08
0.166	4.23± 0.22
0.25	3.69 ± 0.21
0.50	2.62 ± 0.27
1.00	1.31 ± 0.19
2.00	0.44 ± 0.08
4.00	0.08 ± 0.02
8.00	0.00 ± 0.00
12.00	0.00 ± 0.00
24.00	0.00 ± 0.00

Table 5-1. Mean ± SEM plasma ampicillin values following an IV bolus of ampicillin sodium at 2 mg/kg dose rate and continuous IV administration of ampicillin sodium at a 19.19 ± 0.08 μg/min/kg infusion rate. Plasma ampicillin concentrations following disconnection of the peristaltic pump were collected over a 24 hour period.

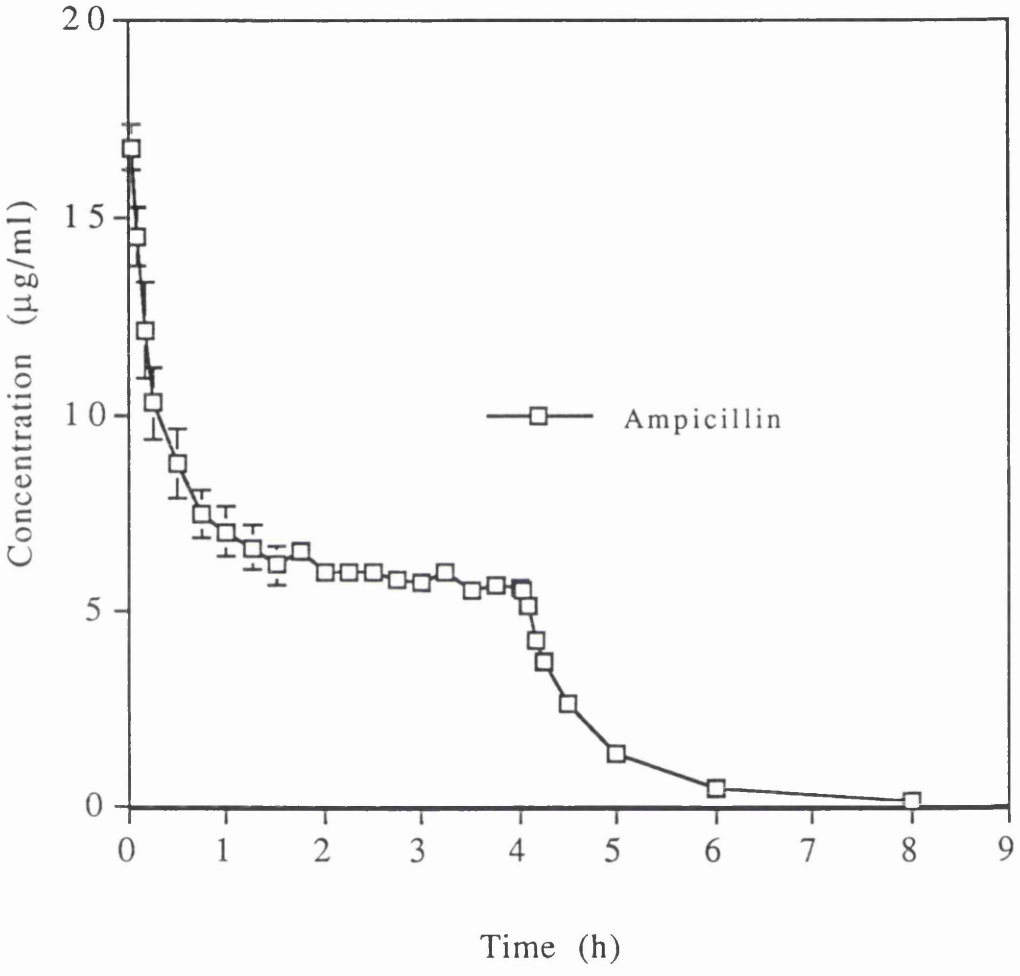


Fig. 5-1. Mean ± SEM plasma concentration of ampicillin during and after IV infusion at a dose rate of 19.19 ± 0.08 µg/min/kg to three horses.

Variable		Mean ± SEM (n=3)
β	(/h)	0.961 ± 0.076
t1/2β	(h) *	0.72
AUC_{obs}	(μg.h/ml)	4.34 ± 0.45
$AUMC_{obs}$	($\mu g.h^2/ml$)	3.83 ± 0.66
MRT	(h)	0.87 ± 0.07

Table 5-2 Pharmacokinetic values obtained after analysis of the plasma ampicillin concentrations recorded in animals 1, 3, and 4 following disconnection of the peristaltic pump.
* Harmonic mean

Variable		Mean ± SEM (n=3)
Cpmax	(μg/ml)	6.73 ± 0.50
Cpmin	(μg/ml)	5.42 ± 0.33
$Cpss_{(actual)}$	(μg/ml)	5.95 ± 0.10
Ro	(μg/min/kg)	19.19 ± 0.08
$Vdss_{(actual)}$	(ml/kg)	204.3 ± 13.9
$Clb_{(actual)}$	(ml/h.kg)	194.7 ± 10.6
$AUC_{obs-total}$	(μg.h/ml)	31.78 ± 2.19

Table 5-3. Pharmacokinetic values obtained after analysis of the plasma ampicillin concentrations recorded in animals 1, 3, and 4 during ampicillin IV constant rate infusion.
$AUC_{obs-total}$ corresponds to the AUC during the infusion and post-infusion periods.

5.3.2 Second Phase

A direct relationship between the increase in the infusion dose rate and the plasma steady-state values obtained (correlation coefficient = 0.994) was obtained (Fig. 5-2). The three different dose rates 13.78, 19.34 and 24.48 produced steady-state plasma concentrations of 4.98 ± 0.11, 6.37 ± 0.08 and 8.25 ± 0.10 μg/ml, respectively (Tables 5-4 and 5-5; Fig. 5-3). In addition, the increase in dose rates correlated very well with the corresponding AUC obtained (correlation coefficient = 0.994) with values of 27.83, 33.89 and 42.10 μg. h/ml, respectively (Table 5-4).

5.4 Discussion

Several experimental and clinical studies have shown that antibiotics should be administered preoperatively rather than during the postoperative period to reduce the incidence of wound infections (Alexander *et al.*, 1960; Bernard & Cole, 1964; Burke, 1961; Polk & Lopez-Mayor, 1969). However, it is unclear which is the most suitable route of administration of antibiotics for the prevention of infection in the contaminated wound. In an experimental study carried out to evaluate the relationship between the route of administration and the wound fluid concentrations, Alexander & Alexander (1976) showed that when ampicillin was given as an intravenous bolus the concentrations in wound fluid were achieved much faster and were higher in the early period when compared to the intramuscular administration or the intravenous continuous infusion but the antibiotic was also eliminated more rapidly from the body when administered by the intravenous route. However, no intravenous loading dose was administered prior to the intravenous infusion and therefore a relatively long period of infusion was necessary to achieve effective plasma and wound fluid concentrations.

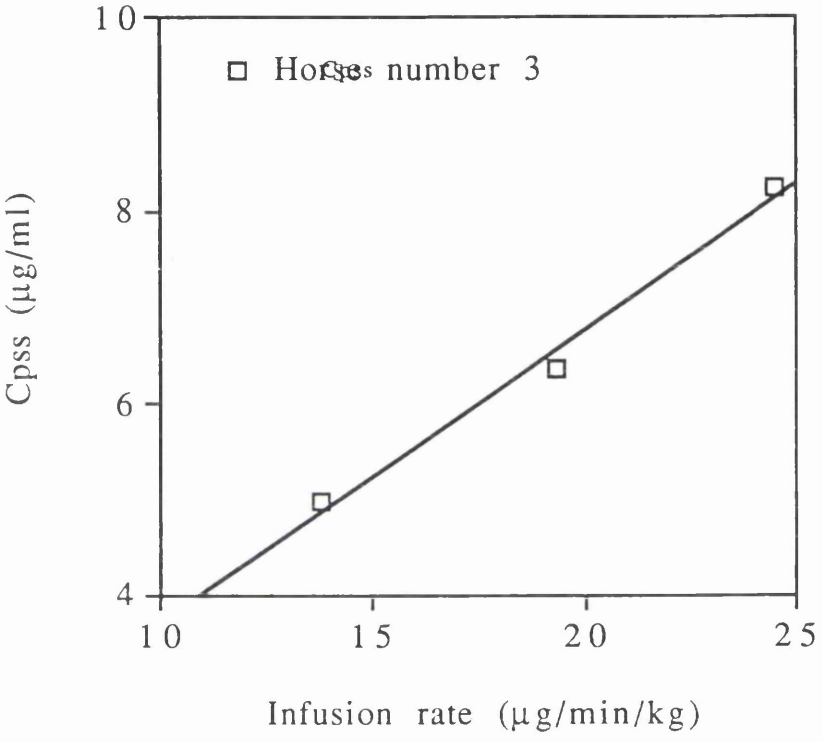


Fig. 5-2. Correlation (0.994) between the infusion rate and the steady-state plasma concentration achieved.

Variable		Animal number		
		3	3	3
Ro	(μg/min/kg)	13.78	19.34	24.48
β	(/h)	0.940	1.006	0.875
Cpmax	(μg/ml)	5.76	6.91	8.90
Cpmin	(μg/ml)	4.53	6.00	7.45
$Cpss_{(actual)}$	(μg/ml)	4.98 ±0.11	6.37 ±0.08	8.25 ±0.10
$Vdss_{(actual)}$	(ml/kg)	176.67	181.09	204.00
$Clb_{(actual)}$	(ml/h.kg)	166.02	182.17	178.03
$AUC_{obs\text{-}total}$	(μg.h/ml)	27.83	33.89	42.10

Table 5-4. Pharmacokinetic values obtained after analysis of the plasma ampicillin concentrations recorded in animal 3 during ampicillin IV constant rate infusion at three different infusion rates.

$AUC_{obs\text{-}total}$ corresponds to the AUC during the infusion and post-infusion periods.

	Infusion period		
	Infusion rate (μg/min/kg)		
Time (h)	13.78	19.34	23.48
0.033	17.85	17.76	19.45
0.083	15.26	15.26	16.49
0.166	12.91	13.02	14.11
0.25	10.67	11.60	12.45
0.50	8.26	8.62	9.81
0.75	7.20	7.70	9.09
1.00	6.11	7.23	8.38
1.25	5.76	6.91	8.56
1.50	5.33	6.62	8.22
1.75	5.27	6.71	8.01
2.00	5.18	6.42	7.97
2.25	5.21	6.49	7.45
2.50	4.78	6.26	8.25
2.75	4.63	6.15	8.31
3.00	4.81	6.00	8.28
3.25	4.53	6.48	8.90
3.50	4.99	6.07	8.33
3.75	4.68	6.15	8.24
4.00	4.63	6.19	8.73
Time (h)	Post-infusion period		
0.033	4.34	5.73	7.39
0.083	3.79	5.19	5.95
0.166	3.36	4.59	5.58
0.25	3.07	3.97	4.87
0.50	2.02	2.89	3.39
1.00	1.08	1.54	2.19
2.00	0.31	0.50	0.69
4.00	0.06	0.08	0.15
8.00	0.00	0.00	0.00
12.00	0.00	0.00	0.00

Table 5-5 Ampicillin plasma concentrations in animal number 3 following an IV bolus of ampicillin at a 2 mg/kg dose rate and constant rate infusions at three different rates.

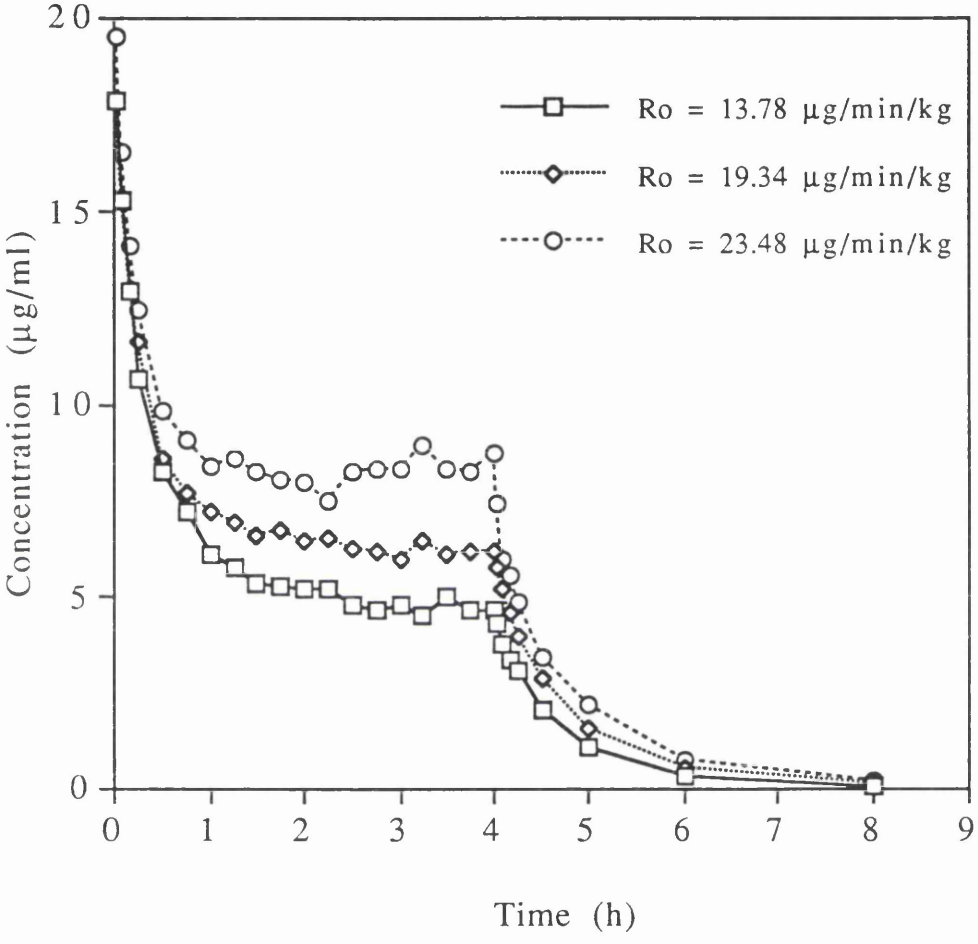


Fig. 5-3. Plasma ampicillin concentrations during and after IV infusions at three different rates to animal number 3.

These results led them to conclude that when rapid and sustained wound fluid concentrations are required an intravenous bolus should be followed either by an intramuscular injection or an intravenous continuous infusion.

When drugs which obey first-order kinetics are administered by continuous intravenous infusion, a period corresponding to 5 times the elimination half-life of the drug is necessary to reach 96.9% of the steady-state plasma concentration. This is the reason why an intravenous loading dose is used when rapid steady-state concentrations are required. No matter what quantity of drug is administered continuously, it will take this time to reach this fraction of plateau concentration if no bolus is administered at the beginning of the infusion. The amount of drug administered during the infusion will determine the concentration at steady-state but not the rate at which steady-state is achieved (Baggot, 1977c). Since the elimination half-life of ampicillin in horses is 0.75 hours (Horspool *et al.*, 1992) it would have taken 3.75 hours to almost attain the plateau level. Administration of an initial loading dose may remove the lag period. Two types of loading dose can be administered immediately before the start of an intravenous infusion. An initial loading dose, D1, aimed at achieving an immediate plasma concentration equal to the calculated Cpss. This is calculated by multiplying Cpss by the volume of the central compartment, Vc, into which drug initially distributes. However, using this regime the plasma concentration equal to the Cpss at the time zero will decline below steady state levels as drug distributes through other body tissues, reach a minimum and increase gradually until Cpss is achieved. On the other hand, a loading dose D 2, calculated by multiplying the Cpss by the Vdss will produce an initial peak above the Cpss level but the plasma concentration will not fall below the Cpss at any time. With this latter method the lipid solubility of the drug to be infused is important. For instance, highly lipid-soluble drugs have large volumes of distribution, Vdss, and therefore the loading dose will produce a high ratio of initial to steady-state plasma concentration (Cpo : Cpss). This is an important factor when dealing with lipid-soluble toxic drugs since the initial plasma concentrations could produce undesirable side

effects. To overcome this problem a model for achieving steady-state plasma concentrations of lipid-soluble drugs with narrow therapeutic window rapidly and safely was developed (Rigg & Wong, 1981). This model, is based on the administration of an initial loading dose where the initial concentration, Cpo, is equal to Cpss, followed by a constant rate infusion and an infusion rate decreasing exponentially with time. However, in most circumstances and if the drug to be infused is relatively non-toxic, a loading dose corresponding to an intermediate value between D1 and D2 is generally administered (Gibaldi & Perrier, 1975). Considering the maximum desirable Cpss during the infusion (in this study) (10μg/ml), the two possible loading doses D1=1.676 mg/kg and a D2= 2.108 mg/kg were calculated (Vc =167.6 ml/kg and Vdss = 210.8 ml/kg) and it was decided to administer an intermediate loading dose corresponding to 2 mg/kg.

In the first phase of the experiment, the intravenous bolus produced a peak plasma concentration of 16.75 ± 0.59 μg/ml (2 min sample) and the plasma concentration declined progressively until a steady-state concentration was achieved at approximately 1.25 hours (Fig. 5-1). From that moment the concentrations were maintained at a steady-state level of $Cpss_{(actual)}$ = 5.95 ± 0.10 μg/ml (range: 6.73 ± 0.50 μg/ml to 5.42 ± 0.33 μg/ml) until the infusion pump was disconnected at 4 hours. Once the infusion pump was disconnected, the concentration of antibiotic declined in an exponential manner and the last sample in which ampicillin could be detected was four hours after the end of the infusion period. The analysis of the curve after disconnecting the peristaltic pump allowed the calculation of the actual elimination rate constant of the drug , β (actual) and of the actual elimination half-life of the drug, t1/2 β (actual) together with the MRT. These results which are shown in Table 5-2, appeared to be in agreement with values obtained in other experiments where ampicillin was administered as an IV bolus dose (Chapters 3, 4 and 6).

When the estimated Cpss was calculated from the pharmacokinetic values of ampicillin sodium in the horse and the dose rate administered, a Cpss (estimate) = 19.19 / 210.8 x

0.0154 = 5.91 μg/ml was obtained. The similarity between the Cpss (estimate) = 5.91 and the Cpss (actual) = 5.95 shows that from a single intravenous plasma disposition study a dose rate for continuous infusion can be predicted accurately for this drug. In addition the AUC values obtained in the three horses showed very slight differences. Similarly, the values obtained during the infusion for the $Clb_{(actual)}$ and $Vdss_{(actual)}$ also appeared to be within the range encountered in previous studies.

In the second phase of the experiment, a linear relationship was observed between the dose rate administered, and both the plasma concentrations at steady-state and the AUC obtained. This was reflected in high correlation coefficients (0.994) between the dose administered and the corresponding $Cpss_{(actual)}$ and the $AUC_{obs\text{-}total}$.

In both phases of the experiment, the plasma concentrations obtained during the infusion were high enough to kill susceptible bacteria and some moderately susceptible bacteria (Adamson *et al.*, 1985; Prescott & Baggot, 1988c). Ampicillin sodium is a highly water-soluble antibiotic and is thought to cross phospholipid membranes very poorly and therefore does not penetrate readily into cells. However, the fact that it is only 6.8 to 8.0 per cent plasma protein-bound in the horse (Dürr, 1976), means that its concentration in the extracellular fluid (ECF), where most infections occur, is almost equal to the concentration in plasma since the free drug diffuses through capillary pores and equilibrates in the ECF (Keen, 1989). This is reflected in a small steady state volume of distribution (204.3 ± 13.9 ml/kg), very similar to the value of ECF volume of most tissues (200 ml/kg). Therefore, it is likely that the concentrations obtained in plasma would be very similar to those in most tissue fluids.

The present study indicates that administration by continuous infusion is accurate and usable; however, an IV bolus of 10 mg/kg would have yielded concentrations above or equal to the Cpss of the first phase for a period of almost two hours (Horspool *et al.*, 1992). Thus a single IV bolus administered at a high enough dose level (mg/kg) or an IV bolus followed by an IM injection, would produce similar plasma concentrations to the

ones obtained here. As ampicillin toxicity is very low, high peak concentrations are unlikely to produce side effects.

5.5 Conclusion

For practical purposes and when non-toxic antimicrobials such as ampicillin are administered, simpler routes of administration such as the intravenous, or intravenous and intramuscular administration may produce similar results and would be more appropriate under field conditions for prophylaxis during surgery. However, for drugs with a narrow therapeutic window which produce toxic side effects when high peak concentrations are achieved, intravenous infusion may be appropriate. In addition, the combination of an IV bolus dose followed by a constant IV infusion has been advocated as the most suitable method of administration of prophylactic antimicrobials in those patients suffering from shock, where the impaired tissue perfusion would limit the absorption of antimicrobials administered IM (Alexander & Alexander, 1976)

Chapter 6

Studies on bacampicillin hydrochloride in ponies and horses

6.1 Introduction

Various studies carried out in the equine have shown the unsuitability of the oral route for the administration of penicillins in this species. Following oral administration of different penicillins to adult horses, the poor absorption of these antibiotics was observed and in many cases the presence of gastrointestinal disturbances was also recorded (Wilson *et al.*, 1988; Baggot *et al.*, 1990; Ensink *et al.*, 1992; Horspool, 1992).

In human antibiotic therapy, the development of different penicillin prodrugs for oral administration led to a marked improvement of their bioavailability. Numerous experimental studies carried out in humans have shown that the greater oral bioavailability of these prodrugs when compared to their corresponding active compounds, is responsible for the presence of higher peak plasma and tissue concentrations together with a faster absorption rate. In addition, the enhanced absorption has also been shown to be responsible for a reduced incidence of disturbances of the intestinal flora ecosystem (Sjovall *et al.*, 1986).

One of these prodrugs, pivampicillin, was recently investigated in the horse and appeared to be absorbed after oral administration, to a much larger extent than other penicillins (Ensink *et al.*, 1992).

In the following studies, bacampicillin hydrochloride, an ampicillin prodrug, was investigated as a possible alternative for the administration of penicillins orally to horses and ponies.

6.2 Materials and methods

6.2.1 Animals

Three Thoroughbred geldings (numbers 1, 2 and 5) weighing 615, 607 and 612 kg respectively and four ponies (numbers 8, 9, 10, and 11) with weights of 294, 168, 232 and 237 kg were used in the study.

6.2.2 Intravenous administration of ampicillin sodium

Ampicillin sodium was administered intravenously at a dose rate of 10 mg/kg. Blood samples were subsequently taken from the contralateral jugular vein at 2, 5, 10, 15, 30 and 45 minutes and at one, 1.5, 2, 4, 8, 12 and 24 hours, stored and analysed as described previously (General Materials and Methods).

6.2.3 Administration of bacampicillin hydrochloride

Bacampicillin hydrochloride (Ambaxin, Upjohn Limited, Crawley, England) was suspended in water (500 ml for horses and 250 ml for ponies) and administered intragastrically via stomach tube at a dose rate of 13.52 mg/kg (equivalent of 10 mg/kg of ampicillin sodium). Heparinised blood samples were then collected at 15, 30 and 45 minutes and one hour, 1.5, 2, 4, 6, 8, 12 and 24 hours after bacampicillin administration. After centrifugation at 1700 x *g* for 15 minutes, plasma was collected and stored at -20 º C until analysis which was carried out on the following day.

6.2.4 Administration of bacampicillin hydrochloride and dichlorvos

Dichlorvos, 0 - (2 , 2- dichloro - vinyl) 0, 0- dimethyl phosphate, (Atgard® vet., Fermenta Animal Health Europe S. A., Belgium), was mixed in a small volume (200 - 400 g) of coarse mix containing a blend of molasses and soya oil and fed to the horses and ponies at a dose rate of 40 mg/kg bodyweight. Samples for determination of plasma pseudocholinesterase and erythrocyte acetylcholinesterase were collected in syringes containing EDTA (EDTA KE/2.7 ml Monovette, Sarstedt Limited, Leicester, England) four days prior to dichlorvos administration and at one, 2, 3, 4, 7, 10, 20, 30, 45, 60, and 90 days after its administration. Following sample collection the haematocrit values were measured by using a microhaematocrit centrifuge (Hawkesley Microhaematocrit Centrifuge, Hawkesley & Sons, Lancing, U. K) and the values recorded for retrospective determination of erythocyte acetylcholinesterase. A 0.1 ml volume of blood was removed from

each sample and the remainder centrifuged at 1700 x *g* for 15 minutes, whole blood and plasma were subsequently stored at -20 º C until analysis.
Bacampicillin was administered one day after dichlorvos administration at a dose rate of 13.52 mg/kg intragastrically and blood samples were collected as described previously (See 6.2.3 Administration of bacampicillin hydrochloride).

6.2.5 Administration of bacampicillin hydrochloride and probenecid

Bacampicillin hydrochloride was administered intragastrically at a dose rate of 13.52 mg/kg immediately after the administration of probenecid by the same route at a dose rate of 75 mg/kg. Blood samples were collected and stored subsequently as described previously (See 6.2.3 Administration of bacampicillin hydrochloride).

6.2.6 Plasma ampicillin concentrations

Plasma ampicillin concentrations after administration of bacampicillin hydrochloride and ampicillin sodium were determined by the agar diffusion microbiological assay using *Bacillus subtilis* as described previously.

6.2.7 Determination of plasma pseudocholinesterase and erythrocyte acetylcholinesterase

A commercially available colorimetric method (Cholinesterase (PTC), SIGMA Chemical Co. Ltd, Dorset, England) was used to determine plasma and erythrocyte esterase concentrations. The estimation of enzymatic activity was based on the Ellman reaction (Dietz *et al.*, 1973) and was carried out by measuring spectrophotometrically (SP8-500 UV/Visible Spectrophotometer) the hydrolysis of propionylthiocholine into thiocholine at 405 nm. The measurements were carried out in plasma and in blood haemolysate, the latter being carried out by adding 1.9 ml of sterile distilled water to 0.1 ml of whole blood collected before

centrifugation of the sample. The plasma pseudocholinesterase concentrations together with the haemolysate concentrations and the haematocrit values permitted determination of the erythocyte acetylcholinesterase concentrations.

6.2.8 Plasma probenecid concentrations

Plasma probenecid concentrations were determined by high performance liquid chromatography (HPLC) at a wavelength of 254 nm as described previously (See General Materials and Methods)

6.2.9 Pharmacokinetic analysis

Plasma ampicillin concentrations after IV administration, were analysed by compartmental model analysis and non-compartmental analysis. When ampicillin was measured in plasma after intragastric bacampicillin administration (experiments 6.2.3, 6.2.4 and 6.2.5), the results were analysed by noncompartmental analysis. Probenecid plasma concentrations were analysed by noncompartmental analysis.

6.2.10 Statistical analysis

A Wilcoxon signed-rank test was used to compare the pharmacokinetic variables obtained after bacampicillin hydrochloride administration during the various experiments.

6.2.11 *In vitro* evaluation of bacampicillin metabolism

Equimolar standards of bacampicillin hydrochloride (Sigma Chemical Co Ltd., Dorset, UK) and ampicillin (equivalent of 1, 5, 10 and 20 μg/ml ampicillin) were prepared in phosphate buffer (pH = 7.00), whole equine blood, plasma, equine caecal fluid and equine caecal fluid filtered (bacteria free) with a 0.2 μm filter (Acrodisc, Gelman Sciences, Michigan, USA). The standards were analysed by microbiological assay as described previously (see General materials and methods).

6.3 Results

6.3.1 Intravenous administration of ampicillin sodium

The administration of ampicillin sodium as an IV bolus produced plasma concentrations that were best described by a biexponential disposition curve. Ampicillin was rapidly eliminated from the body after administration, with the last concentration detected 8 hours after administration in three animals (animals 1, 2, and 11) (Table 6-1; Appendix D Table D-1 ; Fig 6-1).

The rapid elimination was reflected in the elimination half-life (t1/2 β) of 0.664 hours and the total body clearance of 372.30 ± 36.79 ml/h.kg (Table 6-2; Appendix D Table D-2). In addition, when the data was analysed by noncompartmental analysis, the rapid elimination of ampicillin was reflected by a mean residence time, MRT of 46.67 ± 7.19 minutes and a Clb_{obs} of 365.40 ± 34.70 ml/h.kg (Table 6-3; Appendix D Table D-3).

The values obtained for the volumes of distribution at steady-state, Vdss = 293.51 ± 18.04 ml/kg and $Vdss_{obs}$ = 262.80 ± 17.40 ml/kg (Tables 6-2 and 6-3; Appendix D Tables D-2 and D-3) reflected a limited distribution of the antibiotic into body tissues, and suggested that ampicillin was probably confined to the extracellular fluid.

6.3.2 Administration of bacampicillin hydrochloride

When bacampicillin was administered intragastrically, absorption of the antibiotic was rapid as reflected in mean ± SEM plasma ampicillin concentration of 3.80 ± 0.41 μg/ml, 15 minutes after administration (Table 6-4; Appendix D Table D-4; Fig. 6-2). This rapid absorption was also perceived in a mean ± SEM peak plasma concentration Cmax of 6.08 ± 0.54 μg/ml achieved at tmax = 42.86 ± 8.92 minutes after administration (Table 6-5).

Once the peak plasma concentration was reached, ampicillin concentrations declined and were detected in all animals 4 hours after administration but only in two animals (animals 1 and 2) 8 hours after administration (Appendix D Table D-4).

Time (h)	Mean ± SEM (n=7)
0.033	57.85 ± 2.67
0.083	42.83 ± 2.24
0.166	31.23 ± 1.29
0.25	25.83 ± 1.32
0.5	16.12 ± 1.21
0.75	9.80 ± 0.98
1	6.70 ± 0.72
1.5	3.86 ± 0.68
2	2.32 ± 0.49
4	0.48 ± 0.21
8	0.06 ± 0.03
12	0.00 ± 0.00
24	0.00 ± 0.00

Table 6-1. Mean ± SEM plasma ampicillin concentrations after an IV bolus of ampicillin sodium at a 10 mg/kg dosage rate.

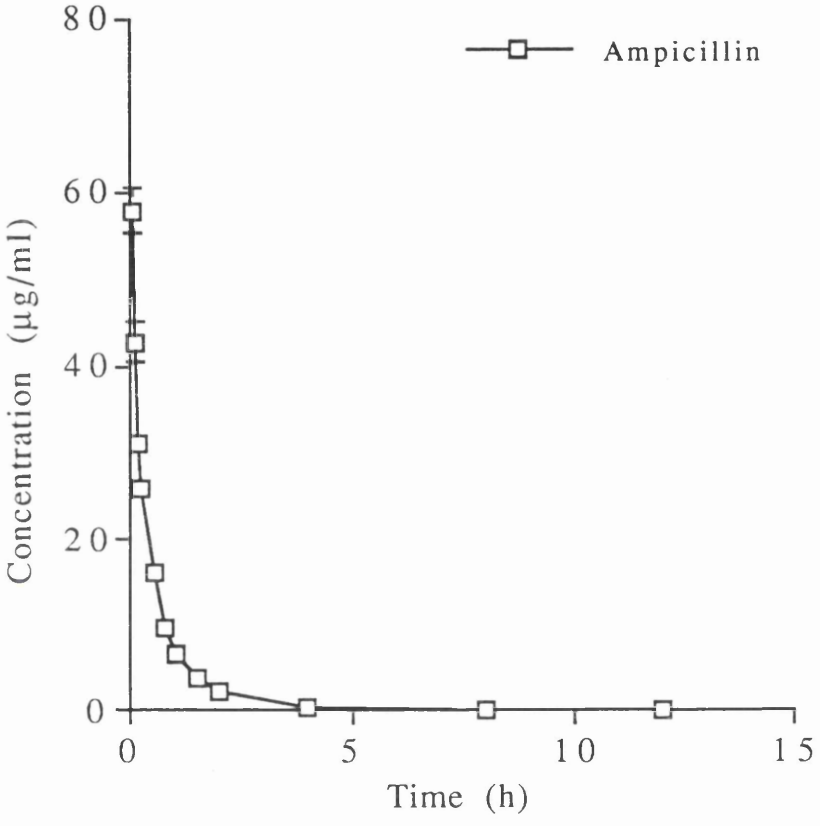


Figure 6-1. Ampicillin plasma concentrations (mean ± SEM) following administration of an IV bolus at a 10 mg/kg dose rate to horses and ponies (n=7).

Variable		Mean ± SEM (n=7)
α	(/h)	10.93 ± 3.22
β	(/h)	1.04 ± 0.144
t1/2 α	(h)	0.068
t1/2 β	(h)	0.664
Cp0	(μg/ml)	74.65 ± 9.06
AUC	(μg.h/ml)	28.35 ± 2.57
Vc	(ml/kg)	142.93 ± 12.68
Vd_{area}	(ml/kg)	377.84 ± 32.05
Vdss	(ml/kg)	293.51 ± 18.04
Clb	(ml/h.kg)	372.30 ± 36.79
k_{21}	(/h)	3.68 ± 0.87
k_{el}	(/h)	2.80 ± 0.43
k_{12}	(/h)	4.76 ± 2.12

Table 6-2. Pharmacokinetic values obtained by compartmental analysis of ampicillin plasma concentration after an IV bolus of ampicillin sodium at a 10 mg/kg dosage rate.

Variable		Mean ± SEM (n=7)
AUC_{obs}	(μg.h/ml)	28.78 ± 2.56
$AUMC_{obs}$	($\mu g.h^2$/ml)	24.13 ± 5.83
MRT	(min)	46.67 ± 7.19
$Vdss_{obs}$	(ml/kg)	262.80 ± 17.40
Clb_{obs}	(ml/h.kg)	365.40 ± 34.70

Table 6-3. Pharmacokinetic values obtained after non-compartmental analysis of ampicillin plasma concentration after an IV bolus of ampicillin sodium at a 10 mg/kg dosage rate.

Time (h)	Bacampicillin (n=7)	Bacampicillin and Probenecid (n=6)	Bacampicillin and Dichlorvos (n=7)
0.25	3.80 ± 0.41	0.39 ± 0.11	3.79 ± 1.12
0.30	5.79 ± 0.48	1.25 ± 0.14	5.75 ± 0.87
0.75	5.96 ± 0.60	2.16 ± 0.27	6.01 ± 0.52
1	4.93 ± 0.53	2.42 ± 0.26	6.31 ± 0.61
1.5	3.15 ± 0.69	2.38 ± 0.37	4.41 ± 0.51
2	2.33 ± 0.67	2.10 ± 0.30	3.01 ± 0.35
4	0.81 ± 0.27	0.99 ± 0.30	0.66 ± 0.27
6	0.30 ± 0.12	0.58 ± 0.25	0.10 ± 0.07
8	0.03 ± 0.00	0.36 ± 0.18	0.00 ± 0.00
12	0.00 ± 0.00	0.34 ± 0.19	0.00 ± 0.00
24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 6-4. Mean ± SEM Plasma ampicillin concentrations after intragastric administration of bacampicillin, bacampicillin and probenecid, and bacampicillin and dichlorvos. Bacampicillin was administered at a 13.52 mg/kg dose rate in the three phases of the study.

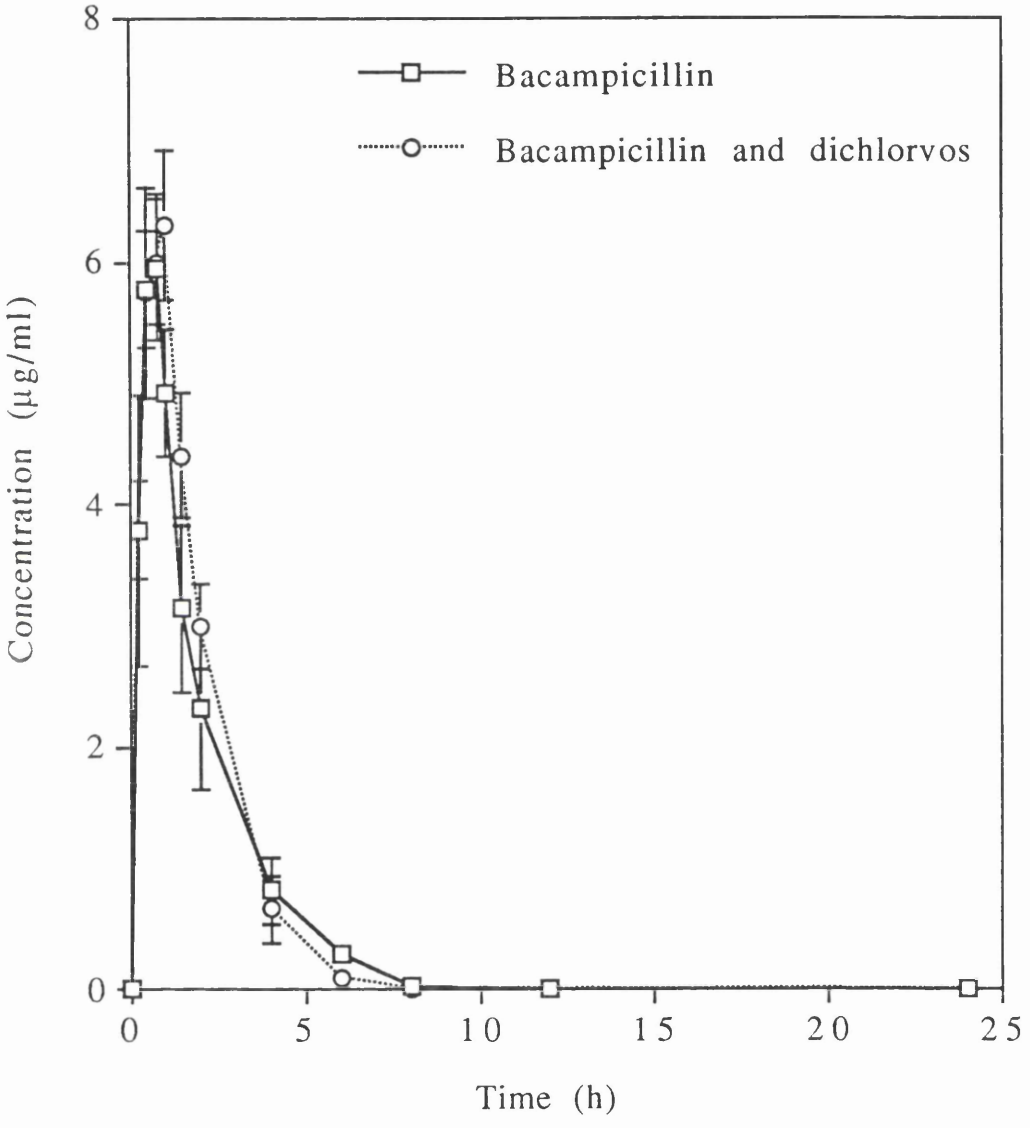


Fig. 6-2. Ampicillin plasma concentrations (mean ± SEM) following oral administration of bacampicillin (n=7) and bacampicillin and dichlorvos (n=7) to horses and ponies.

Variable	Bacampicillin (n=7)	Bacampicillin and Dichlorvos (n=7)	Bacampicillin and Probenecid (n=6)
Cmax (μg/ml)	6.08 ± 0.54*	6.95 ± 0.47*	2.61 ± 0.32
tmax (min)	42.86 ± 8.92	45.00 ± 7.32	65.00 ± 8.37
β (/h)	0.757 ± 0.104	0.949 ± 0.109*	0.38 ± 0.10
AUC_{obs} (μg.h/ml)	12.29 ± 2.26	13.82 ± 1.29	11.31 ± 2.93
$AUMC_{obs}$(μg.h^2/ml)	21.67 ± 5.88*	22.26 ± 4.42*	58.60 ± 28.00
MRT (min)	95.82 ± 9.67*	95.10 ± 10.30*	255.5 ± 60.1
F(%)	41.01	48.02	37.72
MAT (min)	49.15 ± 5.05	-	-

Table 6-5. Mean ± SEM pharmacokinetic values for ampicillin after intragastric administration of bacampicillin, bacampicillin and dichlorvos and bacampicillin and probenecid. Bacampicillin was administered at a 13.52 mg/kg dose rate in the three phases of the study.

* Statistically different ($P<0.05$) from bacampicillin with probenecid.

When pharmacokinetic analysis was carried out, an MRT of 95.82 ± 9.67 minutes was found. In addition, an oral bioavailability of 41.01% was obtained when the AUC_{obs} after oral administration was divided by the AUC_{obs} after IV administration of ampicillin. The mean absorption time MAT, for bacampicillin using the active ampicillin derivative was calculated to be 49.15 ± 5.05 minutes (Table 6-5; Appendix D Table D-5).

6.3.3 Administration of bacampicillin hydrochloride and dichlorvos

The oral administration of dichlorvos produced a marked reduction of plasma pseudocholinesterase and erythrocyte acetylcholinesterase concentration in all animals. Plasma and erythrocyte cholinesterase concentrations were measured three days prior to dichlorvos administration and immediately before administration. The pre values allowed calculation of a mean ± SEM value for normal plasma and erythrocyte cholinesterase. Assuming that pre values corresponded to a 100 % activity of plasma and erythrocyte cholinesterases, bacampicillin hydrochloride was administered at 13.52 mg/kg (Day 1 after dichlorvos administration), when there was a reduction of 96.26 % in plasma cholinesterase activity and 98.75 % in the erythrocyte cholinesterase activity (Table 6-6). The cholinesterase concentrations subsequently increased in a logarithmic manner (Figs. 6-3 and 6-4) with 90.72 % of the plasma pseudocholinesterase activity and 99.06 % of the erythocyte acetylcholinesterase activity present 90 days after dichlorvos administration (Table 6-6; Appendix D Tables D-6 and D-7). The inhibition of cholinesterase activity did not affect the behaviour of bacampicillin after its administration. This was reflected in similar plasma ampicillin concentrations when bacampicillin was administered alone or following dichlorvos administration (Table 6-4; Fig. 6-2; Appendix D Table D-8). In addition, none of the pharmacokinetic values calculated for bacampicillin after dichlorvos administration, were statistically different from those obtained after bacampicillin administration alone (Table 6-5; Appendix D Tables D-5 and D-9).

Day	Plasma esterase levels (U/L)	Activiy (%)	Erythrocyte esterase levels (U/L)	Activiy (%)
Pre	4307.0 ± 264.0	100.00	8114.0 ± 614.0	100.00
1	161.2 ± 11.9	3.74	101.7 ± 20.9	1.25
2	256.7 ± 24.8	5.96	212.6 ± 39.3	2.62
3	454.0 ± 52.2	10.54	590.6 ± 99.6	7.28
4	613.2 ± 75.6	14.24	1099.8 ± 190.4	13.55
7	1249.7 ± 165.7	29.01	2219.2 ± 339.3	27.35
10	1835.3 ± 267.6	42.61	3647.5 ± 551.9	44.96
20	2529.1 ± 425.3	58.72	4767.8 ± 743.4	58.76
30	2705.1 ± 290.2	62.81	5254.4 ± 698.5	64.76
45	3169.8 ± 403.4	73.60	5959.5 ± 889.5	73.45
60	3427.6 ± 379.1	79.58	6897.5 ± 691.3	85.01
90	3907.3 ± 377.7	90.72	8038.0 ± 887.7	99.06

Table 6-6. Plasma and erythrocyte cholinesterase activity before and after administration of dichlorvos orally at a 40 mg/kg dose rate.

Pre value represents the Mean ± SEM of values obtained 4 days prior to dichlorvos administration.

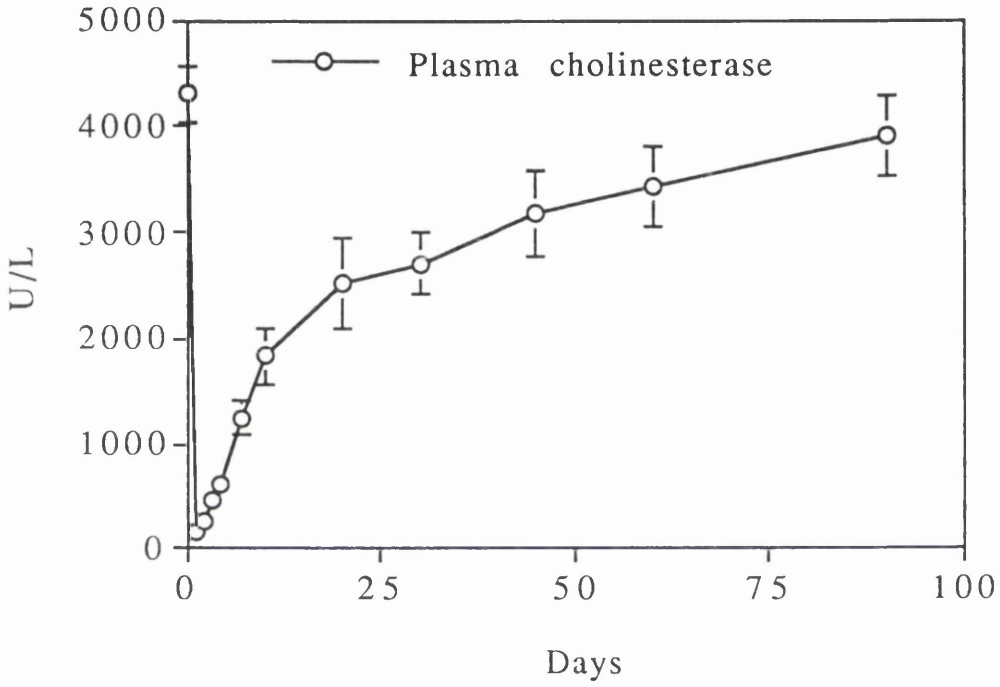


Fig. 6-3. Plasma cholinesterase levels (mean ± SEM) following oral administration of dichlorvos to ponies and horses at a 40 mg/kg dosage rate. Value on day 0 calculated as mean ± SEM of values obtained during four pre days.

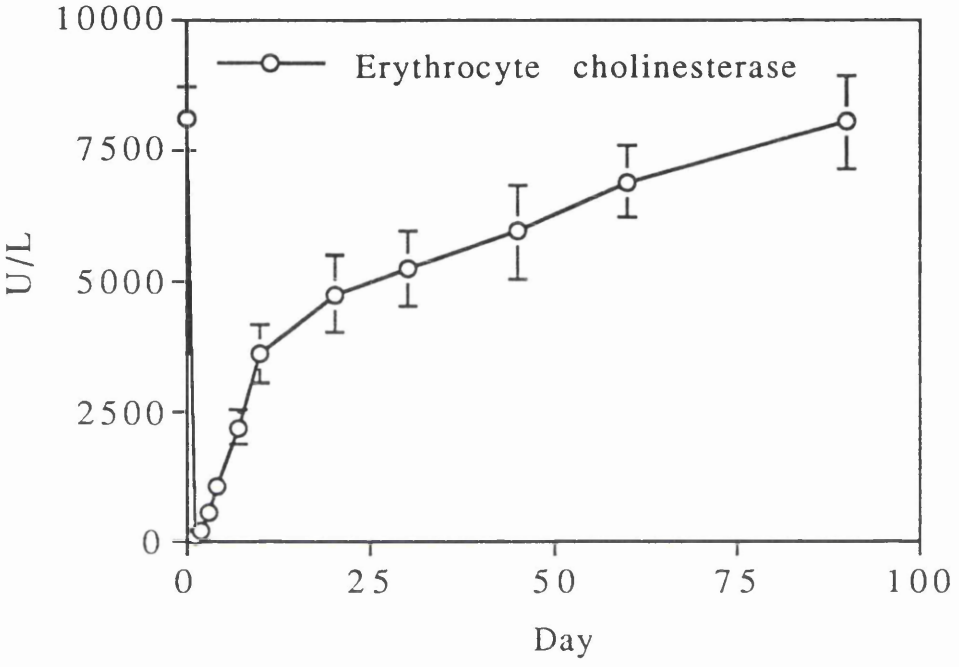


Fig. 6-4. Erythrocyte cholinesterase levels (mean ± SEM) following oral administration of dichlorvos to ponies and horses at a 40 mg/kg dosage rate.
Value on day 0 calculated as mean ± SEM of values obtained during four pre days.

6.3.4 Administration of bacampicillin hydrochloride and probenecid

The presence of probenecid impaired the absorption of bacampicillin as reflected in lower plasma ampicillin concentrations (Table 6-4; Fig. 6-5). There was a significantly ($P<0.05$) lower peak plasma concentration of ampicillin when bacampicillin was administered with probenecid than when bacampicillin was administered alone (Cmax. = 2.61 ± 0.32 μg/ml vs Cmax = 6.08 ± 0.54 μg/ml) (Table 6-5). The Cmax for ampicillin after concurrent bacampicillin and dichlorvos administration was statistically higher than the value obtained when bacampicillin was administered concomitantly with probenecid (Cmax = 6.95 ± 0.47 μg/ml vs Cmax. = 2.61 ± 0.32 μg/ml). However, the presence of probenecid prolonged the elimination rate of ampicillin present in plasma. This was reflected in a mean ± SEM ampicillin concentration of 0.34 ± 0.19 μg/ml, 12 hours following administration of bacampicillin and probenecid (Table 6-4; Appendix D Table D-10). The pharmacokinetic analysis also revealed a prolongation of the antibiotic presence in the body as reflected in a significantly ($P<0.05$) longer MRT when probenecid was present (MRT = 255.5 ± 60.1 vs 95.82 ± 9.67 minutes). The longer MRT was the result of a significant ($P<0.05$) increase of the $AUMC_{obs}$ of ampicillin in the presence of probenecid (Table 6-5; Appendix D Table D-11). Also, the MRT and AUMCobs values for bacampicillin in the presence of probenecid, were statistically higher ($P<0.05$) than the values achieved when dichlorvos and bacampicillin were administered together (Table 6-5). Probenecid was rapidly absorbed from the gastrointestinal tract after administration. Plasma probenecid concentrations of 54.45 ± 7.59 μg/ml were measured 15 minutes after administration and after 24 hours, probenecid levels of 28.51 ± 9.76 μg/ml could still be detected (Table 6-7; Appendix D Table D-12; Fig. 6-6). Peak plasma probenecid concentrations (Cmax) of 153.7 ± 20.3 μg/ml were reached at 165.0 ± 63.8 minutes (Table 6-8). In addition, non-compartmental analysis of plasma probenecid concentrations, yielded an MRT value of 981.60 ± 309.00 minutes (Table 6-8; Appendix D Table D-13).

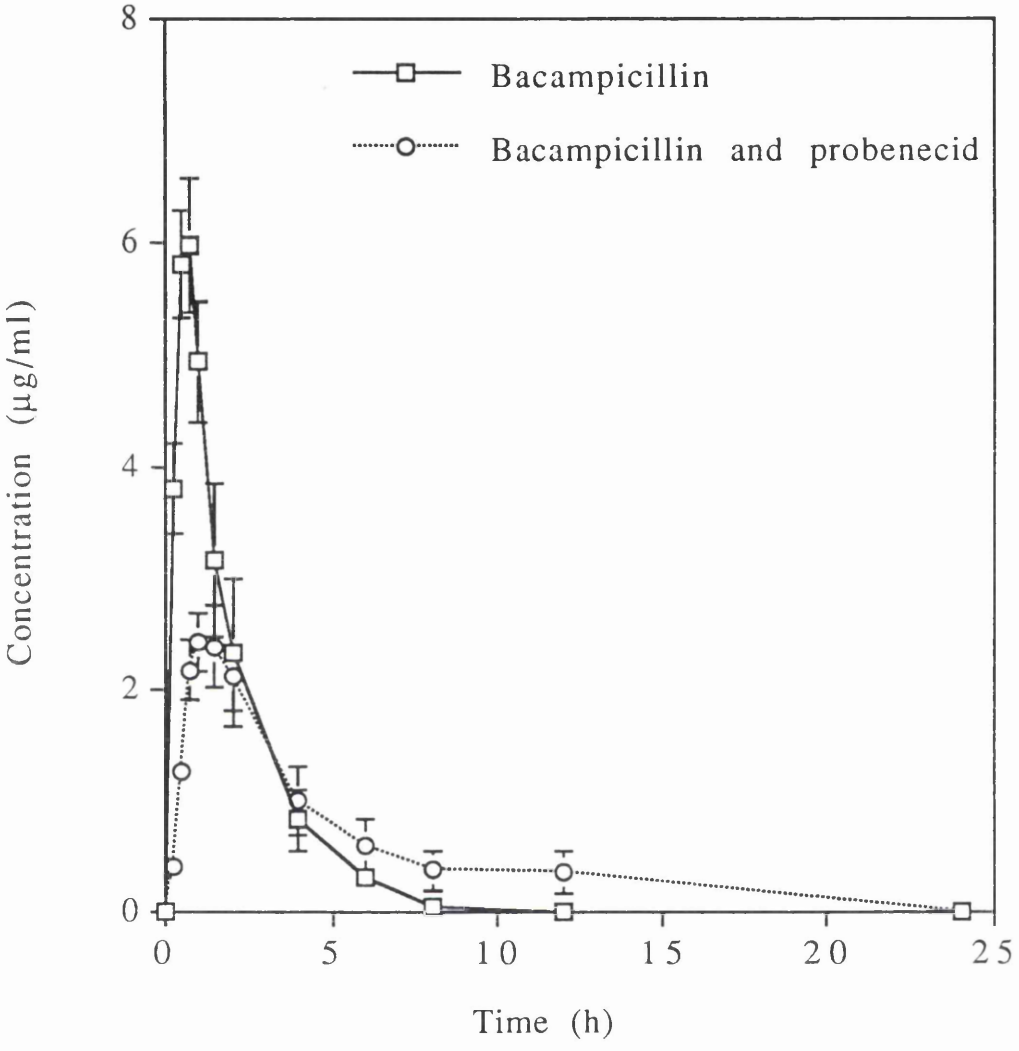


Fig. 6-5. Ampicillin plasma concentrations (mean ± SEM) following oral administration of bacampicillin (n=7), and bacampicillin and probenecid (n=6) to horses and ponies.

Time (h)	Mean ± SEM (n=6)
0.25	54.45 ± 7.59
0.30	100.47 ± 17.87
0.75	119.04 ± 24.60
1	128.40 ± 25.80
1.5	140.85 ± 20.55
2	140.54 ± 17.30
4	99.66 ± 8.27
6	92.08 ± 10.60
8	79.16 ± 13.36
12	62.36 ± 14.81
24	28.51 ± 9.76

Table 6-7. Mean ± SEM plasma probenecid concentrations after intragastric administration of bacampicillin hydrochloride at a 13.52 mg/kg dose rate and probenecid at a 75 mg/kg dose rate.

Variable		Mean ± SEM (n=7)
Cmax	(μg/ml)	153.70 ± 20.30
tmax	(min)	165.00 ± 63.80
β	(/h)	0.0799 ± 0.0188
AUC_{obs}	(μg.h/ml)	3147.00 ± 1115.00
$AUMC_{obs}$	($\mu g.h^2/ml$)	79582.00 ± 55397.00
MRT	(min)	981.60 ± 309.00

Table 6-8. Pharmacokinetic values of probenecid after intragastric administration at a 75 mg/kg dose rate in combination with the intragastric administration of bacampicillin hydrochloride at a 13.52 mg/kg dose rate.

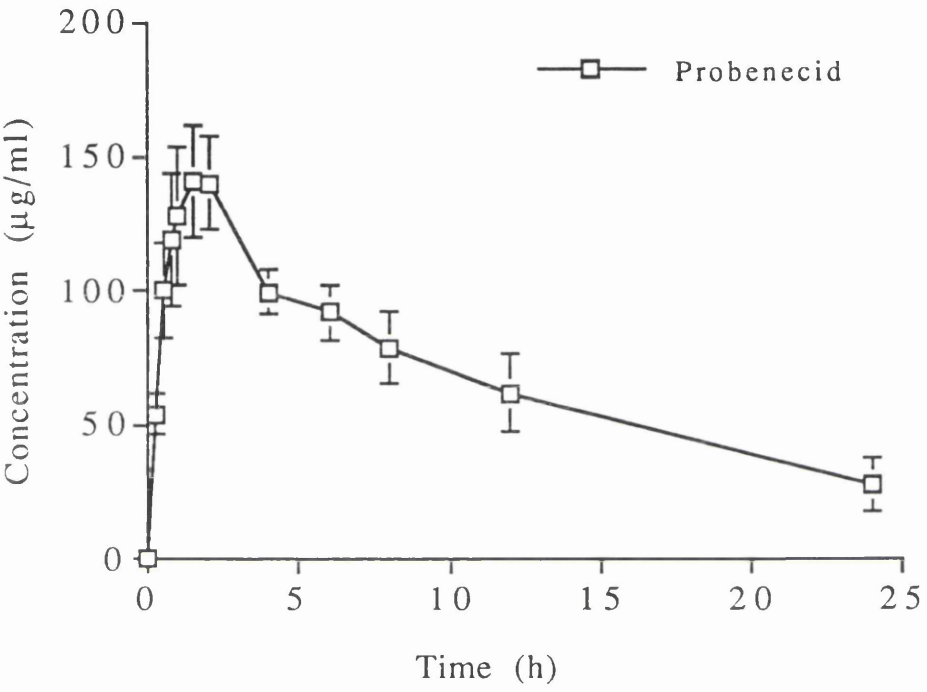


Fig. 6-6. Mean ± SEM plasma probenecid concentrations following intragastric administration of probenecid at a 75 mg/kg dose rate and bacampicillin hydrochloride at a 13.52 mg/kg dose rate to horses and ponies (n=6).

Following the intragastric administration of bacampicillin with probenecid animals had a reduced appetite and passed dark solid faeces for 12 hours after the administration. In addition, animals number 1, 10 and 11, showed abdominal discomfort and periods of sternal recumbency. The animals appeared normal by 48 hours after administration. Routine bacteriological examination of faecal samples from all animals 24 hours after bacampicillin and probenecid administration was characterised by heavy growth of lactose fermenting *E. coli* .

One of the animals was excluded from the calculations (used in tables 6-4 and 6-5) since the stomach tube got blocked during the administration of bacampicillin and probenecid. The experiment was not repeated in this animal since potential gastrointestinal disturbances had been identified in the initial study.

6.3.5 *In vitro* evaluation of bacampicillin metabolism

The diameter of the bacterial growth inhibition zones for bacampicillin and ampicillin standards made up in various body fluids, were compared to the zones of inhibition for equivalent ampicillin concentrations made up in phosphate buffer (pH = 7.00) and the recovery was then calculated. Ampicillin recoveries from ampicillin standards made up in different body fluids ranged from a minimum of 94.27 % in filtered caecal fluid to a maximum of 102.07 % in blood.

Ampicillin recoveries from bacampicillin standards indicated that 75.44 % bacampicillin was transformed into active ampicillin when the standards were made up in phosphate buffer. The transformation of the ester in equine caecal fluid was of 82.85 % when the caecal fluid was filtered and of 81.78 % when no filtration was carried out. In addition, bacampicillin standards made up in whole blood and plasma were transformed into active ampicillin to a 89.89 % and 93.23 %, respectively (Table 6-9).

	Concentration (µg/ml)				
	1.0	5.0	10.0	20.0	Mean
Ampicillin in Plasma	97.71 (n=4)	98.08 (n=4)	98.84 (n=4)	98.37 (n=4)	98.25 (n=16)
Bacampicillin in Plasma	90.87 (n=4)	93.26 (n=4)	94.46 (n=4)	94.35 (n=4)	93.23 (n=16)
Ampicillin in Blood	103.76 (n=4)	101.18 (n=4)	102.35 (n=4)	100.99 (n=4)	102.07 (n=16)
Bacampicillin in Blood	82.76 (n=4)	90.45 (n=4)	92.57 (n=4)	93.79 (n=4)	89.89 (n=16)
Ampicillin in C.fluid	90.61 (n=4)	96.33 (n=4)	96.85 (n=4)	97.42 (n=4)	95.30 (n=16)
Bacampicillin in C.fluid	70.32 (n=4)	83.02 (n=4)	86.24 (n=4)	87.55 (n=4)	81.78 (n=16)
Ampicillin in C. fluid filtered	88.82 (n=4)	94.70 (n=4)	95.50 (n=4)	98.06 (n=4)	94.27 (n=16)
Bacampicillin in C. fluid filtered	72.41 (n=4)	84.53 (n=4)	85.60 (n=4)	88.88 (n=4)	82.85 (n=16)
Ampicillin in Buffer	100.00 (n=4)	100.00 (n=4)	100.00 (n=4)	100.00 (n=4)	100.00 (n=16)
Bacampicillin in Buffer	63.52 (n=4)	76.42 (n=4)	79.41 (n=4)	82.42 (n=4)	75.44 (n=16)

Table 6-9. *In vitro* recoveries (in %) of ampicillin and bacampicillin standards (1, 5, 10 and 20 µg/ml ampicillin equimolars) in different fluids.
The recoveries were calculated after comparing the zones of bacterial growth inhibition produced by ampicillin and bacampicillin standards in different fluids to zones of inhibition of ampicillin standards made up in phosphate buffer.

6.4 Discussion

The administration of different penicillins to adult horses by the oral route has been shown to be of no value for the treatment of infections. Studies carried out to evaluate the extent of absorption of various penicillins in the horse, demonstrated that when penicillin G was administered to adult horses, only 2.87 % of the administered dose was absorbed from the gastrointestinal tract (Baggot *et al.*, 1990). Penicillin V, an antibiotic administered orally in humans, appeared to be even less well absorbed than penicillin G when given to adult horses, with an absolute bioavailability of 1.65 % (Baggot *et al.*, 1990). In addition, the administration of these two antibiotics, produced severe colic and diarrhoea in most of the animals. When amoxycillin, a broad spectrum semisynthetic penicillin, was administered to adult horses at a 20 mg/kg dose rate, the oral bioavailability was 10.4 %, (Wilson *et al.*, 1988), or 5.3 % (Ensink *et al.*, 1992). Consequently no suitable penicillin for oral antibiotherapy in the adult horse has been identified. However, a recent study carried out in adult horses, demonstrated that pivampicillin, the pivaloxyl-oxymethyl-ester of ampicillin, was absorbed after intragastric administration to a much larger extent than those penicillins previously investigated. When pivampicillin was administered at 19.19 mg/kg (equivalent of 15 mg/kg ampicillin) to starved horses and fed horses, an oral bioavailability of 30.9 % and of 35.9 % respectively were observed (Ensink *et al.*, 1992). Bacampicillin, the α-ethoxycarbonyloxyethyl ester of ampicillin, has a significantly greater oral bioavailability than ampicillin itself when administered orally to humans (Neu, 1981). In the present study and to evaluate the extent of absorption of bacampicillin from the equine gastrointestinal tract in the various experiments, an IV bolus of ampicillin sodium at a 10 mg/kg dose rate was administered to all animals. The results obtained for the elimination half-life, ($t_{1/2}\ \beta$ = 0.66 hours) were similar to previous reports where ampicillin sodium administered at a 10 mg/kg dose rate yielded a $t_{1/2}\ \beta$ of 0.75 hours in horses and $t_{1/2}\ \beta$ of 0.68 hours in ponies (Horspool *et al.*, 1992). In addition, a $t_{1/2}\ \beta$ of 0.70 hours was found for ampicillin when

administered to horses in a previous study (See Chapter 4). Other pharmacokinetic parameters calculated also seemed to be in agreement with previous experiments, including total body clearance and volumes of distribution, and confirmed that ampicillin is rapidly excreted from the body after IV administration in the equine and that its physicochemical characteristics restrict its distribution to the extracellular fluid.

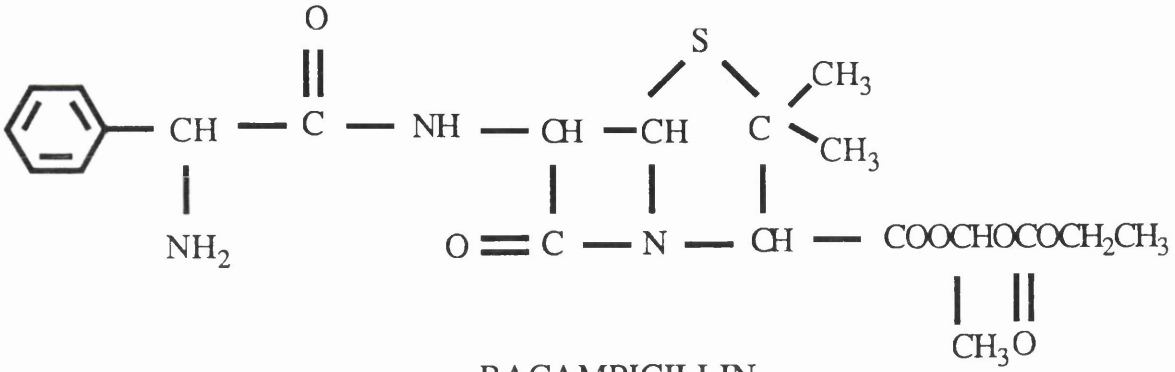
In the present experiments, when bacampicillin hydrochloride was administered to fed horses at a 13.52 mg/kg dose rate (equimolar dose of 10 mg/kg ampicillin sodium), an oral bioavailability of 41.01 % was achieved. This compares with 3.5 % when ampicillin was administered intragastrically to ponies (Horspool, 1992). Thus administration of ampicillin as an ester prodrug, (*ie* bacampicillin), produces a substantial and significant increase in the the extent of absorption. A study carried out in humans by Simon and others (1978) reported an oral bioavailability of 35 % when ampicillin was administered orally, which was increased to 95 % when bacampicillin was administered by the same route. Increases in the bioavailability of ampicillin when administered in its esterified form were also reported by Magni and others (1978) with a bioavalability 2 to 3 times higher for bacampicillin compared to ampicillin and by Rozencweig and others (1976) who found an increase in the bioavailability of 30 to 40 %.

The esterification of the ampicillin molecule has also been shown to be responsible for faster absorption in man when administered orally. Bacampicillin was shown to be absorbed five times faster (Rozencweig *et al.*, 1976) and to produce a much sharper and higher peak plasma concentration than parent ampicillin after oral administration (Swahn, 1976; Sjovall *et al.*, 1978; Ehrnebo *et al.*, 1979; Scheife and Neu, 1982). Another study demonstrated tmax intervals of 0.75 and 1 hour for bacampicillin when doses of 400 mg and 800 mg were administered to humans and of 1.5 and 2 hours when ampicillin was administered at 500 mg and 1000 mg (Magni *et al*,. 1978). Similarly, Swahn (1976) recorded higher peak plasma concentrations achieved faster when bacampicillin was administered orally than when the active compound (*ie* ampicillin) was administered by the same route (tmax = 53 min

vs tmax = 100 min). Also Simon and others, (1978) observed that bacampicillin reached peak plasma concentrations faster than ampicillin (tmax = 60 min vs tmax = 150 min) following oral administration of both compounds. The results of this study in the horse seem to be similar to those obtained in humans, since peak plasma ampicillin concentrations were achieved at 42.86 ± 8.92 minutes after intragastric administration of bacampicillin.

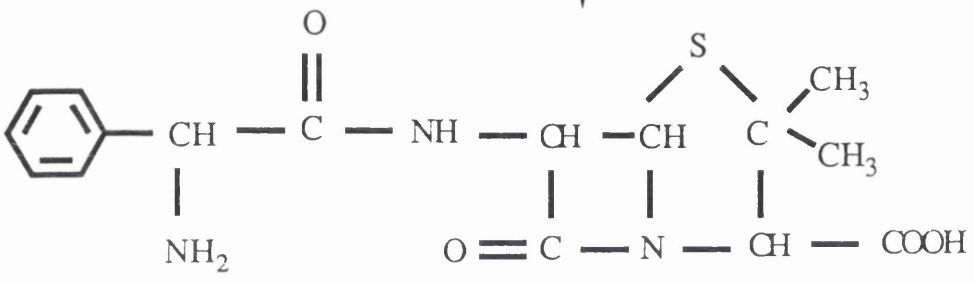
The esterification of ampicillin is responsible for the higher and faster absorption of bacampicillin from different parts of the gastrointestinal tract. When radiolabelled, [^{35}S] bacampicillin, was administered intragastrically to humans, measurement of bacampicillin in gastrointestinal aspirates demonstrated that 20 % of the dose was absorbed from the stomach, 65 % had been absorbed by gut proximal to and including the duodenum and 71 % in the gut proximal to and including jejunum (Swahn, 1976). Soon after absorption, bacampicillin was shown to be metabolised into ampicillin, acetaldehyde, carbon dioxide and ethanol by small intestine and blood esterases (Fig. 6-7). Studies carried out in rats showed that when pivampicillin was administered, it penetrated into the epithelial cells in the ester form and was subsequently hydrolysed by non-specific esterases present in the apical cytoplasm of the cells (Shindo *et al.*, 1978). When bacampicillin was administered intragastrically in humans, metabolism occurred very rapidly since no unchanged bacampicillin could be detected in plasma 10 minutes after administration (Neu, 1981; Sjovall *et al.*, 1985).

In the present study, plasma ampicillin concentrations of 3.80 ± 0.41 μg/ml were measured 15 minutes after bacampicillin administration to horses and ponies indicating rapid absorption of the ester. A mean ± SEM peak plasma concentration Cmax = 6.08 ± 0.54 μg/ml was subsequently reached at 42.86 ± 8.92 minutes and ampicillin concentrations could still be detected in plasma eight hours after bacampicillin administration. When the results of this study were compared to those obtained after intragastric administration of pivampicillin to horses at a 19.19 mg/kg dose rate (equivalent of 15 mg/kg of ampicillin) (Ensink *et al.*, 1992), it appeared that bacampicillin absorption was greater and faster than that of pivampicillin.



BACAMPICILLIN

ESTERASES



AMPICILLIN

+

CH_3CHO CO_2 CH_3CH_2OH

ACETALDEHYDE CARBON DIOXIDE ETHANOL

Fig. 6-7. Transformation of bacampicillin into ampicillin, acetaldehyde, carbon dioxide and ethanol.

Mean maximum peak plasma concentrations of ampicillin of 3.80 and 5.12 were achieved when the pivampicillin was administered to starved and fed horses respectively (Ensink *et al.*, 1992). These values were lower than those obtained following bacampicillin administration to fed horses and ponies during this study (Cmax = 6.08 ± 0.54 μg/ml) especially when it is considered that the equimolar dose of ampicillin was lower (15 mg/kg equimolar dose of ampicillin vs 10 mg/kg equimolar dose of ampicillin). It is unlikely that the ponies in this study were the reason for the higher peak plasma concentration achieved, since the individual higher peak plasma values following bacampicillin administration were obtained in the three horses (animals 1, 2 and 5) (Appendix D Table D-4). In addition, bacampicillin was absorbed faster than pivampicillin as reflected in a tmax of 42.86 minutes for bacampicillin and of 1 hour for pivampicillin.

The measurement of ampicillin concentrations after IV ampicillin administration and intragastric bacampicillin administration, permitted calculation of the mean residence time (MRT) and the mean absorption time (MAT). An MRT of 46.67 ± 7.19 minutes was obtained for ampicillin when administered IV and an MRT of 95.82 ± 9.67 minutes was calculated for ampicillin in plasma after bacampicillin administration. The MAT of 49.15 ± 5.05 minutes for bacampicillin (as ampicillin), suggests flip-flop kinetics where the MAT is greater than the MRT following IV administration.

The oral administration of dichlorvos has been shown to produce a reduction in plasma and red blood cell esterases levels in dogs (Ward & Glicksberg, 1971) and pigs (Bossen *et al.*, 1973). In addition, plasma esterases were also reduced in horses fed with dichlorvos (Snow, 1974). Since it has been shown that bacampicillin is metabolised into ampicillin by esterases (Swahn, 1976), the effects of a reduction of the esterase levels on the metabolism and fate of bacampicillin were evaluated.

The results showed that the oral administration of dichlorvos at 40 mg/kg reduced dramatically plasma esterase levels 24 hours after the administration. A depression of plasma esterase activity of 96.26 % was found 24 hours after administration and by day 90 after administration, 90.72 % of the original plasma esterase activity had returned. A mean depression of plasma esterase

levels of 79.28 % was recorded in horses by Snow (1974), 24 hours after oral dichlorvos administration. These lower levels can explained by the lower dichlorvos intake in the study carried out by Snow (1974) (Mean ingested dose of 30.57 mg/kg vs Mean ingested dose of 40 mg/kg in the present study).

Erythrocyte esterase levels were also markedely depressed in all animals 24 hours after dichlorvos administration during the present experiment (Table 6-6; Appendix D Table D-7). Only 1.25 % of the pre-value erythrocyte esterase activity could be measured 24 hours after dichlorvos administration, but the activity returned progressively to normal and by day 90 after administration an activity of 99.06 % was recorded. These results are in disagreement with the study carried out by Snow (1974), where a much smaller depression of erythrocyte esterase activity (89.5 % activity 24 hours after dichorvos) was detected in two animals in which it was measured. Snow (1974) administered a lower dose of organophosphate; however, the differences are greater than those which could be ascribed the lower dose.

The marked depression in both plasma and erythrocyte esterase activities achieved during the present experiment, did not affect the pharmacokinetic behaviour of bacampicillin. It was anticipated that a reduction in plasma and erythrocyte esterase activity could slow down the hydrolysis of bacampicillin into ampicillin and possibly prolong the ampicillin persistence in the body. However, it was impossible to tell from these results whether the drug present in plasma and measured in the microbiological assay was in the bacampicillin or the ampicillin form at the time of sample collection. Despite the fact that bacampicillin lacks antimicrobial activity *per se*, the *in vitro* study revealed that when bacampicillin standards were incubated overnight by microbiological assay in phosphate buffer (pH = 7.00), a 75.44 % of bacampicillin was tranformed into ampicillin (Table 6-9). This could be the result of the overnight incubation temperature (31ºC), the presence of esterases produced by the test bacteria, *B. subtilis*, in the agar plates, or the phosphate buffer pH (pH = 7.00).

However, the almost identical shape of the ampicillin plasma concentration versus time curves during both experiments

(bacampicillin alone and bacampicillin after dichlorvos), suggest that bacampicillin was hydrolysed into ampicillin despite the minimal plasma and erythrocyte esterase activity at the time of bacampicillin administration. It may be that despite the major reduction in plasma and red blood cell esterase concentrations, at the time of bacampicillin administration, there still was some activity (3.74 % for plasma and 1.25 % for erythrocytes) sufficient to convert bacampicillin into ampicillin. Also various other experimental studies have demonstrated the presence of esterase activity within the gastrointestinal tract, the liver and the kidney (Swahn, 1976; Fukuda *et al.*, 1978; Shindo *et al.*, 1978). Esterase levels in those tissues could not be measured during this experiment and it is not known whether they were reduced when dichlorvos was administered and if so to what extent.

The plasma ampicillin concentration versus time curve obtained for ampicillin after bacampicillin and dichlorvos administration, was characterised by a peak plasma ampicillin concentration of 6.95 ± 0.47 μg/ml achieved 45 minutes after administration of bacampicillin. The oral bioavailabilty for bacampicillin during this experiment was 48.02 %, slightly greater than the value obtained when bacampicillin was administered alone, but not statistically different. The MRT obtained when bacampicillin was administered after dichlorvos (MRT - 95.10 ± 10.30 min) was almost identical to the value obtained when bacampicillin was administered alone (MRT - 95.82 ± 9.67 min), suggesting that the reduction in plasma and erythrocyte esterases did not affect the disposition of bacampicillin.

When probenecid was administered at 75 mg/kg to the animals, it was extensively absorbed from the gastrointestinal tract and a Cmax of 153.70 ± 20.30 μg/ml was reached 165 minutes after administration. The peak plasma concentration measured was similar to that obtained in a previous study (Chapter 4) where a Cmax of 188.6 ± 19.27 μg/ml was measured when probenecid was administered intragastrically at the same dosage. The time at which Cmax was reached appeared to be longer in this experiment (tmax 165.00 ± 63.80) minutes than in the previous experiment (tmax = 120.00 ± 21.21) minutes. This longer tmax

value in the present study appeared to be the result of one animal (animal number 2) having a tmax of 8 hours.

The measurement of ampicillin in plasma after bacampicillin and probenecid administration showed that the presence of probenecid at the time of administration, appeared to limit the absorption of the antibiotic from the gastrointestinal tract as reflected in statistically lower ($P<0.05$) ampicillin peak plasma concentration. Since probenecid is a competitive inhibitor of penicillin excretion in the kidney, it may be that impaired absorption of bacampicillin was related to the presence of an active carrier mechanism for penicillin in the small intestine or that the presence of both compounds resulted in some interaction that reduced the absorption of the antimicrobial. Sjovall and others (1985) observed that when increasing doses of bacampicillin were administered to humans, there was a saturable increase in peak plasma concentration, plasma AUC and urinary excretion of ampicillin. They postulated that bacampicillin and other aminopenicillins are absorbed by a saturable specialized transport mechanism as well as by passive diffusion. Studies carried out in everted gut sac and enterocyte brush-border membrane vesicles, for the evaluation of intestinal absorption of beta-lactam antibiotics confirmed the existence of an active transport mechanism for these antibiotics in the small intestine. Nakashima and others (1984) recorded mixed type kinetics (saturable and non-saturable processes) for beta-lactam antibiotics in the rat jejunum and observed that the carrier was common for various dipeptides and the antibiotics. This carrier system, which transfers dipeptides and beta-lactam antibiotics was shown to be dependent on a proton influx from the intestinal lumen into the epithelial cells (Okano *et al.*, 1986). The proton influx is generated from a Na^+/H^+ exchange pump which ensures the constant presence of protons and consequently an acidic microclimate in the vicinity of the brush-border membrane of enterocytes (Lucas, 1983; Shiau *et al.*, 1985). This mechanism has been shown to be present in rats and humans (Kleinman *et al.*, 1988) and might be responsible for the good oral bioavailabilities of beta-lactams in these species. Whether the poor oral bioavailabilities of beta-lactam antibiotics in the horse are caused

by the lack of a suitable acidic microclimate in the proximity of brush-border membranes in this species is unknown.

The presence of symptoms of gastointestinal disturbance with reduced appetite and mild colic after administration of bacampicillin and probenecid, may have been the result of a reduced absorption of bacampicillin in the small intestine and consequently higher concentrations of the antiobic present in the large intestine. The oral administration of probenecid is unlikely to be the cause of the observed gastrointestinal disturbance as this drug is well tolerated by the oral route in the horse (Donecker *et al.*, 1986). When bacampicillin was administered in combination with probenecid to human beings, adverse effects including nausea and diarrhoea were observed (Wagner *et al.*, 1981). However, ampicillin concentrations were not measured following administration of bacampicillin and probenecid, and the study did not evaluate the presence of adverse effects for bacampicillin administered alone.

In this study, the disturbance of the bacterial ecosystem as reflected in profuse cultures of *E. coli* must have occurred after transformation of bacampicillin into ampicillin since bacampicillin lacks antibacterial activity *per se*. This result is in disagreement with some studies which claim that the lack of antibacterial activity of bacampicillin is responsible for a lower incidence of intestinal microflora disturbances (Sjovall *et al.*, 1986). The lower incidence of microflora disturbances recorded after bacampicillin administration is likely to be caused by the improved oral bioavailability of this compound and thus smaller concentrations reaching the large intestine. The hydrolysis of bacampicillin into ampicillin has previously been shown to occur in the gastrointestinal tract of humans with almost all bacampicillin remaining unchanged in gastric aspirates, but 50 to 100 % degradation in duodenal and upper jejunal aspirates (Swahn, 1976). In addition, incubation of ^{35}S-pivampicillin and ^{35}S-bacampicillin in buffer at different pH's showed that both esters remained in the original form in the range of pH 1.0 - 5.0. However marked degradation into ampicillin occurred when the pH was increased, and less than 50% of pivampicillin and bacampicillin remained intact when a pH of 7.0 was reached

(Swahn, 1976). These results are in agreement with the study carried out by Sommers and others (1984) who found that a reduction in the gastric acidity induced by the administration of ranitidine, an H2-receptor antagonist, and sodium bicarbonate markedly decreased the oral bioavailability of bacampicillin when administered to humans. Also the *in vitro* evaluation of bacampicillin metabolism in duodenal aspirates demonstrated that the lower the pH of the aspirate, the smaller the degradation of bacampicillin, but in addition when the specimens were boiled, the extent of degradation was drastically reduced (Swahn, 1976). These results appear to indicate that the degradation of bacampicillin into ampicillin may be the result of a combination of various factors including a higher pH in the lower parts of the intestine and the presence of esterases. The *in vitro* study (Table 6-9) demonstrated that bacampicillin was metabolised in equine caecal fluid up to 81.78 % and bacteria-free caecal fluid up to 82.85 %, presumably as a result of the more alkaline pH in the large intestine and the presence of esterases. It is likely that bacteria present in the equine large intestine are able to synthesise esterases that could have contributed to the degradation of bacampicillin into ampicillin in this study. The production of esterases by bacteria has been shown to occur in the bovine rumen, particularly among strains capable of digesting starch and cellulose (Fay *et al.*, 1990). It is therefore likely that bacterial strains with similar characteristics to those encountered in the rumen are present in the equine caecum and could have contributed to the bacampicillin hydrolysis.

During the present experiment, the fraction of bacampicillin absorbed from the gastrointestinal tract after bacampicillin and dichlorvos administration, appeared to remain in the body for a prolonged period. This was reflected in an MRT of 255.5 minutes, which is over 2.5 times longer than the value obtained when bacampicillin was administered on its own (MRT - 95.82 minutes). The prolongation of the ampicillin persistence was also reflected in a mean ± SEM plasma concentration of 0.34 ± 0.19 μg/ml, 12 hours after administration. In addition, reduced absorption of bacampicillin should have resulted in a smaller AUCobs unless the antibiotic persisted for a longer period, this

appeared to be the case since the AUCobs value after bacampicillin and probenecid of 12.29 μg.h/ml was very similar to the value obtained when the antibiotic was administered alone (AUC_{obs} = 11.31μg.h/ml)
These results are in agreement with a previous study (See Chapter 4) and confirm that ampicillin renal excretion can be effectively delayed when probenecid is administered concurrently.

6.5 Conclusion

The administration of bacampicillin hydrochloride to ponies and horses produced the highest bioavailability ever recorded for a penicillin in the adult horse following intragastric administration. The high peak ampicillin plasma concentrations and the persistence of the antibiotic 6 to 8 hours after administration suggest that bacampicillin hydrochloride could be administered at 8 to 12 hour intervals by the oral route and would achieve high enough concentrations for the treatment of infections caused by susceptible microorganisms. The co-administration of probenecid with bacampicillin limited the absorption of the antibiotic from the gastrointestinal tract and led to gastrointestinal disturbances in the animals making this combination unsuitable for therapy. In addition, the reduction of plasma and erythrocyte esterase activity following dichlorvos administration, did not affect the disposition and fate of bacampicillin.
The results obtained following bacampicillin administration are of potential interest, since there is a lack of suitable preparations for the oral administration of antibiotics to horses and ponies. The advantages of oral administration compared to the intramuscular or the intravenous injection of antibiotics are that the owner may administer the drug and the possibility of reactions at the injection site is removed. These practical advantages together with the achievement of good concentrations observed, make bacampicillin a potential candidate for the future development of an oral penicillin preparation for use in *equidae*.

Chapter 7

Sensitivities of equine bacterial isolates to different antibiotics

7.1 Introduction

For successful antibiotic therapy, a combination of pharmacokinetic information concerning the antibiotic to be administered and the sensitivity of the bacteria responsible for the infection to that particular antibiotic is required. Pharmacokinetic studies provide *in vivo* information about plasma and tissue concentrations of the drug administered over a period of time. On the other hand, bacterial sensitivity tests determine if the infectious agent is susceptible or not to the antibiotic administered (qualitative tests) and if so, what is the *in vitro* antibiotic concentration necessary to kill or inactivate it (quantitative tests).

The fact that qualitative sensitivity tests, of which disc-diffusion test is the most common, are easy to perform and inexpensive, makes this method more widely used than quantitative sensitivity tests used to determine the minimum inhibitory concentration (MIC). Qualitative tests do not provide the information necessary to establish a therapeutic regime as the concentrations required to inactivate or destroy the bacteria cannot be predicted from the results. However the data provided by the MIC tests combined with pharmacokinetic information enables an appropriate therapeutic regime to be established (dose, route of administration and dosage interval).

Although the MIC of a particular bacterial species is seldom required by the clinician it is nevertheless very useful to carry out MIC tests on as many pathogenic bacteria as possible and classify them into groups such as susceptible, moderately susceptible and resistant to different antibiotics. This information provides criteria neccessary to establish appropriate treatment and in addition, indicates changes of sensitivity patterns during the emergence of resistant strains. Since sensitivity patterns change, it is advisable to select appropriate antibiotics in accordance with up-to-date bacterial sensitivity patterns.

In the horse, many studies have been carried out to evaluate the sensitivity patterns of equine pathogenic bacteria (Adamson *et al.*, 1985; Ensink *et al.*, 1993; Hirsh *et al.*, 1985; Hirsh & Jang, 1987; Knight & Hietala, 1978; Prescott, 1981; Snyder *et al.*, 1987).

In the present study, a number of bacteria were isolated from equine clinical specimens over a three-year period. Following culture and identification of the bacteria, disc-diffusion and MIC tests to a range of antibiotics were carried to define the sensitivity patterns of six commonly encountered equine pathogens.

7.2 Materials and methods

7.2.1 Bacterial isolation and identification.

Ninety-five bacterial isolates were obtained following routine bacteriological examination of equine clinical specimens from different anatomical sites and pathological conditions. The initial examination consisted of an examination of a gram-stained smear followed by culture of the sample on sheep blood agar aerobically (7 % sheep blood in Blood Agar base number 2 CM271, Oxoid, Unipath Ltd, Basingstoke, England) MacConkey agar aerobically (MacConkey CM7, Oxoid), horse blood agar anaerobically (7 % horse blood in Blood Agar base number 2 CM271, Oxoid) and occasionally "chocolate" agar microaerophilically at 37º C for 24 to 48 hours. The isolation of equine bacterial pathogens for retrospective *in vitro* sensitivity determination focussed on six different bacterial species commonly encountered in equine pathology, these were *Actinobacillus equuli* (n=12), *Escherichia coli* (n=23), *Pseudomonas* species (n=13), *Salmonella typhimurium* (n=11), *Staphylococcus aureus* (n=16) and *Streptococcus zooepidemicus* (n=20). The identification of bacteria was carried out using a biochemical characterisation and identification system, (API system, API Laboratory Products LTD, Grafton Way, Basingstoke, Hants, UK). The identification of *S. zooepidemicus* was also confirmed using sugar fermentation tests (trehalose, sorbitol, mannitol, salicin, lactose, raffinose, inulin and esculin) and evaluation of haemolysis type in sheep blood agar plates. During the identification of *Salmonella* species, the sample was incubated in iodine containing tetrathionate broth (0.2 ml iodine per 10 ml of tetrathionate) (Tetrathionate broth base CM29, Oxoid, Unipath Ltd, Basingstoke, England) overnight and

then cultured in SS agar (*Salmonella-Shigella*) (SS Agar CM533, Oxoid). In addition, a urease production test was carried out together with overnight incubation in TSI (Triple Sugar Iron agar) slopes (TSI agar CM 277, Oxoid).

7.2.2 Bacterial sensitivity tests.

7.2.2.1 Disc-diffusion sensitivity tests.

Following bacterial isolation and identification, the Kirby-Bauer or disc-diffusion sensitivity test was carried out. The bacterial isolate was subcultured onto D.S.T. agar (Diagnostic Sensitivity Agar CM 261, Oxoid, Unipath Ltd, Basingstoke, England) and 9 discs (Oxoid, Antimicrobial Susceptibility Test Discs, Unipath Ltd, Basingstoke, England) corresponding to different antibiotics were placed onto the inoculated plate. The discs contained specific concentrations of the antibiotics: ampicillin (10 μg), penicillin G (10 μg), gentamicin (10 μg), erythromycin (10 μg), streptomycin (10 μg), trimethoprim-sulphamethoxazole (25 μg), oxytetracycline (30μg), lincomycin (2μg) and chloramphenicol (30 μg).
Following a 24 hour incubation at 37 º C, the zones of bacterial growth inhibition surrounding the different antibiotic discs were measured. A 3 mm or greater zone of bacterial growth inhibition from the edge of the disc indicated that the bacteria tested were susceptible to that particular antimicrobial. No attempts were made to give a quantitative estimation of the sensitivity, and the evaluation of the growth inhibition size classified bacteria as susceptible (S) or resistant (R).

7.2.2.2 Minimum inhibitory concentrations (MIC)

The MICs were carried out by the broth dilution method in commercially available susceptibility testing plates (Agnum urinary plates and ME gram-negative urinary plates, Sensititre Plates for Susceptibility Testing, Sensititre LTD, East Grinstead, England). A number of original isolates (n = 21) could not be tested for amoxycillin/clavulanic acid, ciprofloxacin, carbenicillin,

ceftriaxone and ceftizoxime, since the original susceptibility testing plates (Agnum urinary plates) were no longer commercially available. The plates were a micro-version of the broth dilution method and contained different antimicrobial agents at appropriate dilutions distributed in 92 wells. In addition 3 positive control wells not containing any antibiotic were used to check that the test organism grew satisfactorily in the absence of any antimicrobial.

Following identification and subculture of bacteria, a small number of colonies were collected and diluted in demineralised water until a 0.5 MacFarland turbidity standard, equivalent to 10^8 cfu/ml (colonies forming units / ml) was reached. A 10 µl volume from the dilution was then transferred and mixed thoroughly into 10 ml of supplemented Mueller Hinton broth with TES (n-tris[Hydroxymethyl]methyl-2-aminoethanesulfonic acid; 2-([2-Hydroxy-1, 1-bis (hydroxymethyl) - ethyl] amino) ethanesulfonic acid) (Sensititre Ltd Product, The Manor, Crawley, West Sussex, England) buffer. Each well of the plate was subsequently inoculated with 50 µl of the seeded broth and then incubated for 24 hours at 37 º C. Following the incubation period, the MIC value for each antibiotic was determined as the lowest antibiotic concentration capable of inhibiting bacterial growth. The antibiotics tested and the appropriate concentration range are shown in table 7-1.

7.3 Results

Equine bacterial isolates were largely recovered from respiratory, gastrointestinal and musculoskeletal sites (Table 7-2; Appendix E Table E-1). Gram-positive microorganisms, *S. aureus* and *S. zooepidemicus* were isolated mainly from samples of respiratory origin with 50 % of both isolates recovered from this source. They also could be found in quite high numbers in samples of musculoskeletal infection and in wounds. *S. typhimurium* and *A. equuli* isolates were found largely in samples of gastrointestinal origin as reflected in 81.82 % and 66.67 % of the isolates recovered from this source respectively.

Antibiotic	Concentration range (μg/ml)
Ampicillin	1-2-4-8-16-32-64-128
Amoxycillin/Clavulanic acid	8/4-16/8-32/16-64/32
Carbenicillin	16-32-64-128
Mezlocillin	16-32-64-128
Cefoxitin	1-2-4-8-16-32-64-128
Ceftizoxime	16-32-64-128
Ceftriaxone	8-16-32-64
Cephalothin	1-2-4-8-16-32-64-128
Amikacin	1-2-4-8-16-32-64
Gentamicin	0.5-1-2-4-8-16-32
Tobramycin	0.5-1-2-4-8-16-32
Tetracycline	2-4-8-16
Trimethoprim/Sulfamethoxazole	2/38-4/76-8/152-16/304
Norfloxacin	1-2-4-8-16-32-64-128
Ciprofloxacin	0.06-0.12-0.25-0.5-1-2-4-8

Table 7-1 Concentration ranges of the antibiotics tested with the broth dilution method.

	Isolates origin					
Isolates	MS/W	R	G.I	G.U	O	(n=)
A. equuli	25.00	8.33	66.67	0.00	0.00	12
E. coli	34.78	8.70	26.09	30.43	0.00	23
Pseudomonas spp.	15.38	53.85	30.77	0.00	0.00	13
S. typhimurium	0.00	0.00	81.82	0.00	18.18	11
S. aureus	25.00	50.00	6.25	0.00	18.75	16
S. zooepidemicus	30.00	50.00	0.00	10.00	10.00	20

Table 7-2. Origin of bacterial isolates expressed as percentage of the total for each isolate.
MS/W: Musculoskeletal/Wounds. R: Respiratory. G.I: Gastrointestinal. G.U: Genitourinary. O: Others.

Finally *Pseudomonas* spp. were isolated from various sources although the majority of isolates (53.85%) were found in samples of respiratory origin (Table 7-2; Appendix E Table E-1).
Qualitative sensitivity patterns given by the Kirby-Bauer sensitivity discs were obtained for all bacteria (Table 7-3; Appendix E Table E-2). The MIC_{50} and MIC_{90} values for equine bacterial isolates to the antibiotics tested were calculated. The MIC_{50} is the antibiotic concentration at which 50 % of the isolates were inhibited and the MIC_{90} the antibiotic concentration at which 90 % of the isolates were inhibited (Tables 7-4, 7-5, 7-6; 7-7). In addition, MIC values obtained were expressed as cumulative percentages of sensitive bacteria to the various antibiotics (Appendix E Tables E-3 and E-4).

7.4 Discussion

Antibiotic pharmacokinetic studies provide information on the plasma and tissue concentration profiles of the drugs studied. However, a study of the sensitivity patterns of the commonly encountered pathogens is neccessary to evaluate whether the achievable plasma and tissue concentrations are sufficient to successfully kill the bacteria. Qualitative sensitivity tests for antimicrobials such as the Kirby-Bauer disc sensitivity test are commonly carried out in practice and indicate whether the organism responsible for the infection is sensitive or resistant to the various antibiotics tested. However, these tests do not provide a realistic approach and can sometimes be misleading, since no information on the amount of the antibiotic necessary to kill the bacteria is given. On the other hand, minimum inhibitory concentrations (MIC) provide *in vitro* quantitative data (in μg/ml) on the susceptibility of the various pathogens. This information can be related to the plasma and tissue concentration data obtained during pharmacokinetic studies.
For practical purposes, MIC data can be expressed in terms of percentages (MIC_{50} and MIC_{90}) giving quantitative information on the susceptibility and also indicating the presence of resistance of a particular bacterial species to a specific antibiotic.

Isolates	Antibiotic								
	AMP (10)	GEN (10)	OXT (30)	ER (10)	PEN (10)	LIN (2)	STR (10)	CHL (30)	T/S (25)
A. equuli	83.3	50	100	83.3	91.7	100	41.7	100	91.7
E. coli	52.2	100	56.5	0	0	0	43.5	87.0	60.9
Pseudomonas sp	0	100	15.4	0	0	0	53.8	30.8	7.7
S. typhimurium	18.2	100	0	0	0	0	9.1	18.2	36.4
S. aureus	68.7	93.7	87.5	100	62.5	100	100	100	100
S zooepidemicus	100	15	100	95	100	100	100	100	95

Table 7-3 Percentage of bacterial isolates sensitive to different antibiotics tested with the disc-diffusion method.
AMP: Ampicillin; GEN: Gentamicin; OXT: Oxytetracycline; ER: Erythromycin; PEN: Penicillin G; LIN: Lincomycin; STR: Streptomycin; CHL: Chloramphenicol; T/S: Trimethoprim /Sulphamethoxazole.
Numbers in brackets correspond to the antibiotic concentrations in μg.

Penicillins	AMP		AMOX/ C.A		CARB		MEZ	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
A. equuli	≤1	≤1	≤8/4	≤8/4	≤16	≤16	≤16	≤16
E. coli	4	>128	≤8/4	16/8	≤16	>128	≤16	>128
Pseudomonas sp	>128	>128	>64/32	>64/32	32	64	32	128
S. typhimurium	>128	>128	32/16	32/16	>128	>128	>128	>128
S. aureus	≤1	4	≤8/4	≤8/4	≤16	≤16	≤16	≤16
S. zooepidemicus	≤1	≤1	≤8/4	≤8/4	≤16	≤16	≤16	≤16

Table 7-4. MIC_{50} and MIC_{90} values for equine bacterial isolates to different penicillins.
AMP: Ampicillin; AMOX/ C.A: Amoxycillin/ Clavulanic acid;
CARB: Carbenicillin; MEZ: Mezlocillin.

Cephalosporins	CEFOX		CEFTIZ		CEFTR		CEPHA	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
A. equuli	≤1	≤1	≤16	≤16	≤8	≤8	≤1	≤1
E. coli	4	8	≤16	≤16	≤8	≤8	8	64
Pseudomonas spp	>128	>128	32	128	16	64	>128	>128
S. typhimurium	8	8	≤16	≤16	≤8	≤8	16	16
S. aureus	2	4	≤16	≤16	≤8	≤8	≤1	≤1
S. zooepidemicus	≤1	≤1	≤16	≤16	≤8	≤8	≤1	≤1

Table 7-5 MIC_{50} and MIC_{90} values for equine bacterial isolates to different cephalosporins.
CEFOX: Cefoxitin; CEFTIZ: Ceftizoxime; CEFTR: Ceftriaxone; CEPHA: Cephalothin

Aminoglycosides	AMI		GENT		TOBR	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
A. equuli	4	8	1	2	1	1
E. coli	2	2	≤0.5	1	1	1
Pseudomonas spp	4	4	2	8	≤0.5	2
S. typhimurium	≤1	2	≤0.5	≤0.5	1	1
S. aureus	2	4	≤0.5	≤0.5	≤0.5	≤0.5
S. zooepidemicus	≤1	4	≤0.5	1	≤0.5	1

Table 7-6 MIC_{50} and MIC_{90} values for equine bacterial isolates to different aminoglycosides.
AMI: Amikacin; GENT: Gentamicin; TOBR: Tobramycin.

Miscellaneous	TETR		TMP/S		NORF		CIPR	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
A. equuli	≤2	≤2	≤2/38	≤2/38	≤1	≤1	≤0.06	≤0.06
E. coli	≤2	>16	≤2/38	>16/304	≤1	≤1	≤0.06	≤0.06
Pseudomonas sp	>16	>16	8/152	>16/304	≤1	8	0.25	2
S. typhimurium	16	>16	>16/304	>16/304	≤1	≤1	≤0.06	≤0.06
S. aureus	≤2	>16	≤2/38	≤2/38	≤1	≤1	0.12	0.25
S. zooepidemicus	≤2	≤2	≤2/38	≤2/38	4	8	1	1

Table 7-7 MIC_{50} and MIC_{90} values for equine bacterial isolates to tetracycline, trimethoprim/sulphonamide and fluoroquinolones.

TETR: Tetracycline; TMP/S: Trimethoprim/ Sulphonamide; NORF: Norfloxacin; CIPR: Ciprofloxacin.

In the present study, MIC data for the penicillins appeared to indicate that this group of antibiotics was a suitable choice for the treatment of gram-positive infections caused by *S. aureus* and *S. zooepidemicus* (Table 7-4). This result appeared to contradict the results obtained by the disc-diffusion test where over 30 % of the *S. aureus* tested were resistant to ampicillin (Table 7-3). In addition, *A. equuli*, a gram negative equine pathogen appeared to be susceptible to all penicillins tested during the study. The presence of resistance to penicillins was particularly obvious among the *E. coli* tested. This was reflected in low concentrations for the MIC_{50} and very high concentrations for the MIC_{90} except for the amoxycillin/ clavulanic acid combination. These differences in susceptibility of *E. coli* isolates to a particular antibiotic are in agreement with the results reported by Ensink and others (1993). They found that susceptibility of *E. coli*, *Salmonella*, *Klebsiella* spp and coagulase positive *Staphylococcus* spp to different antimicrobials varied extensively between isolates. On this basis they recommended that MIC determination should be carried out particularly on those isolates where variation in the susceptibility pattern is most likely to occur.

In this study, *Pseudomonas* spp. was resistant both to ampicillin and amoxycillin/clavulanic acid combinations and high concentrations of carbenicillin and mezlocillin, two penicillins used for the treatment of *Pseudomonas* infections, were necessary to kill the bacteria. *S. typhimurium* appeared resistant to all penicillins tested except to the amoxycillin/clavulanic acid combination where an MIC_{90} of 32/16 μg/ml was recorded. The lower antibiotic concentrations required to kill *Salmonella*, when the amoxycillin was combined with clavulanic acid, a beta-lactamase inhibitor, suggest that this bacteria was resistant to the other penicillins by producing beta-lactamase enzyme.

The cephalosporins appeared to be very effective antibiotics for the treatment of infections caused by *S. aureus*, *S. zooepidemicus* and *A. equuli*. In addition, *E. coli* and *S. typhimurium* isolates were sensitive to cefoxitin, a second generation cephalosporin, and to third generation cephalosporins ceftizoxime and ceftriaxone. Cephalothin, a first generation cephalosporin, appeared to have some activity against *S. typhimurium* with

MIC_{50} and MIC_{90} values of 16 μg/ml but resistance among *E. coli* isolates appeared to be common as reflected in an MIC_{90} value of 64 μg/ml. Finally, *Pseudomonas* spp. were resistant to the first and second generation cephalosporins and only ceftriaxone appeared to have activity against this bacteria although high concentrations (MIC_{50} = 16 μg/ml and MIC_{90} = 64 μg/ml) were required for effect (Table 7-5).

The aminoglycosides tested, amikacin, gentamicin and tobramycin, appeared to be effective against most bacteria tested including gram positive bacteria, *S. typhimurium* and *E. coli*. *Pseudomonas* spp was sensitive to this group of antibiotics and a gentamicin concentration of 8 μg/ml was sufficient to kill 90 % of the isolates. The aminoglycosides, and particularly gentamicin and tobramycin, were effective against *A. equuli* (Table 7-6).

The results obtained for tetracycline showed the presence of resistance to this antibiotic particularly among *E. coli*, *S. typhimurium*, and *Pseudomonas* spp. On the other hand, *A. equuli*. and *S. zooepidemicus* appeared to be particularly sensitive to this antibiotic (Table 7-7). The trimethoprim/ sulfamethoxazole combination was effective against the gram positive pathogens and *A. equuli*. but of limited value for the treatment of salmonella and pseudomonas infections. 65.2 % of the *E. coli* isolates tested were inactivated by concentrations of less than or equal to 2/38 μg/ml of the trimethoprim /sulphonamide combination, the remaining *E. coli* isolates were resistant (Table 7-7; Appendix E Table E-3).

The two quinolone antibacterials, norfloxacin and ciprofloxacin, were tested for their activity against equine pathogens. The results showed that these two antibiotics were highly effective against *A. equuli*, *E. coli* and *S. typhimurium* at low concentrations (≤1 μg/ml for norfloxacin and ≤0.06 μg/ml for ciprofloxacin). A large percentage of *Pseudomonas* spp isolates also appeared sensitive to the quinolones at low concentrations (Table 7-7). Norfloxacin appeared to be effective against *S. aureus* but less so against *S. zooepidemicus* as reflected in an MIC_{50} of 4 μg/ml and MIC_{90} of 8 μg/ml for the latter pathogen. Lower concentrations of ciprofloxacin were neccessary to kill the gram-positive bacteria.

A more practical way to classify bacteria according to their susceptibility can be applied when pharmacokinetic information about the antibiotic is available. The bacteria can be classified into four categories according to its susceptibility in relation to the pharmacokinetic profile of the antibiotic. This four-category guideline system suggested by Ericsson and Sherris (1971) and the National Committee for Clinical Laboratory Standards (NCCLS) (1983), classifies bacterial pathogens into four:

1) Susceptible, when the infecting organism is usually inhibited by concentrations of a particular antibiotic that are attained in tissues by the usual dosage.

2) Moderately susceptible, the infecting organism is only inhibited by blood or tissue concentrations achieved with maximum dosage.

3) Resistant, the organism is resistant to normal achievable or tolerated concentrations of the drug.

4) Conditionally susceptible, the organism causes infection in sites where tissue or body fluid concentrations of drug greatly exceed those present in most areas of the body.

Although the guidelines for the interpretation of MIC data for all antibiotics in veterinary medicine are not available, pharmacokinetic studies carried out with some antimicrobials in the horse have allowed the creation of guidelines for ampicillin, gentamicin, amikacin and tetracycline among the antimicrobials included in the present study (Table 7-8) (Prescott & Baggot, 1988c).

Antibiotic	Susceptible	Moderately Susceptible	Resistant	Conditionally Susceptible
AMP	≤1	2-16	>16	32-128
GENT	≤2	4-8	>8	16-64
AMI	≤4	8-16	>16	32-128
TETRA	≤1	2-4	>4	8-128

Table 7-8. Suggested guidelines for the interpretation of MIC (μg/ml) data in *equidae* (Prescott & Baggot, 1988c).

According to these guidelines, 100% of *A. equuli* and *S. zooepidemicus* isolates and 68.7 % of *S. aureus* isolates were sensitive to ampicillin (≤1 μg/ml) with the rest of the *S. aureus* isolates falling into the moderately susceptible category (2-16 μg/ml). Also, 52.2% of *E. coli* and 18.2 % of *S. typhimurium* isolates were moderately susceptible. The rest of the isolates including all *Pseudomonas* sp were resistant to ampicillin (Appendix E Table E-3).

One hundred per cent of all bacterial species isolates and 53.8 % *Pseudomonas* spp. were classified as sensitive (≤2 μg/ml) to gentamicin and up to 38.5 % of *Pseudomonas* spp fell into the moderately susceptible category (4-8 μg/ml). Similar results were found for amikacin, with 100 % of *E. coli*, *S. typhimurium*, *S. aureus* and *S. zooepidemicus* susceptible (≤4 μg/ml) to this aminoglycoside. In addition, 16.7 % of *A. equuli* fell into the moderately susceptible category (8-16 μg/ml) and 7.6 % *Pseudomonas* spp were resistant. Finally, 56.5 % of the *A. equuli*, 9.1% of the *S. typhimurium* isolates, 87.5 % of the *S. aureus* and 95 % S. *zooepidemicus* were classified as belonging to the susceptible and moderately susceptible category (up to 4 μg/ml). The other isolates were resistant to amikacin (Appendix E Table E-3).

These results, obtained by interpretation of the MIC data, were compared to those obtained with the disc-diffusion method. The ampicillin sensitivity patterns were almost identical by both methods. However, *A. equuli* and *S. zooepidemicus* appeared to be more resistant to gentamicin when tested by the Kirby-Bauer disc-diffusion method, since 50 % of the *A. equuli* isolates were sensitive to gentamicin when tested by the disc-diffusion test and 100 % were sensitive when tested by the broth dilution method. Similarly, only 15 % of the *S. zooepidemicus* isolates were sensitive to gentamicin by the Kirby-Bauer method compared to a 100 % according to these MIC results. Finally the tetracycline sensitivity results appeared to be quite similar by both methods, except for the *Pseudomonas* isolates where 15.4 % sensitive to tetracycline when tested with the discs and 0 % when tested by the broth dilution method. In addition, the MIC results for *S. typhimurium* showed that 9.1 % of the isolates fell into the

tetracycline susceptible and moderately susceptible category but none of the isolates appeared susceptible to this antibiotic when tested by the disc method.
Although the *in vitro* determination of sensitivity patterns does not take into account a number of factors that occur *in vivo,* such as the bactericidal activity of the host immune system or the enhanced killing of antibiotic-damaged bacteria by macrophages, MIC determination is an important guide for the establishment of an appropriate antimicrobial therapy (Prescott & Baggot, 1985). The UK Veterinary Products Committee recommends that peak plasma concentrations of at least twice the MIC should be achieved and that plasma concentrations greater than or equal to the MIC should be maintained for at least half the interdosing interval.

7.5 Conclusion

The lack of comprehensive clinical trials in veterinary medicine, has made the use of MIC data together with antimicrobial pharmacokinetic information (plasma and tissue disposition), the basis for the establishment of antibacterial therapy.
The MIC quantitative susceptibility method gives a more precise description of the bacterial susceptibility than the disc-diffusion method, and it can also be related directly to the pharmacokinetic parameters of the antibiotic. However, it is important to emphasize that MIC data should be interpreted cautiously since the results are in agreement with the free non-protein bound fraction of the antibiotic and not with the total drug concentration (Derendorf, 1989). In addition, factors occuring *in vivo* relating to the immune system are not taken into account when testing bacterial susceptibility *in vitro.*
Despite these limitations, MIC values for bacteria responsible for the infection should be determined since they indicate which is the most suitable antibiotic to be administered and reflect the emergence and incidence of bacterial resistance to the various antimicrobials.

Chapter 8

General discussion

When ampicillin sodium was administered IV to horses and ponies, it was eliminated rapidly from the body, thus an elimination half-life and MRT of less than one hour, and high total body clearance value were obtained. The rapid elimination of ampicillin in the horse represents a therapeutic drawback, since frequent doses of the antimicrobial have to be administered in order to maintain sufficiently high plasma and tissue concentrations.

A major objective of the present study was to modify the absorption and elimination patterns of ampicillin in the horse in order to achieve a prolonged persistence of the antibiotic in the body after administration and consequently reduce the frequency of administration.

The attempt to modify the excretion pattern of ampicillin in the horse by altering the urinary pH had no effect. This may have been attributable to the chemical structure of ampicillin, containing two ionisable groups (an amino and a carboxyl group) within its molecule. The presence of at least one of the groups in the ionised form would limit the ability of the molecule to cross phospholipid, barriers such as those present in the renal tubule and therefore impair the transfer of ampicillin from the urine into plasma. However, alterations of urinary pH have been shown to have marked effects on the plasma disposition of some drugs excreted partly in the urine and this has been demonstrated for the sulphonamides in the dog (Baggot, 1968). In the horse, Gelså (1979) observed that trimethoprim and sulphadoxine were excreted in the urine by glomerular filtration, active tubular secretion and back-diffusion. The mechanisms responsible for the excretion of both compounds, were determined by measuring the creatinine clearance (an indicator of the glomerular filtration rate), the plasma concentration of unbound drug, the urinary flow rate and the urinary pH. Both the urinary pH and and the plasma concentration of unbound drug had an effect on the excretion rate of trimethoprim and sulphadoxine. In addition, the urinary flow rate appeared to have an influence on the excretion of trimethoprim. It would therefore be interesting to investigate whether, the plasma concentration of the drug and the urinary

flow rate would have an effect on the excretion rate of ampicillin in the horse.

The present studies also revealed that although no changes in plasma disposition were found following alterations of the urinary pH, these changes had an effect on the degradation of the antibiotic in the urine, since ampicillin was markedly degraded in alkaline urine. It would therefore be interesting, to investigate the extent of degradation of ampicillin and other penicillins *in vivo* at different urinary pH values and determine whether special considerations should be taken during the treatment of urinary tract infections with these antibiotics.

The concurrent administration of ampicillin and probenecid proved to be an effective way of prolonging the persistence of the antibiotic in the equine body. This was reflected in a prolonged elimination half-life and MRT of ampicillin together with a reduction in the total body clearance when ampicillin was administered after an intragastric dose of probenecid. In addition, the presence of probenecid appeared to reduce the distribution of ampicillin in the body as reflected in smaller volumes of distribution values for the antibiotic in the presence of probenecid. This finding seems to be in agreement with results observed in other species (Gibaldi & Schwartz, 1968; Galtier & Alvinerie, 1979; Juzwiak *et al.*, 1989; Villa *et al.*, 1991) and appears to be the result of probenecid limiting the access of penicillins in some tissues such as the liver and kidney parenchyma (Bergholz *et al.*, 1980). It would be interesting to compare ampicillin concentrations in these organs following the administration of ampicillin and ampicillin in conjunction with probenecid. The results would confirm the effects of probenecid on the distribution of ampicillin and indicate whether an adjustment of the dose would be required to treat infections localised in the organs, where the distribution of the antibiotic might have been impaired.

Prolonged persistence of ampicillin within the equine body was achieved by administration as an IV infusion over a 4-hour period. The system enabled accurate control of the amount of antibiotic administered to each individual during the procedure. However, the impracticality of the method makes it useful only in

those situations where the antibiotic to be administered is potentially toxic at high concentrations. It may also be a suitable method for the administration of antibiotics prophylactically during surgical procedures particularly in those situations where a compromised vascular supply (*ie* shocked patient) would limit the absorption of drugs administered by other routes (Alexander & Alexander, 1976).

Oral administration was investigated as a method of delaying absorption, and consequently elimination, of ampicillin in the body. In the neonatal foal, the greater permeability of the gastrointestinal tract enables the administration of drugs that are poorly absorbed in the adult (Baggot & Short, 1984). This was observed by Baggot and others (1988a) following oral administration of amoxycillin (30 mg/kg) to 5-to 10-day old foals in that oral bioavailability was 42.7 %. However, in the adult horse, a maximum oral bioavailability of 10.4 % for a penicillin was recorded following oral administration of amoxycillin (Wilson *et al.*, 1988). Recently Ensink and others (1992) obtained a bioavailability of 35.9 % following intragastric administration of pivampicillin, an ampicillin prodrug, to adult animals. In this study, the intragastric administration of bacampicillin, another ampicillin prodrug designed for oral administration, yielded the highest oral bioavailability of active ampicillin (41.0 %) obtained for any penicillin administered *per os* to the adult horse. It would therefore appear that the administration of ampicillin prodrugs could play a role in the future of oral antibacterial therapy in the horse where there is a substantial lack of suitable oral preparations.

However, it is important to point out that despite the results achieved following pivampicillin and bacampicillin administration to horses, the bioavailabilities values remain relatively low when compared to those obtained in humans for the same antibiotics. For instance, when pivampicillin and bacampicillin were administered to humans, 83 % and 71 % of the dose were absorbed respectively (Swahn, 1976). Another study showed that up to 98 % of the bacampicillin dose administered to humans was absorbed from the gastrointestinal tract (Rozencweig *et al.*, 1976). Similarly, the oral administration of ampicillin and amoxycillin to

humans is characterised by greater bioavailabilities than are achieved in the horse. A maximum oral bioavailability of 10.4 % and of 3.5 % following oral administration of amoxycillin and ampicillin to horses have been recorded (Wilson *et al.*, 1988; Horspool, 1992). Comparatively, a larger fraction of the antibiotic is absorbed from the gastrointestinal tract when administered to humans. For instance 44 % of ampicillin was absorbed from the gastrointestinal tract in humans (Swahn, 1975) and more than 70 % of amoxycillin when administered by the same route (Arancibia *et al.*, 1980). Thus despite being in their ionised form, ampicillin and amoxycillin are absorbed substantially from the gastrointestinal tract, and this may be attributed to the presence of an active transport carrier system capable of transferring the aminopenicillins from the intestinal lumen into the systemic circulation (Westphal *et al.*, 1991). This was demonstrated for amoxycillin and bacampicillin in man where both antibiotics appeared to have dose-dependent oral bioavailabilities (Sjovall *et al.*, 1985). Also, several *in vitro* studies have demonstrated the presence of a carrier-mediated process involved in the absorption of amino-β-lactam antibiotics in addition to passive transfer mechanisms (Nakashima *et al.*, 1984). The carrier system has been shown to be driven by a H^+ gradient, resulting from a Na^+/H^+ exchange system, and is responsible for the absorption of dipeptides from the intestinal lumen (Okano *et al.*, 1986). Similarly, the dipeptide carrier system has been shown to be present in renal brush border membranes of the rat and is thought to be responsible for tubular reabsorption of aminocephalosporins (Inui *et al.*, 1983; Inui *et al.*, 1984).

In the equine, the aminopenicillins and the ampicillin esters are absorbed to a much less extent than in man. This could be attributed to the antibiotics binding to food particles, however it seems unlikely in this case since a higher oral bioavailability was obtained for pivampicillin when the antibiotic was administered to fed horses than when it was administered to starved horses (Ensink *et al.*, 1992). Whether the equine lacks the H^+ gradient and the carrier system responsible for the partial transfer of aminopenicillins in humans is not known. However, it would be interesting to investigate the presence of these carriers in the

equine small intestine in order to clarify the mechanisms of penicillin absorption and give an explanation for their low bioavailability following oral administration in the equine.

In order to slow down the transformation of bacampicillin into ampicillin following administration, an attempt was made to reduce the enzymatic activity responsible for the conversion of the inactive compound into ampicillin. Despite a massive reduction in both plasma and erythrocyte esterase activity following the administration of dichlorvos, the metabolism of bacampicillin was not altered, suggesting that the prodrug is not only converted by plasma and erythrocyte esterases but also possibly by other factors including the more alkaline pH of the plasma and esterases present in the intestinal wall and other tissues.

These studies also suggested that although bacampicillin may play a role in the future treatment of equine infectious diseases, further studies should be conducted to investigate the possibility of gastrointestinal side effects following its administration to horses and ponies since mild gastrointestinal disfunction was observed. The co-administration of probenecid and bacampicillin resulted in the presence of mild gastrointestinal disturbances. These were related to a decrease in the absorption of the antibiotic from the intestinal lumen and consequently a larger proportion passing into the large intestine. Swahn (1976) demonstrated a correlation between the alkalinity of the media and the extent of degradation of bacampicillin, and it may be that the pH of the large intestinal contents (pH= 6.8-7.2) contributed to transform the inactive bacampicillin into the active ampicillin leading to a disturbance of the microbial ecosystem and the presence of side effects. It would be interesting to investigate whether bacampicillin was also transformed into ampicillin by the esterases produced by bacteria present in the equine large intestine. Fay and others (1990) reported the production of esterases by bacteria in the bovine rumen and it is therefore quite likely that this occurs in the equine caecum and colon where the bacterial fermentation processes take place.

The susceptibility of equine pathogens to ampicillin was tested *in vitro* using MIC tests. Ampicillin appeared very effective for the

treatment of diseases caused by *S. zooepidemicus* and *A. equuli*. However, a large proportion of *E. coli* (47.8 %) and of *S. typhimurium* (81.8 %) isolated were resistant (MIC > 16µg/ml) to the antibiotic. Hirsh and Jang (1987) found that 26 % of *E. coli* and 55 % of *S. typhimurium* were resistant to ampicillin in 1987. The larger proportion of resistant strains found during the present survey indicate an increase in the proportion of these two gram-negative pathogens resistant to ampicillin. Nevertheless, interpretation of MIC data should be done cautiously since major variations in bacterial sensitivity patterns can occur in different geographical areas. The presence of resistant stains among certain bacteria indicates that susceptibility testing should be carried out routinely particularly on those isolates where the susceptibility cannot be predicted (Ensink *et al.*, 1993). It is important to bear in mind that *in vitro* susceptibility data do not take into account many of the circumstances that occur in the animal and should therefore be interpreted on that basis. Unfortunately information on antimicrobial dosages provided by clinical trials is very limited in the veterinary field and consequently most antibiotic dosages are based on a combination of pharmacokinetic data and sensitivity patterns.

References

Abraham, E. P. & Duthie, E. S. (1946) Effect of pH of the medium on activity of streptomycin and penicillin and other chemotherapeutic substances. *The Lancet*, **1**,455-459

Adamson, P. J. W., Wilson, W. D., Hirsh, D. C., Baggot, J. D. & Martin, L. D. (1985) Susceptibility of equine bacterial isolates to antimicrobial agents. *American Journal of Veterinary Research*, **46**, 447-450

Alexander, J. W. & Alexander, N. S. (1976) The influence of route of administration on wound fluid concentration of prophylactic antibiotics. *The Journal of Trauma*, **16**, 488-495

Alexander, J. W., McGloin, J. J. & Altemeier, W. A. (1960) Penicillin prophylaxis in experimental wound infection. *Surgical Forum*, **11**, 299-300

Andersson, G., Ekman, L., Månsson, I., Persson, S., Rubarth, S. & Tufvesson, G. (1971) Lethal complications following administration of oxytetracycline in the horse. *Nordisk Veterinarmedicin*, **23**, 9-22

Arancibia, A., Guttmann, J., Gonzalez, D. & Gonzalez, C. (1980) Absorption and disposition kinetics of amoxycillin in normal human subjects. *Antimicrobial Agents and Chemotherapy*, **17**, 199-202

Arbeiter, K., Awad-Maselmeh, M., Kopschitz, M. M., Lonin, D. & Willinger, H. (1976) Estimation of antibiotic levels in uterine tissue and blood plasma of the mare following parenteral administration of penicillin and ampicillin. *Wiener Tierarztliche Monatsschrift*, **63**, 298-302

Aronson, A. L. & Brownie, C. F. (1982) Guidelines for rational antimicrobial therapy. In: R. A. Mansmann, E. S. Mc Allister & P. W. Pratt (Eds). Equine Medicine and Surgery, Third edition, Volume 1, pp. 149-153. American Veterinary Publications, Santa Barbara, California, USA.

Asbury, A. C. (1986) Antibiotic use in reproductive disease. In: *Proceedings of the thirty-second annual convention of the American Association of Equine Practitioners*, **32**, pp. 233-239, American Association of Equine Practitioners. Nashville, Tennessee, USA.

Baggot, J. D. (1968) Influence of urinary pH on persistence of plasma levels of sulphonamides in dogs. *The Veterinary Record*, **82**, 266-267

Baggot, J. D. (1977a) Principles of pharmacokinetics. In: J. D. Baggot. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology, pp. 144-189. W. B. Saunders Company, London, UK.

Baggot, J. D. (1977b) Mechanisms of drug excretion. In: J. D. Baggot. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology, pp. 113-143. W. B. Saunders Company, London, UK.

Baggot, J. D. (1977c) Some important principles of drug dosage. In: J. D. Baggot. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology, pp. 190-218. W. B. Saunders Company, London, UK.

Baggot, J. D. (1989) Comparative absorption and disposition of drugs: horses compared with dogs and ruminant species. In: T. M. Dyke (Ed). *Proceedings of eleventh Bain-Fallon Memorial Lectures. Equine pharmacology and therapy*, pp.159-171. Australian Equine Veterinary Association. Coff's Harbour, Australia.

Baggot, J. D. (1992) Bioavailability and bioequivalence of veterinary drug dosage forms, with particular reference to horses: an overview. *Journal of Veterinary Pharmacology and Therapeutics*, **15**, 160-173

Baggot, J. D., Davis, L. E., Murdick, P. W., Ray, R. S. & Noonan, J. S. (1972) Certain aspects of amphetamine elimination in the horse. *American Journal of Veterinary Research*, **33**, 1161-1164

Baggot, J. D., Love, D. N., Love, R. J., Raus, J. & Rose, R. J. (1990) Oral dosage of penicillin V in adult horses and foals. *Equine Veterinary Journal*, **22**, 290-291

Baggot, J. D., Love, D. N., Stewart, J. & Raus, J. (1988 a) Bioavailability and disposition kinetics of amoxicillin in neonatal foals. *Equine Veterinary Journal*, **20**, 125-127

Baggot, J. D. & Prescott, J. F. (1987) Antimicrobial selection and dosage in the treatment of equine bacterial infections. *Equine Veterinary Journal*, **19**, 92-96

Baggot, J. D. & Short, C. R. (1984) Drug disposition in the neonatal animal, with particular reference to the foal. *Equine Veterinary Journal*, **16**, 364-367

Baggot, J. D., Wilson, W. D. & Hietala, S. (1988 b) Clinical pharmacokinetics of metronidazole in horses. *Journal of Veterinary Pharmacology and Therapeutics*, **11**, 417-420.

Bartlett, S. P. & Burton, R. C. (1983) Effects of prophylactic antibiotics on wound infection after elective colon and rectal surgery: 1960 to1980. *The American Journal of Surgery*, **145**, 300-309

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turck, M. (1966) Antibiotic susceptibility testing by a standarized single disk method. *The American Journal of Clinical Pathology*, **45**, 493-496

Beech, J., Leitch, M., Kohn, C. W., Weinstein, A. & Gallagher, M. (1979) Serum and synovial fluid levels of sodium ampicillin and ampicillin trihydrate in horses. *Journal of Equine Medicine and Surgery*, **3**, 350-354

Bennett, J. V., Brodie, J. L., Benner, E. J. & Kirby, W. M. M. (1966) Simplified, accurate method for antibiotic assay of clinical specimens. *Applied Microbiology*, **14**, 170-177

Bergholz, H., Erttmann, R. R. & Damm, K. H. (1980) Effects of probenecid on plasma/tissue distribution of 14C-benzylpenicillin in rats. *Experientia*, **36**, 333-334

Bernard, H. R. & Cole W. R. (1964) The prophylaxis of surgical infection: The effect of prophylactic antimicrobial drugs on the incidence of infection following potentially contaminated operations. *Surgery*, **56**, 151-157

Beyer, K. H., Russo, H. F., Tillson, E. K., Miller, A. K., Verwey, W. F. & Gass, S. R. (1951) "Benemid", p-(di-n-propylsulfamyl)-benzoic acid: its renal affinity and its elimination. *American Journal of Physiology*, **166**, 625-640

Bogan, J. A. (1983) Absorption and distribution. In: J. A. Bogan, P. Lees and A. T. Yoxall. (Eds). Pharmacological Basis of Large Animal Medicine., pp. 3-22. Blackwell Scientific Publications, Oxford, UK.

Bossen, F., Karlog, O. & Rasmussen, F. (1973) The cholinesterase activity in red blood cells and blood plasma after oral intake of Atgard vet.® (dichlorvos) in pigs. *Nordisk Veterinarimedicin*, **25**, 584-587

Bowman, K. F., Dix, L. P., Riond, J. L. & Riviere, J. E. (1986) Prediction of pharmacokinetic profiles of ampicillin sodium, gentamicin sulfate, and combination ampicillin sodium-gentamicin sulfate in serum and synovia of healthy horses. *American Journal of Veterinary Research*, **47**, 1590-1596

Brown, M. P. (1986) Antibiotic prophylaxis in equine surgery - fact or fiction? In: *Proceedings of the thirty second annual convention of the American Association of Equine Practitioners*, **32**, pp.205-208, American Association of Equine Practitioners. Nashville, Tennessee, USA.

Brown, M. P., Gronwall, R., Kroll, W. R. & Beal, C. (1984) Ampicillin trihydrate in foals: serum concentrations and clearance after a single oral dose. *Equine Veterinary Journal*, **16**, 371-373

Brown, M. P., Stover, S. M., Kelly, R. H. & Farver, T. B. (1982) Body fluid concentrations of ampicillin trihydrate in 6 horses after a single intramuscular dose. *Equine Veterinary Journal*, **14**, 83-85.

Brumbaugh, G. W. (1986) Antibiotic combinations. In: *Proceedings of the thirty-second annual convention of the American Association of Equine Practitioners*, **32**, pp. 201-204, American Association of Equine Practitioners. Nashville, Tennessee, USA.

Brumbaugh, G. W. (1987) Rational selection of antimicrobial drugs for treatment of infections of horses. *Veterinary Clinics of North America: Equine Practice, Clinical Pharmacology*, **3**, 191-220

Burke, J. F. (1961) The effective period of preventive antibiotic action in experimental incisions and dermal lesions. *Surgery*, **50**,161-168

Clark, C. H. (1980) Prophylactic use of antibiotics in surgery. Part II. *Modern Veterinary Practice*, **61**,122-126

Clark, E. S. & Becht, J. L. (1987) Clinical pharmacology of the gastrointestinal tract. *Veterinary Clinics of North America: Equine practice, Clinical Pharmacology*, **3**, 101-122

Cook, W. R. (1973) Diarrhoea in the horse associated with stress and tetracycline therapy. *The Veterinary Record*, **93**, 15-17

Curry, S. H. (1980) Chemical introduction: drug sources, drug classification and chemical properties of drugs. In: Stephen. H. Curry. (Ed). Drug Disposition and Pharmacokinetics. With a consideration of pharmacological and clinical relationships. Third edition, pp. 1-21. Blackwell Scientific Publications, Oxford, UK.

Derendorf, H. (1989) Pharmacokinetic evaluation of β-lactam antibiotics. *Journal of Antimicrobial Chemotherapy*, **24**, 407-413

Dietz, A. A., Rubinstein, H. M. & Lubrano, T. (1973) Colorimetric determination of serum cholinesterase and its genetic variants by the propionyl-thiocholine-dithiobis [nitrobenzoic acid] procedure. Clinical Chemistry, **19**,1309-1313

Donahue, J. M. (1986) Emergence of antibiotic-resistant *Salmonella agona* in horses in Kentucky. *Journal of the American Veterinary Medical Association,* **188**, 592-94

Donecker, J. M., Sams, R. A. & Ashcraft, S. M. (1986) Pharmacokinetics of probenecid and the effect of oral probenecid administration on the pharmacokinetics of cefalozin in mares. *American Journal of Veterinary Research,* **47**, 89-95

Dürr, A. (1976) Comparison of the pharmacokinetics of penicillin G and ampicillin in the horse. *Research in Veterinary Science*, **20**, 24-29

Eagle, H., Levy, M. & Fleischman, R. (1952) The effect of the pH of the medium on the antibacterial action of penicillin, streptomycin, chloramphenicol, terramycin, and bacitracin. *Antibiotics and Chemotherapy*, **2**, 563-575

Ehrnebo, M., Nilsson, S. O. & Boreus, L. O. (1979) Pharmacokinetics of ampicillin and its prodrugs bacampicillin and pivampicillin in man. *Journal of Pharmacokinetics and Biopharmaceutics*, **7**, 429-451

English, P. B. & Roberts, M. C. (1979) Antimicrobial chemotherapy in the horse: I. Pharmacological considerations. *Journal of Equine Medicine and Surgery*, **3**, 259-268

English, P. B. & Roberts, M. C. (1983) Adverse reactions to antimicrobial agents in the horse. *Veterinary Research Communications*, **7**, 207-210

Ensink, J. M., Klein, W. R., Mevius, D. J., Klarenbeek, A. & Vulto, A. G. (1992) Bioavailability of oral penicillins in the horse: a comparison of pivampicillin and amoxicillin. *Journal of Veterinary Pharmacology and Therapeutics*, **15**, 221-230

Ensink, J. M., Van Klingeren, B., Houwers, D. J., Klein, W. R. & Vulto, A. G. (1993) In-vitro susceptibility to antimicrobial drugs of bacterial isolates from horses in The Netherlands. *Equine Veterinary Journal*, **25**, 309-313

Ericsson, H. M. & Sherris, J. C. (1971) Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathologica Microbiologica Immunologica Scandinavica* [B], **217**, 1-90

Evans, J. A. & Lambert, M. B. T. (1974) Estimation of procaine in urine of horses. *The Veterinary Record*, **95**, 316-318

Evans, L. H., Stewart, J. V. & Huebner, R. A. (1971) Ampicillinemia following single intramuscular injection of sodium ampicillin in horses. *Veterinary Medicine / Small Animal Clinician*, **66**, 374-381

Fay, J. P., Jakober, K. D. & Cheng, K.-J. (1990) Esterase activity of pure cultures of rumen bacteria as expressed by the hydrolysis of *p*-nitrophenylpalmitate. *Canadian Journal of Microbiology*, **36**, 585-589

Firth, E. C., Klein, W. R., Nouws, J. F. M. & Wensing, Th. (1988) Effect of induced synovial inflammation on pharmacokinetics and synovial concentration of sodium ampicillin and kanamycin sulfate after systemic administration in ponies. *Journal of Veterinary Pharmacology and Therapeutics*, **11**, 56-62

Firth, E. C., Nouws, J. F. M., Driessens, F., Schmaetz, P., Peperkamp, K. & Klein, W. R. (1986) Effect of the injection site on the pharmacokinetics of procaine penicillin G in horses. *American Journal of Veterinary Research*, **47**, 2380-2384

Foster, J. W. & Woodruff, H. B. (1943) Microbiological aspects of penicillin. I. Methods of assay. *Journal of Bacteriology*, **46**, 187-202

Fukuda, K., Imai, M., Shindo, H. & Mizuhira, V. (1978) Histochemical study of non-specific esterase activity in the liver, kidney and small intestine from nine different mammalian species. *Acta Histochemica et Cytochemica*, **11**, 78-89

Furr, M. O. (1989) Antibiotic therapy in neonatal septicemia. *Equine Practice*, **11**, 19-23

Gabel, A. A. (1977) Intravenous injections-complications and their prevention. In: *Proceedings of Twenty-third Annual Convention of the American Association of Equine Practitioners*, **23**, pp. 29-33, American Association of Equine Practitioners, Vancouver, British Columbia, Canada.

Galbraith, E. A. (1984) Factors affecting the absorption of drugs after subcutaneous, intramuscular and topical administration in sheep and cattle. MSc. Thesis. pp. 8-34, University of Glasgow, UK.

Galtier, P. & Alvinerie, M. (1979) Enhancement of ampicillin bioavailability in pigs. *Journal of Veterinary Pharmacology and Therapeutics,* **2**, 181-186

Galtier, P. & Charpenteau, J. L. (1979) Pharmacokinetics of ampicillin in pigs. *Journal of Veterinary Pharmacology and Therapeutics*, **2**, 173-180

Gans, J. H. & Mercer, P. F. (1984) The kidneys. In: Melvin. J. Swenson. (Ed). Duke's Physiology of domestic animals, Tenth edition, pp. 507-536. Comstock Publishing Associates. A division of Cornell University Press. London, UK.

Gelså, H. (1979) The renal clearance of inulin, creatinine, trimethoprim and sulphadoxine in horses.*Journal of Veterinary Pharmacology and Therapeutics*, **2**, 257-264

Gibaldi, M. & Perrier, D (1975) Multicompartmental models. In: M. Gibaldi & D. Perrier (Eds). Pharmacokinetics. pp. 45-111. Marcel Dekker, Inc., New York, USA.

Gibaldi, M. & Schwartz, M. A. (1968) Apparent effect of probenecid on the distribution of penicillins in man. *Clinical Pharmacology and Therapeutics*, **9**, 345-349

Gibaldi, M., Davidson, D., Plaut, M. E. & Schwartz, M. A. (1970) Modification of penicillin distribution and elimination by probenecid. *International Journal of Clinical Pharmacology*, **3**, 182-189

Gorbach, S. L. (1983) Prophylactic antibiotics. In: U. F. Gruber & S. L. Gorbach. (Eds). *Proceedings of 13th International Congress of Chemotherapy. Symposium: Perioperative prevention of infection with antibiotics.* pp. 6-12. Vienna, Austria.

Gronwall, R. & Brown, M. P. (1988) Probenecid infusion in mares: effect on para-aminohippuric acid clearance. *American Journal of Veterinary Research*, **49**, 250-253

Guerrini, V. H., Filippich, L. J., English, P. B., Schneider, J., Cao, G. R. & Bourne, D. W. A. (1985) Pharmacokinetics of probenecid in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **8**, 128-135

Guglielmo, B. J., Hohn, D. C., Koo, P. J., Hunt, T. K., Sweet, R. L. & Conte, J. E. (1983) Antibiotic prophylaxis in surgical procedures. A critical analysis of the literature. *Archives of Surgery*, **118**, 943-953

Gundert-Remy, U. & Weber, E. (1981) Degradation of Azlocillin and Mezlocillin I. Behaviour in biological material and buffer. *Arzneimittelforschung*, **31**, 2041-2044

Haginaka, J. & Wakai, J. (1986) Liquid chromatographic determination of ampicillin and its metabolites in human urine by postcolumn alkaline degradation. *Journal of Pharmacy and Pharmacology*, **39**, 5-8

Hillidge, C. J. (1987) Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. *Veterinary Microbiology*, **14**, 337-342

Hirsh, D. C. & Jang, S. S. (1987) Antimicrobic susceptibility of bacterial pathogens from horses. *Veterinary Clinics of North America: Equine Practice, Clinical Pharmacology*, **3**, 181-190

Hirsh, D. C., Indiveri, M. C., Jang, S. S. & Biberstein, E. L. (1985) Changes in prevalence and susceptibility of obligate anaerobes in clinical veterinary practice. *Journal of the American Veterinary Medical Association*, **186**, 1086-1089

Hirschmann, J. V. & Inui, T. S. (1980) Antimicrobial prophylaxis: a critique of recent trials. *Reviews of Infectious Diseases*, **2**, 1-19

Horspool, L. J. I. (1992) Antimicrobial disposition and interactions with gastrointestinal microflora in *Equidae*. PhD Thesis. pp. 137-179. University of Glasgow, UK.

Horspool, L. J. I., Sarasola, P. & McKellar, Q. A. (1992) Disposition of ampicillin sodium in horses, ponies and donkeys after intravenous administration. *Equine Veterinary Journal*, Supplement **11**, 59-61

Hou, J. P. & Poole, J. W. (1969) The amino acid nature of ampicillin and related penicillins. *Journal of Pharmaceutical Sciences*, **58**, 1510-1515

Houston, T., Chay, S., Woods, W. E., Combs, G., Kamerling, S., Blake, J. W., Edmundson, A. G., Vessiney, R. & Tobin, T. (1985) Phenylbutazone and its metabolites in plasma and urine of thoroughbred horses: population distributions and effects of urinary pH. *Journal of Veterinary Pharmacology and Therapeutics*, **8**, 136-149

Ingham, B. & Large, B. J. (1969) An introduction to allergy and its treatment. *Equine Veterinary Journal*, **1**, 279-282

Inui, K-I., Okano, T., Takano, M., Kitazawa, S. & Hori, R. (1983) Carrier-mediated transport of amino-cephalosporins by brush border membrane vesicles isolated from rat kidney cortex. *Biochemical Pharmacology*, **32**, 621-626

Inui, K-I., Okano, T., Takano, M., Saito, H. & Hori, R. (1984) Carrier-mediated transport of cephalexin via the dipeptide transport system in rat brush-border membrane vesicles. *Biochimica et Biophysica Acta*, **769**, 449-454

Jenkins, W. L. (1984) Concurrent use of corticosteroids and antimicrobial drugs in the treatment of infectious diseases in large animals. *Journal of the American Veterinary Medical Association*, **185**, 1145-1149

Juzwiak, J. S., Brown, M. P., Gronwall, R. & Houston, A. E. (1989) Effect of probenecid administration on cephapirin pharmacokinetics and concentrations in mares. *American Journal of Veterinary Research*, **50**, 1742-1747

Kampmann, J., Mølholm Hansen, J., Siersbœk-Nielsen, K. & Laursen, H. (1972) Effect of some drugs on penicillin half-life in blood. *Clinical Pharmacology and Therapeutics*, **13**, 516-519

Katzung, B. G. (1984) Introduction. In: Bertram G. Katzung. (Ed). Basic and Clinical Pharmacology. 2nd Ed. pp.1-8. Lange Medical Publications, Los Altos, California, USA.

Keefe, T. J., Simmons, R. D. & Southard, M. A. (1980) Ampicillin sodium (Amp-Equine®) as a parenteral antibiotic for horses. *Veterinary Medicine / Small Animal Clinician*, **75**, 1727-1731

Keen, P. (1989) Tissue distribution of antibacterials as related to blood levels. *Pig Veterinary Journal*, **24**, 24-29

Keen, P. & Livingston, A. (1983) Adverse reactions to drugs. *In Practice*, **5**, 174-180

Kinabo, L. D. B. & McKellar, Q. A. (1989) Current models in pharmacokinetics: applications in veterinary pharmacology. *Veterinary Research Communications*, **13**, 141-157

Kirk, R. W. (1986) Prophylactic use of antimicrobial agents in surgical patients. In: R. W. Kirk (Ed). Current veterinary therapy IX Kirk, pp. 24-26. W. B. Saunders Company, Philadelphia, USA.

Kleinman, J. G., Harig, J. M., Barry, J. A. & Ramaswamy, K. (1988) Na^+ and H^+ transport in human jejunal brush-border membrane vesicles. *American Journal of Physiology*, **255**, G206-211

Knight, H. D. (1975) Antimicrobial agents used in the horse. In: *Proceedings of the twenty-first annual convention of the American Association of Equine Practitioners*, **21**, pp.131-144, American Association of Equine Practitioners. Boston, Massachusetts, USA.

Knight, H. D. & Hietala, S. (1978) Antimicrobial susceptibility patterns in horses. In: *Proccedings of the 2nd Equine Pharmacology Symposium. of the American Association of Equine Practitioners*, pp. 63-88, American Association of Equine Practitioners. Columbus, Ohio, USA.

Knudsen, E. (1960) Renal clearance studies on the horse. II. Penicillin, sulfathiazole and sulfadimidine. *Acta Veterinaria Scandinavica*, **1**, 188-200

Koritz, G. D. (1984) Relevance of peak and trough concentrations to antimicrobial drug therapy. *Journal of the American Veterinary Medical Association*, **185**, 1072-1075

Koterba, A., Torchia, J., Silverthorne, C., Ramphal, R., Merritt, A. M. & Manucy, J. (1986) Nosocomial infections and bacterial antibiotic resistance in a university equine hospital. *Journal of the American Veterinary Medical Association*, **189**, 185-191

Levy, G. (1965) Effect of probenecid on blood levels and urinary recovery of ampicillin. *The American Journal of Medical Sciences*, **250**, 174-176

Linton, A. H. (1982) Antibiotic resistance in veterinary practice. *In Practice*, **4**, 11-15

Long, P. E., Guarnieri, J. A., Herbst, D. V. & Teske, R. H. (1983) Disposition of ampicillin administered intravenously and intratracheally to young calves. *Journal of Veterinary Pharmacology and Therapeutics*, **6**, 273-279

Love, D. N., Rose, R. J., Martin, I. C. A. & Bailey, M. (1981) Serum levels of amoxycillin following its oral administration to Thoroughbred foals. *Equine Veterinary Journal*, **13**, 53-55

Love, D. N., Rose, R. J., Martin, I. C. A. & Bailey, M. (1983) Serum concentrations of penicillin in the horse after administration of a variety of penicillin preparations. *Equine Veterinary Journal*, **15**, 43-48

Lucas, M. (1983) Determination of acid surface pH *in vivo* in rat proximal jejunum. *Gut*, **24**,734-739

Magni, L., Sjovall, J. & Syvalahti, E. (1978) Comparative clinical pharmacology of bacampicillin and high oral doses of ampicillin. *Infection*, **6**, 283 - 289

Mair, T. S. & Yeo, S. P. (1987) Equine pleuropneumonia: The importance of anaerobic bacteria and the potential value of metronidazole in treatment. *The Veterinary Record*, **121**, 109-110

Mandell, G. L. & Sande, M. A. (1980) Antimicrobial agents. Penicillins and Cephalosporins. In: Alfred Goodman Gilman, Louis S. Goodman & Alfred Gilman. (Eds). The Pharmacological Basis of Therapeutics, Sixth edition, pp. 1126-1161. Bailliere Tindal, London, UK.

Montesissa, C., Carli, S., Sonzogni, O., Garlappi, R. & Sericola, A. (1988) Pharmacokinetics of sodium amoxicillin in horses. *Research in Veterinary Science*, **44**, 233-236

Moore, R. M., Schneider, R. K., Kowalski, J., Bramlage, L. R., Mecklenburg, L. M. & Kohn, C. W. (1992) Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979-1989. *Equine Veterinary Journal*, **24**, 450-456

Mortimer, P. R., Mackie, D. B. & Haynes, S. (1969) Ampicillin levels in human bile in the presence of biliary tract disease. *British Medical Journal*, **3** , 88-89

Moss, M. S. (1976) The metabolism and urinary and salivary excretion of drugs in the horse and their relevance to detection of dope. In: *Drug metabolism - from microbe to man.* Taylor and Francis, London. Symposium on drug metabolism 1976, pp. 263-280. University of Surrey, UK.

Moss, M. S. & Haywood, P. E. (1973) Persistence of phenylbutazone in horses producing acid urines. *The Veterinary Record*, **93**, 124-125

Mou, T. W. (1962) Effect of urine pH on the antibacterial activity of antibiotics and chemotherapeutic agents. *The Journal of Urology*, **87**, 978-987

Nakashima, E., Tsuji, A., Mizuo, H. & Yamana, T. (1984) Kinetics and mechanism of *in vitro* uptake of amino-β-lactam antibiotics by rat small intestine and relation to the intact-peptide transport system. *Biochemical Pharmacology*, **33**, 3345-3352

Ndikuwera, J. & Winstanley, E. W. (1987) The use and misuse of antibiotics in the treatment of wounds. *Irish Veterinary Journal*, **41**, 223-226

Neu, H. C. (1981) The pharmacokinetics of bacampicillin. *Reviews of Infectious Diseases*, **3**, 110-116

Okano, T., Inui, K-I., Takano, M. & Hori, R. (1986) H^+ gradient-dependent transport of aminocephalosporins in rat intestinal brush-border membrane vesicles. Role of dipeptide transport system. *Biochemical Pharmacology*, **35**, 1781-1786

Palmer, G. H., Bywater, R. J. & Stanton, A. (1983) Absorption in calves of amoxicillin, ampicillin, and oxytetracycline given in milk replacer, water, or an oral rehydration formulation. *American Journal of Veterinary Research*, **44**, 68-71

Park, J. T. (1987) Murein synthesis. In: F. C. Neidhard (Ed). *Escherichia coli* and *Salmonella typhimurium*, cellular and molecular biology, Volume1. pp. 663-671. American Society for Microbiology, Washington D. C., USA.

Paul, J. W. (1987) Drug interactions and incompatibilities. *Veterinary Clinics of North America: Equine Practice, Clinical Pharmacology*, **3**, 145-151

Piperno, E., Ellis, D. J., Getty, S. M. & Brody, T. M. (1968) Plasma and urine levels of phenylbutazone in the horse. *Journal of the American Veterinary Medical Association*, **153**, 195-198

Polk, H. C. JR. & Lopez-Mayor, J. F. (1969) Postoperative wound infection: A prospective study of determinant factors and prevention. *Surgery*, **66**, 97-103

Powers, J. (1990) Statistical analysis of pharmacokinetic data. *Journal of Veterinary Pharmacology and Therapeutics*, **13**, 113-120

Powers, T. E., Varma, K. J. & Powers, J. D. (1984) Selecting therapeutic concentrations: minimum inhibitory concentrations vs subminimum or supraminimum inhibitory concentrations. *Journal of the American Veterinary Medical Association*, **185**, 1062-1067

Prescott, J. F. (1981) The susceptibility of isolates of *Corynebacterium equi* to antimicrobial drugs. *Journal of Veterinary Pharmacology and Therapeutics* **4**, 27-31

Prescott, J. F. & Baggot, J. D. (1985) Antimicrobial susceptibility testing and antimicrobial drug dosage. *Journal of the American Veterinary Medical Association*, **187**, 363-368

Prescott, J. F. & Baggot, J. D. (1988a) Principles of antimicrobial drug disposition. In: J. F. Prescott & J. Desmond Baggot. Antimicrobial Therapy in Veterinary Medicine, pp. 29-53. Blackwell Scientific Publications, Oxford, UK.

Prescott, J. F. & Baggot, J. D. (1988b) Beta-lactam antibiotics: Penicillins, Cephalosporins, and newer antibiotics. In: J. F. Prescott & J. Desmond Baggot. Antimicrobial Therapy in Veterinary Medicine, pp. 71-109. Blackwell Scientific Publications, Oxford, UK.

Prescott, J. F. & Baggot, J. D. (1988c) Antimicrobial susceptibility and drug dosage prediction. In: J. F. Prescott & J. Desmond Baggot. Antimicrobial Therapy in Veterinary Medicine, pp. 9-16. Blackwell Scientific Publications, Oxford, UK.

Pugh, D. M. (1977) Pharmacokinetic studies and amoxicillin. *Veterinary Medicine Small / Animal Clinician*, **72** (April Supplement), 704-719

Puyt, J. D. (1990) Ampicilline. In: Section nationale des Groupements techniques vétérinaires du Syndicat national des vétérinaires practitiens français (Eds). *Antibiothérapie chez les équidés* G.T.V. 90-1-Eq-028. p. 51, Paris, France.

Rahal, J. J. Jr. (1978) Antibiotic combinations: the clinical relevance of synergy and antagonism. *Medicine*, **57**, 179-195

Raisbeck, M. F., Holt, G. R. & Osweiler, G. D. (1981) Lincomycin-associated colitis in horses. *Journal of the American Veterinary Medical Association*, **179**, 362-363

Rang, H. P. & Dale, M. M. (1987a) Absorption, distribution and fate of drugs. In: H. P. Rang & M. M. Dale (Eds). Pharmacology. pp. 57-89. Churchill Livingstone, Edinburgh, UK.

Rang, H. P. & Dale, M. M. (1987b) Antibacterial agents. In: H. P. Rang & M. M. Dale (Eds). Pharmacology. pp. 626-646. Churchill Livingstone, Edinburgh, UK.

Rang, H. P. & Dale, M. M. (1987c) The kidney. In: In: H. P. Rang & M. M. Dale (Eds). Pharmacology. pp. 309-329. Churchill Livingstone, Edinburgh, UK.

Reif, J. S. (1979) Epidemiology of equine infectious respiratory disease. *Veterinary Clinics of North America: Large Animal Practice*, **3** , 3-15

Ricketts, S. W. & Hopes, R. (1983) The use of antibiotics in equine practice. In: J. A. Bogan, P. Lees and A.T. Yoxall. (Eds). Pharmacological Basis of Large Animal Medicine., pp. 70-86. Blackwell Scientific Publications, Oxford, UK.

Ricketts, S. W. & Hopes, R. (1984) Selection of antibiotics for use in equine practice. *The Veterinary Record*, **114**, 544-546

Riegelman, S. & Collier, P. (1980) The application of statistical moment theory to the evaluation of *in vivo* dissolution time and absorption time. *Journal of Pharmacokinetics and Biopharmaceutics*, **8**, 509-534

Rigg, J. R. A. & Wong, T. Y. (1981) A method for achieving rapidly steady-state blood concentrations of I.V. drugs. *British Journal of Anaesthesia*, **53**, 1247-1257

Rozencweig, M., Staquet, M. & Klastersky, J. (1976) Antibacterial activity and pharmacokinetics of bacampicillin and ampicillin. *Clinical Pharmacology and Therapeutics* , **19**, 592-597

Russell, A. D. & Chopra, I. (1990 a) Mode of action of antibiotics and their uptake into bacteria. In: Russell, A. D. and Chopra, I (Eds). Understanding Antibacterial action and Resistance. pp. 39-94. Ellis Horwood Limited.

Russell, A. D. & Chopra, I. (1990 b) Genetic and biochemical basis of resistance to chemotherapeutic antibiotics. In: Russell, A. D. and Chopra, I (Eds). Understanding Antibacterial action and Resistance. pp. 146-181. Ellis Horwood Limited.

Sams, R. A. (1987) Principles of drug disposition in the horse.*Veterinary Clinics of North America: Equine Practice, Clinical Pharmacology*, **3**, 221-250

Sande, M. A. & Mandell, G. L. (1985) Antimicrobial agents. general considerations. In: Alfred Goodman Gilman, Louis S. Goodman, Theodore W. Rall, & Ferid Murad (Eds). The Pharmacological basis of therapeutics. pp. 1066-1094. Macmillan Publishing Company. New York, USA.

Sandholm, M., Kaartinen, L. & Pyorala, S. (1990) Bovine mastitis-why does antibiotic therapy not always work? An overview. *Journal of Veterinary Pharmacology and Therapeutics*, **13**, 248-260

Sato, G., Nakaoka, Y., Marufuji, S., Ishiguro, N. & Shinagawa, M. (1988) A plasmid profile study of *Salmonella typhimurium* strains isolated from horses in Japan. In: D. G. Powell (Ed). *Proceedings of the Fifth International Conference of Equine Infectious Diseases*. pp. 223-30. The University Press of Kentucky, Lexington, Kentucky, USA.

Scheife, R. T. & Neu, H. C. (1982) Bacampicillin hydrochloride: chemistry, pharmacology and clinical use. *Pharmacotherapy*, **2**, 313-321

Sedman, A. J. & Wagner, J. G. (1976) CSTRIP, a Fortran IV computer programme for obtaining initial polyexponential parameter estimates. *Journal of Pharmaceutical Sciences*, **65**, 1006-1010

Shiau, Y. F., Fernandez, P., Jackson, M. J. & McMonagle, S. (1985) Mechanisms maintaining a low-pH microclimate in the intestine. *American Journal of Physiology*, **248**, G 608-17

Shindo, H., Fukuda, K., Kawai, K. & Tanaka, K. (1978) Studies on intestinal absorption of pivampicillin and species difference in the intestinal esterase activity. *Journal of Pharmacobio-Dynamics.*, **1**, 310-323

Simon, C., Malerczyk, V. & Klaus, M. (1978) Absorption of bacampicillin and ampicillin and penetration into body fluids (skin blister fluid, saliva, tears) in healthy volunteers. *Scandinavian Journal of Infectious Diseases Supplement*, **14**, 228 - 232

Sjovall, J., Alvan, G. & Westerlund, D. (1985) Dose-dependent absorption of amoxycillin and bacampicillin. *Clinical Pharmacology and Therapeutics*, **38**, 241-250

Sjovall, J., Huitfeldt, B., Magni, L. & Nord, C. E. (1986) Effect of beta-lactam prodrugs on human intestinal microflora. *Scandinavian Journal of Infectious Diseases Supplement*, **49**, 73-84

Sjovall, J., Magni, L. & Bergan, T. (1978) Pharmacokinetics of bacampicillin compared with those of ampicillin, pivampicillin and amoxycillin. *Antimicrobial Agents and Chemotherapy*, **13**, 90-96

Snow, D. H. (1974) Studies on the action of an anthelmintic preparation of dichlorvos in horses. *The Veterinary Record*, **95**, 231-233

Snyder, J. R., Pascoe, J. R. & Hirsh, D. C. (1987) Antimicrobial susceptibility of microorganisms isolated from equine orthopedic patients. *Veterinary Surgery*, **16**, 197-201

Sommers, De. K., Van Wyk, M, Moncrieff, J. & Schoeman, H. S. (1984) Influence of food and reduced gastric acidity on the bioavailability of bacampicillin and cefuroxime axetil. *British Journal of Clinical Pharmacology*, **18**, 535-539

Spratt, B. G. (1975) Distinct penicillin binding proteins involved in the division, elongation and shape of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences (USA)*, **72**, 2999-3003

Spurlock, S. L. & Wilcke, J. R. (1986) Penicillins and cephalosporins. In: *Proceedings of the thirty-second annual convention of the American Association of Equine Practitioners*, **32**, pp. 175-182, American Association of Equine Practitioners, Nashville, Tennessee, USA.

Step, D. L., Blue, J. T. & Dill, S. G. (1991) Penicillin-induced hemolytic anemia and acute hepatic failure following treatment of tetanus in a horse. *Cornell Veterinarian*, **81**, 13-18

Stevenson, A. J., Weber, M. P., Todi, F., Mendonca, M., Fenwick, J. D., Kwong, E., Young, L., Leavitt, R., Nespolo, R., Beaumier, P., Timmings, S. & Kacew, S. (1990) The influence of furosemide on plasma elimination and urinary excretion of drugs in standardbred horses. *Journal of Veterinary Pharmacology and Therapeutics*, **13**, 93-104

Stover, S. M., Brown, M. P., Kelly, R. H. & Farber, T. B. (1981) Sodium oxacillin in the horse: serum, synovial fluid, peritoneal fluid and urine concentrations after single dose intramuscular administration. *American Journal of Veterinary Research*, **42**, 1826-1828

Sullins, K. E., Messer, N. T. & Nelson, L. (1984) Serum concentration of penicillin in the horse after repeated intramuscular injections of procaine penicillin G alone or in combination with benzathine penicillin and/or phenylbutazone. *American Journal of Veterinary Research*, **45**, 1003-1007

Sutherland, R. & Rolinson, G. N. (1978) Methods of antibiotic assay. Penicillins. In: D. S. Reeves, I. Phillips, J. D. Williams & R. Wise (Eds). Laboratory methods in antimicrobial chemotherapy. pp.171-178. Churchill Livingstone, Edinburgh, UK.

Swahn, Å. (1975) On the absorption and metabolism of ^{35}S-ampicillin. *European Journal of Clinical Pharmacology*, **9**, 117-124

Swahn, Å. (1976) Gastrointestinal absorption and metabolism of two ^{35}S-labelled ampicillin esters. *European Journal of Clinical Pharmacology*, **9**, 299-306

Sweeney, C. R., Sweeney, R. W. & Divers, T. J. (1987) *Rhodococcus equi* pneumonia in 48 foals: response to antimicrobial therapy. *Veterinary Microbiology*, **14**, 329-336

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. *American Journal of Veterinary Research*, **47**, 1726-1729

Tipper, D. J. & Strominger, J. L. (1965) Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alnyl-D-alanine. Proceedings of the US National Academy of Sciences. **54**, 1133-1141

Tobin, T. (1979) An overview of the major antibiotics in equine medicine. In: *Proceedings of the twenty-fifth annual convention of the American Association of Equine Practitioners*, **25**, pp. 491-502. American Association of Equine Practitioners, Miami Beach, USA.

Tobin, T., Blake, J. W., Sturma, L., Arnett, S. & Truelove, J. (1977) Pharmacology of procaine in the horse: Pharmacokinetics and behavioral effects. *American Journal of Veterinary Research*, **38**, 637-647

Tobin, T. & Woods, W. E. (1979) Pharmacology review: drug metabolism and elimination in horses. *Journal of Equine Medicine and Surgery*, **3**, 150-156

Tomasz, A. (1979) From penicillin-binding proteins to the lysis and death of bacteria: a 1979 view. *Review of Infectious Diseases*, **1**, 434-467

Traver, D. S. & Riviere, J. E. (1981) Penicillin and ampicillin therapy in horses. *Journal of the American Veterinary Medical Association*, **178**, 1186-1189

Traver, D. S. & Riviere, J. E. (1982) Ampicillin in mares: a comparison of intramuscular sodium ampicillin or sodium ampicillin-ampicillin trihydrate injection. *American Journal of Veterinary Research*, **43**, 402-404

van den Bogaard, A. E. J. M. Jr. & Weidema, W. F. (1985) Antimicrobial prophylaxis in veterinary surgery. *Journal of the American Veterinary Medical Association*, **186**, 990-992

van Miert, A. S. J. P. A. M. (1988) *Veterinary Antimicrobial Therapy Guide*. Alfasan Nederland b. v., pp 16 -58. Holland.

van Scoy, R. E. & Wilkowske, C. J. (1983) Prophylactic use of antimicrobial agents. *Mayo Clinic Proceedings*, **58**, 241-245

Villa, R., Carli, S., Montesissa, C. & Sonzogni, O. (1991) Influence of probenecid on cephalexin pharmacokinetics in sheep. In: C. Friis, N. Gyrd-Hansen, P. Nielsen and F. Rasmussen (Eds). *Proceedings of the 5th Congress of the European Association for Veterinary Pharmacology and Toxicology.* pp.124-126. Copenhagen, Denmark.

Wagner, K. F., Counts, G., Holmes, K. K., Sparling, P. F., Gentry, L. O. & Edwards, L. (1981) Clinical evaluation of single-dose oral bacampicillin or ampicillin in therapy of uncomplicated gonorrhea. *Reviews of Infectious Diseases*, **3**, 166-169

Ward, F. P. & Glicksberg, C. L. (1971) Effects of dichlorvos on blood cholinesterase activity in dogs. *Journal of the American Veterinary Medical Association*, **158**, 457-461

Watanakunakorn, C. (1971) Penicillin combined with gentamicin or streptomycin: Synergism against Enterococci. *The Journal of Infectious Diseases*, **124**, 581-586

Waxman, D. J., Yocum, R. R. & Stominger, J. L. (1980) Penicillins and cephalosporins are active site-directed acylating agents: evidence in support of the substrate analogue hypothesis. *Philosophical Transactions of the Royal Society of London. [Biology]*, **289**, 257-271

Westphal, J. F., Deslandes, A., Brogard, J. M. & Carbon, C. (1991) Reappraisal of amoxycillin absorption kinetics. *Journal of Antimicrobial Chemotherapy.* **27**, 647-654

Whelton, A., Walker, W. G. & Carter, G. G. (1967) The influence of nonionic diffusion on renal concentration and excretion of antibiotics. In: *Proceedings of the Fifty-ninth Annual Meeting. American Society for clinical Investigation. Journal of Clinical Investigation*, **46**, 1132-1133

White, G. & Prior, S. D. (1982) Comparative effects of oral administration of trimethoprim / sulphadiazine or oxytetracycline on the faecal flora of horses. *The Veterinary Record*, **111**, 316-318

Wilson, W. D., Spensley, M. S., Baggot, J. D. & Hietala, S. K. (1988) Pharmacokinetics and estimated bioavailability of amoxicillin in mares after intravenous, intramuscular, and oral administration. *American Journal of Veterinary Research* **49**, 1688-1694

Wood, T., Weckman, T. J., Henry, P. A., Chang, S.-L., Blake, J. W. & Tobin, T. (1990) Equine urine pH: normal population distributions and methods of acidification. *Equine Veterinary Journal*, **22**, 118-121

Wright, A. J. & Wilkowske, C. J. (1983) The penicillins. *Mayo Clinic Proceedings*, **58**, 21-32

Yamaoka, K., Nakagawa, T. & Uno, T. (1978) Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *Journal of Pharmacokinetics and Biopharmaceutics*, **6**, 165-175

Zarowny, D., Ogilvie, R., Tamblyn, D., MacLeod, C. & Ruedy, J. (1974) Pharmacokinetics of amoxicillin. *Clinical Pharmacology and Therapeutics*, **16**, 1045-1051

Zertuche, J. M. L, Brown, M. P., Gronwall, R., & Merritt, K. (1992) Effect of probenecid on the pharmacokinetics of flunixin meglumine and phenylbutazone in healthy mares. *American Journal of Veterinary Research*, **53**, 372-374

Ziv, G. & Horsey, J. (1979) Elevation and prolongation of serum ampicillin and amoxycillin concentrations in calves by the concomitant administration of probenecid. *Journal of Veterinary Pharmacology and Therapeutics*, **2**, 187-194

Ziv, G., Neumann, J., Fridman, J., Ziv, E., Singer, N. & Meshorer, A. (1979) Effects of probenecid on blood levels and tissue distribution of ampicillin in fowls and turkeys. *Avian Diseases*, **24**, 927-939

Appendices

Appendix A

Studies on the effects of urinary pH on plasma pharmacokinetics of ampicillin in the horse

	Animal number			
Time (h)	1*	3*	4*	5
0.033	52.19	61.79	59.49	55.43
0.083	43.41	54.27	50.22	41.62
0.25	34.71	38.01	37.84	30.57
0.5	27.18	25.94	29.58	15.65
0.75	16.03	18.26	21.38	10.08
1	7.01	14.11	14.39	7.89
1.5	5.33	6.95	8.35	3.81
2	3.21	3.39	4.16	2.33
4	0.41	0.39	1.27	0.47
8	0.03	0.00	0.13	0.00
12	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00

Table A-1. Plasma ampicillin concentrations after IV administration of ampicillin sodium at a 10 mg/kg dosage rate to horses with normal urine.
* Results from a previous study (Horspool *et al.*, 1992).

	Animal number			
Time (h)	1	2	3	6
0.033	51.46	61.46	57.11	71.29
0.083	43.78	55.58	51.28	50.30
0.25	32.18	47.48	34.34	31.37
0.5	20.91	26.61	19.92	25.51
0.75	14.75	14.37	14.06	13.43
1	12.97	10.85	12.04	10.57
1.5	8.01	7.50	5.72	10.28
2	5.17	3.55	3.69	3.71
4	1.68	0.64	0.75	0.61
8	0.08	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00

Table A-2. Plasma ampicillin concentrations after IV administration of ampicillin sodium at a 10 mg/kg dosage rate to horses with alkaline urine.

	Animal number			
Time (h)	1	2	3	6
0.033	64.24	61.26	72.21	68.10
0.083	59.04	51.69	71.03	62.92
0.25	35.70	37.39	41.37	39.30
0.5	23.17	22.19	27.28	25.73
0.75	17.62	14.91	16.62	15.97
1	11.79	10.74	12.34	9.66
1.5	6.67	5.00	6.42	4.98
2	4.11	3.19	4.04	2.74
4	0.86	0.60	0.65	0.34
8	0.07	0.10	0.06	0.00
12	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00

Table A-3. Plasma ampicillin concentrations after IV administration of ampicillin sodium at a 10 mg/kg dosage rate to horses with acidic urine.

	Animal number			
Variable	1*	3*	4*	5
α (/h)	1.98	6.66	1.84	4.40
β (/h)	0.80	1.28	0.57	0.93
t1/2 α (h)	0.35	0.104	0.377	0.157
t1/2 β (h)	0.87	0.568	1.213	0.741
Cp0 (μg/ml)	53.17	67.92	59.67	59.88
AUC (μg.h/ml)	38.09	41.27	47.94	28.76
Vc (ml/kg)	188.1	147.2	167.6	167.0
Vd_{area} (ml/kg)	329.8	198.7	365.3	371.8
Vdss (ml/kg)	229.9	186.0	231.3	275.0
Clb (ml/h.kg)	262.6	242.3	208.6	347.7
k_{21} (/h)	1.129	4.937	0.834	1.979
k_{el} (/h)	1.396	1.646	1.245	2.082
k_{12} (/h)	0.251	1.299	0.320	1.279

Table A-4. Pharmacokinetic variables after compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10 mg/kg dose rate to horses with normal urine.
* Results from a previous study (Horspool *et al.*, 1992).

	Animal number			
Variable	1*	3*	4*	5
AUC_{obs} (μg.h/ml)	36.43	42.27	48.62	29.37
$AUMC_{obs}$(μg.h^2/ml)	29.07	31.30	51.94	21.96
MRT (min)	47.89	44.43	64.10	44.87
CLb_{obs}(ml/h.kg)	274.49	236.59	205.67	340.53
$Vdss_{obs}$ (ml/kg)	219.07	175.20	219.73	254.69

Table A-5. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10mg/kg dose rate to horses with normal urine.
* Results from a previous study (Horspool *et al.*, 1992)

	Animal number			
Variable	1	2	3	6
α (/h)	4.25	2.77	4.41	15.15
β (/h)	0.71	0.96	0.88	1.04
t1/2 α (h)	0.163	0.250	0.157	0.046
t1/2 β (h)	0.974	0.722	0.783	0.669
Cp0 (μg/ml)	55.72	68.04	65.21	96.26
AUC (μg.h/ml)	42.02	44.02	36.80	39.26
Vc (ml/kg)	179.5	147.0	153.3	103.9
Vd_{area} (ml/kg)	334.6	236.6	307.0	247.3
Vdss (ml/kg)	286.2	186.6	245.2	223.9
Clb (ml/h.kg)	238.0	227.2	271.7	256.3
k_{21} (/h)	2.282	1.721	2.202	6.365
k_{el} (/h)	1.326	1.546	1.772	2.467
k_{12} (/h)	1.357	0.464	1.319	7.355

Table A-6. Pharmacokinetic variables after compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10 mg/kg dose rate to horses with alkaline urine.

	Animal number			
Variable	1	2	3	6
AUC_{obs} (μg.h/ml)	44.08	43.42	38.26	41.23
$AUMC_{obs}$(μg.h^2/ml)	53.82	33.57	32.86	34.21
MRT (min)	73.26	46.39	50.94	49.77
CLb_{obs}(ml/h.kg)	226.84	230.33	261.36	242.52
$Vdss_{obs}$ (ml/kg)	276.97	178.04	224.50	201.18

Table A-7. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10mg/kg dose rate to horses with alkaline urine.

	Animal number			
Variable	1	2	3	6
α (/h)	3.73	2.73	4.44	4.84
β (/h)	0.74	0.64	0.80	1.20
t1/2 α (h)	0.186	0.254	0.156	0.143
t1/2 β (h)	0.934	1.080	0.870	0.576
Cp0 (μg/ml)	70.20	64.36	83.95	77.14
AUC (μg.h/ml)	42.93	39.35	45.59	38.59
Vc (ml/kg)	142.5	155.4	119.1	129.6
Vd_{area} (ml/kg)	313.8	396.1	275.4	215.6
Vdss (ml/kg)	238.7	252.0	210.6	180.1
Clb (ml/h.kg)	232.9	254.1	219.3	259.1
k_{21} (/h)	1.694	1.072	1.920	2.912
k_{el} (/h)	1.635	1.636	1.840	1.999
k_{12} (/h)	1.145	0.666	1.470	1.133

Table A-8. Pharmacokinetic variables after compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10 mg/kg dose rate to horses with acidic urine.

	Animal number			
Variable	1	2	3	6
AUC_{obs} (μg.h/ml)	43.35	38.97	45.92	39.34
$AUMC_{obs}$(μg.h^2/ml)	40.74	34.17	38.14	25.87
MRT (min)	56.40	52.60	49.83	39.46
CLb_{obs}(ml/h.kg)	230.68	256.61	217.77	254.19
$Vdss_{obs}$ (ml/kg)	216.84	225.05	180.75	167.26

Table A-9. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10mg/kg dose rate to horses with acidic urine.

Appendix B

Studies on the effects of probenecid on ampicillin pharmacokinetics in the horse

	Animal number			
Variable	1	2	5	7
Cmax (μg/ml)	150.00	163.40	232.70	208.30
tmax (min)	90.00	90.00	120.00	180.00
AUC_{obs} (μg.h/ml)	1579.71	2509.24	2719.32	1305.13
$AUMC_{obs}$(μg.h^2/ml)	12628.35	31388.50	33516.54	7823.04
MRT (h)	7.99	12.51	12.32	5.99

Table B-1. Pharmacokinetic variables after non-compartmental analysis of probenecid plasma concentrations following an IV bolus of ampicillin sodium at a 10mg/kg dose rate and intragastric administration of probenecid at a 75 mg/kg dose rate.

	Animal number			
Time (h)	1	2	5	7
0.25	54.48	66.44	77.36	54.06
0.5	97.81	97.31	132.07	111.93
1	122.63	98.23	180.29	185.8
1.5	133.80	128.03	232.70	208.30
2	115.02	163.40	196.20	195.55
3	150.00	154.68	189.87	153.17
5	119.05	160.17	168.77	94.42
9	69.03	124.67	95.36	37.14
13	46.33	62.03	68.34	25.18
25	6.38	28.47	32.28	3.64

Table B-2. Plasma probenecid concentrations (μg/ml) following an intragastric administration of probenecid at 75 mg/kg dosage rate.

	Animal number			
Time (h)	1	2	5	7
0.033	52.19	55.43	65.54	59.14
0.083	43.41	41.62	48.99	43.06
0.25	34.71	30.57	31.60	35.06
0.5	27.18	15.65	18.34	26.47
0.75	16.03	10.08	14.16	19.84
1	7.01	7.89	11.14	15.69
1.5	5.33	3.81	5.65	9.15
2	3.21	2.33	3.00	5.29
4	0.41	0.47	0.53	0.95
8	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00

Table B-3. Plasma ampicillin concentrations (μg/ml) following an IV administration of ampicillin sodium at a 10 mg/kg dosage rate.

	Animal number			
Time (h)	1	2	5	7
0.033	90.86	72.29	85.50	86.70
0.083	71.17	64.60	71.15	64.90
0.25	53.28	45.55	63.16	53.96
0.5	44.47	31.98	42.07	38.88
0.75	33.65	25.62	32.60	35.82
1	27.52	21.59	29.34	30.33
1.5	20.42	17.10	21.27	21.53
2	14.78	11.52	14.02	17.53
4	5.24	3.59	4.31	5.45
8	0.80	0.60	0.65	0.38
12	0.06	0.06	0.04	0.02
24	0.00	0.00	0.00	0.00

Table B-4 Plasma ampicillin concentrations (μg/ml) following an IV administration of ampicillin sodium at a 10 mg/kg dosage rate and an intragastric administration of probenecid at a 75 mg/kg dosage rate.

	Animal number			
Variable	1	2	5	7
α (/h)	2.085	4.400	8.810	31.50
β (/h)	0.974	0.935	1.065	0.977
t1/2 α (h)	0.332	0.157	0.079	0.022
t1/2 β (h)	0.711	0.741	0.651	0.710
Cp0 (μg/ml)	53.16	59.87	76.28	93.5
AUC (μg.h/ml)	36.98	28.76	35.12	45.35
Vc (ml/kg)	188.10	167.03	131.10	107.00
Vd_{area} (ml/kg)	277.60	372.00	267.70	225.80
Vdss (ml/kg)	215.90	275.10	233.70	218.00
Clb (ml/h.kg)	270.50	347.70	284.80	220.50
k_{21} (/h)	1.413	1.977	4.320	143925
k_{el} (/h)	1.438	2.082	2.170	2.061
k_{12} (/h)	0.209	1.279	3.380	15.49

Table B-5. Pharmacokinetic variables after compartmental analysis of ampicillin plasma concentrations following an IV bolus of ampicillin sodium at a 10 mg/kg dose rate.

	Animal number			
Variable	1	2	5	7
α (/h)	12.650	4.460	4.160	26.210
β (/h)	0.554	0.526	0.585	0.649
t1/2 α (h)	0.055	0.155	0.166	0.026
t1/2 β (h)	1.251	1.318	1.185	1.067
Cp0 (μg/ml)	112.24	79.38	90.95	125.98
AUC (μg.h/ml)	100.07	77.29	95.84	96.11
Vc (ml/kg)	89.10	126.00	109.90	79.40
Vd_{area} (ml/kg)	180.40	246.00	178.40	160.30
Vdss (ml/kg)	172.30	218.40	162.80	156.30
Clb (ml/h.kg)	99.90	129.30	104.30	104.10
k_{21} (/h)	6.248	2.285	2.566	12.981
k_{el} (/h)	1.121	1.027	0.949	1.312
k_{12} (/h)	5.833	1.676	1.233	12.575

Table B-6. Pharmacokinetic variables after compartmental analysis of ampicillin plasma concentrations following an IV bolus of ampicillin sodium at a 10 mg/kg dose rate and intragastric administration of probenecid at a 75 mg/kg dose rate.

	Animal number			
Variable	1	2	5	7
AUC_{obs} (μg.h/ml)	35.93	29.36	35.55	46.70
$AUMC_{obs}$(μg.h^2/ml)	27.08	21.95	27.64	44.03
MRT (min)	45.22	44.85	46.65	56.57
CLb_{obs}(ml/h.kg)	278.32	340.60	281.29	214.12
$Vdss_{obs}$ (ml/kg)	209.76	254.43	218.56	201.91

Table B-7. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations following an IV bolus of ampicillin sodium at a 10mg/kg dose rate.

	Animal number			
Variable	1	2	5	7
AUC_{obs} (μg.h/ml)	102.17	79.69	99.48	104.65
$AUMC_{obs}$(μg.h^2/ml)	169.61	127.10	151.94	164.92
MRT (min)	99.60	95.69	91.64	94.56
CLb_{obs}(ml/h.kg)	97.88	125.49	100.52	95.56
$Vdss_{obs}$ (ml/kg)	162.48	199.53	153.80	150.98

Table B-8. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations following an IV bolus of ampicillin sodium at a 10mg/kg dose rate and intragastric administration of probenecid at a 75 mg/kg dose rate.

Appendix C

Intravenous infusions of ampicillin in horses.

	Infusion period		
	Animal number		
Time (h)	1	3	4
0.033	15.72	17.76	16.76
0.083	13.00	15.26	15.16
0.166	9.68	13.02	13.66
0.25	8.54	11.60	10.75
0.50	7.30	8.62	10.39
0.75	6.30	7.70	8.38
1.00	5.83	7.23	7.98
1.25	5.47	6.91	7.49
1.50	5.17	6.62	6.73
1.75	5.80	6.71	7.03
2.00	5.41	6.42	6.04
2.25	5.37	6.49	6.01
2.50	5.55	6.26	6.19
2.75	5.01	6.15	6.16
3.00	5.27	6.00	5.79
3.25	5.40	6.48	6.05
3.50	5.02	6.07	5.39
3.75	5.26	6.15	5.58
4.00	4.87	6.19	5.61
Infusion rate (μg/min/kg)	19.07	19.34	19.15

Table C-1 Ampicillin plasma concentrations during IV constant rate infusion over a 4 hour period.

	Post-infusion period		
	Animal number		
Time (h)	1	3	4
0.033	5.67	5.73	5.09
0.083	5.13	5.19	4.94
0.166	3.82	4.59	4.28
0.25	3.28	3.97	3.82
0.50	2.09	2.89	2.90
1.00	0.93	1.54	1.47
2.00	0.28	0.50	0.54
4.00	0.05	0.08	0.11
8.00	0.00	0.00	0.00
12.00	0.00	0.00	0.00
24.00	0.00	0.00	0.00

Table C-1 (cont). Ampicillin plasma concentrations following disconnection of the peristaltic pump after a 4 hour infusion period.

		Animal number		
Variable		1	3	4
β	(/h)	1.065	1.006	0.812
$t1/2\beta$	(h)	0.65	0.69	0.85
AUC_{obs}	(μg.h/ml)	3.43	4.78	4.80
$AUMC_{obs}$	($\mu g.h^2$/ml)	2.55	4.21	4.73
MRT	(h)	0.74	0.88	0.99

Table C-2. Pharmacokinetic variables obtained after analysis of the plasma ampicillin concentrations recorded in animals 1, 3, and 4 following disconnection of the peristaltic pump.

Variable		Animal number		
		1	3	4
Cpmax	(μg/ml)	5.80	6.91	7.49
Cpmin	(μg/ml)	4.87	6.00	5.39
$Cpss_{(actual)}$	(μg/ml)	5.30±0.07	6.37±0.08	6.17±0.18
Ro	(μg/min/kg)	19.07	19.34	19.15
$Vdss_{(actual)}$	(ml/kg)	202.68	181.09	229.20
$Clb_{(actual)}$	(ml/h.kg)	215.85	182.17	186.22
$AUC_{obs\text{-}total}$	(μg.h/ml)	27.40	33.89	34.03

Table C-3 Pharmacokinetic variables obtained after analysis of the plasma ampicillin concentrations recorded in animals 1, 3, and 4 during ampicillin IV constant rate infusion.
$AUC_{obs\text{-}total}$ corresponds to the AUC during the infusion and post-infusion periods.

Appendix D

Studies on bacampicillin hydrochloride in ponies and horses.

	Animal number						
Time (h)	1	2	5	8	9	10	11
0.033	48.94	53.13	54.32	65.52	53.17	63.58	66.32
0.083	39.31	43.09	40.98	35.70	39.35	48.60	52.79
0.166	28.49	33.96	27.80	30.92	30.27	30.18	37.41
0.25	28.07	28.54	22.42	28.14	20.54	23.75	28.94
0.5	19.74	19.97	15.60	14.97	10.70	14.78	17.09
0.75	13.64	11.02	9.92	10.00	5.18	8.39	10.47
1	8.97	8.72	6.30	6.61	3.43	5.55	7.30
1.5	6.32	6.14	3.06	3.07	1.37	3.14	3.90
2	3.43	4.57	1.76	1.65	0.68	1.81	2.33
4	1.00	1.51	0.20	0.17	0.04	0.25	0.22
8	0.05	0.24	0.00	0.00	0.00	0.00	0.11
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D-1 Plasma ampicillin concentrations after IV administration of ampicillin sodium at a 10 mg/kg dosage rate.

	Animal number						
Variable	1	2	5	8	9	10	11
α (/h)	8.730	3.550	10.694	28.800	6.263	7.570	5.800
β (/h)	0.798	0.496	1.280	1.350	1.546	1.120	0.720
t1/2 α (h)	0.079	0.195	0.065	0.024	0.111	0.092	0.119
t1/2 β (h)	0.869	1.397	0.541	0.512	0.448	0.621	0.963
Cp0 (μg/ml)	57.74	56.84	67.56	125.75	61.42	75.77	77.49
AUC(μg.h/ml)	35.33	37.19	24.98	25.67	18.13	24.77	32.35
Vc (ml/kg)	173.20	175.90	148.00	79.50	162.80	132.0	129.1
Vd_{area}(ml/kg)	354.70	541.90	312.70	287.00	356.80	361.60	429.40
Vdss(ml/kg)	320.80	384.30	271.10	252.40	251.90	268.70	305.40
Clb (ml/h.kg)	283.00	268.90	400.40	389.60	551.50	403.60	309.10
k_{21} (/h)	4.26	1.152	5.061	7.958	2.858	2.762	1.743
k_{el} (/h)	1.630	1.528	2.705	4.899	3.387	3.058	2.395
k_{12} (/h)	3.63	1.365	4.208	17.298	1.563	2.862	2.381

Table D-2. .Pharmacokinetic values after compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10 mg/kg dose rate.

	Animal number						
Variable	1	2	5	8	9	10	11
AUC_{obs} (μg.h/ml)	34.99	38.91	25.00	27.09	18.66	25.80	30.99
$AUMC_{obs}$ (μg.h^2/ml)	36.94	52.64	16.04	15.82	8.17	16.24	23.04
MRT (min)	63.33	81.17	38.49	35.04	26.27	37.76	44.61
CLb_{obs} (ml/h.kg)	285.77	256.98	399.96	369.18	535.77	387.54	322.69
$Vdss_{obs}$ (ml/kg)	301.63	347.64	256.55	215.63	234.56	243.92	239.93

Table D-3. Pharmacokinetic values after non-compartmental analysis of ampicillin plasma concentrations after an IV bolus of ampicillin sodium at a 10 mg/kg dose rate.

	Animal number						
Time (h)	1	2	5	8	9	10	11
0.25	5.21	3.04	3.62	5.28	2.41	3.22	3.84
0.30	7.55	6.67	6.46	5.24	3.59	5.37	5.64
0.75	7.65	7.02	7.37	5.27	3.21	4.94	6.29
1	6.38	6.72	4.62	5.04	2.46	4.98	4.28
1.5	3.55	7.04	2.90	2.53	1.43	2.05	2.56
2	3.15	6.00	2.01	1.14	0.73	1.76	1.55
4	1.57	1.74	1.36	0.18	0.08	0.28	0.47
6	0.56	0.47	0.08	0.00	0.00	0.00	0.09
8	0.12	0.11	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D-4. Plasma ampicillin concentrations after intragastric administration of bacampicillin hydrochloride at a 13.52 mg/kg dose rate.

	Animal number						
Variable	1	2	5	8	9	10	11
Cmax (μg/ml)	7.65	7.04	7.37	5.28	3.59	5.37	6.29
tmax (min)	45	90	45	15	30	30	45
AUC_{obs} (μg.h/ml)	17.82	22.42	12.96	8.87	5.00	9.07	9.91
$AUMC_{obs}$(μg.h^2/ml)	38.22	47.17	23.49	10.15	5.68	12.31	14.68
MRT (min)	128.66	126.22	108.72	68.64	68.17	81.49	88.85
F(%)	50.92	57.62	51.84	32.74	26.79	35.15	31.98
MAT (min)	65.33	45.05	70.23	33.60	41.90	43.73	44.24

Table D-5. Pharmacokinetic values after non-compartmental analysis of ampicillin plasma concentrations following an intragastric dose of bacampicillin hydrochloride at a 13.52 mg/kg dose rate.

	Animal number						
Day	1	2	5	8	9	10	11
Pre (-3)	2064.4	3267.4	4663.5	6089.3	5450.7	3579.3	5435.9
Pre (-2)	2153.5	2970.4	5287.3	5851.7	5331.9	3727.8	5762.6
Pre (-1)	2227.8	3074.4	5584.3	5361.6	5539.8	3817.0	5911.1
Pre (0)	1797.1	2717.9	4544.7	5450.7	5391.3	2777.3	4752.6
1	118.8	163.4	163.4	193.1	148.5	133.8	207.9
2	207.9	222.8	311.9	237.6	163.4	341.6	311.9
3	326.7	356.4	549.5	638.6	326.7	356.4	623.8
4	430.7	371.3	772.3	802.0	505.0	534.7	876.3
7	935.7	965.4	1559.5	2109.0	1099.0	920.8	1158.5
10	1158.5	1203.0	1930.8	3237.7	1856.5	2005.0	1455.5
20	1262.4	1886.2	2836.7	4752.6	2420.9	1826.8	2717.9
30	1722.8	1826.0	3312.0	3727.8	3029.8	2287.2	3029.8
45	1930.8	2153.5	3282.3	4143.7	3163.5	2613.9	4901.2
60	1782.2	3185.7	3445.7	4589.3	3623.9	2747.6	4619.0
90	2272.4	3044.7	4121.4	4856.6	4173.4	3104.1	4916.0

Table D-6. Plasma cholinesterase values (U/L) before and after administration of an oral dose of dichlorvos at a 40 mg/kg dose rate.
Dichlorvos was administered immediately after blood sample collection on day 0.

	Animal number						
Day	1	2	5	8	9	10	11
Pre (-3)	3525.2	5001.5	9608.4	8686.5	12128.4	7460.6	10001.2
Pre (-2)	4037.9	4619.9	9061.4	8109.2	11927.2	7233.5	11827.8
Pre (-1)	3074.3	4625.2	4404.5	8083.4	12332.1	7920.9	13000.5
Pre (0)	2920.6	3926.3	7501.9	8111.5	12696.3	6505.2	11859.6
1	70.9	126.2	92.7	78.8	34.2	99.1	209.8
2	207.9	148.5	107.0	381.2	101.8	239.1	302.7
3	465.4	237.6	648.2	565.6	530.1	578.7	1108.9
4	644.8	390.3	1481.1	1673.3	925.2	950.5	1633.2
7	1589.2	954.5	2756.9	3619.6	2940.7	1939.6	2119.2
10	2257.5	1493.8	3334.9	5425.9	5094.2	4540.9	3385.1
20	2235.0	3154.5	4259.2	8020.1	6318.9	4007.9	5378.8
30	4218.0	2347.8	6701.1	4886.3	7880.7	4420.1	6326.9
45	3856.4	3481.5	4826.1	7344.7	8539.9	4459.9	9208.2
60	4777.4	8338.3	6950.7	6784.2	9114.5	4224.6	8092.6
90	6453.2	5773.7	9271.9	7142.2	11510.3	5282.9	9936.0

Table D-7. Erythrocyte cholinesterase values (U/L) before and after administration of an oral dose of dichlorvos at a 40 mg/kg dose rate.
Dichlorvos was administered immediately after blood sample collection on day 0.

	Animal number						
Time (h)	1	2	5	8	9	10	11
0.25	8.20	0.00	0.75	4.60	6.49	2.61	3.88
0.30	9.28	2.08	4.01	7.02	6.34	5.23	6.33
0.75	8.23	4.49	5.99	7.31	4.48	5.72	5.86
1	8.38	6.42	6.08	8.06	3.46	5.96	5.83
1.5	5.64	6.09	4.39	3.78	2.14	5.12	3.74
2	3.86	4.47	3.34	2.18	1.83	2.70	2.67
4	0.57	2.25	0.33	0.13	0.31	0.33	0.67
6	0.07	0.50	0.00	0.00	0.07	0.03	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table D-8. Plasma ampicillin concentrations after intragastric administration of bacampicillin hydrochloride at a 13.52 mg/kg dose rate following an oral administration of dichlorvos at a 40 mg/kg dose rate.

	Animal number						
Variable	1	2	5	8	9	10	11
Cmax (μg/ml)	9.28	6.42	6.08	8.06	6.49	5.96	6.33
tmax (min)	30	60	60	60	15	60	30
AUC_{obs} (μg.h/ml)	18.50	18.62	11.95	12.59	9.76	12.28	13.02
$AUMC_{obs}$(μg.h^2/ml)	22.75	47.62	18.30	14.81	13.05	17.72	21.58
MRT (min)	83.51	153.44	91.85	70.56	80.22	86.59	99.42
F(%)	52.87	47.85	47.80	46.47	52.30	47.60	42.01

Table D-9. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations following an intragastric dose of bacampicillin hydrochloride at a mg/kg dose rate and an oral dose of dichlorvos at a 40 mg/kg dosage rate

	Animal number					
Time (h)	1	2	8	9	10	11
0.25	0.77	0.61	0.15	0.06	0.332	0.44
0.30	1.71	1.45	1.22	0.65	1.20	1.25
0.75	3.46	1.78	2.30	1.79	1.88	1.80
1	3.17	1.59	3.20	2.01	2.36	2.20
1.5	3.16	1.47	3.70	1.57	2.54	1.87
2	2.52	1.32	3.30	1.68	2.24	1.56
4	2.41	0.65	0.92	0.46	1.11	0.41
6	1.81	0.60	0.23	0.29	0.34	0.19
8	1.03	0.48	0.00	0.09	0.08	0.13
12	0.71	0.27	0.00	0.00	0.00	0.05
24	0.00	0.00	0.00	0.00	0.00	0.00

Table D-10. Plasma ampicillin concentrations after intragastric administration of bacampicillin hydrochloride at a 13.52 mg/kg dose rate and an probenecid at a 75 mg/kg dose rate.

	Animal number					
Variable	1	2	8	9	10	11
Cmax (μg/ml)	3.46	1.78	3.7	2.01	2.54	2.20
tmax (min)	45	45	90	60	90	60
AUC_{obs} (μg.h/ml)	25.42	10.50	10.51	6.06	8.91	6.47
$AUMC_{obs}$(μg.h^2/ml)	190.61	76.85	24.18	16.53	23.67	19.73
MRT (min)	449.82	439.23	138.01	163.74	159.43	182.88
F(%)	72.65	26.98	38.80	32.46	34.53	20.88

Table D-11. Pharmacokinetic variables after non-compartmental analysis of ampicillin plasma concentrations following an intragastric dose of bacampicillin hydrochloride at a 13.52 mg/kg dose rate and probenecid at a 75 mg/kg dosage rate.

	Animal number					
Time (h)	1	2	8	9	10	11
0.25	60.98	45.58	81.41	28.97	65.51	44.16
0.30	110.54	69.35	178.38	66.61	111.69	66.23
0.75	118.38	76.96	234.71	87.98	122.28	73.94
1	139.65	78.47	242.10	79.36	144.65	86.26
1.5	149.62	87.68	224.22	106.59	167.02	109.95
2	132.27	104.03	208.43	111.84	175.97	110.71
4	129.30	105.02	101.40	77.54	108.86	75.83
6	111.67	122.86	67.15	64.66	111.35	74.82
8	101.61	131.80	60.01	43.11	58.79	79.66
12	63.35	123.63	53.16	21.55	33.80	78.66
24	24.52	67.43	47.36	13.23	8.69	9.81

Table D-12. Plasma probenecid concentrations after intragastric administration of bacampicillin hydrochloride at a 13.52 mg/kg dose rate and probenecid at a 75 mg/kg dose rate.

	Animal number					
Variable	1	2	8	9	10	11
Cmax (μg/ml)	149.62	131.80	242.10	111.84	175.97	110.71
tmax (min)	90.00	480.00	60.00	120.00	120.00	120.00
AUC_{obs} (μg.h/ml)	2378.21	2982.69	8601.69	1573.87	1431.74	1914.58
$AUMC_{obs}$ ($\mu g.h^2/ml$)	26166.7	46194.9	355463.7	22435.4	10796.3	16431.9
MRT (min)	660.0	929.4	2479.2	855.0	452.4	514.8

Table D-13. Pharmacokinetic variables after non-compartmental analysis of probenecid plasma concentrations following an intragastric dose of bacampicillin hydrochloride at a 13.52 mg/kg dose rate and probenecid at a 75 mg/kg dosage rate

Appendix E

Sentivities of equine bacterial isolates to different antibiotics.

Bacterial isolate	Origin	Disease/Symptoms
A. equuli 1	Wound	Abdominal wound
A. equuli 2	LRT	COPD
A. equuli 3	Faeces	Grass Sickness
A. equuli 4	Ileum	Colic
A. equuli 5	Faeces	Chronic Weight Loss
A. equuli 6	Joint	Arthritis
A. equuli 7	Faeces	Chronic Weight Loss
A. equuli 8	Faeces	Chronic Weight Loss
A. equuli 9	Faeces	Chronic Weight Loss
A. equuli 10	Femoral head	Necrosis
A. equuli 11	Faeces	Chronic Weight Loss
A. equuli 12	Faeces	Chronic Diarrhoea
E. coli 1	LRT	COPD
E. coli 2	Wound	Postoperative infection
E. coli 3	Urine	Renal failure
E. coli 4	Wound	Navicular bursa puncture
E. coli 5	Cervix	Chronic endometritis
E. coli 6	Cervix	Chronic endometritis
E. coli 7	Cervix	Chronic endometritis
E. coli 8	Faeces	Diarrhoea
E. coli 9	LRT	Pneumonia
E. coli 10	Cervix	Necrotic vaginitis
E. coli 11	Bone	Osteomyelitis
E. coli 12	Wound	Delayed closure(canon)
E. coli 13	Uterus	Infertility
E. coli 14	Faeces	Colic
E. coli 15	Faeces	Chronic Diarrhoea

Table E-1. Bacterial isolates, their origin, and related disease.
URT: Upper respiratory tract. LRT: Lower respiratory tract.
COPD: Chronic obstructive pulmonary disease.

Bacterial isolate	Origin	Disease/Symptoms
E. coli 16	Wound	Postopeartive infection
E. coli 17	Ileum	Colic
E. coli 18	Faeces	Diarrhoea
E. coli 19	Wound	Elbow wound infection
E. coli 20	Faeces	ChronicWeight Loss
E. coli 21	Femoral head	Necrosis
E. coli 22	Wound	Postoperative wound
E. coli 23	Endometrium	Vaginal Discharge
Ps. aeruginosa 1	URT	Guttural pouch empyema
Ps. aeruginosa 2	Caecum	Recurrent colic
Ps. aeruginosa 3	Faeces	Chonic Weight Loss
Ps. aeruginosa 4	Faeces	Chronic Weight Loss
Ps. fluorescens 1	URT	Influenza
Ps. fluorescens 2	URT	Influenza
Ps. fluorescens 3	URT	Influenza
Ps. fluorescens 4	Wound	Osteomyelitis
Ps. fluorescens 5	Wound	Granulating wound
Ps. fluorescens 6	URT	Sinusitis
Ps. fluorescens 7	URT	Sinusitis
Ps. maltophilia	URT	Influenza
Ps. putrefasciens	Faeces	Colitis
S. typhimurium 1	Abscess	Thoracic abscess
S. typhimurium 2	Faeces	Salmonellosis
S. typhimurium 3	Faeces	Salmonellosis
S. typhimurium 4	Faeces	Salmonellosis
S. typhimurium 5	Faeces	Salmonellosis
S. typhimurium 6	Spleen	Salmonellosis
S. typhimurium 7	Faeces	Salmonellosis
S. typhimurium 8	Faeces	Chronic Diarrhoea
S. typhimurium 9	Faeces	Diarrhoea
S. typhimurium 10	Faeces	Diarrhoea
S. typhimurium 11	Faeces	Salmonellosis

Table E-1 (cont.). Bacterial isolates, their origin, and related disease.
URT: Upper respiratory tract. LRT: Lower respiratory tract.
COPD: Chronic obstructive pulmonary disease.

Bacterial isolate	Origin	Disease/Symptoms
S. aureus 1	Abscess	Thoracic abscess
S. aureus 2	Wound	Postoperative infection
S. aureus 3	Joint	Purulent hygroma
S. aureus 4	Bone	Osteomyelitis
S. aureus 5	URT	Sinusitis
S. aureus 6	Skin	Scabs (Hepatopathy)
S. aureus 7	URT	Influenza
S. aureus 8	URT	Influenza
S. aureus 9	URT	Influenza
S. aureus 10	URT	Influenza
S. aureus 11	URT	Influenza
S. aureus 12	URT	Influenza
S. aureus 13	URT	Nasal discharge
S. aureus 14	Abscess	Fetlock wound
S. aureus 15	Skin	Mud fever (fetlock)
S. aureus 16	Faeces	Chronic Weight Loss
S. zooepidemicus 1	LRT	COPD
S. zooepidemicus 2	Wound	Shoulder fistula
S. zooepidemicus 3	LRT	Pneumonia
S. zooepidemicus 4	URT	Purulent nasal discharge
S. zooepidemicus 5	Cervix	Chronic endometritis
S. zooepidemicus 6	LRT	Pneumonia
S. zooepidemicus 7	Skin	Scabs (Hepatopathy)
S. zooepidemicus 8	Wound	Frontal carpus wound
S. zooepidemicus 9	Cervix	Chronic endometritis
S. zooepidemicus 10	URT	Influenza
S. zooepidemicus 11	URT	Influenza
S. zooepidemicus 12	URT	Influenza
S. zooepidemicus 13	URT	Influenza
S. zooepidemicus 14	Conjunctiva	Conjunctivitis
S.zooepidemicus 15	Wound	Right hock wound
S. zooepidemicus 16	Wound	Hock wound

Table E-1 (cont.). Bacterial isolates, their origin, and related disease. URT: Upper respiratory tract. LRT: Lower respiratory tract. COPD: Chronic obstructive pulmonary disease.

Bacterial isolate	Origin	Disease/Symptoms
S. zooepidemicus 17	URT	Purulent nasal discharge
S. zooepidemicus 18	Wound	Inner thigh wound
S. zooepidemicus 19	Wound	Elbow wound
S. zooepidemicus 20	LRT	Respiratory noise

Table E-1 (cont.). Bacterial isolates, their origin, and related disease. URT: Upper respiratory tract. LRT: Lower respiratory tract. COPD: Chronic obstructive pulmonary disease.

	AMP	GEN	OXT	ER	PEN	LIN	STR	CHL	T/S
A. equuli 1	S	S	S	S	S	R	S	S	S
A. equuli 2	S	S	S	S	S	R	S	S	S
A. equuli 3	S	S	S	S	S	R	S	S	S
A. equuli 4	S	S	S	S	S	R	S	S	S
A. equuli 5	S	R	S	S	S	R	R	S	R
A. equuli 6	S	R	S	S	S	R	R	S	S
A. equuli 7	S	R	S	S	S	R	R	S	S
A. equuli 8	S	R	S	R	S	R	R	S	S
A. equuli 9	S	S	S	S	S	R	R	S	S
A. equuli 10	S	R	S	S	S	R	R	S	S
A. equuli 11	R	R	S	S	S	R	R	S	S
A. equuli 12	R	S	S	R	R	R	S	S	S
E. coli 1	S	S	S	R	R	R	S	S	S
E. coli 2	R	S	R	R	R	R	R	R	R
E. coli 3	S	S	R	R	R	R	S	S	S
E. coli 4	R	S	R	R	R	R	R	R	R
E. coli 5	S	S	S	R	R	R	S	S	S
E. coli 6	R	S	S	R	R	R	R	R	R
E. coli 7	S	S	S	R	R	R	S	S	S
E. coli 8	R	S	S	R	R	R	R	S	R
E. coli 9	R	S	R	R	R	R	R	S	R
E. coli 10	S	S	S	R	R	R	S	S	S
E. coli 11	R	S	R	R	R	R	R	S	R
E. coli 12	R	S	R	R	R	R	R	S	R
E. coli 13	S	S	S	R	R	R	S	S	S
E. coli 14	R	S	R	R	R	R	R	S	S
E. coli 15	S	S	S	R	R	R	S	S	S
E. coli 16	R	S	R	R	R	R	R	S	R
E. coli 17	S	S	S	R	R	R	R	S	S
E. coli 18	S	S	S	R	R	R	R	S	S
E. coli 19	S	S	S	R	R	R	S	S	S
E. coli 20	S	S	S	R	R	R	R	S	S
E. coli 21	S	S	S	R	R	R	S	S	S
E. coli 22	R	S	R	R	R	R	S	S	R
E. coli 23	R	S	R	R	R	R	R	S	S

Table E-2. Disc-diffusion sensitivity results.
S : SENSITIVE; R : RESISTANT

	AMP	GEN	OXT	ER	PEN	LIN	STR	CHL	T/S
Ps. aeruginosa 1	R	S	S	R	R	R	R	S	R
Ps. aeruginosa 2	R	S	S	R	R	R	S	S	R
Ps. aeruginosa 3	R	S	R	R	R	R	R	R	R
Ps. aeruginosa 4	R	S	R	R	R	R	R	R	R
Ps. fluorescens 1	R	S	R	R	R	R	R	R	R
Ps. fluorescens 2	R	S	R	R	R	R	R	R	R
Ps. fluorescens 3	R	S	R	R	R	R	R	R	R
Ps. fluorescens 4	R	S	R	R	R	R	S	R	R
Ps. fluorescens 5	R	S	R	R	R	R	S	R	R
Ps. fluorescens 6	R	S	R	R	R	R	S	R	R
Ps. fluorescens 7	R	S	R	R	R	R	S	R	R
Ps. maltophilia	R	S	R	R	R	R	S	S	S
Ps. putrefaciens	R	S	R	R	R	R	S	S	R
S. typhimurium 1	S	S	R	R	R	R	R	S	S
S. typhimurium 2	R	S	R	R	R	R	R	R	R
S. typhimurium 3	R	S	R	R	R	R	R	R	R
S. typhimurium 4	R	S	R	R	R	R	R	R	R
S. typhimurium 5	R	S	R	R	R	R	R	R	R
S. typhimurium 6	R	S	R	R	R	R	R	R	R
S. typhimurium 7	R	S	R	R	R	R	R	R	R
S. typhimurium 8	R	S	R	R	R	R	R	R	S
S. typhimurium 9	R	S	R	R	R	R	R	R	R
S. typhimurium 10	R	S	R	R	R	R	R	R	S
S. typhimurium 11	S	S	R	R	R	R	S	S	S
S. aureus 1	R	S	S	S	R	S	S	S	S
S. aureus 2	S	S	S	S	R	S	S	S	S
S. aureus 3	S	S	S	S	S	S	S	S	S
S. aureus 4	R	S	S	S	R	S	S	S	S
S. aureus 5	S	S	R	S	S	S	S	S	S
S. aureus 6	S	S	S	S	S	S	S	S	S
S. aureus 7	S	S	S	S	S	S	S	S	S
S. aureus 8	S	S	S	S	S	S	S	S	S
S. aureus 9	R	S	S	S	R	S	S	S	S
S. aureus 10	S	R	S	S	S	S	S	S	S
S. aureus 11	S	S	S	S	S	S	S	S	S
S. aureus 12	R	S	S	S	R	S	S	S	S
S. aureus 13	S	S	S	S	S	S	S	S	S

Table E-2. Disc-diffusion sensitivity results.
S : SENSITIVE; R : RESISTANT

	AMP	GEN	OXT	ER	PEN	LIN	STR	CHL	T/S
S. aureus 14	S	S	S	S	S	S	S	S	S
S. aureus 15	S	S	S	S	S	S	S	S	S
S. aureus 16	R	S	R	S	R	S	S	S	S
S. zooepidemicus 1	S	S	S	R	S	S	R	S	R
S. zooepidemicus 2	S	R	S	S	S	S	R	S	S
S. zooepidemicus 3	S	R	S	S	S	S	R	S	S
S. zooepidemicus 4	S	R	S	S	S	S	R	S	S
S. zooepidemicus 5	S	R	S	S	S	S	R	S	S
S. zooepidemicus 6	S	R	S	S	S	S	R	S	S
S. zooepidemicus 7	S	S	S	S	S	S	R	S	S
S. zooepidemicus 8	S	R	S	S	S	S	R	S	S
S. zooepidemicus 9	S	R	S	S	S	S	R	S	S
S. zooepidemicus 10	S	R	S	S	S	S	R	S	S
S. zooepidemicus 11	S	R	S	S	S	S	R	S	S
S. zooepidemicus 12	S	R	S	S	S	S	R	S	S
S. zooepidemicus 13	S	R	S	S	S	S	R	S	S
S. zooepidemicus 14	S	R	S	S	S	S	R	S	S
S. zooepidemicus 15	S	R	S	S	S	S	R	S	S
S. zooepidemicus 16	S	R	S	S	S	S	R	S	S
S. zooepidemicus 18	S	R	S	S	S	S	R	S	S
S. zooepidemicus 19	S	R	S	S	S	S	R	S	S
S. zooepidemicus 20	S	R	S	S	S	S	R	S	S

Table E-2 (cont.). Disc-diffusion sensitivity results.
S : SENSITIVE; R : RESISTANT

AMP: Ampicillin; GEN: Gentamicin; OXT: Oxytetracycline; ER: Erythromycin; PEN: Penicillin G; LIN: Lincomycin; STR: Streptomycin; CHL: Chloramphenicol; T/S: Trimethoprim /Sulphamethoxazole.

Ampicillin	Range (µg/ml)							
	n	≤1	2	4	8	16	32	64	128
A. equuli	12	100	-	-	-	-	-	-	-
E. coli	23	0	21.7	52.2	52.2	52.2	52.2	52.2	56.5
Pseudomonas spp	13	0	0	0	0	0	7.69	7.69	7.69
S. typhimurium	11	0	18.2	18.2	18.2	18.2	18.2	18.2	18.2
S. aureus	16	68.7	87.5	93.7	100	-	-	-	-
S. zooepidemicus	20	100	-	-	-	-	-	-	-

Amoxycillin / Clavulanic Acid	Range (µg/ml)				
	n	≤8/4	16/8	32/16	64/32
A. equuli	10	100	-	-	-
E. coli	17	82.3	94.1	94.1	100
Pseudomonas spp	11	0	9.1	18.2	18.2
S. typhimurium	10	10	30	100	-
S. aureus	12	100	-	-	-
S. zooepidemicus	14	100	-	-	-

Carbenicillin	Range (µg/ml)				
	n	≤16	32	64	128
A. equuli	10	100	-	-	-
E. coli	17	58.8	58.8	58.8	58.8
Pseudomonas spp	11	18.2	63.6	90.9	100
S. typhimurium	10	10	10	10	10
S. aureus	12	100	-	-	-
S. zooepidemicus	14	100	-	-	-

Mezlocillin	Range (µg/ml)				
	n	≤16	32	64	128
A. equuli	12	100	-	-	-
E. coli	23	65.2	73.8	78.1	86.7
Pseudomonas spp	13	38.46	76.9	84.6	92.3
S. typhimurium	11	27.3	27.3	27.3	27.3
S. aureus	16	100	-	-	-
S. zooepidemicus	20	100	-	-	-

Table E-3 . Cumulative percentages of bacterial sensitivities.

Cefoxitin	Range (µg/ml)								
	n	≤1	2	4	8	16	32	64	128
A. equuli	12	91.7	100	-	-	-	-	-	-
E. coli	23	4.3	43.4	86.9	95.6	100	-	-	-
Pseudomonas spp	13	0	0	0	0	0	0	7.7	7.7
S. typhimurium	11	0	18.2	45.4	90.8	100	-	-	-
S. aureus	16	25	68.7	100	-	-	-	-	-
S. zooepidemicus	20	100	-	-	-	-	-	-	-

Ceftizoxime	Range (µg/ml)				
	n	≤16	32	64	128
A. equuli	10	100	-	-	-
E. coli	17	100	-	-	-
Pseudomonas spp	11	9.1	63.6	81.8	100
S. typhimurium	10	100	-	-	-
S. aureus	12	100	-	-	-
S. zooepidemicus	14	100	-	-	-

Ceftriaxone	Range (µg/ml)				
	n	≤8	16	32	64
A. equuli	10	100	-	-	-
E. coli	17	94.1	94.1	94.1	94.1
Pseudomonas spp	11	45.4	72.7	81.8	90.9
S. typhimurium	10	100	-	-	-
S. aureus	12	100	-	-	-
S. zooepidemicus	14	100	-	-	-

Cephalothin	Range (µg/ml)								
	n	≤1	2	4	8	16	32	64	128
A. equuli	12	91.7	100	-	-	-	-	-	-
E. coli	23	0	0	4.3	52.1	82.5	86.8	91.1	95.4
Pseudomonas spp	13	0	0	0	0	0	0	0	0
S. typhimurium	11	0	9.1	36.4	45.5	90.9	100	-	-
S. aureus	16	100	-	-	-	-	-	-	-
S. zooepidemicus	20	100	-	-	-	-	-	-	-

Table E-3 (cont). Cumulative percentages of bacterial sensitivities.

Amikacin	Range (µg/ml)							
	n	≤1	2	4	8	16	32	64
A. equuli	12	0	16.7	83.3	100	-	-	-
E. coli	23	21.7	95.6	100	-	-	-	-
Pseudomonas spp	13	7.7	38.5	92.4	92.4	92.4	100	-
S. typhimurium	11	72.7	100	-	-	-	-	-
S. aureus	16	49.9	81.1	100	-	-	-	-
S. zooepidemicus	20	55	65	100	-	-	-	-

Gentamicin	Range (µg/ml)							
	n	≤0.5	1	2	4	8	16	32
A. equuli	12	41.7	83.3	100	-	-	-	-
E. coli	23	82.6	95.7	100	-	-	-	-
Pseudomonas spp	13	0	0	53.8	84.6	92.3	100	-
S. typhimurium	11	100	-	-	-	-	-	-
S. aureus	16	93.7	100	-	-	-	-	-
S. zooepidemicus	20	75	95	100	-	-	-	-

Tobramycin	Range (µg/ml)							
	n	≤0.5	1	2	4	8	16	32
A. equuli	12	8.3	91.7	100	-	-	-	-
E. coli	23	30.4	91.3	100	-	-	-	-
Pseudomonas spp	13	53.8	84.6	92.3	92.3	92.3	100	-
S. typhimurium	11	18.2	100	-	-	-	-	-
S. aureus	16	93.7	100	-	-	-	-	-
S. zooepidemicus	20	70	90	100	-	-	-	-

Tetracycline	Range (µg/ml)				
	n	≤2	4	8	16
A. equuli	12	100	-	-	-
E. coli	23	52.2	56.5	56.5	60.8
Pseudomonas spp	13	0	0	0	7.7
S. typhimurium	11	0	9.1	9.1	54.5
S. aureus	16	87.5	87.5	87.5	87.5
S. zooepidemicus	20	95	95	100	-

Table E-3 (cont). Cumulative percentages of bacterial sensitivities.

Trimethoprim/ Sulfamethoxazole	Range (µg/ml)				
	n	≤2/38	4/76	8/152	16/304
A. equuli	12	100	-	-	-
E. coli	23	65.2	65.2	65.2	65.2
Pseudomonas spp	13	15.4	38.5	69.3	77.0
S. typhimurium	11	36.4	36.4	36.4	36.4
S. aureus	16	100	-	-	-
S. zooepidemicus	20	100	-	-	-

Norfloxacin	Range (µg/ml)								
	n	≤1	2	4	8	16	32	64	128
A. equuli	12	100	-	-	-	-	-	-	-
E. coli	23	95.6	100	-	-	-	-	-	-
Pseudomonas spp	13	61.5	69.2	84.6	100	-	-	-	-
S. typhimurium	11	100	-	-	-	-	-	-	-
S. aureus	16	100	-	-	-	-	-	-	-
S. zooepidemicus	20	0	0	50	95	100	-	-	-

Ciprofloxacin	Range (µg/ml)								
	n	≤0.06	0.12	0.25	0.5	1	2	4	8
A. equuli	10	100	-	-	-	-	-	-	-
E. coli	17	100	-	-	-	-	-	-	-
Pseudomonas spp	11	0	9.1	54.5	63.6	81.8	100	-	-
S. typhimurium	10	100	-	-	-	-	-	-	-
S. aureus	12	0	50	100	-	-	-	-	-
S. zooepidemicus	14	0	0	0	21.4	92.8	-	-	-

Table E-3 . Cumulative percentages of bacterial sensitivities.

Table E-4. Individual MIC values for all antibiotics tested.

BACTERIAL ISOLATES	ANTIBIOTIC														
	AMPI	MEZ	AMOX / C.A	CEPHA	CEFOX	NORF	CIPR	TETR	CARB	CEFTR	CEFTIZ	TMP/S	GENT	TOBR	AMI
A. equuli 1	≤1	≤16	-	≤1	≤1	≤1	-	≤2	-	-	-	≤2/38	1	1	4
A. equuli 2	≤1	≤16	-	≤1	≤1	≤1	-	≤2	-	-	-	≤2/38	2	2	8
A. equuli 3	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	4
A. equuli 4	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	4
A. equuli 5	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	4
A. equuli 6	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	1	1	4
A. equuli 7	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	1	1	2
A. equuli 8	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	2	1	8
A. equuli 9	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	4
A. equuli 10	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	1	1	4
A. equuli 11	≤1	≤16	≤8/4	≤1	≤1	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	1	1	4
A. equuli 12	≤1	≤16	≤8/4	2	2	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	<0.5	2
E. coli 1	4	≤16	-	16	8	≤1	-	≤2	-	-	-	≤2/38	≤0.5	≤0.5	2
E. coli 2	>128	>128	-	64	4	≤1	-	>16	-	-	-	>16/304	≤0.5	1	2
E. coli 3	2	≤16	-	8	2	≤1	-	>16	-	-	-	≤2/38	≤0.5	1	2
E. coli 4	>128	>128	-	128	8	2	-	>16	-	-	-	>16/304	1	2	4
E. coli 5	2	≤16	-	8	2	≤1	-	4	-	-	-	≤2/38	≤0.5	1	2
E. coli 6	>128	≤16	-	16	4	≤1	-	≤2	-	-	-	>16/304	≤0.5	1	2
E. coli 7	4	≤16	≤8/4	8	4	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	2
E. coli 8	>128	32	16/8	8	2	≤1	≤0.06	≤2	>128	<8	≤16	>16/304	≤0.5	≤0.5	2
E. coli 9	>128	128	≤8/4	8	2	≤1	≤0.06	>16	>128	>64	≤16	≤2/38	≤0.5	1	2
E. coli 10	2	≤16	≤8/4	16	4	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	2	1	2
E. coli 11	>128	≤16	≤8/4	8	2	≤1	≤0.06	>16	>128	<8	≤16	>16/304	≤0.5	1	2
E. coli 12	128	≤16	64/32	>128	16	≤1	≤0.06	>16	≤16	<8	≤16	>16/304	≤0.5	2	2
E. coli 13	4	≤16	≤8/4	32	4	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	1	1	≤1
E. coli 14	>128	128	16/8	16	4	≤1	≤0.06	>16	>128	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
E. coli 15	2	≤16	≤8/4	16	2	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1

BACTERIAL ISOLATES	AMPI	MEZ	AMOX / C.A	CEPHA	CEFOX	NORF	CIPR	TETR	CARB	CEFTR	CEFTIZ	TMP/S	GENT	TOBR	AMI
E. coli 16	>128	32	≤8/4	16	≤1	≤1	≤0.06	>16	>128	<8	≤16	>16/304	≤0.5	≤0.5	2
E. coli 17	4	≤16	≤8/4	16	4	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	2
E. coli 18	4	≤16	≤8/4	8	4	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	2
E. coli 19	4	≤16	≤8/4	8	2	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	1	2
E. coli 20	2	≤16	≤8/4	4	2	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	1	≤0.5	≤1
E. coli 21	4	≤16	≤8/4	8	4	≤1	≤0.06	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	2
E. coli 22	>128	64	≤8/4	8	4	≤1	≤0.06	>16	>128	<8	≤16	>16/304	≤0.5	1	2
E. coli 23	>128	>128	≤8/4	8	2	≤1	≤0.06	16	>128	<8	≤16	≤2/38	≤0.5	1	≤1
P. aeruginosa 1	>128	32	-	>128	>128	≤1	-	>16	-	-	-	4/76	2	1	2
P. aeruginosa 2	>128	128	-	>128	>128	2	-	16	-	-	-	≤2/38	2	2	4
P. aeruginosa 3	>128	≤16	>64/32	>128	>128	≤1	0.25	>16	64	16	32	8/152	4	1	4
P aeruginosa 4	>128	≤16	>64/32	>128	>128	≤1	0.12	>16	64	16	64	4/78	8	1	4
P. fluorescens 1	>128	32	>64/32	>128	>128	≤1	0.25	>16	32	<8	32	16/304	2	≤0.5	4
P. fluorescens 2	>128	≤16	>64/32	>128	>128	≤1	0.25	>16	32	<8	32	8/152	2	≤0.5	2
P. fluorescens 3	>128	≤16	>64/32	>128	>128	≤1	0.25	>16	32	<8	32	8/152	4	≤0.5	2
P. fluorescens 4	>128	32	>64/32	>128	>128	4	2	>16	<16	16	32	>16/304	2	≤0.5	2
P. fluorescens 5	>128	32	>64/32	>128	>128	8	2	>16	32	<8	≤16	>16/304	4	≤0.5	4
P. fluorescens 6	>128	32	>64/32	>128	>128	≤1	0.25	>16	64	<8	64	4/78	2	≤0.5	4
P. fluorescens 7	>128	64	>64/32	>128	>128	4	1	>16	128	32	128	>16/304	2	≤0.5	≤1
P. maltophilia	>128	>128	32/16	>128	>128	8	1	>16	≤16	>64	128	≤2/38	16	16	32
P. putrefaciens	32	≤16	16/8	>128	64	≤1	0.5	>16	32	64	32	8/152	4	1	4
S. typhimurium 1	2	4	-	4	4	≤1	-	4	-	-	-	≤2/38	≤0.5	1	2
S. typhimurium 2	>128	>128	32/16	32	16	≤1	≤0.06	16	>128	<8	≤16	>16/304	≤0.5	1	≤1
S. typhimurium 3	>128	>128	32/16	16	8	≤1	≤0.06	16	>128	<8	≤16	>16/304	≤0.5	1	≤1
S. typhimurium 4	>128	>128	32/16	16	8	≤1	≤0.06	16	>128	<8	≤16	>16/304	≤0.5	1	≤1
S. typhimurium 5	>128	>128	32/16	16	8	≤1	≤0.06	16	>128	<8	≤16	>16/304	≤0.5	1	≤1
S. typhimurium 6	>128	>128	32/16	16	8	≤1	≤0.06	16	>128	<8	≤16	>16/304	≤0.5	1	≤1
S. typhimurium 7	>128	>128	32/16	16	8	≤1	≤0.06	>16	>128	<8	≤16	>16/304	≤0.5	1	≤1

BACTERIAL ISOLATES	AMPI	MEZ	AMOX / C.A	CEPHA	CEFOX	NORF	CIPR	TETR	CARB	CEFTR	CEFTIZ	TMP/S	GENT	TOBR	AMI
S. typhimurium 8	>128	≤16	16/8	2	2	≤1	≤0.06	>16	>128	≤8	≤16	≤2/38	≤0.5	≤0.5	2
S. typhimurium 9	>128	>128	32/16	8	4	≤1	≤0.06	>16	>128	≤8	≤16	>16/304	≤0.5	1	≤1
S. typhimurium 10	>128	>128	16/8	4	2	≤1	≤0.06	>16	>128	≤8	≤16	≤2/38	≤0.5	1	2
S. typhimurium 11	2	≤16	≤8/4	4	4	≤1	≤0.06	>16	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 1	2	≤16	-	≤1	2	≤1	-	≤2	-	-	-	≤2/38	≤0.5	≤0.5	4
S. aureus 2	8	≤16	-	≤1	4	≤1	-	≤2	-	-	-	≤2/38	1	1	2
S. aureus 3	≤1	≤16	-	≤1	2	≤1	-	≤2	-	-	-	≤2/38	≤0.5	≤0.5	≤1
S. aureus 4	2	≤16	-	≤1	4	≤1	-	≤2	-	-	-	≤2/38	≤0.5	≤0.5	2
S. aureus 5	≤1	≤16	≤8/4	≤1	2	≤1	0.25	>16	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 6	≤1	≤16	≤8/4	≤1	2	≤1	0.12	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 7	≤1	≤16	≤8/4	≤1	≤1	≤1	0.12	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S aureus 8	≤1	≤16	≤8/4	≤1	≤1	≤1	0.12	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 9	2	≤16	≤8/4	≤1	4	≤1	0.25	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	2
S. aureus 10	≤1	≤16	≤8/4	≤1	2	≤1	0.25	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	4
S. aureus 11	≤1	≤16	≤8/4	≤1	≤1	≤1	0.12	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 12	4	≤16	≤8/4	≤1	4	≤1	0.25	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	2
S. aureus 13	≤1	≤16	≤8/4	≤1	4	≤1	0.25	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	2
S. aureus 14	≤1	≤16	≤8/4	≤1	2	≤1	0.25	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 15	≤1	≤16	≤8/4	≤1	2	≤1	0.12	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. aureus 16	≤1	≤16	≤8/4	≤1	≤1	≤1	0.12	>16	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 1	≤1	≤16	-	≤1	≤1	8	-	8	-	-	-	≤2/38	≤0.5	1	4
S. zooepidemicus 2	≤1	≤16	-	≤1	≤1	4	-	≤2	-	-	-	≤2/38	2	1	4
S. zooepidemicus 3	≤1	≤16	-	≤1	≤1	4	-	≤2	-	-	-	≤2/38	1	1	4
S. zooepidemicus 4	≤1	≤16	-	≤1	≤1	16	-	≤2	-	-	-	≤2/38	1	2	4
S. zooepidemicus 5	≤1	≤16	-	≤1	≤1	4	-	≤2	-	-	-	≤2/38	1	2	4
S. zooepidemicus 6	≤1	≤16	-	≤1	≤1	8	-	≤2	-	-	-	≤2/38	1	1	4
S. zooepidemicus 7	≤1	≤16	≤8/4	≤1	≤1	8	1	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 8	≤1	≤16	≤8/4	≤1	≤1	4	1	≤2	≤16	≤8	≤16	≤2/38	≤0.5	≤0.5	≤1

BACTERIAL ISOLATES	AMPI	MEZ	AMOX / C.A	CEPHA	CEFOX	NORF	CIPR	TETR	CARB	CEFTR	CEFTIZ	TMP/S	GENT	TOBR	AMI
S. zooepidemicus 9	≤1	≤16	≤8/4	≤1	2	8	2	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 10	≤1	≤16	≤8/4	≤1	≤1	8	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	2
S. zooepidemicus 11	≤1	≤16	≤8/4	≤1	≤1	4	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	2
S. zooepidemicus 12	≤1	≤16	≤8/4	≤1	≤1	4	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	4
S. zooepidemicus 13	≤1	≤16	≤8/4	≤1	≤1	8	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 14	≤1	≤16	≤8/4	≤1	≤1	4	0.5	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 15	≤1	≤16	≤8/4	≤1	≤1	8	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 16	≤1	≤16	≤8/4	≤1	≤1	4	0.5	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 17	≤1	≤16	≤8/4	≤1	≤1	4	0.5	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S zooepidemicus 18	≤1	≤16	≤8/4	≤1	≤1	8	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 19	≤1	≤16	≤8/4	≤1	≤1	8	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1
S. zooepidemicus 20	≤1	≤16	≤8/4	≤1	≤1	4	1	≤2	≤16	<8	≤16	≤2/38	≤0.5	≤0.5	≤1

AMP: Ampicillin. MEZ: Mezlocillin. AMOX/ C. A: Amoxycillin/ Clavulanic Acid. CEPHA: Cephalothin. CEFOX: Cefoxitin. NORF: Norfloxacin. CIPR: Ciprofloxacin. TETR: Tetracycline. CARB: Carbenicillin. CEFTR: Ceftriaxone. CEFTIZ: Ceftizoxime TMP/S: Trimethoprim/ Sulfamethoxazole. GENT: Gentamicin. TOBR: Tobramycin. AMI: Amikacin.