EFFECTS OF IVERMECTIN ON ANTIPYRINE AND ERYTHROMYCIN DISPOSITION IN CATTLE

By

DEANNA MARIE BOHLEN

Bachelor of Science

Northwestern Oklahoma State University

Alva, Oklahoma

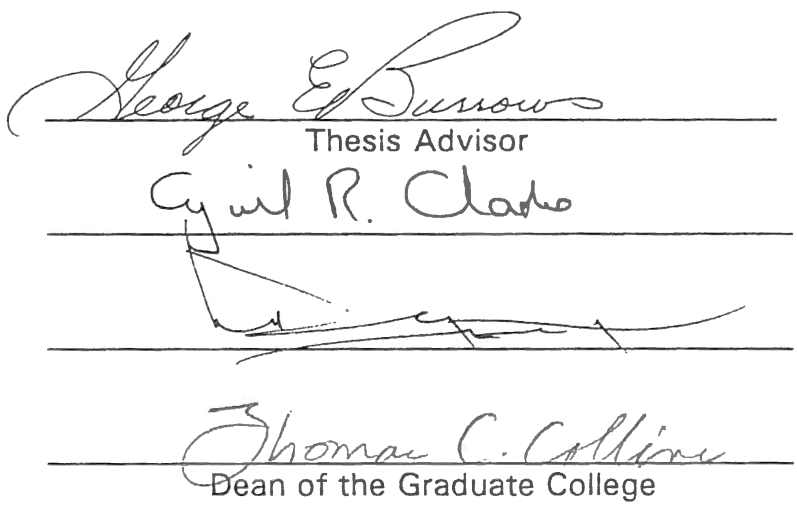
1986

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements of
the Degree of
MASTER OF SCIENCE
May, 1993

OKLAHOMA STATE UNIVERSITY

EFFECTS OF IVERMECTIN ON ANTIPYRINE AND ERYTHROMYCIN DISPOSITION IN CATTLE

Thesis Approved:



Thesis Advisor

Dean of the Graduate College

PREFACE

After many trials and tributes, I finally completed my research and thesis. All the work could not have been completed without the aid to a wonderful group of people.

I wish to express my sincere gratitude to my major professor, Dr. George E. Burrows for his continued support, inspiration, guidance, and most of all patience and belief in me. To Dr. Cyril R. Clarke and Dr. Lawrence D. Yates, I wish to extend a sincere thank-you for the constant support, patience and encouragement to think. To Dr. Patty Ewing for her technical help, thank-you. I would like to express my sincerest gratitude to Hamid R. Amouzadeh for his assistance and friendship. To Mike Van Koevering, Mark Kreul, Fred Alavi, and Jackie Cherry, I am very grateful for the friendship and support you each have given me. My deepest gratitude to my family, Dr. David H., and Dr. David S., Kathy, Alex, and Cary Bohlen, for the continued support, love, inspiration and encouragement. To Dr. Charles R. Gasaway, the person who helped me realize my potential and convinced me to attend graduate school, I wish to extend a sincere thank-you.

I wish to dedicate this thesis to the memory of my mother, Marjorie M. R. Bohlen.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

CHAPTER II

LIST OF FIGURES

Figure Page

CHAPTER I

APPENDIX

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Biotransformation of Xenobiotics

Biotransformation or metabolism is a process by which a foreign chemical is subjected to chemical change by an animal's hepatic drug metabolizing enzyme system. Biotransformation is usually a biphasic process in which the initial phase (phase I) consists of oxidative, reductive, or hydrolytic *reactions. These reactions transform* a drug by exposing existing polar groups or introducing into the drug molecule, functional groups such as -OH or =O. These functional groups enable the compound to undergo synthetic or conjugation reactions in phase II. Conjugation involves endogenous substances such as glucuronic acid, acetate (acetylation), sulfate (sulfuric acid ester formation) and various amino acids which react with xenobiotics resulting in the formation of water soluble conjugated products. These products are excreted by transfer across hepatic, renal, and intestinal membranes. The excretion of conjugated drugs is enhanced by their ability to bind to transport carriers (Sipes & Gandolfi, 1986).

The liver is the major site of biotransformation for many xenobiotics. However, other tissues such as lung, kidney, placenta, white blood cells, and gut may play an important role in metabolism. Most phase I reactions are

catalyzed or mediated by microsomal enzyme systems located in the smooth endoplasmic reticulum of hepatocytes. Microsomal enzyme systems are composed of mixed-function oxidases of which cytochrome P-450 are predominant. When reduced, cytochrome P-450 (Fe^{2+}) forms a ligand with carbon monoxide and maximally absorbs light at 450 nm. Thus the name cytochrome P-450 was derived. Microsomal cytochrome P-450 are hemeproteins with a prosthetic group containing iron. This group forms coordinate bonds by sharing electrons with the four nitrogen atoms of a tetrapyrrole ring and two other ligands. The ligands are a thiolate anion and either oxygen, carbon monoxide, or cyanide. The apparent monomeric molecular weight of cytochrome P-450 enzymes is 45,000 to 60,000 (Guengerich & Liebler, 1985; Sipes & Gandolfi, 1986; Riviere & Cabanne, 1987).

NADPH-cytochrome P-450 reductase and cytochrome P-450 comprise the enzyme system. Also associated with the reaction are cytochrome b_5 and cytochrome b_5 reductase, but the function of cytochrome b_5 and its associated reductase in the cytochrome P-450 mediated reaction has not been clearly established. This reaction occurs when substrate (RH) combines with cytochrome P-450 (Fe^{3+}) in an oxidized state to form a substrate-cytochrome P-450 complex. The complex then accepts an electron from NADPH, following which NADPH-cytochrome P-450 reductase is released. This results in reduction of iron in cytochrome P-450 heme moiety to Fe^{2+}. After combining with molecular oxygen, oxygenated substrate-cytochrome P-450 complex

accepts another electron from NADPH via cytochrome b_5 reductase. Both electrons are thought to be transferred to molecular oxygen resulting in an unstable and highly reactive oxygen species. One of the reactive oxygen atoms is reduced to water, while the other is introduced into the substrate. The oxidized form of cytochrome P-450 is regenerated after dissociating from the oxygenated substrate (Figure 1)(Sipes & Gandolfi, 1986).

Substrates of cytochrome P-450 can be divided into endogenous and exogenous compounds (xenobiotics). Endogenous compounds include fatty acids, prostaglandins, cholesterol, steroids, and lipid-soluble vitamins (hydroxylation of vitamin D). Examples of exogenous compounds are drugs, *pesticides, and organic pollutants,* which are mainly lipophilic (Riviere & Cabanne, 1987).

In each animal species, the cytochrome P-450 occur in multiple isozymic forms differing with regard to the specificity of the reactions they catalyze and the rates of substrate metabolism. Different sites on a single compound may be metabolized by different isozymes and chemical stereoisomers may be metabolized differently by the various isozymes. Potentially toxic chemicals may be detoxified by one isozyme and bioactivated by another isozyme, or one isozyme can carry out both types of competing reactions. (Guengerich & Liebler, 1985).

Individual isozymes can be induced or inhibited by xenobiotics, leading to an increase in catalytic activity of the isozymes (induction) or a decrease in activity (repression of hemoprotein synthesis, competitive or noncompetitive

inhibition, limited supply of a cofactor). Cytochromes that metabolize exogenous substrates are very sensitive to inducers (Riviere & Cabanne, 1987). A xenobiotic may also serve as a substrate for the same isozyme which it induces. The number of molecules of a specific enzyme can increase in response to an enzyme-inducing agent. The activity level of as many as seven different forms of P-450 can be increased by a xenobiotic while at the same time decreasing the activity level of another (Guengerich & Liebler, 1985). Pharmacologically, the induction of liver microsomal enzymes is important. As the rate of biotransformation is altered, so is the duration and intensity of drug action (Conney, 1967). There are several common features of enzyme inducing agents including; lipophilicity, ability to bind to cytochrome P-450 and relatively long biological half-life, however, not all agents that have these properties are enzyme inducers (Anadon, 1982). Polycyclic aromatic hydrocarbons, known inducers, enter cells and bind to cytosolic receptors. These agents are translocated to the nucleus where specific genes for transcription are activated. Activation increases the level of mRNA coding for individual P-450's. For an individual P-450's, the degree of induction by a single chemical can range from less than 2- to as much as 100-fold (Guengerich & Liebler, 1985). Treatment with phenobarbital, another known inducer, results in proliferation of the smooth endoplasmic reticulum and increased concentration of microsomal proteins (Sipes & Gandolfi, 1986).

Phenobarbital and 3-methylcholanthrene-like compounds are the two traditional classes of inducers. Studies with pregnenolone-16α-carbonitrile

(PCN), isosafrole, and polyhalogenated biphenyls indicate that the binary classification of inducers is too simplistic (Dannan et al, 1983). The activity of hepatic microsomal enzymes have been shown to be stimulated by more that 200 drugs, insecticides, carcinogens, and other chemicals. In the organism, there is no apparent characteristic pharmacological relationship between either the actions or chemical structures of these compounds and their ability to induce enzymes (Conney, 1967). The induction time course varies with the inducing agents. Therapeutic doses of most inducing agents produce their maximum effect within 2 weeks. Rifampin, a potent inducer, can produce noticeable changes in activity of hepatic drug metabolizing enzymes within 48 *hours* (Park & Breckenridge, 1981). Antipyrine, a much less potent inducer, can produce a change following administration for a period of 14 days (Ohnhaus et al, 1979; Ohnhaus & Park, 1979).

Phenobarbital is characterized by its ability to stimulate the metabolism of a large number of substrates by various pathways. It has been demonstrated that administration of phenobarbital to laboratory animals increases the metabolism of the anticoagulant drugs, bishydroxycoumarin and warfarin. This results in a decreased anticoagulant effect of a given dose of these agents (Gelehrter, 1976). Phenobarbital differs from other enzyme inducing agents by increasing liver mass which is paralleled by an increase in liver blood flow (Park & Breckenridge, 1981). Phenobarbital decreases the storage of dieldrin (HEOD) in rat body fat probably by an induction of hepatic microsomal enzymes. HEOD is metabolized to a more polar compound that can

be excreted by the kidney. Phenobarbital has also been shown to induce hepatic microsomal enzymes in ruminants indicating that it may increase the excretion rate of HEOD in cattle as well as rats (Cook & Wilson, 1970).

Organophosphates act as indirect inhibitors of cholinesterases. Hepatic microsomal enzymes may bioactivate thioorganophosphates to toxic analogues or detoxify them to inactive or less active compounds. The effect hepatic microsomal enzyme induction will have on the toxicity of a particular organophosphate is not predictable. Hepatic microsomal enzyme inducing agents have been found to increase hepatic carboxylesterase activity resulting in reduced toxicity of many organophosphates. In cattle, pretreatment with the hepatic microsomal enzyme inducers dieldrin and phenobarbital increased the toxic effect of diazinon and increased liver carboxylesterase activity. An increase in the toxicity of organophosphates such as guthion, azinophosmethyl, scharadon, and dimethoate have also been reported under similar conditions. Conversely, the toxicity of parathion, malathion, EPN, mipafox, demetilan, bidrin and phosphamidon was decreased following pretreatment with phenobarbital or dieldrin (Abdelsalam & Ford, 1986).

Ethanol and rifampin are examples of other hepatic microsomal enzyme inducers. Chronic ethanol consumption increases the metabolism of ethanol and a variety of other drugs including tolbutamide, barbiturates, phenytoin, and warfarin. An increased tolerance of alcoholic patients to barbiturates and other sedatives may be accounted for by an increased rate of metabolism. Drug metabolism can also be inhibited by a single large dose of ethanol which may

account for the enhanced sensitivity to barbiturates seen in intoxicated persons (Gelehrter, 1976). The antibiotic rifampin, a potent inducer, has been shown to increase cytochrome P-450 content of human liver but does not influence liver blood flow. Elimination of a wide range of drugs has been reported to be accelerated by rifampin: antipyrine, warfarin, digitoxin, and cortisol. Rifampin has also been reported to stimulate its own clearance (Park & Breckenridge, 1981).

The therapeutic implications of enzyme induction and inhibition depends largely on the relative biological activity of the drug and its metabolite(s). In most cases the metabolites are therapeutically less active than the parent drug. Generally, the extent and duration of drug action are potentiated by enzyme inhibition and reduced by enzyme induction. These effects are reversible and withdrawal of the inducing or inhibiting agent results in reversal to the original rate of biotransformation (Park & Breckenridge, 1981).

Most studies on hepatic microsomal enzyme system-catalyzed metabolism of drugs have been restricted to laboratory animals such as rats and rabbits. Relatively little is known about comparative drug metabolism in food-producing mammalian species (Dalvi et al, 1987). Often, wide differences in physiological or metabolic capabilities exist among species that are classified as closely related phylogenetically. This is especially so in the biotransformation and disposition of xenobiotics (Smith et al, 1984). Inter-species variations make it difficult to extrapolate information from one species to another. This difficulty may be due to the presence of multiple enzyme forms and the role of

extra-hepatic tissues such as the rumen wall which is the site of significant biotransformation (Watkins & Klaassen, 1986). Cattle differ widely from rats in having lower activity of glutathione-S-transferase for sulfobromophthalein and 1,2-dichloro-4-nitrobenzene and remarkably high activities of styrene oxide hydrolase, ethoxyresorufin o-deethylase, 2-naphthol sulfotransferase and p-aminobenzoic acid acetyltransferase (Smith et al, 1984).

Antipyrine

Antipyrine is widely used as a model drug for studying changes in hepatic drug metabolism. Antipyrine is rapidly absorbed through the gut and then distributed in total body water. There is negligible plasma protein binding of antipyrine. Metabolism of antipyrine is almost exclusively by the hepatic microsomal enzyme system and is not influenced by hepatic blood flow. Phase 1 metabolism is the rate limiting step in antipyrine biotransformation. Total body clearance for antipyrine is equal to antipyrine metabolic clearance (Park & Breckenridge, 1981; Depelchin et al, 1988). Metabolism of antipyrine occurs by a number of different isozymes of cytochrome P-450 in rats (Inaba et al, 1980). In calves, however, hydroxylation is the major metabolic pathway of antipyrine. Biotransformation of antipyrine in man involves N-methylation and hydroxylation (Depelchin et al, 1988). Changes in antipyrine disposition may be used to measure hepatic microsomal enzyme induction in an organism and plasma half-life or clearance of antipyrine is often used to assess an individual's capacity to metabolize other drugs (Park & Breckenridge, 1981). For example, the half-lives of antipyrine are considerably longer in patients with liver disease

than in patients not suffering from liver disease (Inaba et al, 1980). In a given subject, tests consist of measuring antipyrine clearance in a controlled, environmentally stable state and again after a single environmental change is imposed. These tests have been applied to identify and quantitate the impact of many conditions and factors on hepatic metabolism of antipyrine. Under stable environmental condititions, disposition of antipyrine is highly reproducible, therefore, each subject can serve as its own control (Vesell, 1979; Depelchin et al, 1987).

A wide range of inducing agents such as drugs, pesticides, and nutrients have been shown to increase antipyrine clearance in humans and other animals (Park & Breckenridge, 1981). Phenobarbital has been shown to shorten the half-life of antipyrine (Inaba et al, 1980). Dexamethasone phosphate has been shown to stimulate the metabolism of antipyrine by activating cytochrome P-450. This increase in metabolism results in a shortening of antipyrine elimination half-life in calves (Depelchin et al, 1987). Griseofulvin stimulates an increase in liver weight which results in induction of antipyrine metabolism (Depelchin et al, 1987). Promethazine, a phenothiazine derivative, significantly increased clearance of antipyrine. However, the decrease in the half-life of antipyrine was not significant since there was an observed change in the apparent volume of distribution (Taylor & Houston, 1985). Phenytoin has been shown to significantly reduce the half-life of antipyrine in epileptic subjects (Park & Breckenridge, 1981).

Antipyrine has been reported to be a mild enzyme inducer in man, which

is one of the disadvantages to its use as a model drug. Daily administration of antipyrine for a 14 day period produces an induction of hepatic microsomal enzyme system. Therefore, long term administration of antipyrine can stimulate its own metabolism and has been shown to increase the rate of elimination of diazepam and warfarin. Since antipyrine requires 14 days of daily administration to produce an induction, intermittent administration of antipyrine is unlikely to result in a change in the hepatic microsomal enzyme systems (Ohnhaus et al, 1979; Park & Breckenridge, 1981).

Erythromycin

The macrolide antibiotic, erythromycin, is used in the treatment of gram-positive and some gram-negative bacterial infections. Erythromycin is highly lipid soluble, widely distributed in the body (Burrows, 1980), and slightly bound to serum proteins (Ziv & Sulman, 1972). Metabolism of erythromycin occurs primarily in the liver by demethylation and is followed by biliary excretion (Burrows, 1980; Manzo et al, 1980).

In the rat, hepatic microsomal metabolism and biliary excretion of erythromycin was increased by phenobarbital. This increase in metabolism and excretion results in markedly lower concentrations of erythromycin in tissues and serum (Manzo et al, 1980). Theophylline was found to cause a 30% decrease in area under the serum erythromycin concentration time curve, possibly resulting from increased hepatic blood flow (Ludden, 1985).

Derivatives of erythromycin have been reported to be potent inducers of hepatic cytochrome P-450 (Sartori et al, 1985). The elimination of

methylprednisolone has been reported to be inhibited by erythromycin (Reisz et al, 1983).

Avermectins

Avermectins are natural products of the microorganism *Streptomyces avermitilis.* This microbe was isolated at Kitasato Institute from a soil sample collected in Japan. Studies using the product indicated at least an eight-fold increase in activity against *Nematospiraoides dubis* in mice and there was no notable toxicity. The complex, which was named avermectin, was then isolated and identified (Burg et al., 1979).

Avermectins are glycosidic derivatives of pentacyclic 16-member lactones but lack the antibacterial properties associated with macrolide antibiotics. The compounds were isolated by solvent extraction of the mycelia. Reverse phase high pressure liquid chromatography separation and mass spectra examination indicated the existence of two series of compounds designated A and B. The A-series compounds have a methoxy group at the 5-position and are generally less effective than those of the B-series. For this reason, the A-series have never been developed for use. Within the two series, there exists two structural subsets designated 1 and 2 and the presence of minor homologues a and b. The eight components were designated A_{1a} through B_{2b} (Albers-Schönberg et al., 1981; Campbell et al, 1983). The compounds of subset-1 posses an olefinic bond between carbon 22 and 23 while in subset-2, this linkage is hydrated with the hydroxyl group found at the 23 position. The difference in conformation has a profound effect on the ring

bearing these functionalities, causing subtle changes in bioactivity. Upon oral administration, avermectin B_1 had been determined to be more active than avermectin B_2 while the converse was true when each was administered parenterally. The nonproprietary name ivermectin contains at least 80 percent 22, 23-dihydroavermectin B_{1a} and not more than 20 percent 22, 23-dihydroavermectin B_{1b} (Chabala et al., 1980).

Ivermectin has low solubility in water and is lipophilic. In cattle the half-life of ivermectin was found to be 2.8 days, with a large volume of distribution (1.91/kg) and a rapid distribution phase. This large volume of distribution results in a shift in the equilibrium distribution from the central compartment toward the peripheral compartment (Lo et al, 1985). Fat and liver contain the highest tissue residue levels while brain and blood have the lowest. Depletion half-lives of ivermectin in fat and liver were 4-8 days (Chiu et al, 1990). The major tissue residue of ivermectin in liver, fat, muscle and kidney was the unaltered drug (Chiu et al, 1986). In cattle, ivermectin is biotransformed in the liver to produce polar metabolites which are esterified to nonpolar entities. These nonpolar entities are stored in fat and can be readily hydrolyzed back to the polar metabolites. Levels of the nonpolar metabolites could be detected in fat tissues of steers after the animals were withdrawn from the drug for 28 days (Chiu et al, 1988). The major route of excretion was in feces. In cattle, high levels of ivermectin found in bile suggest that biliary excretion is an important route of elimination (Chiu et al, 1990).

In mammals, ivermectin has a wide margin of safety and when

administered at the recommended dose there are no indications of adverse effects. However, at 40 times the recommended dose, toxicity and death were observed. No fetal abnormalities were seen in cattle treated subcutaneously during pregnancy at twice the recommended dosage (Campbell, 1981; Campbell et al, 1983).

Experimental Goals and Design

As discussed previously, xenobiotics that have a relatively long biological half-life and are lipophilic are thought to have the potential to act as enzyme inducers (Anadon, 1982). Ivermectin is lipophilic and has a relatively long half-life in cattle and other animals. Based upon these properties, ivermectin appears to be a potential enzyme inducer. Therefore, this study was carried out to ascertain the effect of ivermectin on biotransformation of model drugs in cattle. One of the model drugs, erythromycin was selected because it is one of the drugs of choice for treatment of bovine respiratory diseases and might be used in conjunction with ivermectin in animals newly introduced to feedyards. It is also biotransformed in the body and subject to possible changes in hepatic mixed-function oxidase activity. Antipyrine was used because it is widely accepted as a model drug to determine changes in hepatic microsomal metabolism.

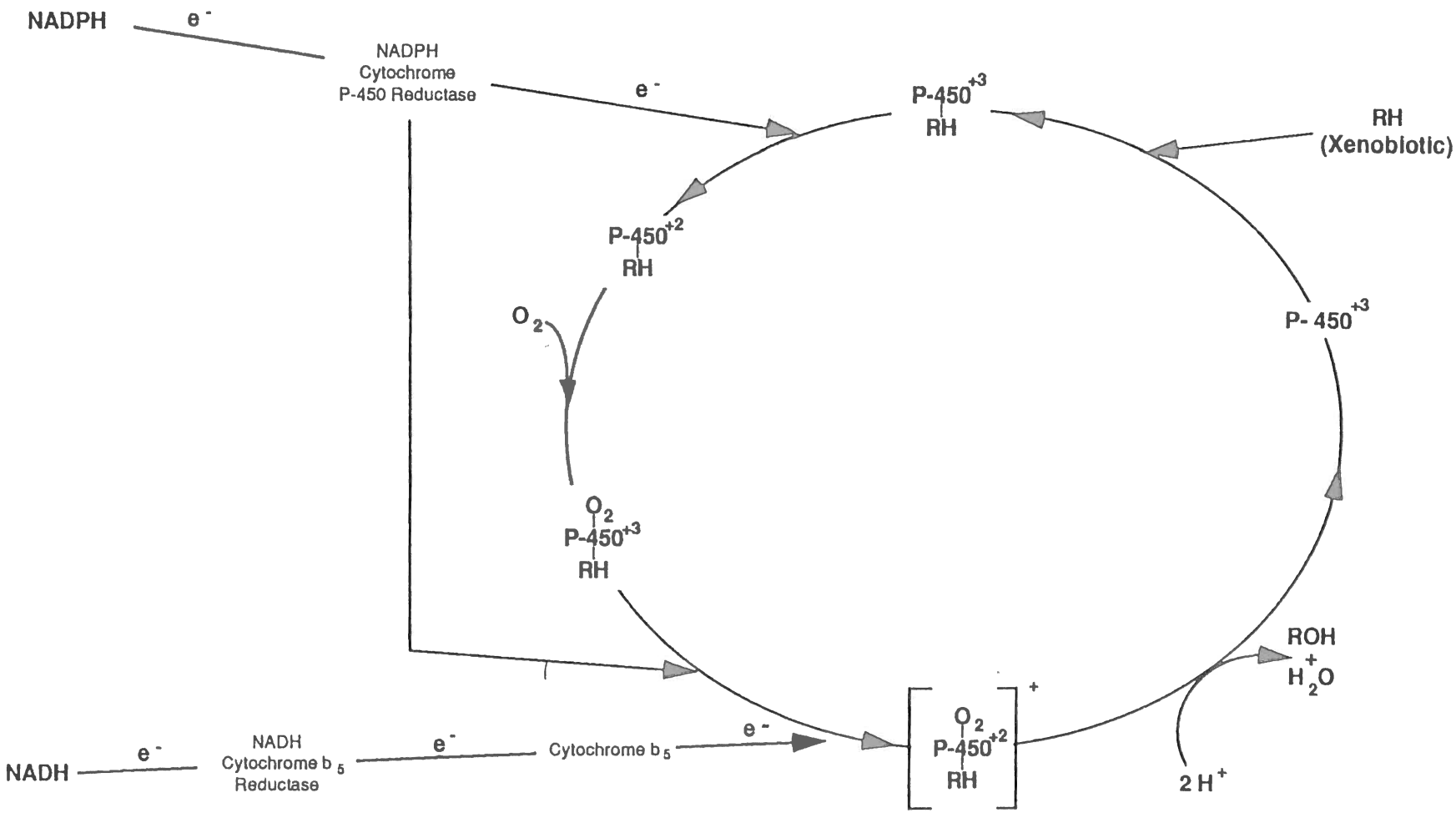


Figure 1. Oxidation of a xenobiotic by cytochrome P-450 electron transport systems (adapted from Sipes & Gandolfi, 1986).

References

Abdelsalam, E.B. & Ford, E.J.H. (1986) Effect of pretreatment with hepatic microsomal enzyme inducers on the toxicity of diazinon in calves. *Research in Veterinary Science*, **41**, 336-339.

Albers-Schönberg, G., Arison, B.H., Chabala, J.C. *et al.* (1981) Avermectins. Structure determination. *Journal of American Chemical Society*, **103**, 4216-4221.

Anadon, A. (1982) Enzyme induction and inhibition. In *Veterinary Pharmacology and Toxicology*. Eds Ruckebusch, Y., Toufain, P.L. & Konritz, G.D., pp. 523-537. AVI, Westport, Connecticut.

Burg, R.W., Miller, B.M., Baker, E.E. *et al* (1979) Avermectins, new family of potent anthelmintic agents: Producing organism and fermentation. *Antimicrobial Agents and Chemotherapy*, **15**, 361-367.

Burrows, G.E. (1980) Pharmacotherapeutics of macrolides, lincomycins, and spectinomycin. *Journal of American Veterinary Medicine Association*, **176**, 1072-1077.

Campbell, W.C. (1981) An introduction to the avermectins. *New Zealand Veterinary Journal*, **29**, 174-178.

Campbell, W.C. Fisher, M.H., Stapley, E.O., Albers-Schönberg, G. & Jacob, T.A. (1983) Ivermectin: A potent new antiparasitic agent. *Science*, **221**, 823-828.

Chabala, J.C., Mrozik, H., Tolman, R.L. *et al.* (1980) Ivermectin, a new broad-spectrum antiparasitic agent. *Journal of Medicinal Chemisty*, **23**, 1134-1136.

Chiu, S-H.L., Sestokas, E., Taub, R., Buhs, R.P. *et al.* (1986) Metabolic disposition of ivermectin in tissues of cattle, sheep, and rats. *Drug Metabolism and Disposition*, **14**, 590-600.

Chiu, S-H.L., Carlin, J.R., Taub, R. *et al.* (1988) Comparative metabolic disposition of ivemectin in fat tissues of cattle, sheep, and rats. *Drug Metabolism and Disposition*, **16**, 728-736.

Chiu, S-H.L., Green, M.L., Baylis, F.P. *et al.* (1990) Absorption, tissue distribution, and excretion of tritium-labeled ivermectin in cattle, sheep, and rat. *Journal of Agriculture and Food Chemistry*, **38**, 2072-2078.

Conney, A.H. (1967) Pharmacological implications of microsomal enzyme induction. *Pharmacology Reviews*, **19**, 317-366.

Cook, R.M. & Wilson, K.A. (1970) Metabolism of xenobiotics in ruminants. *Journal of Agriculture and Food Chemistry*, **18**, 441-442.

Dalvi, R.R., Nunn, V.A. & Juskevich, J. (1987) Hepatic cytochrome P-450 dependent drug metabolizing activity in rats, rabbits and several food-producing species. *Journal of Veterinary Pharmacology and Therapeutics*, **10**, 164-168.

Dannan, G.A., Guengerich, F.P., Kaminsky, L.S. & Aust, S.D. (1983) Regulation of cytochrome P-450. *The Journal of Biological Chemistry*, **258**, 1282-1288.

Depelchin, B.O., Bloden, S. & Ansay, M. (1987) Antipyrine disposition in calves. I. Effects of some drugs. *Journal of Veterinary Pharmacology and Therapeutics*, **10**, 49-53.

Depelchin, B.O., Bloden, S., Michaux, C. & Ansay, M. (1988) Effects of age, sex and breed on antipyrine disposition in calves. *Research in Veterinary Science*, **44**, 135-139.

Gelehrter, T.D. (1976) Enzyme induction (Second of three parts). *New England Journal of Medicine*, **294**, 589-595.

Guengerich, F.P. & Liebler, D.C. (1985) Enzymatic activation of chemicals to toxic metabolites. *CRC Critical Reviews in Toxicology*, **14**, 259-307.

Inaba, T., Lucassen, M. & Kalow, W. (1980) Antipyrine metabolism in the rat by three hepatic monooxygenases. *Life Sciences*, **26**, 1977-1983.

Lo, P-K.A., Fink, D.W., Williams, J.B. & Blodinger, J. (1985) Pharmacokinetic studies of ivermectin: Effects of formulation. *Veterinary Research Communications*, **9**, 251-268.

Ludden, T.M. (1985) Pharmacokinetic interactions of the macrolide antibiotics. *Clinical Pharmacokinetics*, **10**, 63-79.

Manzo, L., Gregotti, C., Richelmi, P., Di Nucci, A. & Bertè, F. (1980) Effects of phenobarbitone on the distribution, metabolism and biliary excretion of erythromycin in rats. *Chemotherapy*, **26**, 164-170.

Ohnhaus, E.E., Park, B.K., Colombo, B.K. & Heizmann, P. (1979) The effect of enzyme inducition on diazepam metabolism in man. *British Journal of Pharmacology*, **8**, 557-563.

Ohnhaus, E.E. & Park, B.K. (1979) Measurement of urinary 6-ß-hydroxycortisol excretion as an in vivo parameter in the clinical assessment of the microsomal enzyme-inducing capacity of antipyrine, phenobarbitone and rifampicin. *European Journal of Clinical Pharmacology*, **15**, 139-145.

Park, B.K. & Breckenridge, A.M. (1981) Clinical implication of enzyme induction and enzyme inhibition. *Clinical Pharmacokinetics*, **6**, 1-24.

Reisz, G., Pingleton, S.K., Melethil, S. & Ryan, P.B. (1983) The effect of erythromycin on theophylline pharmacokinetics in chronic bronchitis. *American Review of Respiratory Diseases*, **127**, 581-584.

Riviere, J-L. & Cabanne, F. (1987) Animal and plant cytochrome P-450 system. *Biochimie*, **69**, 743-752.

Sartori, E., Delaforge, M., Mansuy, D. & Beaune, P. (1985) Some erythromycin derivatives are strong inducers in rats of a cytochrome P-450 very similar to that induced by 16 α-pregnenolone carbonitrile. *Biochemical and Biophysical Research Communications*, **128**, 1434-1439.

Sipes, I.G. & Gandolfi, A.J. (1986) Biotransformation of toxicants. In *Casarett and Doull's Toxicology*, 3rd edn. Eds Klassen, C.D., Amdur, M.O. & Doull, J., pp. 64-98. Macmillan, New York.

Smith, G.S., Watkins, J.B., Thompson, T.N., Rozman, K. & Klaassen, C.D. (1984) Oxidative and conjugative metabolism of xenobiotics by livers of cattle, sheep, swine and rats. *Journal of Animal Science*, **58**, 386-395.

Taylor, G. & Houston, J.B. (1985) Changes in the disposition of promethazine during multiple dosing in the rabbit. *Journal of Pharmacy and Pharmacology*, **37**, 243-247.

Vesell, E.S. (1979) The antipyrine test in clinical pharmacology: Conceptions and misconceptions. *Clinical Pharmacology and Therapeutics*, **26**, 275-286.

Watkins, J.B. & Klaassen, C.D. (1986) Xenobiotic biotransformation in livestock: Comparison to other species commonly used in toxicity testing. *Journal of Animal Science*, **63**, 933-942.

Ziv, G. & Sulman, F.G. (1972) Binding of antibiotics to bovine and ovine serum. *Antimicrobial Agents and Chemotherapy*, **2**, 206-213.

CHAPTER II

EFFECTS OF IVERMECTIN ON ANTIPYRINE AND ERYTHOMYCIN DISPOSION IN CATTLE

Introduction

Drug interactions commonly occur when two or more drugs are administered simultaneously or when animals are exposed to xenobiotics present in food, water, and air. Such interactions may modify the biological activity of a xenobiotic by altering absorbtion, distribution, metabolism, or elimination. Interactions are particularly likely when a xenobiotic is capable of inducing or inhibiting hepatic metabolic enzymes (Hayes & Borzelleca, 1985). As the rate of biotransformation is altered, the duration and intensity of drug action may be affected (Conney, 1967). Lipophilicity, binding to cytochrome P-450, and relatively long half-life are three common characteristics of hepatic metabolic enzyme inducers such as phenobarbital (Anadon, 1982).

Ivermectin, a macrolactone with antiparasitic properties, is a lipophilic compound which is metabolized in the liver and excreted in the bile of cattle (Campbell et al, 1983). Chiu et al, (1990) reported that ivermectin has a relatively long half-life (2.8 days); the depletion half-life in fat and liver residues was 4-8 days and metabolites have been detected in fat tissues of steers 28 days after withdrawal (Chiu et al, 1988). These dispositional characteristics,

which are similar to those of recognized enzyme inducers, suggest that ivermectin may induce the metabolism of concurrently administered drugs.

In order to evaluate the enzyme inducing potential for ivermectin, two model drugs were selected. The objective of the study was to determine whether ivermectin acts as an enzyme inducer or inhibitor by studying the effect of intramuscular administration of ivermectin on the pharmacokinetic disposition of the two model drugs, erythromycin and antipyrine. Erythromycin is a macrolide antibiotic commonly used in cattle. It is eliminated by hepatic metabolism and biliary excretion (Burrows, 1980; Manzo et al, 1980) and represents a clinically relevant drug which may be affected by concurrent *administration of ivermectin*. Antipyrine is eliminated exclusively via hepatic biotransformation and is widely used as an experimental model drug for identifying changes in the rate of drug metabolism (Park & Breckenridge, 1981; Depelchin, 1988).

Materials and Methods

Experiment 1

Five mixed-breed heifer calves of approximately 5 months of age and initial body weights of 65.9 to 115.9 kg were maintained on prairie hay and a commercial grain mixture containing 14% protein (A&M Milling, Stillwater, OK) and water ad libitum. Calves were penned outdoors.

A cross-over experimental design was used to investigate the effect of ivermectin on erythromycin pharmacokinetics. Calves were divided into two

groups. Three calves in group 1 received a single i.v. dose of erythromycin (Gallimycin 200mg/ml, CEVA Labs, Overland Park, KS) (15mg/kg body wt.) via a jugular catheter to establish baseline parameters. Two weeks later, group 1 calves were given a single i.m. dose of ivermectin (1% w/v cattle injection, Ivomec, MSD Agvet, Rahway, NJ) (1mg/5kg body wt.) and a single i.m. dose of erythromycin (15mg/kg body wt.). On days 2, 4, and 13 following the treatment with ivermectin, each calf received the same dose of erythromycin i.v. via a jugular catheter. The two calves in group 2 were initially given a single i.m. dose of ivermectin (1mg/5kg body wt.) and a single i.m. dose of erythromycin (15mg/kg body wt.). Thereafter, erythromycin was administered i.v. via a jugular catheter on days 2, 4, and 13 following the initial ivermectin dose. Two weeks after the end of the 13 day sampling period, group 2 calves were given the single i.v. dose of erythromycin to establish baseline parameters. Because of an adverse response in some of the calves given erythromycin i.v. on day 2, it was thereafter diluted 1:1 with saline and administered in small increments over a minute on days 4 and 13. Blood was collected from a contralateral jugular catheter at 0, 2, 5, 10, 20, 30, 45 and 60 minutes and 2, 3, 4, 6, 8, 12, and 16 hours following each i.v. administration of erythromycin. Serum was harvested and stored at -20°C until analysis.

Experiment 2

Five bull and one heifer Hereford calves with an initial body weight of

75.0 to 140.9 kg were maintained and housed as previously described.

Antipyrine (10% solution, Sigma Chemical Co., St. Louis, MO) (20 mg/kg body wt.) was administered to each calf via a jugular catheter. Blood was collected from a contralateral indwelling jugular catheter at 0, 2, 5, 10, 15, 20, 30, 45, 60 and 90 minutes and 2, 3, 4, 5, 6, 7, and 8 hours after administration of antipyrine. Serum was harvested and stored as previously described. Two weeks later, each calf was given ivermectin i.m. (1% w/v cattle injection, MSD Agvet, Rahway, NJ) (1mg/5kg body wt.) on day 1. On days 3 and 17, each calf was administered antipyrine i.v., blood was collected as previously described. Serum was harvested and frozen at -20° until assayed.

Drug Assay

Erythromycin. Erythromycin concentrations were determined using the microbial assay method of Bennett et al, (1966); an agar-gel diffusion method employing *Micrococcus luteus* ATCC 9341 as the test organism (Burrows, 1985) with a detectability level of approximately 0.3 μg/ml. Standard solutions were prepared in calf serum by appropriate serial dilutions of drug. Standards and serum samples were analyzed in duplicate with samples undiluted and diluted to 1:2 with serum. The measured width of zones from serum samples were converted to concentrations, using standard curves developed for each 81-well agar plate.

Antipyrine. Antipyrine concentrations in serum were quantitated using a

modification of the method reported by Clarke et al, (1985). Briefly, 40-μl of 5 N sodium hydroxide and 2 ml of ethyl acetate containing 2.5 ppm phenacetin as the internal standard were added to 0.2 ml of serum and vortexed immediately for 30 seconds. The combination was separated by centrifugation at 600 x g for 5 minutes and the organic supernatant transferred to a chromatography vial. Standards (0, 0.5, 1, 2.5, 5, 10, 25, 50 ppm) were prepared by dissolving appropriate amounts of antipyrine in 0.2 ml of serum. The test sera and standards were subjected to the same analytic procedure. Antipyrine concentrations were quantitated by injecting 4-μl samples of supernatant into a gas chromatograph (Tracor 565, Austin, TX) with a 30m, 0.53*mm* ID *megabore* capillary column with a 1.0 μm film of 50% phenylmethyl polysiloxane (DB17, J&W Scientific, Folsom, CA). The column, injection port, and nitrogen-phosphorus detector temperatures were 180, 230, and 250° C, respectively. The flow rate for helium carrier gas was 10 ml/min; for detector hydrogen, 2.5ml/min; and for air, 120 ml/min.

Pharmacokinetics

Coefficients and exponents of the disposition curves were obtained using a computer program for modeling and simulation of pharmacokinetic data (Bourne, 1986). Determination of appropriate compartmental model was based on examination of individual concentration-time curve fits and goodness of fit parameters.

Statistical Analysis

Pharmacokinetic parameters for which a normal distribution cannot be assumed (elimination half-life, volume of distribution, total body clearance) were rank transformed (Conover & Iman, 1981) prior to analysis. The ranked and mean nonranked parameter values were analyzed by the general linear model and Dunnett's test ($p < 0.05$) to compare treatment to baseline values. For experiment 1, pre-ivermectin and post-ivermectin baseline ß and B values were analyzed using the general linear model and LS means to determine the validity of the cross-over design (Steel & Torrie, 1980).

Results

The serum concentration-time disposition data were described using a two-compartment open model and the bi-exponential equation:

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

where Cp is the concentration in serum at time t; A and B are the biphasic zero-time intercepts; e is the natural logarithm base; and α and β are the rate constants related to the disposition and elimination phases, respectively (Baggot, 1977).

Pharmacokinetic parameters describing erythromycin disposition are presented in Table 1. The semi-logarithmatic plots of the concentration-time curves for individual treatments are presented in the appendix (figures 1-4).

There were no significant differences between baseline values and those calculated after ivermectin treatment, except for an increase in total body

clearance rate on day 4. The cross-over design pretreatment and posttreatment baseline values were not significantly different. Immediately following administration of erythromycin, an adverse systemic effect was observed in some calves characterized by dyspnea, polypnea, and tremors. This effect was alleviated when the erythromycin was diluted with saline and administered slowly in small increments.

The pharmacokinetic values determined for antipyrine are presented in Table 2 and the semi-logarithmatic concentration-time curves of the individual treatments are presented in the appendix (figures 5-8). There were no differences between antipyrine kinetics when baseline and post ivermectin parameters were compared.

Discussion

The parmacokinetic values for erythromycin and antipyrine are consistent with published values (Baggot & Gingerich, 1976; Burrows, 1985; Burrows et al, 1989; Tufenkji et al, 1988) and in general were not affected by ivermectin. The only significant difference noted was for erythromycin total body clearance on day 4. However, this apparent increase in $Cl_{ß}$ may not be meaningful. The slope of the elimination phase (ß) and volume of distribution (V_d), which are used in the determination of the clearance rate, were apparently increased on day 4 but the changes were not significant. An additional factor contributing to the difference in clearance rate may be the change in the method of administration of erythromycin. Erythromycin given for baseline determination

and on day 2, was administered as an i.v. bolus while on days 4 and 13 it was diluted 1:1 with saline and administered in small increments over one minute. Since the erythromycin was administered slowly on day 4, the distribution phase may have been lengthened accordingly, thereby producing an apparent increase in the volume of distribution (V_d), and thus making the elimation rate appear to be increased. The combination of these effects might lead to an apparent significant increase in the clearance rate (Cl_B). The change in the method of erythromycin administration was made in an attempt to prevent the peracute systemic reactions observed. The calves showed signs of dyspnea, polypnea, and tremors immediately after rapid i.v. administration of the *concentrated* preparation. The dilution of erythromycin and slow administration in small increments alleviated the reaction and the calves showed no adverse signs when this method was employed on days 4 and 13. This acute reaction to antibiotics is similar to that reported previously for chloramphenicol and oxytetracycline (Gross et al, 1981; Burrows, 1984; Burrows et al, 1988; Sangiah & Burrows, 1989) and erythromycin (Burrows et al, 1989).

Drug elimination is dependent upon several processes. In the case of antipyrine, elimination follows hepatic uptake, biotransformation and subsequent clearance in urine. Phase 1 metabolism is the rate limiting step in antipyrine elimination, which is independent of hepatic blood flow (Vesell, 1979). Thus, the lack of effect of ivermectin on disposition of antipyrine is likely reflective of an absence of effect on basic hepatic biotransformation pathways including cytochrome P-450. This may be further focused

specifically on hydroxylation pathways. Whereas antipyrine metabolism in man and laboratory animals results in several important metabolites, ie 3-hydroxymethyl-antipyrine, 4-hydroxyantipyrine and norantipyrine (Teunissen et al, 1983a; Teunissen et al, 1983b) only 4-hydroxyantipyrine is of significance in ruminants (Tufenkji et al, 1988). Ivermectin apparently does not affect this hydroxylation pathway. The N-demethylation pathway involved with biotransformation of antipyrine to norantipyrine is not an important pathway in calves.

Erythromycin is more useful as a model drug for the purpose of evaluating the N-demethylation pathway. In rats, erythromycin is taken up by hepatocytes, undergoes N-demethylation and is eliminated in bile, probably as conjugated metabolites (Lee et al, 1956). In addition, substantial amounts of active parent drug are also eliminated in bile and urine (Twiss et al, 1956; Wyman et al, 1968). Although these pathways have not been confirmed for cattle, there is little reason to believe they are less functional than in rats since N-demethylase activity for other substrates is quite comparable between cattle and rats (Smith et al, 1984; Watkins & Klaassen, 1986). Futhermore, liver disease limiting antipyrine disposition also limits erythromycin disposition (Burrows et al, 1992). Thus, it appears that neither the antipyrine hydroxylation pathway nor the erythromycin N-demethylation pathway is affected by ivermectin. However, in the case of erythromycin, an additional factor of importance is the role of active biliary secretion of both the parent compound and the metabolite. Nevertheless, the more important overall effects

indicate a lack of interaction.

Based on the present results and until further investigations are carried out, there is little reason for undue concern regarding the potential for ivermectin to affect disposition of other drugs with which it might be concurrently administered. However, to more conclusively establish a lack of effect by ivermectin on xenobiotic disposition, the pharmacokinetics of representative agents, subject to other biotransformation pathways such as deacylation, nitro reduction, and oxidation should be determined in the presence of ivermectin. Compounds of particular importance include anesthetics, antibiotics subject to biotransformation, steroidal hormones and various antiparasitic agents.

The results point out the limitations of using generalities to predict specific drug interactions. In this case, high lipid solubility with extensive biotransformstion and very long $t_{1/2\beta}$ led to the prediction that the disposition of other extensively biotransformed drugs would be affected. The lack of effect may be indicative of an absence of influence on biotransformation in general, however, erythromycin and antipyrine are representative of only two of several pathways. While it is probably reasonable to assume that results based on two divergent biotransformation and elimination pathways are indicative of an overall lack of disposition interaction potential, some caution is warranted. Because of the many different isozymes of cytochrome P-450, broad extrapolation may not be entirely justified.

In the present studies, the objective was to determine if ivermectin

influenced the disposition of antipyrine and erythromycin. The converse may also be an important consideration, particularly since erythromycin has been shown to inhibit and in a few cases induce hepatic biotransformation (Ludden, 1985; Sartori, 1985). These effects require prolonged exposure with repeated dosage longer than 5 days, in contrast to the present studies where erythromycin was given intermittently.

Table 1. A comparison of the mean ±SD or median ±mdev pharmacokinetic values for erythromycin (15mg/kg BW) i.v. using a two compartmental model following treatment with ivermectin (1 mg/5kg BW) i.m. (n=5)

Determinants	Treatments with ivermectin			
	before ivermectin	3 days after	5 days after	14 days after
A, μg/ml	52.89 ± 47.46	45.94 ± 14.68	65.42 ± 87.41	64.69 ± 34.97
B, μg/ml	10.40 ± 1.83	7.59 ± 4.57	5.26 ± 3.98	8.54 ± 6.83
α, h^{-1}	9.36 ± 8.25	10.31 ± 5.83	15.51 ± 12.89	18.30 ± 4.45
ß, h^{-1}	0.22 ± 0.06	0.24 ± 0.07	0.25 ± 0.10	0.13 ± 0.12
$t_{1/2\beta}$, h^{-1}*	2.94 ± 1.95	2.41 ± 0.81	2.98 ± 1.03	3.57 ± 2.88
C^0_p, μg/ml*	42.39 ± 28.24	47.98 ± 12.12	33.47 ± 46.71	89.30 ± 24.46
V_c, ml/kg*	353.84 ± 111.42	312.64 ± 54.79	448.18 ± 236.33	167.98 ± 126.31
K_{21}, h^{-1}	1.78 ± 1.05	1.53 ± 0.82	1.60 ± 0.68	2.00 ± 0.49
K_{el}, h^{-1}	1.23 ± 1.07	1.66 ± 0.51	2.42 ± 1.94	1.37 ± 0.88
K_{12}, h^{-1}	6.57 ± 6.79	7.36 ± 4.94	11.74 ± 10.67	15.08 ± 4.83
$V_{d(area)}$, ml/kg*	1244.50 ± 168.72	1431.11 ± 1265.01	3055.71 ± 1484.48	1789.50 ± 1790.02
$V_{d(ss)}$, ml/kg*	1074.74 ± 153.67	1288.45 ± 947.62	2499.22 ± 1239.77	1591.10 ± 1749.80
Cl_β, ml/h/kg*	285.89 ± 49.80	429.30 ± 144.67	709.87 ± 382.50**	347.18 ± 59.94

Median deviation determined by $\Sigma|x-m|/n$; *median values; **significantly different from the control ($p \leq 0.05$). Bagott, J.D. (1977)

Table 2. A comparison of the mean ± SD or median ± mdev pharmacokinetic values for antipyrine (20mg/kg BW) i.v. using a two compartmental model following treatment with ivermectin (1mg/5kg BW) i.m. (n = 6)

	Treaments with ivermectin		
Determinants	before ivermectin	3 days after	17 days after
A, μg/ml	51.00 ± 17.28	76.25 ± 35.20	63.22 ± 25.13
B, μg/ml	28.49 ± 10.67	30.58 ± 5.97	29.07 ± 9.79
α, h^{-1}	11.24 ± 8.94	9.70 ± 7.25	12.10 ± 6.68
ß, h^{-1}	0.27 ± 0.08	0.25 ± 0.03	0.29 ± 0.09
$t_{1/2ß}$, h^{-1}*	2.56 ± 0.63	2.84 ± 0.27	2.54 ± 0.66
$C°_p$, μg/ml*	76.38 ± 17.84	93.93 ± 19.33	106.10 ± 24.28
V_c, ml/kg*	263.22 ± 66.63	213.17 ± 30.71	188.82 ± 77.83
K_{21}, h^{-1}	4.18 ± 3.36	2.72 ± 0.94	3.86 ± 1.85
K_{el}, h^{-1}	0.74 ± 0.24	0.82 ± 0.31	0.91 ± 0.43
K_{12}, h^{-1}	6.60 ± 5.71	6.40 ± 6.24	7.62 ± 4.68
$V_{d(area)}$, ml/kg*	679.51 ± 211.87	634.88 ± 80.67	615.33 ± 201.52
$V_{d(ss)}$, ml/kg*	618.36 ± 212.46	597.45 ± 72.79	574.81 ± 187.7
$Cl_ß$, ml/h/kg*	175.22 ± 45.11	156.87 ± 15.39	198.66 ± 72.93

*median values; median deviation determined by $\Sigma|x-m|/n$; Bagott, J.D. (1977)

References

Anadon, A. (1982) Enzyme induction and inhibition. Eds Ruckebusch, Y., Toutain, P.L., & Koritz, G.D.; In *Veterinary Pharmacology and Toxicology*. AVI Publishing Company, Westport.

Baggot, J.D. (1977) *Principles of drug disposition in domestic animals: The basis of veterinary clinical pharmacology*. WB Saunders Company, Philadelphia.

Baggot, J.D. & Gingerich, D.A. (1976) Pharmacokinetic interpretation of erythromycin and tylosin activity in serum after intravenous administration of a single dose to cows. *Research in Veterinary Science*, **21**, 318-323.

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.

Bourne, D.W. (1986) Multi-forte, a microcomputer program for modeling and simulation of pharmacokinetic data. *Computer Methods and Programs in Biomedicine*, **23**, 277-281.

Burrows, G.E. (1980) Pharmacotherapeutics of macrolides, lincomycins and spectinomycin. *Journal of American Veterinary Medicine Association*, **176**, 1072-1077.

Burrows, G.E., Tyler, R.D., Craigmill, A.L. & Barto, P.B. (1984) Chloramphenicol and the neonatal calf. *American Journal of Veterinary Research*, **45**, 1586-1591.

Burrows, G.E. (1985) Effects of experimentally induced *Pasteurella haemolytica* pneumonia on the pharmacokinetics of erythromycin in the calf. *American Journal of Veterinary Research*, **46**, 798-803.

Burrows, G.E., Tyler, R.D., Sangiah, S. & Keeton, R.D. (1988) Experimental chloramphenicol intoxication in neonatal calves: Intravenous administration. *Research in Veterinary Science*, **45**, 101-106.

Burrows, G.E., Griffin, D.D., Pippin, A. & Harris, K. (1989) A comparison of the various routes of administration of erythromycin in cattle. *Journal of Veterinary Pharmacology and Therapeutics*, **12**, 289-295.

Burrows, G.E., Tasler, J., Boray, J.C. & Egerton, J.R. (1992) Antipyrine, erythromycin and oxytetracycline disposition in experimental fasciolosis. *Research in Veterinary Science*, in press.

Campbell, W.C. Fisher, M.H., Stapley, E.O., Albers-Schönberg, G. & Jacob, T.A. (1983) C: A potent new antiparasitic agent. *Science*, **221**, 823-828.

Chiu, S-H.L., Carlin, J.R., Taub, R. *et al.* (1988) Comparative metabolic disposition of ivemectin in fat tissues of cattle, sheep and rats. *Drug Metabolism and Disposition*, **16**, 728-736.

Chiu, S-H.L., Green, M.L., Baylis, F.P. *et al.* (1990) Absorption, tissue distribution, and excretion of tritium-labeled C in cattle, sheep, and rat. *Journal of Agricultural and Food Chemistry*, **38**, 2072-2078.

Clarke, C.R., Short, C.R., Hsu, R-C. & Baggot, J.D. (1985) Pharmacokinetics of gentamicin in the calf: Developmental changes. *American Journal of Veterinary Research*, **46**, 2461-2466.

Conney, A.H. (1967) Pharmacological implications of microsomal enzyme induction. *Pharmacology Reviews*, **19**, 317-366.

Conover, W.J. & Iman, R.L. (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician*, **35**, 124-130.

Gross, D.R., Dodd, K.T., Williams, J.D. & Adams, H.R. (1981) Adverse cardiovascular effects of oxytetracycline preparations and vehicles in intact awake calves. *American Journal of Veterinary Research*, **42**, 1371-1377.

Hayes, J.R. & Borzelleca, J.F. (1985) Nutrient interaction with drugs and other xenobiotics. *Journal of the American Dietetic Association*, **85**, 335-339.

Lee, C.C, Anderson, R.C. & Chen, K.K. (1956) The excretory products of N-methyl-^{14}C-erythromycin in rats. *Journal of Pharmacology and Experimental Therapeutics*, **117**, 274-280.

Ludden, T.M. (1985) Pharmacokinetic interaction of the macrolide antibiotics. *Clinical Pharmacokinetics*, **10**, 63-79.

Sangiah, S. & Burrows, G.E. (1989) Hypotension produced by rapid intravenous administration of chloramphenicol in anesthetized dogs. *Research in Veterinary Science*, **46**, 62-67.

Sartori, E., Delaforge, M., Mansuy, D. & Beaune, P. (1985) Some erythromycin derivatives are strong inducers in rats of a cytochrome P-450 very similar to that induced by 16 α-pregnenolone carbonitrile. *Biochemical and Biophysical Research Communications*, **128**, 1434-1439.

Smith, G.S., Watkins, J.B., Thompson, T.N., Rozman, K. & Klaassen, C.D. (1984) Oxidative and conjugative metabolism of xenobiotics by livers of cattle, sheep, swine and rats. *Journal of Animal Science*, **58**, 386-395.

Steel, R.G.D. & Torrie, J.H. (1980) *Principles and procedures of statistics: A biometrical approach.* 2nd ed. McGraw-Hill Book Company, New York.

Teunissen, M.W.E., Meerburg-Van Der Torren, J.E., Vermeulen, N.P.E. & Breimer, D.D. (1983a) Automated high-performance liquid chromatographic determination of antipyrine and its main metabolites in plasma, saliva and urine, including 4,4'-dihydroxyantipyrine. *Journal of Chromatography*, **278**, 367-378.

Teunissen, M.W.E., Joeres, R.P., Vermeulen, N.P.E. & Breimer, D.D. (1983b) Influence of 9-hydroxyellipticine and 3-methylcholanthrene treatment on antipyrine metabolite formation in rats *in vivo*. *Xenobiotica*, **13**, 223-231.

Tufenkji, A.E., Alvinerie, M., Pineau, T., Boulard, C. & Galtier, P. (1988) Incidence of a subclinical fascioliasis on antipyrine clearance and metabolite excretion in sheep. *Xenobiotica*, **18**, 357-364.

Twiss, J.R., Berger, W.V., Gillette, L., Aronson, A.R. & Siegel, L. (1956) The biliary excretion of erythromycin. *Surgery, Gynecology & Obstetrics*, **26**, 355-357.

Vesell, E.S. (1979) The antipyrine test in clinical pharmacology: Conceptions and misconceptions. *Clinical Pharamcology and Therapeutics*, **26**, 275-286.

Watkins, J.B. & Klaassen, C.D. (1986) Xenobiotic biotransformation in livestock: Comparison to other species commonly used in toxicity testing. *Journal of Animal Science*, **63**, 933-942.

Wyman, J.B., Bartholomew, L.G., Karlson, A.G., Wakim, K.G. & Cain, J.C. (1968) Pancreatic, hepatic, and renal excretion of antibiotics in dogs. *Archives of Surgery*, **97**, 46-50.

CHAPTER III

SUMMARY AND CONCLUSIONS

The parmacokinetic values for erythromycin and antipyrine are consistent with published values (Baggot & Gingerich, 1976; Burrows, 1985; Burrows et al, 1989; Tufenkji et al, 1988) and in general were not affected by ivermectin. The only significant difference noted was for erythromycin total body clearance on day 4. However, this apparent increase in Cl_{β} may not be meaningful. The slope of the elimination phase (ß) and volume of distribution (V_d), which are used in the determination of the clearance rate, were apparently increased on day 4 but the changes were not significant. An additional factor contributing to the difference in clearance rate may be the change in the method of administration of erythromycin. Erythromycin given for baseline determination and on day 2, was administered as an i.v. bolus while on days 4 and 13 it was diluted 1:1 with saline and administered in small increments over one minute. Since the erythromycin was administered slowly on day 4, the distribution phase may have been lengthened accordingly, thereby producing an apparent increase in the volume of distribution (V_d), and thus making the elimation rate appear to be increased. The combination of these effects might lead to an apparent significant increase in the clearance rate (Cl_{β}). The change in the method of erythromycin administration was made in an attempt to prevent the

peracute systemic reactions observed. The calves showed signs of dyspnea, polypnea, and tremors immediately after rapid i.v. administration of the concentrated preparation. The dilution of erythromycin and slow administration in small increments alleviated the reaction and the calves showed no adverse signs when this method was employed on days 4 and 13. This acute reaction to antibiotics is similar to that reported previously for chloramphenicol and oxytetracycline (Gross et al, 1981; Burrows, 1984; Burrows et al, 1988; Sangiah & Burrows, 1989) and erythromycin (Burrows et al, 1989).

Drug elimination is dependent upon several processes. In the case of antipyrine, elimination follows hepatic uptake, biotransformation and subsequent clearance in urine. Phase 1 metabolism is the rate limiting step in antipyrine elimination, which is independent of hepatic blood flow (Vesell, 1979). Thus, the lack of effect of ivermectin on disposition of antipyrine is likely reflective of an absence of effect on basic hepatic biotransformation pathways including cytochrome P-450. This may be further focused specifically on hydroxylation pathways. Whereas antipyrine metabolism in man and laboratory animals results in several important metabolites, ie 3-hydroxymethyl-antipyrine, 4-hydroxyantipyrine and norantipyrine (Teunissen et al, 1983a; Teunissen et al, 1983b) only 4-hydroxyantipyrine is of significance in ruminants (Tufenkji et al, 1988). Ivermectin apparently does not affect this hydroxylation pathway. The N-demethylation pathway involved with biotransformation of antipyrine to norantipyrine is not an important pathway in calves.

Erythromycin is more useful as a model drug for the purpose of evaluating the N-demethylation pathway. In rats, erythromycin is taken up by hepatocytes, undergoes N-demethylation and is eliminated in bile, probably as conjugated metabolites (Lee et al, 1956). In addition, substantial amounts of active parent drug are also eliminated in bile and urine (Twiss et al, 1956; Wyman et al, 1968). Although these pathways have not been confirmed for cattle, there is little reason to believe they are less functional than in rats since N-demethylase activity for other substrates is quite comparable between cattle and rats (Smith et al, 1984; Watkins & Klaassen, 1986). Futhermore, liver disease limiting antipyrine disposition also limits erythromycin disposition (Burrows et al, 1992). Thus, it appears that neither the antipyrine hydroxylation pathway nor the erythromycin N-demethylation pathway is affected by ivermectin. However, in the case of erythromycin, an additional factor of importance is the role of active biliary secretion of both the parent compound and the metabolite. Nevertheless, the more important overall effects indicate a lack of interaction.

Based on the present results and until further investigations are carried out, there is little reason for undue concern regarding the potential for ivermectin to affect disposition of other drugs with which it might be concurrently administered. However, to more conclusively establish a lack of effect by ivermectin on xenobiotic disposition, the pharmacokinetics of representative agents, subject to other biotransformation pathways such as deacylation, nitro reduction, and oxidation should be determined in the presence of ivermectin. Compounds of particular importance include anesthetics,

antibiotics subject to biotransformation, steroidal hormones and various antiparasitic agents.

The results point out the limitations of using generalities to predict specific drug interactions. In this case, high lipid solubility with extensive biotransformstion and very long $t_{1/2\beta}$ led to the prediction that the disposition of other extensively biotransformed drugs would be affected. The lack of effect may be indicative of an absence of influence on biotransformation in general, however, erythromycin and antipyrine are representative of only two of several pathways. While it is probably reasonable to assume that results based on two divergent biotransformation and elimination pathways are indicative of an overall lack of disposition interaction potential, some caution is warranted. Because of the many different isozymes of cytochrome P-450, broad extrapolation may not be entirely justified.

In the present studies, the objective was to determine if ivermectin influenced the disposition of antipyrine and erythromycin. The converse may also be an important consideration, particularly since erythromycin has been shown to inhibit and in a few cases induce hepatic biotransformation (Ludden, 1985; Sartori, 1985). These effects require prolonged exposure with repeated dosage longer than 5 days, in contrast to the present studies where erythromycin was given intermittently.

References

Baggot, J.D. & Gingerich, D.A. (1976) Pharmacokinetic interpretation of erythromycin and tylosin activity in serum after intravenous administration of a single dose to cows. *Research in Veterinary Science*, **21**, 318-323.

Burrows, G.E., Tyler, R.D., Craigmill, A.L. & Barto, P.B. (1984) Chloramphenicol and the neonatal calf. *American Journal of Veterinary Research*, **45**, 1586-1591.

Burrows, G.E. (1985) Effects of experimentally induced *Pasteurella haemolytica* pneumonia on the pharmacokinetics of erythromycin in the calf. *American Journal of Veterinary Research*, **46**, 798-803.

Burrows, G.E., Tyler, R.D., Sangiah, S. & Keeton, R.D. (1988) Experimental chloramphenicol intoxication in neonatal calves: Intravenous administration. *Research in Veterinary Science*, **45**, 101-106.

Burrows, G.E., Griffin, D.D., Pippin, A. & Harris, K. (1989) A comparison of the various routes of administration of erythromycin in cattle. *Journal of Veterinary Pharmacology and Therapeutics*, **12**, 289-295.

Burrows, G.E., Tasler, J., Boray, J.C. & Egerton, J.R. (1992) Antipyrine, erythromycin and oxytetracycline disposition in experimental fasciolosis. *Research in Veterinary Science*, in press.

Gross, D.R., Dodd, K.T., Williams, J.D. & Adams, H.R. (1981) Adverse cardiovascular effects of oxytetracycline preparations and vehicles in intact awake calves. *American Journal of Veterinary Research*, **42**, 1371-1377.

Lee, C.C, Anderson, R.C. & Chen, K.K. (1956) The excretory products of N-methyl-^{14}C-erythromycin in rats. *Journal of Pharmacology and Experimental Therapeutics*, **117**, 274-280.

Ludden, T.M. (1985) Pharmacokinetic interaction of the macrolide antibiotics. *Clinical Pharmacokinetics*, **10**, 63-79.

Sangiah, S. & Burrows, G.E. (1989) Hypotension produced by rapid intravenous administration of chloramphenicol in anesthetized dogs. *Research in Veterinary Science*, **46**, 62-67.

Sartori, E., Delaforge, M., Mansuy, D. & Beaune, P. (1985) Some erythromycin derivatives are strong inducers in rats of a cytochrome P-450 very similar to that induced by 16 α-pregnenolone carbonitrile. *Biochemical and Biophysical Research Communications*, **128**, 1434-1439.

Smith, G.S., Watkins, J.B., Thompson, T.N., Rozman, K. & Klaassen, C.D. (1984) Oxidative and conjugative metabolism of xenobiotics by livers of cattle, sheep, swine and rats. *Journal of Animal Science*, **58**, 386-395.

Teunissen, M.W.E., Meerburg-Van Der Torren, J.E., Vermeulen, N.P.E. & Breimer, D.D. (1983a) Automated high-performance liquid chromatographic determination of antipyrine and its main metabolites in plasma, saliva and urine, including 4,4'-dihydroxyantipyrine. *Journal of Chromatography*, **278**, 367-378.

Teunissen, M.W.E., Joeres, R.P., Vermeulen, N.P.E. & Breimer, D.D. (1983b) Influence of 9-hydroxyellipticine and 3-methylcholanthrene treatment on antipyrine metabolite formation in rats *in vivo*. *Xenobiotica*, **13**, 223-231.

Tufenkji, A.E., Alvinerie, M., Pineau, T., Boulard, C. & Galtier, P. (1988) Incidence of a subclinical fascioliasis on antipyrine clearance and metabolite excretion in sheep. *Xenobiotica*, **18**, 357-364.

Twiss, J.R., Berger, W.V., Gillette, L., Aronson, A.R. & Siegel, L. (1956) The biliary excretion of erythromycin. *Surgery, Gynecology & Obstetrics*, **26**, 355-357.

Vesell, E.S. (1979) The antipyrine test in clinical pharmacology: Conceptions and misconceptions. *Clinical Pharamcology and Therapeutics*, **26**, 275-286.

Watkins, J.B. & Klaassen, C.D. (1986) Xenobiotic biotransformation in livestock: Comparison to other species commonly used in toxicity testing. *Journal of Animal Science*, **63**, 933-942.

Wyman, J.B., Bartholomew, L.G., Karlson, A.G., Wakim, K.G. & Cain, J.C. (1968) Pancreatic, hepatic, and renal excretion of antibiotics in dogs. *Archives of Surgery*, **97**, 46-50.

Appendix

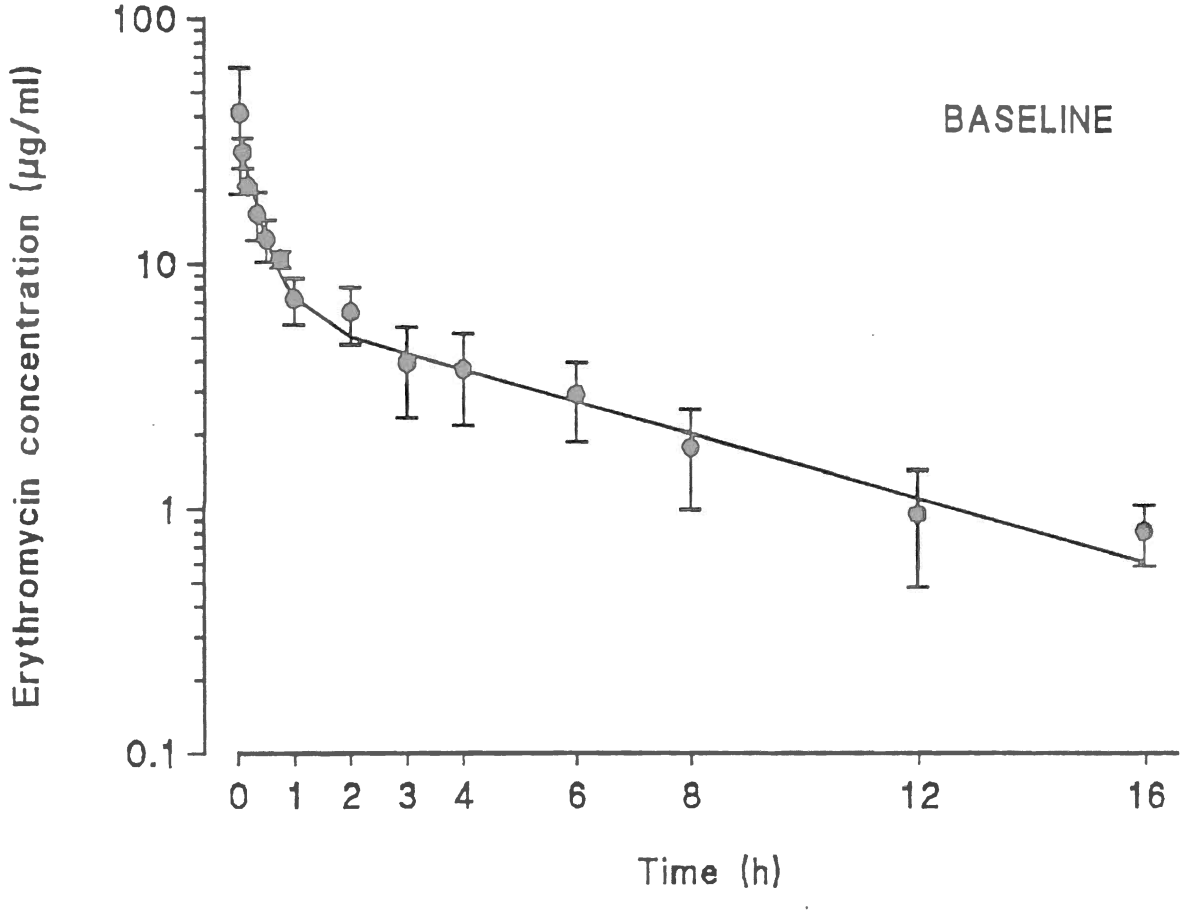


Figure 1. Computer generated curves of erythromycin (15 mg/kg) i.v. versus time for baseline. Points represent mean observed ± SD values in cattle.

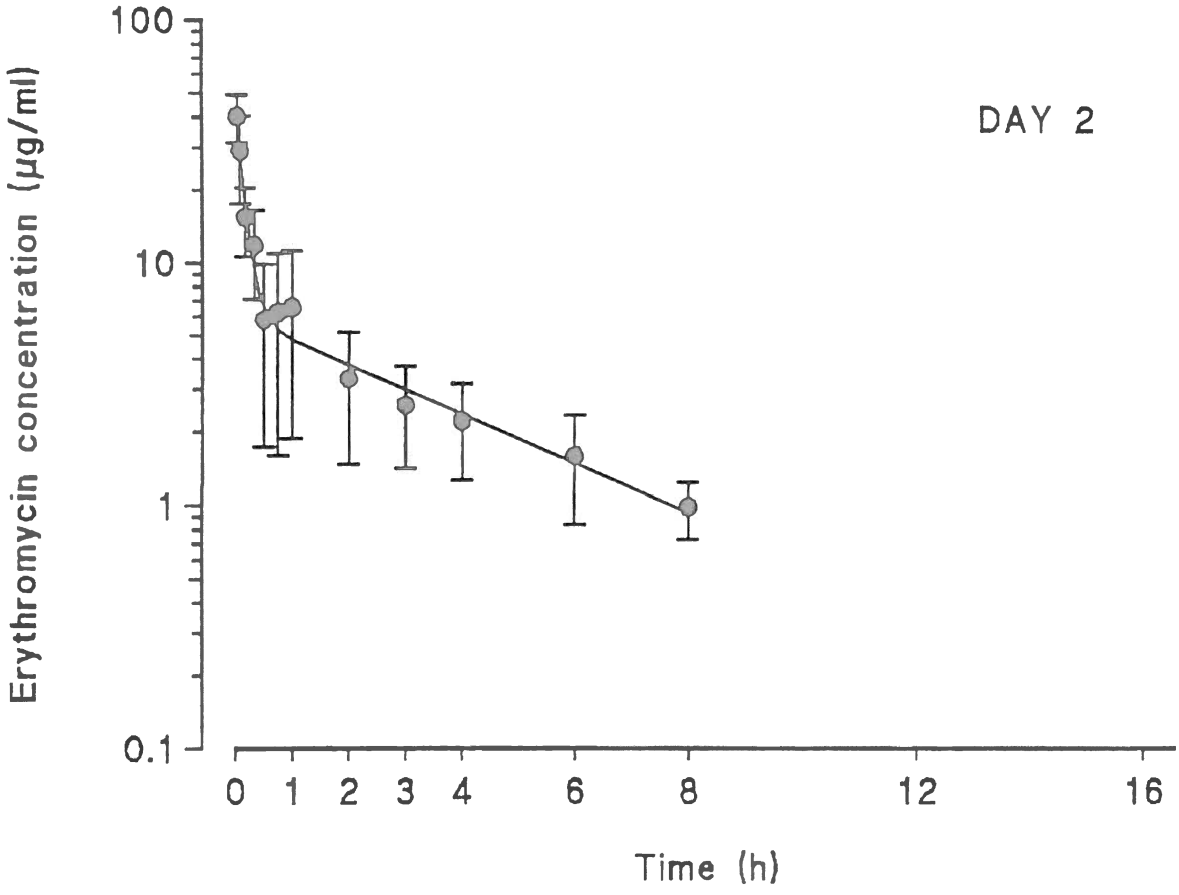


Figure 2. Computer generated curves of erythromycin (15 mg/kg) i.v. versus time for day 2. Points represent mean observed ± SD values in cattle.

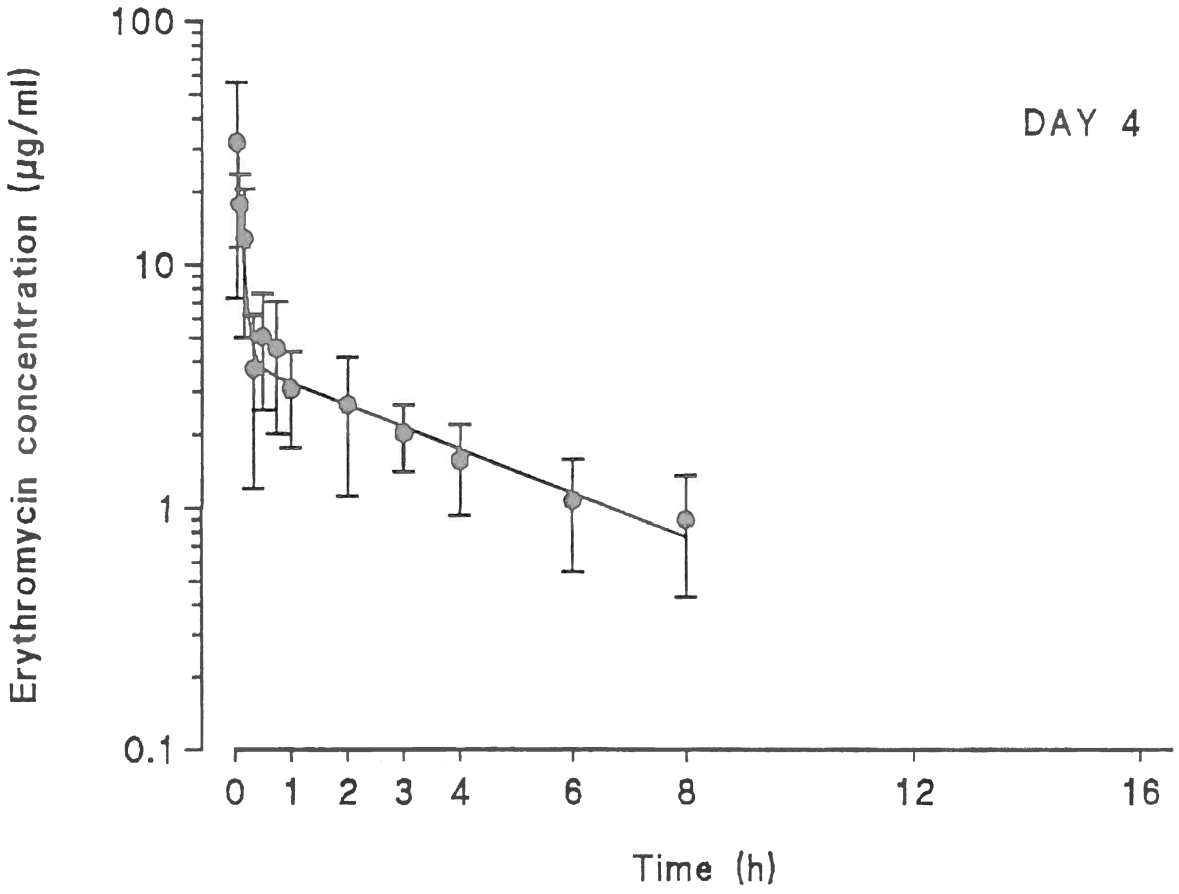


Figure 3. Computer generated curves of erythromycin (15 mg/kg) i.v. versus time for day 4. Points represent mean observed ± SD values in cattle.

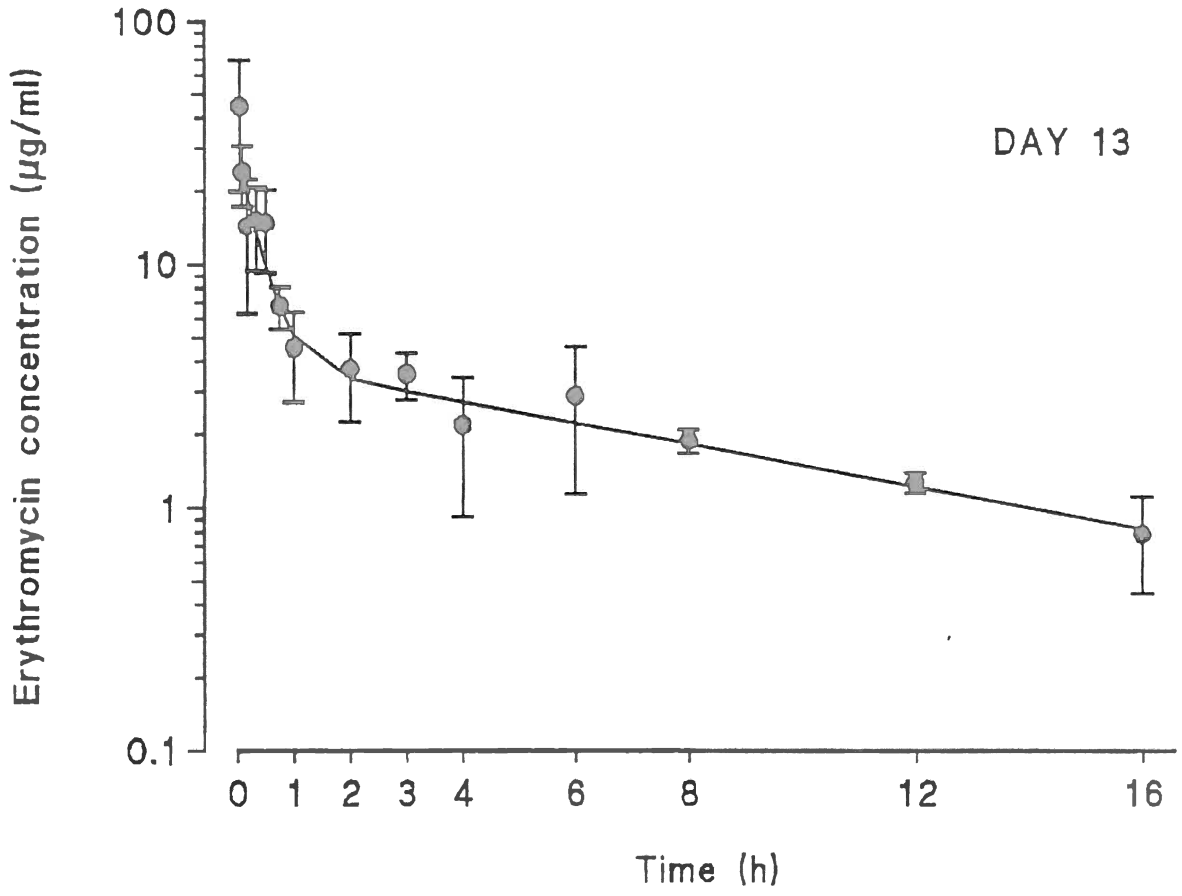


Figure 4. Computer generated curves of erythromycin (15 mg/kg) i.v. versus time for day 13. Points represent mean observed ± SD values in cattle.

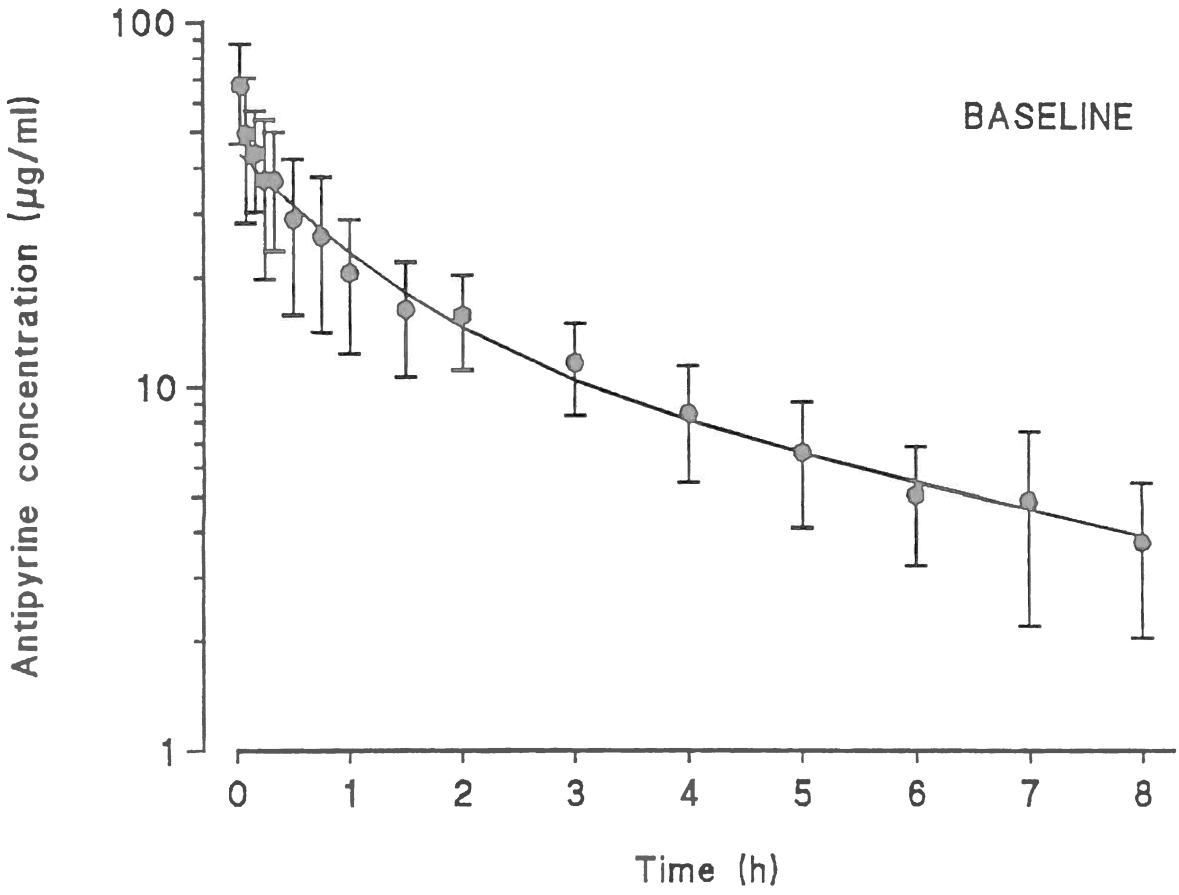


Figure 5. Computer generated curves of antipyrine (20 mg/kg) i.v. versus time for baseline. Points represent mean observed ± SD values in cattle.

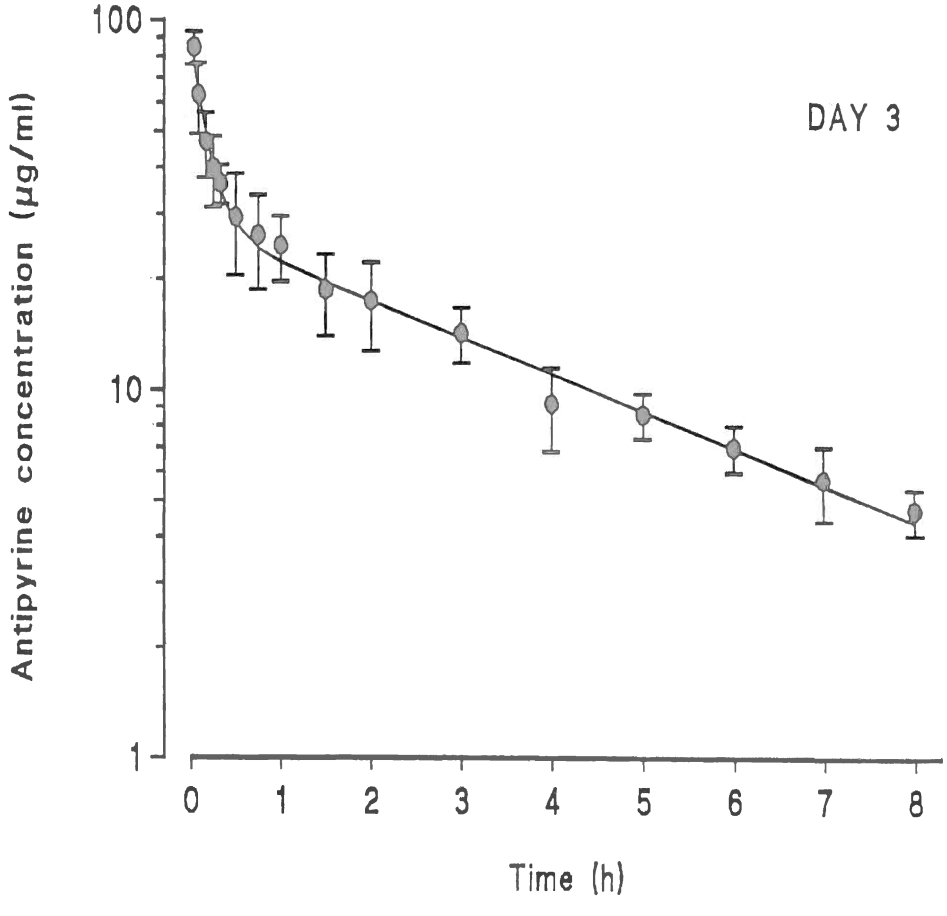


Figure 6. Computer generated curves of antipyrine (20 mg/kg) i.v. versus time for day 3. Points represent mean observed ± SD values in cattle.

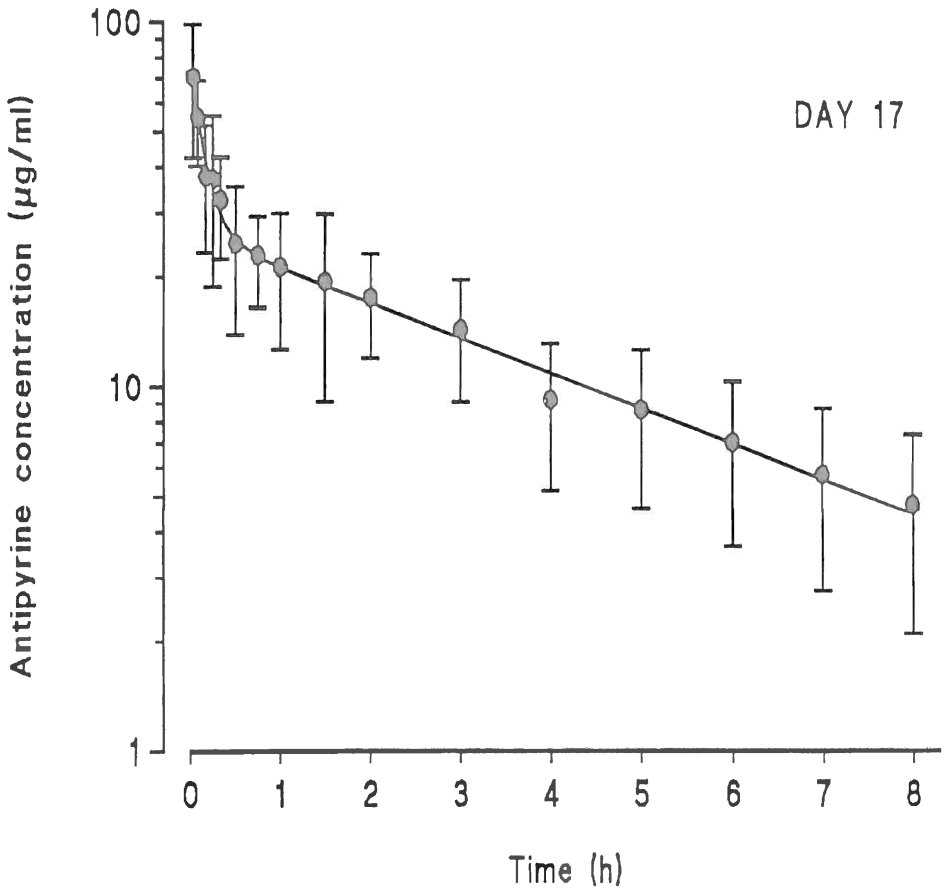


Figure 7. Computer generated curves of antipyrine (20 mg/kg) i.v. versus time for day 17. Points represent mean observed ± SD values in cattle.

VITA

Deanna Marie Bohlen

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF IVERMECTIN ON ANTIPYRINE AND ERYTHROMYCIN DISPOSITION IN CATTLE

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Sumner, Iowa, November 15, 1963, the daughter of Dr. David H. Bohlen and the late Marjorie M.R. Bohlen.

Education: Graduated from Alva High School, Alva, Oklahoma,in May, 1982; received Bachelor of Science Degree in Biology from Northwestern Oklahoma State University in May, 1986; completed requirements for the Master of Science degree at Oklahoma State University in May 1993.

Professional Experience: Technician-Receptionist, Alva Veterinary Clinic, Alva, Oklahoma, May, 1983 to August, 1986; Instructor, Department of Chemistry, Northwestern Oklahoma State University, January, 1990 to May, 1990; Instructor, Department of Nursing, Northwestern Oklahoma State University, June, 1990 to August, 1990; Laboratory Manager, Department of Animal Science, Oklahoma State University, January, 1989 to May, 1992; Sales Representative, Fisher Scientific Company, May 1992 to present.