A comparison of intramammary and intravenous routes of administration of pencillin and the effects of Dimethyl sulfoxide (DMSO) on the distribution of pencillin by both administration routes

Milton W. Orr

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I am submitting herewith a thesis written by Milton W. Orr entitled "A comparison of intramammary and intravenous routes of administration of penicillin and the effects of Dimethyl sulfoxide (DMSO) on the distribution of penicillin by both administration routes." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

B.R. Bell, Major Professor

We have read this thesis and recommend its acceptance:

Robert Walker, Fred Hopkins, M.J. Montgomery

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
To the Graduate Council:

I am submitting herewith a thesis written by Milton W. Orr entitled "A Comparison of Intramammary and Intravenous Routes of Administration of Penicillin and the Effects of Dimethyl Sulfoxide (DMSO) on the Distribution of Penicillin by Both Administration Routes." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

B. R. Bell, Major Professor

We have read this thesis and recommend its acceptance:

[Signatures]

Accepted for the Council:

The Graduate School
A COMPARISON OF INTRAMAMMARY AND INTRAVENOUS ROUTES OF ADMINISTRATION OF PENICILLIN AND THE EFFECTS OF DIMETHYL SULFOXIDE (DMSO) ON THE DISTRIBUTION OF PENICILLIN BY BOTH ADMINISTRATION ROUTES

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Milton W. Orr
August 1984
Sincere appreciation is expressed to Dr. B. R. Bell, faculty advisor and committee chairman, for his guidance in structuring of the thesis and analysis of data. Also, thanks to Dr. Bell for his patience during the writing of the thesis.

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ABSTRACT

The distribution of Penicillin G Potassium administered intra-mammarily (IMM) and intravenously (IV) and the effects Dimethyl sulfoxide (DMSO) has on this distribution was investigated. In this study, cows were divided into four treatment groups. Cows in treatment group one were given penicillin in the right front quarter while cows in treatment group two were given penicillin plus DMSO in the right front quarter. Cows in treatment group three were given penicillin IV and cows in treatment group four were given penicillin plus DMSO IV. Data were obtained from analysis of milk and serum samples collected at 0, 30, 60, 90, 120, 240, 360, and 480 minutes following treatment. Differences in concentration of penicillin in serum and in milk from each quarter were the primary measurement responses.

The use of DMSO with IMM treatments did not significantly affect the concentration of penicillin in the milk. However, when DMSO was added with the IV administration of penicillin, there was a faster rate of removal of penicillin from the serum with a slight increase in penicillin in the milk samples. This increase in milk levels of penicillin was not highly significant. Since in each treatment group involving the use of DMSO increases in concentration of penicillin in milk were slight, it would be difficult to advise the use of DMSO in clinical treatment of mastitis until additional data is available.
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CHAPTER I

INTRODUCTION

The first step in any mastitis control program is to recognize the seriousness of the disease and its associated economic losses. The National Mastitis Council estimates losses from reduced milk production alone at one billion dollars annually in the U.S. (Philpot, 60). Because of these losses, there is considerable economic incentive to investigate products that may enhance the effectiveness of drugs commonly used to treat mastitis. Presently there are many drugs used alone or in combinations for controlling and treating mastitis. Bishop et al. (4) found penicillin to be the most commonly used drug for treating mastitis. However, because penicillin has been so commonly used, many mastitis pathogens have become resistant to penicillin therapy (Bishop et al., 4).

A major consideration in determining an appropriate drug to treat mastitis is the route of administration. Appropriate routes include local, intramammary (IMM) and systemic (intravenous and intramuscular). The advantages of IMM treatment include higher levels of antibiotic at the site of infection, the use of potentially effective drugs that might be toxic if given systemically, and medication at the site of infection to minimize disruption of natural flora at other body sites. The disadvantage, however, is the risk of bacterial contamination of the udder with resistant bacteria by improper treatment procedures. The advantages of systemic treatment include treatment of the whole
animal to prevent systemic colonization by those organisms that might spread from the udder via the vascular system, and less risk of bacterial contamination of the udder by improper sanitation procedures during treatment.

Systemic therapy is usually the treatment of choice in subclinical cases of mastitis (University of Kentucky, 77). However, systemic treatment is usually more difficult to perform and most dairymen prefer IMM treatment to save labor and time and to provide an increased level of antibiotic for a longer period of time. In severe cases of mastitis, both IMM and systemic treatments are used.

Agents that enhance the effectiveness of antibiotics used in the treatment of mastitis have received considerable attention. One such agent is Dimethyl Sulfoxide (DMSO). DMSO has been shown to penetrate into all body sites rapidly. Because of this, many people are following the practice of adding DMSO to mastitis treatments.

This study examines the different responses between IMM and IV routes of administration of penicillin and the effects DMSO has on penicillin concentrations in serum and milk.
CHAPTER II

REVIEW OF LITERATURE

Mastitis has long been recognized as one of the major problems of the dairy industry. Mastitis, both clinical and subclinical, account for the greatest economic loss in the dairy industry (Philpot, 60).

When considering both clinical and subclinical mastitis, it becomes apparent that the economic losses are not easy to recognize. According to Philpot (60), 14% of economic losses attributed to mastitis are due to death and culling, 8% due to discarded milk, 8% due to treatment costs, and 70% due to decreased milk production. Much of the 70% loss due to decreased production is attributed to subclinical mastitis. Janzen (36) and Dobbins (15) agree with Philpot's estimation of losses due to mastitis but also included losses in milk composition, veterinary fees, and added labor in their figures. Gilmore (21) placed a cost of $31.40 per year per cow on loss in productivity due to mastitis. Natzke et al. (51) found that in a lactating cow milk production may be reduced by 10-12% if she has a single infected quarter. He put this loss of productivity plus cost of treatment and culling at 700 Kg of milk per cow per year in an average herd (Natzke, 52). Although the losses attributed to mastitis are significant, Janzen (36) and Dobbins (15) surmised that the economic losses of mastitis were less than the cost of replacing affected animals.
There are many variables associated with mastitis outbreaks. Dodd and Neave (17) found that cows which milk out faster were more susceptible to mastitis indicating that milking time may be a factor in mastitis susceptibility. Cows which milk out faster were found to have more relaxed teat sphincters. This could be an indirect cause of mastitis. Mahle and others (46), by infecting cows with *Staphylococcus aureus* immediately following milking, found an increase in the numbers of infected quarters. They postulated that at this time the teat sphincters were relaxed and allowed the entry of the organism into the teat canal.

Many other factors may affect the incidences of mastitis such as poor environment, poor sanitation, poor detection procedures, and inappropriate treatment. Funk and others (19) found an increase in mastitis susceptibility as age increased. Gonyon and others (23) found that resistance and susceptibility to mastitis in the bovine were not highly heritable.

**I. CAUSATIVE ORGANISMS**

There are many microorganisms that can cause mastitis. The most prevalent of these are *S. aureus*, various *Streptococcus* spp., and certain *coli*form organisms, such as *Escherichia coli* and *Klebsiella pneumoiaie*.

*Staphylococcus aureus* may be present as part of the normal flora of the skin of the cow. This organism can colonize the teat canal. In doing so, scar tissue is formed which can then hinder the action of
drugs used in treatment (Philpot, 60). Dodd (16) states that Staphylococcus spp. infections are best eliminated by control rather than treatment. Control involves proper sanitation and milking procedures.

The most common Streptococcus spp. associated with mastitis include Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis. Streptococcus agalactiae can be found in the air, bedding, milking equipment and on the milker's hands. Mastitis due to this organism generally occurs as a result of contact with contaminated milk (Philpot, 60). Streptococcus dysgalactiae usually emanates from the mouth of carrier animals but may also be isolated from skin and udder lesions (Philpot, 60). Of the streptococci causing mastitis, Philpot (60) found Streptococcus uberis to be the causative organism for most of the infections occurring during the dry period and prior to first calving. S. agalactiae and S. dysgalactiae can be eliminated by effective control measures (Dodd, 16). However, S. uberis is harder to control and to eliminate (Dodd, 16).

Coliform bacteria such as Escherichia coli, Klebsiella pneumoniae, and Enterobacter aerogenes are usually responsible for a small percentage of the cases of mastitis. Coliform mastitis does not respond favorably to control measures (Dodd, 16). These organisms are commonly found in manure, soil, sawdust bedding, and on the skin around the udder.

Controlling mastitis presents a serious problem for the average dairyman. The clinical cases are easier to detect and usually command immediate attention and treatment. However, the clinical cases represent only 30% of the economic losses to dairymen (Philpot, 60). The
subclinical cases of mastitis account for the largest loss to dairymen (70%) and are the most difficult to detect (milk may seem normal in gross appearance) and treat (Philpot, 60). For the control of mastitis, a five-step program has been proposed (Philpot, 60). The program includes: 1) use of functionally adequate milking equipment, 2) dip teats in disinfectant after each milking, 3) promptly treat all clinical cases of mastitis, 4) treat each quarter of each cow at drying off, and 5) cull cows unresponsive to treatment. Dodd (16), in a symposium on mastitis control, found hygiene, antimicrobial therapy, milking machine maintenance, and housing to be important factors in mastitis control. Smith (70) agrees with Dodd that housing as well as postmilking disinfection of teats are invaluable management tools in mastitis control. Philpot and Dodd agree that to achieve any control over mastitis all methods of control must receive direct attention.

II. MASTITIS PREVENTION

Mastitis prevention requires proper environmental conditions, proper milking equipment maintenance and cleaning, teat dipping, dry cow treatment and treatment of clinical mastitis. Since reference to environmental conditions and milking equipment maintenance was made earlier, dry cow treatment and teat dipping will be the focus of this section.

Philpot (60) has suggested that every quarter of every cow be treated with a recommended dry cow treatment at the time of drying off.
This is in agreement with Kingwill (39) and Meek et al. (48), who demonstrated a 70% reduction in the incidence of mastitis over a three-year period using a program of dry cow treatment and teat dipping. Funk et al. (19), in a study comparing five dry cow therapies, found that more cows of the untreated control group exhibited mastitis in one or more quarters than the treated group. Funk et al. (19) further demonstrated that a combination of dry cow treatment and teat dipping were far superior to dry cow treatment only or teat dipping only in mastitis prevention. Pankey et al. (55), comparing three dry cow treatments using S. aureus to experimentally induce mastitis, found that a high persistency product at drying off and a low persistency product one to three days prepartum was equal in efficacy to a high persistency product administered at drying off. A low persistency product one to three days prepartum was not as efficient as the high persistency product. This supports a previous report by Pankey et al. (56) in which two dry cow treatments were compared to untreated controls. The spontaneous recovery rate of the control groups was about 50% that of the treated animals.

III. PENICILLIN IN MASTITIS THERAPY

There are numerous antibiotics available that have been used in the treatment of bovine mastitis. The most commonly used antibiotic is penicillin (Bishop et al., 4).

History

Penicillin is a naturally occurring antibacterial agent derived from the mold Penicillium notatum. Its antimicrobial properties were
first observed in 1928. Its first use in a clinical setting occurred in 1941. Since that time it has been used extensively in the treatment of infectious disease processes of bacterial etiology (Bentley, 3).

Bacterial Resistance

Penicillin has been so commonly used that many bacterial species have developed resistance to the drug (Bishop et al., 4). The mechanism of resistance in most instances is in the production of penicillinase, an enzyme which destroys the antibacterial activity of penicillin by cleaving its Beta-lactam ring.

Mechanism of Action

The mechanism of action of penicillin against susceptible bacteria is inhibition of cell wall synthesis during bacterial replication. As mammalian cells lack cell walls, penicillins are essentially non toxic to them (Oliver, 53).

Routes of Administration

Effective antimicrobial therapy depends on the route of administration and frequency and absorption of the drug used. There are two major routes of administration of antibiotics for the treatment of mastitis. These are local (IMM) and systemic (IV or intramuscular). Local treatment provides higher levels of antibiotic in mammary tissue than systemic treatment (Ziv, 82). In addition, it allows the use of formulations not approved for systemic treatment (Bishop et al., 4).
Factors affecting the efficiency of local treatment include the amount of milk produced (dilution factor), frequency of treatment, presence of scar tissue in the udder, and the chemical properties of the antimicrobial agent and its carrier (Stang, 73). Because of the potential for pooling of the antibiotic within the milk infrequent administration could result in subtherapeutic levels post-milking. To maintain therapeutic levels of a drug requires the administration of the antibiotic at recommended intervals. However, at each treatment caution must be exercised to prevent entry of resistant microorganisms into the mammary tissue (Ziv, 82; Bishop, 4).

Systemic administration of the antibiotic using intravenous injection results in the rapid bioavailability of the antibiotic. This provides immediate high levels of antibiotic systemically which may then diffuse into the mammary tissue without the risk of contaminating the teat and udder with virulent resistant bacteria (Bishop, 4). In addition, systemic treatment allows for the treatment of organisms that might spread from the udder to other parts of the body during the disease process.

Frequency and Absorption

The frequency and duration of treatment are critical in providing suitable concentrations of the active drug at the site of infection. Therapeutic success occurs when the concentration of the antibiotic remains above the minimal inhibitory concentration of the infecting organism for a sufficient length of time (Stang, 73; Gingerich, 22).
The protein binding capacity, lipid solubility, and rate of excretion are all factors that influence the availability of the drug at the site of infection. Drugs that are highly protein bound are bacteriologically inactive and are limited in their distribution which can be beneficial or harmful depending on the situation. Lipid solubility affects the distribution of drugs by dictating their ability to diffuse across cell membranes (Gingerich, 22). Both factors influence the rate of elimination of the antibiotic from the body.

**Route of Elimination**

The route of elimination of penicillin administered IMM is through milk and urine. Penicillin administered into normal bovine udders is eliminated more slowly than when infused into infected quarters (Mercer et al., 49). It is postulated that this is due to: 1) an inflammatory response increases the blood flow in mammary tissue or 2) reduced milk production would have a concentrating effect on the penicillin thereby increasing absorption. Once penicillin enters the bloodstream following IMM infusion, elimination occurs primarily (70%) through the renal system with the drug being excreted in the active form (Goodman and Gilman, 24). However, penicillin residues can be detected in milk 14 days post treatment (Rakel, 62).

In systemic administration of penicillin there is a greater serum level of antibiotic (Goodman and Gilman, 24). This results in a faster rate of elimination than in local treatment. Because of this, systemic treatment should be given once or twice daily depending on dosage and
preparation. As the rate of elimination of penicillin is both dose and carrier related, systemic treatment usually results in a lower level of penicillin in the udder than local treatment (Mercer et al., 50, Ziv, 82).

Summary of Penicillin

Penicillin has been the most widely used drug in mastitis therapy (Bishop, 4). Penicillin inhibits cell wall synthesis which inhibits bacterial replication and increases their susceptibility to phagocytosis. Elimination of the drug is primarily through milk and urine. Bacteria may develop resistance to penicillin when exposed to continual low levels of the drug. Despite the shortcomings of penicillin, it will probably continue to be one of the most used drugs in mastitis therapy.

V. MASTITIS THERAPY WITH DMSO

Little research has been done on the subject of DMSO in mastitis therapy (Medline Search, 30). DMSO has three properties that make it particularly suited for mastitis therapy: 1) it will increase the permeability of tissue to a variety of drugs, 2) it mixes well with pharmaceuticals, and 3) it has antibacterial properties (Black, 5). Wooley et al. (81) reported on the synergistic interaction of DMSO in combination with antibiotics for the treatment of bacterial infections. In 1980, Jaurequi (37) in a study involving 80 cows found that cows treated with penicillin in DMSO had a higher percent of normal
quarters following treatment than did those cows treated with penicillin in oil or water.

VI. DMSO

History

DMSO is a byproduct of the wood pulping industry. It was first identified in 1886 by Alexander Saytzeff. Jacob et al. (34) reported in 1964 that DMSO enhanced the movement of antibiotics in trees and plants resulting in increased growth and less incidence of disease. Herschler et al. (29) in 1964 demonstrated that DMSO had the ability to penetrate unbroken skin and possessed medicinal properties after penetration. He also reported that DMSO penetrated most biological membranes readily and that topical administration of 100% DMSO gave relief from pain and swelling (Herschler et al., 29).

A report in 1965 on possible adverse effects of DMSO on the eyes of experimental animals halted further investigation in the use of DMSO as a drug for human use (Wilson et al., 79). The FDA later altered this ruling to allow for controlled studies. DMSO is currently approved for the topical treatment of horses, dogs, and in certain cases, humans. At present DMSO is available commercially for treatment of horses and dogs by topical application only (Knowles, 42). Investigations are currently underway to explore the possibility of DMSO treatment of arthritis.
VII. CHEMISTRY

Dimethyl Sulfoxide is a clear, colorless liquid represented chemically as:

\[ \text{H}_3\text{C}---\text{S}---\text{CH}_3 \]

This molecular configuration results in a negatively charged oxygen resulting in a highly polar substance. As a highly polar substance DMSO has the ability to dissolve other polar substances very readily. Reactions of ionic materials which proceed slowly in water proceed $10^3$ to $10^9$ times faster in DMSO (Parker, 57). This increase is due to DMSO being an aprotic solvent, a solvent which does not contain an acidic hydrogen attached to an oxygen. An aprotic unlike water solvates cations and leaves anions highly reactive and bases become more basic and nucleophiles become more nucleophilic (Solomons, 72).

DMSO can readily pass through biological membranes (Herschler et al., 29). Although the exact mechanism of cell entry is not known, it probably is related to the molecule's small size, binding ability, and the ability to substitute for water in the cell membrane. In addition, it has been theorized that areas of the epidermis are altered by DMSO to permit passage of substances dissolved in DMSO (Horeta and Weber, 31). This alteration is reversible in that the skin returns to its natural state after the DMSO is removed. This reversal occurs by both diffusion and active transport of DMSO followed by a binding of DMSO and water (Klingman, 41).
The fact that DMSO can substitute for water explains the anti-freeze effect or the ability of the solvent to resist freezing alone or in solution at low temperatures. This attribute of DMSO has led to the use of DMSO in the storage of mammalian cells at ultra low temperatures. This resistance to freezing at ultra low temperatures reduces cell damage upon thawing (Parker, 57).

VIII. METABOLISM AND EXCRETION

DMSO is absorbed rapidly by oral, topical, intravenous, or peritoneal administration. Kolb et al. (43) found that radioactively labeled DMSO was present in human blood within five minutes following cutaneous application. Gerhards and Gibson (20) found that DMSO is metabolized by the liver and is eliminated via urine, feces, and expired breath in the form of Dimethyl Sulphone and Dimethyl Sulfide. Kolb et al. (43) reported that the elimination rate was 10 to 15% via the urine in 24 hours, 3% via the lungs the first 24 hours, and 40% via all routes of elimination during the first week. Tiews et al. (75) found that cattle have the ability to metabolize and eliminate DMSO very quickly. Tiews et al. (75) and Herschler (29) reported that Dimethyl Sulphone and Dimethyl Sulfide are normally found in bovine meat and milk and are probably continually eliminated via the lungs. The excretion of Dimethyl Sulfide is through the lungs resulting in the characteristic garlic odor to the breath (Ramlmer and Zaffaroni, 63). Dimethyl Sulfide and DMSO elimination reach a peak in 6-12 hours and disappear within 28 hours (Tiews et al., 75).
IX. TOXICITY

Data presented in 1965 demonstrated that DMSO could be toxic to humans. This resulted in the removal of DMSO from human use until additional data was obtained. Wood et al. (80) demonstrated that lens changes occurred in rabbits when treated with 8-11 g/kg of DMSO per day. Reactions were noticed 7-10 days after treatment. Haziness and clouding of the lens increased with time until myopia or nearsightedness was noticed. After ten weeks of administration, no cataracts developed and the retina could still be viewed. The effect on vision was primarily the appearance of myopia, which increased in severity with increased concentration and exposure (Rubin, 65). Rubin and Barnett (66) found the same symptoms in swine, dogs, and rabbits with an incomplete regression of symptoms following removal of DMSO. Wood et al. (80) explained these changes as being due to a loss of soluble protein and an increase in the insoluble components. Gordon (25) postulated that lens changes were due to systemic changes in body organs resulting from toxic doses. Additional studies involving histological examination of the lens did not reveal the nature of damage induced by DMSO (Denko et al., 13).

In a study on ocular effects of DMSO in humans, Brobyn (6) found no changes in ocular function at a dose of 1g/kg/day. This dosage was 3-30 times the normal dosage. Brobyn (7) did note that at this dosage there was mild sedation, insomnia, nausea, dizziness, and diarrhea accompanying treatment. The most common effect was the garlic breath odor and dry and scaly skin at the area of application.
In animals, the LD\textsubscript{50} is extremely high. The LD\textsubscript{50} is extremely high as DMSO does not appear to accumulate in the tissue (Wilson \textit{et al.}, 79). DMSO may cause hepato toxicity, kidney failure, and pulmonary edema. Pulmonary edema as a result of DMSO toxicity occurs primarily because of a decrease in heart rate with the subsequent drop in blood pressure and increased vasodilation (Mason, 47). DMSO also causes hemorrhagic gastroenteritis when given at toxic levels (Smith \textit{et al.}, 71).

\section*{X. Pharmacologic Action}

\subsection*{Membrane Transport}

DMSO can readily carry molecules of less than 3000 molecular weight across intact skin. Substances such as hormones can be applied in this manner mimicking a subcutaneous injection of these substances (Tjan and Geinberg, 76). Gorog and Kovacs (26) discovered that corticosteroids, when administered with DMSO, were ten times as effective in relief of arthritis as the corticosteroids alone. The lower dosage of corticosteroids needed with this method of administration may prove valuable in eliminating side effects associated with large doses of corticosteroids. Potassium penicillin G is one substance that readily moves through intact skin under the influence of DMSO (Tjan and Geinberg, 76).

\subsection*{Anti-Inflammatory Action}

Gorog and Kovacs (26) when testing various anti-inflammatory agents found DMSO to be effective in reducing inflammation and platelet
thrombosis. They were also able to demonstrate that the increased vasodilation and blood flow was the main reason for reduction in platelet thrombosis.

Weisman et al. (78) found that cortisone coupled with DMSO was effective in the reduction of the inflammatory response. Jacob (33) demonstrated that steroids such as cortisone are more active in the presence of DMSO and an effective level of cortisone required to stabilize lysosomes is reduced by 10 to 1000 times.

The use of DMSO in treatment of thermal burns is controversial. Ashley et al. (2) found no decrease in inflammation or edema in rats subjected to leg burns. However, Jacob (35) noticed a decrease in blister formation and less severe edema. Discrepancies may be due to the type of burns and the way they were produced.

Vasodilation

Transitory erythema followed by burning and pruritis are noticed following topical application of 90% DMSO. After the initial effects occur, the skin will experience dryness and mild scaling (Arno et al., 1; Steinberg, 74).

The fact that DMSO has the ability to stimulate histamine release seems to be the major cause of skin reactions (Klingman, 41). Bradham and Sample (6) found increases in temperature of the skin, subcutaneous tissue, and muscle following dermal treatment with DMSO. They postulate that this increase is due to release of heat from the reaction of DMSO and cellular water.
Bacteriostatic and Bactericidal

It has been demonstrated that DMSO is bactericidal against certain bacteria (Pottz et al., 61). *Escherichia coli*, *Proteus vulgaris*, and *Salmonella* spp. are killed at 30% concentrations while *Pseudomonas* are killed at concentrations of 10%. *Staphylococcus aureus* required concentrations of 40% to exhibit bactericidal action.

Nerve Blockade

Sams (68) found that the rate of conduction of the sciatic nerve in frogs was reduced by 40% when exposed to 6% DMSO. The effect was reversible by washing the nerve with a buffer, however, at concentrations of 100% DMSO the effect was irreversible. Smaller nerves seem to be the first to exhibit decreases in conductivity (Shealy, 69). This action could explain the pain relief property of DMSO (Jacob, 33).

The tone of smooth muscle is increased when DMSO is given intravenously. Muscle tremors are noticed after the intravenous administration of DMSO. This may be due to cholinesterase inhibition at the peripheral nerves (Sams, 68; Jacob, 33).

Diuresis

Formaneck and Suckert (18) found a tenfold increase in urine volume and a corresponding increase in urine sodium and potassium with high oral or low intravenous doses of DMSO. It is postulated that this phenomenon is due to an increase in vasodilation of the kidney which increases the filtration rate (Karow and Jeske, 38). These changes
were not evident with topical doses. The rate of urine flow returns to near normal levels once DMSO is eliminated from the body.

**Respiratory Stimulant**

DMSO also affects the respiratory system. Distefano and Klahn (14) found that doses of 200 mg/kg of DMSO given intravenously to the cat resulted in apnea which if not corrected immediately resulted in death. A later study, by Peterson and Robertson (59), demonstrated an increase in respiration rate in dogs when 5-10 mg/kg of DMSO in 2.5% saline was injected intravenously. A range of 5-500 g/kg proved toxic when DMSO was administered intravenously (Peterson and Robertson, 59). This dose is low in comparison to toxic levels of other species. De la Torre (8) found that under conditions of oxygen deprivation, such as nitrogen hypoxia, DMSO has a protective effect. DMSO seems to work at the cellular level to reduce tissue destruction associated with hypoxia by making oxygen more available to the cell.

**XI. CENTRAL NERVOUS SYSTEM EFFECTS**

Recent studies have demonstrated that DMSO may be advantageous in treatment of injuries to the Central Nervous System (CNS) (de la Torre, 9; de la Torre and Surgeon, 10; de la Torre et al., 12; Lee, 45). DMSO also has been examined for its usefulness in treating post-operative damage to the CNS (Rucker et al., 67). The mechanism of how this occurs is unclear. DMSO does not appear to protect the gray matter. De la Torre et al. (11) found no differences in necrosis of gray matter
between control animals and DMSO-treated animals when subjected to experimental injury. He noted that DMSO did not seem to stop necrosis of gray matter. However, because of the protection it provided to other nervous tissue he still saw merit for its use in treating nerve related injuries. On the other hand, Parker and Smith (58) using similar studies, concluded that DMSO could not be relied upon in clinical situations to aid in the recovery of damaged nerve tissue.

Laha et al. (44) in studying the effect of DMSO on nerve injuries injected emboli into the carotid artery of dogs to produce embolization. These dogs were divided into four treatment groups. Treatments were 1) 2g/kg DMSO diluted in saline and given intravenously, 2) 30 mg/kg methylprednisolone given intravenously, 3) 2mg/kg methylprednisolone given intravenously, and 4) a control group. Twenty-one days after injection of emboli, brains were examined and those dogs treated with DMSO showed no neurological defects or pathological changes, while definite changes were noticed in the other treatments.

Although DMSO, in the treatment of CNS trauma, has not been clearly demonstrated to protect the gray matter (de la Torre, 11), its protection of white matter has been studied (de la Torre, 9, 11, 12). Factors leading to this protective effect may be the anti-edema effect and anti-thrombotic effect (Panganmala et al., 54).

XII. CONCLUSION OF DMSO

While many theories have been proposed explaining the effects of DMSO, little is known about the exact mode of action. Theories
deal primarily with increased oxygen flow to tissue to maintain normal metabolic activity and reduce damage to cells. Dosages and treatment times vary widely among researchers. More studies are needed to provide insight on dosage levels, intervals, and toxicity levels.
CHAPTER III

MATERIALS AND METHODS

Data used in this study were collected from May to September 1983 from eight cows in the University of Tennessee dairy herd. All cows had similar levels of production and stage of lactation. In some instances a cow was used in two or more treatment groups to substantiate data.

I. EXPERIMENTAL DESIGN

Cows in this study were randomly assigned to one of four treatment groups. Random assignment compensated for variations in body weight and production.

Cows receiving intrammary treatment (groups one and two) were treated in the right front quarter with ten mls of penicillin (10,000 u/ml) in physiological saline (0.85% NaCl). Cows in treatment group one received penicillin only while cows in treatment group two received penicillin followed by 10 mls of 90% DMSO.

Cows in group three were given penicillin intravenously in the jugular vein, at a dosage of 5,000 units of penicillin per pound of body weight. Cows in treatment group four received penicillin followed by DMSO (one ml of 90% DMSO per 100 pounds of body weight).
II. SAMPLE COLLECTION

Serum samples were collected via indwelling cathers, in the jugular vein, at time 0 (just before treatment) and at 30, 60, 90, 120, 240, 360, and 480 minutes following treatment. Blood collection was achieved by drawing a two to three ml sample for discard and then drawing a 10 ml sample which was immediately cooled on ice. The catheter was then flushed with 2 ml of heparinized saline to prevent coagulation.

Milk samples were collected at time 0 (before treatment), 30, 60, 90, 120, 240, 360, and 480 minutes following treatment. Before commencing treatment, each udder was washed with clear warm water to remove manure and debris. Before collection of milk samples, the teat was cleaned with 70% ethanol. One to three streams of foremilk was removed to clear the teat cistern prior to collection of the sample. Approximately five milliliters of milk were collected at each sampling time. Milk samples were immediately cooled and aliquoted to three subsamples before freezing at 0°F.

III. PREPARATION OF PLATES

Antibiotic levels in the serum and milk samples were analyzed using a bioassay. Petri dishes (150 x 25 mm) were inoculated with 30 mls of base E agar and allowed to cool. This layer was overlayed with 15 mls of a seed agar. The seed agar was prepared by inoculating a BHI plate with Sarcinea lutea and incubating it overnight at 37°C.
After overnight incubation at 37°C the organism was removed and suspended in Phosphate Buffered Saline solution (PBS) to a concentration of 50% transmission using a Spectronic 20 Spectrophotometer at 550 nm. This solution was used to inoculate the prepared seed A agar at the rate of three ml/100 ml of seed agar. Caution was exercised to ensure that the temperature of agar A was 44-45°C to prevent a heat kill of the organism. After the addition of the organism, the seed agar was mixed thoroughly using a magnetic stirrer. Fifteen ml were then overlayed onto prewarmed (37°C) base agar. The plates were then used immediately or stored at 4°C until used (less than two weeks).

IV. PREPARATION OF STANDARD CURVE

A standard curve was prepared by using varying concentrations of penicillin to provide a series of zones of inhibition. The zones of inhibition produced by these varying concentrations were used as a base to determine the concentration of penicillin present in milk and serum samples based on the size of zones of inhibition produced by them. The standard concentrations for this study were .75, 1.0, 2.5, 5.0, and 10.0 u/ml. The 2.5 concentration was used as the midpoint. The preparations were absorbed into .25 inch paper discs prepared by BBL laboratories at the level of 25 lambda (.025 ml). The arrangement of discs on the agar plates consisted of six disks placed in circular fashion approximately 2.5 cm from the outer edge of the agar. Each plate held 3 disks containing 2.5 ug of penicillin (midpoint) alternating with 3 disks of one of the concentrations of penicillin used as a standard.
Each plate was run in triplicate for a total of twelve plates, twelve observations for each concentration, and thirty-six observations for the midpoint. The purpose of the midpoint was to allow for variation in zone sizes between plates. An overall average of the midpoint from twelve plates provided the average reference zone size which was then used to adjust the average of the midpoint for each of the other points of the standard curve. The amount of difference between the midpoint average and the average reference zone size was then added or subtracted from the zone size for the other points to produce a corrected zone size for each point. The next step was to obtain a low point (LP) and high point (HP) to plot a linear curve on semi-log paper. Each of the points on the standard curve was assigned a letter: a, b, c, d, or e from lowest to highest. The HP and LP were obtained by the following equations:

\[
LP = \frac{3A + 2B + C - E}{5}
\]

\[
HP = \frac{3E + 2D + C - A}{5}
\]

When the LP and HP were obtained, they were then plotted along with the corrected zone size for each point to obtain a linear curve. The original standard curve was run using saline as the diluent. As the samples to be run would be in DMSO, serum, or milk as the diluent additional curves were run using these. As there was no appreciable difference between the four diluents, saline diluent was used.
V. PREPARATION OF SAMPLES

Serum and milk samples were placed on the paper disc as described above at a volume of 25 lambda. A series of three discs were alternated with three discs of the midpoint concentration of penicillin on each plate. The same procedure was used to correct zone sizes as was used for the standard curve. When the corrected zone sizes were compared to the linear standard curve concentrations for each time period, values were obtained for both milk and serum. These figures allowed for plotting curves for serum concentrations over time and milk concentrations for each quarter over time. These curves provided a means for comparisons of the different treatment groups and evaluation of the efficiency for each treatment in relation to drug diffusion, appearance and disappearance, and persistency of the drug in the body.
CHAPTER IV

RESULTS AND DISCUSSION

Data were collected and analyzed from 8 cows to study the effects of DMSO (dimethyl sulfoxide) on the distribution of penicillin when administered intravenously (IV) or intramammarily (IMM). A secondary purpose was to verify the differing responses of penicillin when given IV versus IMM. Four treatments were utilized with 3 cows assigned to each treatment for a total of 12 cow treatments. Drugs and routes of administration for each treatment are presented in Table 1.

Table 1. Composition of Treatments

<table>
<thead>
<tr>
<th>Trt #</th>
<th>TRT. Route</th>
<th>Composition of Trt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IMM</td>
<td>Penicillin Only</td>
</tr>
<tr>
<td>2</td>
<td>IMM</td>
<td>Penicillin Plus DMSO</td>
</tr>
<tr>
<td>3</td>
<td>IV</td>
<td>Penicillin Only</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>Penicillin Plus DMSO</td>
</tr>
</tbody>
</table>

The differences that occurred between treatments over time are represented graphically. Figures 1-5 represent graphs for each sampling site by treatment and serum graphs for each treatment.
Figure 1. Concentrations of Penicillin G in Milk Samples Collected from Each Quarter in Relation to Time for Treatment Group One (Penicillin only IMM).
Concentrations of Penicillin G in Milk Samples Collected from Each Quarter in Relation to Time for Treatment Group Two (Penicillin plus DMSO IMM).

Figure 2.
Figure 3. Concentrations of Penicillin G in Milk Samples Collected from Each Quarter in Relation to Time for Treatment Group Three (Penicillin only IV).
Figure 4. Concentrations of Penicillin G in Milk Samples Collected from Each Quarter in Relation to Time for Treatment Group Four (Penicillin plus DMSO IV).
Figure 5. Serum Concentration of Penicillin G for Each of the Four Treatment Groups in Relation to Time.
When illustrated graphically, the results of each treatment became more apparent. In treatment groups one and two the response generally was confined to the quarter in which treatment occurred. An exception to this was in group two where penicillin G was detected in the left front quarter following infusion of the right front quarter. This response was noticed in only one cow. This measurement does not follow the results in treatment group one or results in the RR or LR sampling sites of treatment group two. This could be attributed to experimental error in analysis or contamination during sampling. In Figure 2(b), it should be noted that the scale ascends only to .30 units per ml while in Figure 2(a) the scale ascends to 600 units per ml. When compared on the same scale, the curve in Figure 2(b) would be negligible. No response was noticed in serum for treatment groups one or two.

In treatment groups three and four, a crossover from serum to milk was evident as detectable concentrations of penicillin were apparent in each quarter following IV treatment (Figures 3 & 4). These levels varied which may be attributed to differences in production. Serum levels for treatment groups three and four showed the characteristic IV response (Figures 5(c) & 5(d)). It was interesting to note that the shape of the serum curves for treatment groups three and four were practically identical with only the actual levels being different.

Means, standard deviations, standard errors, minimum and maximum values were calculated for each treatment group by sampling site. Sampling sites were right front quarter, left front quarter, right
rear quarter, left rear quarter, and serum. These values are given in Table 2.

Although Table 2 does not take into account the different sampling times, a trend toward larger concentrations of penicillin at the site of administration is evident. In the IMM route of administration, all animals were treated in the right front quarter. Values were largest for treatment group two in the right front quarter indicating that more penicillin remained in the milk when DMSO was added to the treatment. No detectable penicillin entered the vascular system from the udder or appeared in the other quarters following IMM treatment in the RF. One exception was the response in the left front quarter in treatment group two which could have been due to error or contamination during sampling.

In the IV route of administration, the effect was the opposite of IMM. Treatment group three, with no DMSO, exhibited higher levels of penicillin in serum over the period of collection than did treatment group four with DMSO. Statistical means indicated that penicillin left the blood stream more quickly in the presence of DMSO. In both IV treatments, penicillin was detectable in small amounts in milk samples from all quarters.

Data were analyzed using a model that included treatment, time, treatment by time interaction, and production as a covariate. F-values obtained from this model are given in Table 3.

Time of sampling significantly differs in respect to concentration in all sampling sites. Concentration increased or decreased in
Table 2. Mean, Standard Deviation, Standard Error, Minimum and Maximum Values of Concentration of Penicillin by Treatment and Sampling Site

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trt. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>241.9958</td>
<td>243.8747</td>
<td>49.7807</td>
<td>0.0000</td>
<td>700.0000</td>
</tr>
<tr>
<td>LF</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>RR</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>LR</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Serum</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Trt. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>316.6666</td>
<td>192.0821</td>
<td>39.2085</td>
<td>0.0000</td>
<td>630.0000</td>
</tr>
<tr>
<td>LF</td>
<td>0.0600</td>
<td>0.2074</td>
<td>0.0423</td>
<td>0.0000</td>
<td>0.8600</td>
</tr>
<tr>
<td>RR</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>LR</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Serum</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Trt. 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>0.4112</td>
<td>0.5187</td>
<td>0.1058</td>
<td>0.0000</td>
<td>1.3000</td>
</tr>
<tr>
<td>LF</td>
<td>0.4225</td>
<td>0.5036</td>
<td>0.1028</td>
<td>0.0000</td>
<td>1.4100</td>
</tr>
<tr>
<td>RR</td>
<td>0.4271</td>
<td>0.5031</td>
<td>0.1026</td>
<td>0.0000</td>
<td>1.4200</td>
</tr>
<tr>
<td>LR</td>
<td>0.3967</td>
<td>0.5103</td>
<td>0.1042</td>
<td>0.0000</td>
<td>1.4000</td>
</tr>
<tr>
<td>Serum</td>
<td>38.1167</td>
<td>103.4477</td>
<td>21.1162</td>
<td>0.0000</td>
<td>440.0000</td>
</tr>
<tr>
<td>Trt. 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>0.7363</td>
<td>0.5236</td>
<td>0.1069</td>
<td>0.0000</td>
<td>1.5100</td>
</tr>
<tr>
<td>LF</td>
<td>0.5638</td>
<td>0.5005</td>
<td>0.1034</td>
<td>0.0000</td>
<td>1.2800</td>
</tr>
<tr>
<td>RR</td>
<td>0.8392</td>
<td>0.4555</td>
<td>0.0929</td>
<td>0.0000</td>
<td>1.5700</td>
</tr>
<tr>
<td>LR</td>
<td>0.6078</td>
<td>0.4082</td>
<td>0.0833</td>
<td>0.0000</td>
<td>1.0200</td>
</tr>
<tr>
<td>Serum</td>
<td>18.3991</td>
<td>46.6336</td>
<td>9.5190</td>
<td>0.0000</td>
<td>208.0000</td>
</tr>
</tbody>
</table>
Table 3. F-Values and Residual Mean Squares for Each Sampling Site

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>RF</th>
<th>LF</th>
<th>RR</th>
<th>LR</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>7</td>
<td>24.08**</td>
<td>5.02**</td>
<td>3.89**</td>
<td>4.43**</td>
<td>9.97**</td>
</tr>
<tr>
<td>Trt.</td>
<td>3</td>
<td>175.97**</td>
<td>19.52**</td>
<td>46.67**</td>
<td>29.95**</td>
<td>6.35**</td>
</tr>
<tr>
<td>Trt. * Time</td>
<td>21</td>
<td>8.07**</td>
<td>1.79*</td>
<td>1.71</td>
<td>1.67</td>
<td>4.94**</td>
</tr>
<tr>
<td>Prd.</td>
<td>1</td>
<td>19.71**</td>
<td>1.57</td>
<td>1.01</td>
<td>4.87*</td>
<td>0.22</td>
</tr>
<tr>
<td>Error M.S.</td>
<td>63</td>
<td>4361.7614</td>
<td>0.0927</td>
<td>0.0832</td>
<td>0.0733</td>
<td>1251.1528</td>
</tr>
</tbody>
</table>

Model RF LF RR LR Serum = Time Trt Trt*Time Prd.

*P < .05.

**P < .01.
a stepwise fashion during all time periods up until collection ceased at 480 minutes following treatment.

Treatment was also highly significant (P<.01) in regard to concentration at sampling site. It is normal to expect this difference in treatment routes as illustrated in the graphs previously.

The interaction of treatment by time was significant (P<.05) for the left front quarter. This is probably due to a larger crossover of penicillin from blood to mammary tissue in this quarter than for the other quarters. However, the unexpected response of the left front quarter in treatment two may have been due to experimental error as previously mentioned and could have been responsible for some of this significance.

The significance of treatment in this preliminary analysis prompted a more complete analysis. First, treatment routes (IMM vs IV) were contrasted. Secondly, DMSO vs no DMSO was contrasted for IMM and IV treatments. These values are shown in Table 4.

As mentioned earlier, differences between IMM and IV were highly significant. There were also differences between DMSO and no DMSO. While significant differences occurred only in the right rear and left rear quarters in the IV treatments, other sampling sites were approaching significance (P < .10). Again many sampling sites showed a trend toward quicker disappearance of the drug in the presence of DMSO but not to the point of significance (P<.05). Additional analysis was performed using production as a covariate. The values obtained are given in Table 5.
Table 4. F-Values and Residual Mean Squares for Each Sampling Site for Contrasting Treatment Routes

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>RF</th>
<th>LF</th>
<th>RR</th>
<th>LR</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMM vs IV</td>
<td>1</td>
<td>408.14**</td>
<td>55.03**</td>
<td>115.52**</td>
<td>77.87**</td>
<td>15.51**</td>
</tr>
<tr>
<td>DMSO/No DMSO IMM</td>
<td>1</td>
<td>0.36</td>
<td>0.46</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>DMSO/No DMSO IV</td>
<td>1</td>
<td>0.00</td>
<td>2.56†</td>
<td>24.47**</td>
<td>6.87*</td>
<td>3.77†</td>
</tr>
<tr>
<td>Error M.S.</td>
<td>64</td>
<td>5636.9446</td>
<td>0.0935</td>
<td>0.0832</td>
<td>0.0776</td>
<td>1235.8883</td>
</tr>
</tbody>
</table>

*P < .05.
**P < .01.
†P < .10.

Table 5. F-Values and Residual Mean Squares for Each Sampling Site for Contrasting Treatment Routes using Production as a Covariate

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>RF</th>
<th>LF</th>
<th>RR</th>
<th>LR</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMM vs IV</td>
<td>1</td>
<td>540.74**</td>
<td>56.68**</td>
<td>116.53**</td>
<td>85.33**</td>
<td>14.92**</td>
</tr>
<tr>
<td>DMSO/No DMSO IMM</td>
<td>1</td>
<td>2.79</td>
<td>0.14</td>
<td>0.005</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>DMSO/No DMSO IV</td>
<td>1</td>
<td>0.98</td>
<td>3.42†</td>
<td>25.46**</td>
<td>9.79**</td>
<td>3.95†</td>
</tr>
<tr>
<td>Error M.S.</td>
<td>63</td>
<td>4361.7614</td>
<td>0.09272</td>
<td>0.0833</td>
<td>0.0733</td>
<td>1251.1528</td>
</tr>
</tbody>
</table>

*P < .01.
**P < .05.
†P < .10.
Results were similar with the left rear sampling site becoming significant (P < .01). F-values increased in most cases due to a decrease in the error mean squares.

Least squares means were obtained. Levels of significance for the differences between means verify the earlier analyses. Values are given in Table 6.

In looking at the results in Table 6 it became apparent that they substantiated earlier analyses. In the right front quarter, treatment group one and two were different from three and four but not from each other. This substantiated the earlier statement that the route of treatment was statistically different but DMSO vs no DMSO was not.

Results approached significance (P < .10) in the left front quarter between the IV treatments. Results were also significant for all other sampling sites except the right front quarter which was not.

Treatment groups one and two were different from three and four in the RF, LF, and RR but only approached significance in the serum. In the serum samples, treatment groups one and two were different from three and four. Differences between IV treatments approached significance in the LF, were significant in the LR, RR, and serum, while the RF was not significant. Differences between the IMM treatments were not significant. This faster rate of clearance may be due to the drug being carried through the bloodstream to many parts of the body. The drug infused into the udder was in a more localized situation as indicated by the absence of the drug in serum in treatment groups one and two.
Table 6. Least Squares Analysis of Treatments by Sampling Site

<table>
<thead>
<tr>
<th>Site</th>
<th>Trt.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>1</td>
<td>0.0998</td>
<td>0.0001**</td>
<td>0.0001**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0001**</td>
<td>0.0001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.3250</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>1</td>
<td>0.7052</td>
<td>0.0001**</td>
<td>0.0001**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0001**</td>
<td>0.0001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.0001**</td>
<td></td>
<td></td>
<td>0.0691†</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
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</tr>
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<td>0.0001**</td>
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*P < .05.  **P < .01.  †P < .10.
Data from one animal was not included in this study due to a highly unusual response. The response did not correspond to any response seen with any other animal on the same treatment group. Data from a replacement corresponded with other data. The reason for this abnormal response remains unclear. However, due to the consistency of the data obtained from the other animals receiving the same treatment, it was felt that data from this animal represented a sampling error or an unacceptable biological variation.
CHAPTER V

CONCLUSIONS

This study of IV and IMM administration and the effect of DMSO on the dispersion of penicillin resulted in the following conclusions:

1. The concentration of penicillin in milk at various sampling times varied according to route of administration in regard to concentration over time.

2. The addition of DMSO to IMM treatments did not vary the concentrations of penicillin in milk.

3. The addition of DMSO to IV treatments resulted in significantly different serum levels of penicillin although milk levels differed only slightly.
CHAPTER VI

SUMMARY

The data for this study was obtained by collecting milk from each quarter of lactating cows and serum at predetermined time intervals. Four treatments were used. Treatment group one consisted of an intramammary infusion of penicillin. Treatment group two was an intramammary infusion of penicillin followed by an equal volume of 90% DMSO. Treatment group three was an intravenous injection of penicillin while treatment group four was an intravenous injection of penicillin followed by 90% DMSO.

The concentration of penicillin present at a given time was the primary measurement response. The difference between treatment routes was found to be highly significant as was expected. An interesting note, however, was that in the IMM treatment groups the drug was generally confined to the quarter in which treatment occurred, which was the right front quarter. In treatment group two, some migration of the drug to the left front quarter was evident although no drug was present in the serum.

Differences between DMSO and no DMSO in treatment groups one and two were small and were not significant. Both treatment groups in the IMM route of administration showed similar levels and the distribution of penicillin was similar.

In treatment groups three and four which were both IV administration, differences between DMSO and no DMSO were more evident. When DMSO
was added, the levels found in serum were about half that found when no DMSO was added. Levels in other sampling sites were also slightly elevated. In the presence of DMSO penicillin leaves the vascular system in slightly larger amounts. The concentrations found in the mammary system when DMSO was added were not significantly higher than those in treatment three although they approached statistical significance. Possibly a larger dose or a difference in production levels would add to the significance of the study. The results of this study lead to the following conclusions:

1. The distribution of penicillin administered IV differs significantly from IMM administration of penicillin in milk in regard to concentration over time.
2. When adding DMSO in IMM administration of penicillin, concentrations of penicillin in the mammary system do not significantly differ from treatment without DMSO.
3. When adding DMSO in IV administration concentrations of penicillin have larger differences than does IV administration without DMSO although levels in the mammary system differ only slightly.

From the conclusions listed above, the author feels that although DMSO does show some merit for use in certain applications with drugs, more work is indicated before advising its use in clinical treatment of mastitis. Further work should include a more sophisticated method of detecting DMSO in milk and serum. This would provide a means of
determining a definite clearance time for DMSO. Also, cows could be sampled for a longer period of time to determine the time required to reach a level of the drug to be acceptable in milk for sale. Sampling times could also be varied to determine levels between current sampling times. These additions should add credence and understanding to the action of DMSO when used in conjunction with drugs in a mastitis treatment schedule.
BIBLIOGRAPHY


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VITA

Milton Wayne Orr was born in Blount County, Tennessee, on February 1, 1958. Upon graduation from Friendsville High School in 1976, he enrolled in The University of Tennessee, Knoxville, Animal Science Department and received a B.S. in Animal Science in Spring 1981.

After graduation the author decided to further his education by entering graduate school in the Animal Science Department at The University of Tennessee, Knoxville. The Master of Science degree was obtained in the summer of 1984.

As an undergraduate, the author was a member of the Dairy Club and also Alpha Zeta honor society.