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A COMPARISON OF SERUM AND INTRAUTERINE LEVELS OF PENICILIN, AMOXICILLIN AND OXYTETRACYCLINE FOLLOWING INTRAMUSCULAR AND INTRAUTERINE ADMINISTRATION

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Peter C. Mullowney

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DEDICATION

This thesis is dedicated to my wife, Una L. Mullowney, without whose love, encouragement, support and patience this thesis would never have been completed.

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ABSTRACT

Antibiotic levels in the serum and uterine fluid were determined following systemic and intrauterine administration of procaine penicillin G, amoxicillin, oxytetracycline (Liquamycin 100) and long acting oxytetracycline (Liquamycin LA-200). In this study it was determined that serum levels of each antibiotic were significantly greater following intramuscular administration than intrauterine administration. Following intramuscular administration, detectable levels of antibiotics were measured in the uterine fluids in all of the cows treated with amoxicillin, tetracycline and long acting tetracycline but in only 2 of 6 cows treated with procaine penicillin G. The levels in the uterus were below the minimal inhibitory concentration (MIC) for common uterine pathogens for the tetracycline preparations but remained above the mean MIC for Corynebacterium pyogenes, the most common cause of bovine endometritis for up to 36 hours after administration of amoxicillin.

Intrauterine administration of antibiotics resulted in more consistent levels of antibiotics in the uterine fluid and these levels persisted above the mean MIC for up to 36 hours for all common uterine pathogens. Absorption of antibiotic from the uterus into the serum was more consistent with amoxicillin and long acting oxytetracycline than with procaine penicillin G and oxytetracycline. However, in at least one cow treated with each of the antibiotics by the intrauterine route, no measurable levels of antibiotic were detected in the serum.

It was considered important that the antibiotic should be absorbed or secreted into the uterine lumen to ensure penetration into the deep as well as superficial layer of the endometrium in the clinical treatment. As this did not occur to a great extent in these animals the optimal treatment of the condition may involve the treatment by both the intramuscular and intrauterine routes.

It was originally intended that cows should be in the luteal phase of the cycle for the entire experiment. The heat periods of the cows were synchronized with prostaglandin PGF 2α. Each cow was to act as her own control in a series of eight experiments using the four drugs by two different routes--intramuscular and intrauterine and that experiments would not be scheduled during the periods when cows would be in the estrous phase of the cycle. However, following infusion early estrus was induced. This resulted in experiments having to be carried out at different stages of estrous cycles. Blood samples were analyzed for progesterone levels to determine the stage of the cycle. Sufficient numbers were not present in each group to determine if the stage of the cycle had an effect on absorption and secretion of antibiotics.

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CHAPTER 1

INTRODUCTION

Endometritis is a major cause of infertility in the dairy cow. It usually occurs as a result of bacterial invasion of the uterus following retained placenta or other causes of delayed uterine involution such as dystocia, uterine prolapses, abortions, premature births, twin calves, septic metritis or lacerations of the uterus, cervix, vagina or vulva. Bacteria involved in endometritis are frequently a result of the unsanitary treatment of these conditions.

Diagnosis of endometritis is generally based on observing a mucopurulent discharge from the vulva or findings on rectal or vaginal examination. Other diagnostic aids that may be employed include uterine biopsy, which may also aid in determining the prognosis, bacteriological culture of the uterus and cytological examination of the uterine discharge. However, in order to diagnose endometritis the clinician should be familiar with the normal involution of the bovine uterus and factors that affect this in order to avoid overtreatment. At one stage, it was common to treat all animals with intrauterine antibiotics in the immediate postpartum period, in the mistaken belief that this would decrease the incidence of endometritis and thereby decrease the number of days open. Infusion of antibiotics into the uterus is the preferred method of treatment of endometritis by most practitioners. However, this does not always achieve adequate levels throughout the

uterus. Antibiotic levels may be markedly different in samples obtained by uterine biopsy vs. those in the uterine lumen. The stage of the estrous cycle may affect the absorption and effectiveness of any antibiotic treatment. The uterus is more resistant to experimental infection and uterine phagocytosis is more efficient during the estrus stage of the cycle. It is very important that any treatment should not suppress the normal phagocytosis of bacteria in the uterine lumen. Endometritis may cause a lengthening of the estrous cycle due to decreased prostaglandin production in the endometrium not causing lysis of the corpus luteum. This is the probable mechanism that initiates pyometra. Infusion of the uterus may cause a production of prostaglandin, either due to distension or local irritation with a subsequent shortening of the estrous cycle.

Treatments for endometritis have consisted of various antibiotics including amoxicillin, ampicillin, chloramphenicol (which is now illegal to use in food producing animals in the United States), furacin, gentamicin, penicillin, streptomycin, sulfas and tetracycline. Several other medications have also been used as an intrauterine preparation, including iodine in various concentrations.

In some cases the diagnosis of endometritis is not made until purulent material is noted in the vaginal mucus at the time of breeding. Infusions of antibiotics, either 12 hours before breeding or 12 hours postbreeding, have been given and may increase fertility. Following the use of antibiotics in dairy animals, it is important to avoid antibiotic residues in milk. Several studies have shown that following

intrauterine administration antibiotics cannot be detected in the milk. Absorption, however, varies with the stage of the estrous cycle and the presence and severity of metritis. If antibiotics are not absorbed sufficiently to be present in the milk, they may not penetrate the uterine tissue in sufficient quantity to be effective against tissue invading organisms.

Several surveys have shown no difference in clinical response following intrauterine treatment with antibiotics when compared to control animals either infused with saline into the uterus or untreated. Systemic administration of antibiotic will result in antibiotic residues in milk but may be more effective than intrauterine treatment. It is proposed in this study that in order for an antibiotic to be clinically effective following intrauterine administration levels of antibiotics in the serum must be greater than the MIC for the common pathogens causing endometritis. If antibiotics are administered systemically therapeutic levels should be achieved both in the serum and the uterine lumen.

Several studies have been carried out on the pharmokinetics of the antibiotics used in the treatment of bovine endometritis. The purpose of this study is to compare two newer antibiotics; long acting oxytetracycline (Liquamycin LA-200, Pfizer) and amoxicillin trihydrate (Amoxi-Inject, Beecham Laboratories) with the more commonly used oxytetracycline (Liquamycin 100, Pfizer) and Procaine penicillin G. Antibiotics were administered by the intramuscular and intrauterine routes. Antibiotic levels in the serum and uterine lumen were measured at various time intervals up to 72 hours after administration.

CHAPTER 2

LITERATURE REVIEW

Bovine Endometritis

Incidence and Etiology

Endometritis has been stated by many authors to be a common cause of bovine infertility (1). However, in an abbatoir survey of almost 2,500 genital organs, endometritis and metritis were present in only 0.69% (2). In another survey, the incidence was 1.36%. In this study the author suggested that this low incidence might be due to the fact that subclinical cases of endometritis without any apparent gross lesions in the uterus might have been overlooked (3). In one survey of infertility, endometritis was responsible for 12.7% of infertility and 2.6% of all diseases treated. More common causes of infertility include cystic ovaries, subestrus and anestrus (4). In another study, metritis occurred following 12% of calvings (5). In this study, 22% of the cows with retained placenta and metritis were removed from the herds, 10.5% due to infertility, 9.3% for other reasons and 2.3% due to death from septic metritis (5). In some large dairy herds with high producing cows, uterine infection has reached new epizootic proportion with up to 40% of cows being affected (6). In another survey of infertile cows, endometritis was an uncommon finding and hormonal, physiological and nutritional factors were thought to be major causes of infertility (7).

Retained placenta is the major cause for postpartum metritis. This can be caused by stress at calving, dystocia, twin births, toxic mastitis, hypocalcemia, uterine infection associated with abortion caused by leptospirosis, brucellosis, Infectious Bovine Rhinotracheitis (IBR) virus, vibriosis or mycotic infections (8). Nutritional deficiencies such as imbalance in the calcium/phosphorus ratio, Vitamin D deficiency, Vitamin A deficiency, Vitamin E or selenium deficiency and a low protein ration may also result in an increased incidence of retained placenta (8, 9). In two groups of cows, one fed a protein adequate ration and the other a protein deficient, the incidence of uterine infection was 52.5% and 48.1% respectively at 25 days postpartum. However, at 40 days postpartum, 21.7% of the cows in the protein adequate group were infected and 51.9% in the protein deficient (9). As many as 88% of cows with retained placentas have been reported to develop metritis as compared to 8% of cows passing the placenta normally. This often results in an increase in days open and services per conception (10).

The treatment of retained placenta is controversial. Manual removal may result in trauma that predisposes to endometritis, whereas nonremoval may hinder uterine drainage (10). Some studies show that retained placenta has no significant effect on fertility, whereas others show impaired fertility and shortened reproductive life in cows with reproductive tract abnormalities which are more common after retained placenta (5). In North America, retained placenta is seen more commonly in the winter months (5) but this is not observed in some

European countries (11). Cows with metritis had a higher mean days open than nonmetritis cows (165 vs 114) (5). Services per conception, calving to first heat and calving to first service, were also statistically greater in cows affected with metritis (5).

Endometritis is more likely to occur in cows that have calved more than three times, in cows with a previous calving interval of under 365 days, and in high yielding cows (12, 13). Occasionally, endometritis and a subsequent pyometra may develop after breeding or embryonic death (5), in which case vibriosis, trichomoniasis or brucellosis may be suspected (1).

Recovery from endometritis may occur spontaneously or following treatment. In some cases, a mild subclinical endometritis will remain and will be one of the causes of failure to conceive or the repeat breeder syndrome. If the endometritis is severe enough to suppress the endometrial production of prostaglandins, the corpus luteum will not be lysed and an accumulation of pus or mucopurulent matter will occur in the uterus. This condition is diagnosed as pyometra (1). There has been much written in the literature on the differentiation of pyometra and endometritis but they are variations of the same clinical syndrome. True pyometra seldom resolves spontaneously and hormone administration is necessary to cause luteolysis, the induction of estrus and expulsion of the purulent material from the uterine lumen.

The repeat breeding cow is one that has normal estrus cycles but has failed to become pregnant following two or more breedings to a fertile bull. This may be due to either failure of conception or early

embryonic death. A repeat breeding cow may be defined as a cow with a minimum of one calving to exclude congenital abnormalities preventing conception, a maximum age of ten years to exclude infertility resulting from senility, freedom from gross genital abnormalities detectable by rectal palpation and freedom from abnormal discharges from the genital tract (14). There is a 10-15% incidence in both beef and dairy cows of the repeat breeder syndrome (15, 16). Infectious agents and bacterial endometritis are thought to play only a minor role. Other more common causes are fertilization failure caused by ovulation failure, occlusion of the oviduct, abnormal ova and ovarian adhesions. Up to 60% of repeat breeders may be affected with fertilization failure (17). Other causes include either too early or too late, insemination, and use of bulls of low fertility (15). Early embryonic death before day 15 of the cycle results in normal estrus periods and can be due to many causes including fertilization of aged ova. Management factors that can be associated with the repeat breeder syndrome include a seasonal effect, being more common in the fall and winter, climatic conditions, housing, inadequate detection of estrus, skill in artificial insemination, age, milk production and nutrition (15). Genetic factors and hormonal imbalances are responsible for some cases and failure of ovulation, delayed ovulation and congenital or acquired anatomical defects have also been reported as causing the syndrome (15).

In one study bacteria were only found on uterine culture from 11.6% of 69 repeat breeders on day 16 after breeding. This is

believed to be when the major portion of embryonic death occurs (18). The uterus of the repeat breeder animal during the luteal phase of the estrus cycle appears to be more resistant to bacterial invasion than that of the normal first service animal, possibly indicating a deficiency of progesterone action (19). It is also thought unlikely that endometritis is a major cause of the repeat breeder syndrome, as in several studies there was no statistically significant difference in conception rates between cows treated with an infusion of antibiotics and 0.9% saline solution (16).

Uterine flushings from repeat breeders five to seven days post-estrus were lower in protein, sodium, phosphorus and glucose and higher in calcium, potassium and magnesium. Calcium was twelve times higher in uterine flushings from cows with abnormal embryos than those with normal embryos. Zinc, phosphorus and potassium were also elevated. These ions are mainly intracellular and their extracellular presence may indicate disturbed tissue function. Unfortunately, these findings were not correlated with bacterial culture or endometrial biopsy (15).

Diagnosis

Diagnosis of endometritis is made by finding a mucopurulent discharge from the uterus present in the vagina, either discharged onto the tail or by vaginal speculum examination. Rectal examination reveals the uterus to be slightly enlarged, heavy and thick walled. These changes may be more obvious in one horn. Diagnosis on the basis of rectal examination is not always reliable and may result in

unnecessary treatment. Aids to diagnosis include endometrial biopsy and uterine culture (1).

Rectal Palpation

Routine rectal palpation of the uterus is carried out on cows 30 to 40 days postpartum by many veterinarians to diagnose endometritis. A significant correlation between findings on rectal palpation and visual inspection of vaginal discharge and histological examination of uterine biopsies was found in one study (20) but was not noted in several others (21). In one survey of 110 sterile cows, 31 were diagnosed as having clinical abnormalities of the uterus and of these 87% had endometritis, with 57% having an endometritis severe enough to interfere with conception. In 79 cows without clinically detectable abnormalities, 63% had endometritis, of these 37% had endometritis severe enough to interfere with conception (21). There was a higher correlation between clinical and histopathological findings than between clinical and bacteriological findings or between histopathologic and bacteriological findings (22).

Uterine Biopsy

Endometrial biopsy can be used to identify histopathological lesions in the bovine endometrium that could contribute to infertility and can serve as a basis for prognostic evaluations of the repeat breeder cow. It is a safe and easily performed diagnostic technique and repeat biopsies cause no adverse effects on a cow's reproductive capacity (23, 24) and if performed correctly, does not result in colonization of the uterus by exogenous bacteria (25).

Endometrial biopsies are best taken during the luteal stage of the cycle at the 5th day postestrus as the best histological detail is obtained at this time (20). However, at this stage, the uterus is more susceptible to infection and great care should be taken to not introduce contaminants into the uterus during the biopsy procedure. Initially, the reproductive organs should be palpated per rectum, then the vulvar lips and perineum are thoroughly scrubbed with disinfectant soap and warm water, followed by disinfection with 70% isopropyl alcohol and then a tamed iodine solution (26). A sterile glass speculum is then inserted to visually examine the cervix and any discharge present. The biopsy instrument may now be introduced through the speculum or the speculum removed. The hand is again inserted into the rectum, the cervix grasped and a closed sterilized biopsy instrument is passed through the vagina and cervix into the body of the uterus and one of the uterine horns (22). Some authors consider that one sample of biopsy material is sufficient (22), while others consider that a biopsy sample should be taken from each of the uterine horns and another from the body of the uterus (27).

Several different biopsy instruments have been developed. These include the modified Nielsen biopsy rod (22, 28), Holinger specimen and tissue forceps (Pilling Co., Delaware Drive, Fort Washington, PA 19034) (26), a modification of the instrument described by Dubois (30) (United Surgical Supplies Biopsy Instrument, USS, Port Chester, NY) (20), a human rectal biopsy instrument with a specially constructed 18 inch shaft (Edward Weck & Company, Inc., Weck Drive, P.O. Box

12600, Research Triangle Park, NC 27709), Yeomans Uterine Biopsy Forceps (Western Veterinary Supply Co., Lakewood, CO) (23), an alligator type forceps manufactured by Lawton Instrument Co., Fayette, AL (23), and a biopsy punch similar to a Hug's teat tumor extractor manufactured by D. M. Burnside, V.M.D., Quakerstown, PA 18951) (23). Other instruments used include a sliding trocar-canula instrument (24), and the Hauptner uterine biopsy instrument (Hauptner, Hannover W. Germany) (32). The use of several other types of biopsy instruments has also been reported (23, 27, 33-37).

In performing the biopsy a piece of endometrium to be biopsied is either sucked into the instrument or by manual assistance via the rectum the uterine wall is pressed into the jaws of the biopsy device (32). The specimen should be immediately placed in Bouin's fixative solution. This solution is preferred as it results in fewer shrinkage artifacts compared with a softer fixative such as formalin (36). A technique where the biopsy instrument is taken back to the laboratory, where it is disinfected with 70% alcohol and the tip lightly flamed before removal of the biopsy specimen for histological and microbiological examination (22, 28), will give rise to artifacts on histological examination.

Examination of biopsy specimens reveals the presence and extent of endometritis. Endometrial abnormalities associated with infertility include cystic glands with shallow epithelium, infiltration of neutrophils, eosinophils, plasma cells and macrophages, accumulation of lymphocytic follicles and periglandular fibrosis or encapsulation (35).

Studer has devised a grading system for histological evaluation of endomerial biopsies (20). This includes:

Uterine glands. (1) normal; (2) some with increased lumen size; (3) some cystic with shallow epithelium; (4) periglandular fibrosis. Manspeaker further divided periglandular fibrosis into mild, moderate and severe (27).

Lymph nodules. (1) none; (2) one or more.

Polymorphonuclear cells. (1) 0 to 5/high power field (hpf); (2) few to 15/hpf; (3) few to 16 or more/hpf.

Mononuclear cells. (1) 0 to 15/hpf; (2) few to 50/hpf; (3) few to 50 or more/hpf.

Exudate in lumen of glands or lumen of uterus. (1) none; (2) some; (3) much.

Overall inflammatory rating. (1) absent; (2) slight; (3) moderate; (4) severe.

There have been several surveys involving histopathology on samples of uterine tissue obtained from repeat breeder cows (3, 7, 33, 34, 38-43). Findings in these surveys were similar to those of endometrial biopsy (20), but nodules consisting of masses of lymphocytes distributed at random within the otherwise normal endometrial stroma, often surrounded by a fibrous connective tissue capsule and clearly separated from the endometrial stroma were observed (38). These nodules were found in 27% of repeat breeders sampled but were not found in any animals with good breeding history. These nodules may not, however, be a poor prognostic sign, as none of five cows in which the

nodules were present at day 30, postpartum, were bacteriologically positive. The presence of these foci may indicate infection at one time but failure to isolate bacteria in the uteri showing these foci suggests that the infection was chronic or mild or had been cleared (34). Abnormalities including nodules, encapsulated uterine glands and cystic glands were seen in 16% of animals with normal breeding history, and 70% of animals with a poor breeding history (38). It was thought that the encapsulated glands were caused by inflammation resulting from infection, whereas cystic endometrial glands can be produced experimentally following the administration of estrogen, are present in the endometria of cows with cystic ovaries (38) and have a higher incidence in animals fed excessive levels of estrogen in the forage (39). It was also thought that cystic glands were caused by occluded gland ducts (39). Periglandular fibrosis was noted in 33.3% of repeat breeder animals, one pregnant animal and in none of 24 controls (42), but in another survey was present in 97.3% of first calf heifers biopsied in one herd even though the incidence of retained placenta (10%) was considered average (27). There is an apparent relationship between extent of endometrial scarring and services per conception and final conception rate (23). Endometrial scarring is more prevalent in poor breeders than good breeders, and cows that are reproductively normal and calve on an annual basis show lesser degrees of scarring along with some normal sections (23).

Variation in muscle thickness of the uterine wall may occur with age while variations in gland cross section numbers and diameter are

due to individual cow differences, with no significant differences due to age or number of calvings (34). In only one cow out of a hundred and nine repeat breeders were histological changes in the endometrium sufficient to account for the repeat breeding. In forty other cows, more than normal numbers of lymphoid follicles, plasma cells and eosinophils were seen, but all of these suspected abnormal changes might be within the normal physiological limits of the bovine uterus (40). Lesions that were observed in uterine cross section samples obtained from repeat breeder cows at slaughter that would not be observed in mucosal biopsies included an increase in number and size of capillaries and blood vessels. A considerable collection of blood in the stroma outside the blood vessels was frequently observed. Moderate stromal edema was also noted (3). In another survey of the reproductive tracts from 300 repeat breeder cows, there were 59 cases where the uterus was abnormal grossly (43). Sections from 8 of these and 19 of the remainder failed to reveal any endometrial glands or surface epithelium. From 25 of the gross cases and 73 others the sites of the glands were marked in the sections only by masses of inflammatory cells. A further 13 cases showed cystic development of the glands.

In some cases, the endometritis appeared to spread to the oviduct where it caused lesions that could have resulted in blockage. The uterus has a superior power of resolution compared to the oviduct, as evidenced by the lowered incidence of sclerosis and gland site mass lesions in infertile cows having oviduct lesions (43). Conception is

possible after periods of fertility in the presence of moderately severe persistent endometrial lesions; but completion of pregnancy to term, even though possible, is lower than in those with a normal endometrium (43). These findings by Dawson are thought by some authors to represent the endpoint of endometrial histopathology, and that the endometrium is irreparably damaged only in very extreme cases (33). One of the advantages of the in vivo endometrial biopsy over samples obtained at slaughter is that the biopsy specimen can be placed in a fixative solution almost immediately following cessation of blood flow, whereas considerable time may elapse between death and excision of uterine samples in the abbatoir. Artifacts may develop as a result of sampling, storage and fixation of tissue specimens (41).

The level of endometrial damage has been related to the species of bacteria isolated. Most severe changes were noted in those uteri from which Corynebacterium pyogenes was isolated (20). In one study, endometritis was diagnosed in 97.4% of cows from which C. pyogenes had been isolated (33) and was present in subsequent samples in 3 of the 4 cows that were culture negative (33). The presence of bacteria in some samples without corresponding leucocytic foci or any other histological evidence of infection indicates that bacteria may be found in the uterine mucosa or fluid without necessarily invading the endometrium (34). A large number of cows when transiently infected with C. pyogenes during the first 3 weeks postpartum, will eliminate the organisms sufficiently rapidly to prevent the development of significant endometrial lesions. When moderate or severe endometritis is induced

by C. pyogenes, uterine lesions of progressively decreasing severity are detectable for 3 or 4 weeks after clearance of the organism. Bacteria that are transiently present and are isolated during that time might be falsely identified as the etiological agent for the endometritis (14). When purulent exudate from 2 cows with pyometra was infused into 14 other nonlactating cows, an inflamed endometrium was associated with the presence of Pasteurella sp. on day 7 following infusion, but not with the presence of other bacteria including Corynebacterium spp. (44). In a survey of uterine bacterial flora, there was a 100% correlation between presence of inflammation in the endometrium and bacterial isolation of C. pyogenes, Corynebacterium hemolyticum, Pasteurella hemolytica, Pasteurella multocida or Proteus vulgaris (31) (Table 1).

In a survey of 147 infertile cows, the clinical diagnosis of metritis in 92 cows was confirmed histologically in 74 (80.4%) of them. Fifteen cows had histologically positive biopsies without detectable signs. Severe endometritis was associated with Corynebacterium spp. in three cows and moderate endometritis was associated with Staphylococcus spp., Corynebacterium spp., Escherichia coli and Streptococcus spp. in thirteen cows. Histopathologic lesions indicative of mild endometritis were associated with all species isolated. Staphylococcus spp., Corynebacterium spp. and Streptococcius spp. were isolated from 8% of the 57 biopsy specimens that did not have evidence of inflammation (22). These findings are similar to the correlation between bacterial isolation and biopsy results reported by Studer (35, 36).

TABLE 1

PERCENTAGE OF COWS WITH POSITIVE BACTERIAL ISOLATION THAT HAD HISTOLOGICAL LESIONS OF ENDOMETRITIS

Organism	Reference Number		
	32	39 (percent positive)	48
Corynebacterium pyogenes	100%	100%	0%
Corynebacterium hemolyticum	100%	--	--
Mixed Culture Including C. pyogenes	87%	--	--
Pasteurella hemolytica	100%	--	100%
Pasteurella multocida	100%	--	--
Proteus vulgaris	100%	100%	--
Escherichia coli	40%	100%	0%
Streptococcus spp.	30%	75%	--
Staphylococcus aureus	50%	83%	--

It has been determined that the best histological detail is obtained at the 5th day postestrus. The stage of the estrus cycle affects the number of inflammatory cells within the endometrium. It is normal to find a variable number of neutrophils present during relatively short periods preceding and following estrus. These cells are normally absent at other times. However, neutrophils beneath the surface epithelium around glandular ducts and in the superficial stroma, even when present in large numbers, are considered physiologically normal. Neutrophil infiltration of the endometrium during the luteal phase of the cycle is considered abnormal (20).

Endometritis tends to recover spontaneously. The more severe the inflammation, the longer the time required for recovery. The approximate length of time for recovery may be estimated from the severity of endometritis on the biopsy (21).

Culture

A large number of surveys of the bacterial flora of the involuting bovine uterus have been carried out, both in the live animal and following slaughter. The technique of obtaining uncontaminated samples is more difficult in the cow than in the horse and undue emphasis may be placed on the isolation of organisms that may be contaminants. In one series, the culturing technique was validated by sampling a series of cows immediately prior to slaughter and then bringing the genital tract to the laboratory and culturing the uterine contents. There was agreement between antemortem and postmortem isolates in 18 of 20

cases (31). Care was taken to plug the cervix prior to slaughter to prevent contamination of the uterus with vaginal flora. This is quite likely when the cow is hung by its back legs during the slaughtering process. In another study, food dye placed in the vagina just prior to slaughter was found in the uterus of 39% of dairy cows and 23% of beef cows. Bacteria could always be isolated from uteri totally contaminated by dye in contrast to 5-7% in uncontaminated uteri (45).

Techniques for obtaining bacterial specimens varied. Some simply consisted of taking a sample of vaginal flora by inserting a sterile swab into the vagina as far as the urinary meatus (37, 46). Culturing of the cervico-vaginal mucus obtained by inserting a sterile glass tube to which negative pressure was applied has been described (47). Cervical swabs were obtained by inserting a one inch diameter sterile glass speculum into the vagina as far as the cervix and passing a long sterile cotton swab through this to the external os of the cervix (7). Zemjanis recommends culturing vaginal and cervical mucus but cautions that contamination is a serious problem. It might overshadow the presence of slow growing organisms such as Vibrio fetus (Campylobacter fetus ss. fetus) and Listeria monocytogenes and be responsible for false negative results. Frequent contaminants such as Proteus vulgaris and Pseudomonas aeruginosa might be isolated in pure culture and instead of providing supplementary information for differential diagnosis, only serve to confuse the diagnosis further (48). Most sampling techniques involve introducing a sterile swab into the uterus, although passing a guarded pipette into the uterus, infusing 50-100 ml

of physiological saline, massaging slightly and withdrawing the fluid for subsequent culture, has been described (55). Aspiration of uterine mucus has also been described (6). Several guarded culture instruments have been used (9, 10, 20, 36, 44, 49-51).

Other methods of obtaining samples include passing a sterilized biopsy instrument through a sterile vaginal speculum passed as far as the cervix. The biopsy instrument is passed through the cervix, which is fixed in place by rectal palpation, into the uterus and a sample taken and removed. Sterile swabs are used to plate out the sample on bacteriological media (22, 28, 34).

Another method that has been described includes using an instrument for in vivo sampling consisting of an outer sterile stainless steel tube, an inner sterile teflon tube and a sterile steel stillette with a sterile cotton swab was used to sample on the day of slaughter (25).

In using this technique the vulva was washed with antiseptic solution, the lips of the vulva parted, the cervix grasped per rectum and the distal end of the outer tube inserted through the cervix and into the uterus. The proximal end of the outer tube was swabbed with an antiseptic solution and the inner tube containing the retracted swab introduced through the outer tube into the uterus. When the inner tube was at a point about 30 mm anterior to the distal end of the outer tube, the swab was pushed forward to sample the endometrium (25). The uterine flora in these samples corresponded exactly with the uterine flora in samples obtained postmortem aseptically through an incision in the uterine wall (25).

Organisms cultured from the uterus include specific pathogens which have a natural affinity for reproductive organs including Brucella abortus, Leptospira spp., Trichomonas foetus and Vibrio fetus (14). These organisms will not be covered in this review. Other organisms include opportunistic pathogens that invade the uterus in the postpartum period. These bacteria can be isolated from up to 96% of normal cows in the first 30 days postpartum, but are usually eliminated spontaneously, reducing to 30% at 4 weeks and remaining fairly constant at 30% up to week 14 (14). These organisms often will not cause any significant endometritis if eliminated early (14).

Of these organisms C. pyogenes is by far the most common isolate (Table 2). On the other hand organisms isolated from cows with endometritis confirmed by uterine biopsy are listed in Table 3.

A large proportion of these organisms including coliforms and streptococci are primarily digestive tract inhabitants and are probably nonpathogenic contaminants of the genital tract. In a survey where bacteriological samples were taken repetitively from the uteri of 93 cows in 9 dairy herds, C. pyogenes was recovered at least once from 65.6% of the cows. The highest rate of infection was found during the second week postpartum. Intrauterine infection with C. pyogenes invariably induced endometritis and the duration of infection also determined the effect on fertility. In this study transient infection during the immediate postpartum period did not affect fertility (33). However, transient infection at a later period reduced fertility to first service, but rarely to a second or third service. Persistent infection

TABLE 2

UTERINE MICROBIAL FLORA FROM POSTPARTUM COWS

Reference Number	56	10	45	59	54	60	57	7	58	9	61	23	62	21	63
# of Cows Sampled	26	63	300	100	207	316	130	1359	26	100	64	57	25	106	?
% Positive Cultures	35	92	37	62	40	22	58	28	--	83	67	96	60	72	--
Corynebacterium pyogenes	11	12	16	5	--	--	--	--	23	--	12	7	7	16	10
Corynebacterium spp.	11	--	--	17	--	--	53	--	17	--	4	39	--	5	--
Staphylococcus aureus	11	--	7	5	16	25	11	3	--	11	--	29	--	--	1
Staphylococcus epidermidis	11	--	42	23	--	--	11	20	--	--	--	--	--	7	10
Streptococcus spp.	55	19	27	15	75	62	45	29	4	8	--	16	--	21	18
Pasteurella spp.	--	5	--	--	--	--	--	--	--	--	--	--	--	--	--
Escherichia coli	22	47	5	5	1	--	12	12	-	2	--	16	--	36	17
Micrococcus spp.	--	--	--	12	--	--	--	--	--	--	--	2	--	5	10
Proteus spp.	--	12	--	2	--	--	6	2	--	--	--	2	--	4	2
Pseudomonas spp.	--	--	--	18	--	--	--	--	--	--	--	--	--	--	--
Bacillus spp.	11	--	--	--	4	6	21	8	--	--	10	--	--	--	21
Kurthia spp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	6
Neiserria spp.	--	--	1	--	--	--	--	--	22	--	--	--	--	--	1
Diptheroids	--	2	--	6	6	5	--	--	--	--	--	--	--	--	--
Acartophylus spp.	--	--	--	--	--	--	--	--	--	--	--	--	4	--	--
Achromobacter lipolyticum	--	--	--	--	--	--	--	--	4	--	--	--	--	--	--
Acinetobacter spp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1
Actinomyces bovis	--	--	--	2	--	--	--	--	--	--	--	--	--	--	--
Aerobacter aerogenes	--	--	--	2	--	--	--	--	--	--	--	--	--	--	--
Alcaligenes spp.	--	--	--	3	--	--	--	--	--	--	--	--	--	--	--
Arthrobacter simplex	--	--	--	2	--	--	--	--	--	--	--	2	--	--	--
Aspergillus spp.	--	--	--	--	--	2	--	--	--	--	--	--	34	--	--
Bacteroides spp.	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--
Bifidobacterium spp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Branhamella spp.	--	--	--	--	--	--	--	--	--	--	6	--	--	--	2
Candida spp.	--	--	--	--	--	--	--	--	--	--	--	--	4	--	--
Cladosporium spp.	--	--	--	--	--	--	--	--	--	--	--	--	8	--	--
Clostridium spp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
C. pyogenes + Fusobacterium necrophorum	--	--	--	--	--	--	--	--	--	--	--	--	32	--	--
Curvularia spp.	--	--	--	--	--	--	--	--	--	--	--	--	8	--	--
Diplococcus pneumoniae	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--
Enterobacter hafnia	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--
Fusidium spp.	--	--	--	--	--	--	--	--	--	--	--	--	4	--	--
Fusobacterium spp.	--	--	--	--	--	--	--	--	--	--	10	--	4	--	--
Gaffyka tetragena	--	--	--	--	2	11	--	3	4	--	--	--	--	--	--
Listeria spp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Mycobacterium flavum	--	--	--	5	--	--	--	--	--	--	--	--	--	--	--
Moraxella spp.	--	2	--	--	--	--	--	--	--	--	--	--	4	--	--
Mucor spp.	--	--	--	--	--	--	--	--	--	--	--	--	4	--	--
Nocardia spp.	--	--	--	--	--	--	--	--	--	--	--	--	4	--	--
Penicillium spp.	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--
Peptococcus indoliticus	--	--	--	--	--	--	--	2	--	--	--	--	--	--	--
Mycoplasma spp.	--	--	--	--	--	--	--	--	--	5	--	--	--	--	--
Propionobacterium spp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Salmonella spp.	--	--	--	2	--	--	--	1	--	--	--	--	--	--	--
Sarcinia lutea	--	--	--	--	--	--	--	--	--	--	--	--	4	--	--
Scopuloriopsis spp.	--	--	--	2	--	--	--	7	--	--	--	2	--	--	--
Vibrio fetus	--	--	--	--	1	10	--	8	--	--	--	--	--	--	--
Vibrio spp.															

TABLE 3

ORGANISMS ISOLATED FROM COWS WITH METRITIS

Reference Number	10	32	23	6
Number of Cows Sampled	19	216	52	59
Percent Positive Cultures	100	32.4	100	95
Corynebacterium spp.	37	26	48	51
Streptococcus spp.	16	16	17	15
Escherichia coli	74	7	17	--
Staphylococcus spp.	--	4	31	8
Proteus spp.	26	1	2	4
Pasteurella spp.	5	13	--	--
Bacillus spp.	--	--	--	20
Micrococcus spp.	--	--	2	5
Pseudomonas spp.	--	--	--	7
Listeria spp.	--	--	--	4
Moraxella spp.	--	--	--	3
Aerobacter spp.	--	--	2	--

induced chronic purulent endometritis or pyometra accompanied by anestrus. The factors which determined the duration of intrauterine infection with C. pyogenes were not determined (33). Anaerobic culture of uterine swabs was not carried out in all cases. However, the most common anaerobe isolated in one survey was Fusobacterium necrophorum and the most common aerobe C. pyogenes (57). When only C. pyogenes or F. necrophorum were isolated from the uterus, cows had either mild or no clinical signs of infection. However, in cows infected with both organisms, severe clinical signs of infection including excessive vulvar discharge, uterine abscesses and pelvic adhesions were present. This suggests that a pathogenic synergism between C. pyogenes and F. necrophorum might have caused the increased severity of postpartum uterine infections and the subsequent detrimental effect on return to estrus and conception (57). Cows fed on a protein deficient diet had a higher number of positive bacterial isolations of these organisms at 40 days postpartum, 51.9% vs 21.7% of cows in the protein adequate group (9).

In a series of 93 cows repetitively sampled during the nongravid period, 14 cows developed persistent purulent endometritis and failed to complete uterine involution and were chronically infertile. The remaining 79 cows had completed uterine involution prior to 50 days postpartum; 47 of these cows subsequently were fertile and 32 infertile, either due to having had at least 3 unsuccessful services or deranged ovarian activity. Fertility was not influenced by the variable uterine flora or by the endometritis which was found in all the cows

within the first two weeks postpartum. Ninety-two percent of all cows were bacteriologically positive; 7% in the first week and 96% in the second. Cows which had uterine infection due to C. pyogenes subsequent to day 21 developed severe endometritis and were almost always infertile to the first service (53). Fertility to subsequent services was not necessarily impaired in those cows which eliminated C. pyogenes sufficiently early to allow complete uterine involution prior to day 50 (53). The percentage of cows positive on bacterial culture in all cows decreased from 92% on week 1 and 96% on week 2 to 77%, 64%, 30%, 30% and 25% on weeks 3, 4, 5, 6 and 7 respectively. In the two groups of infertile cows the infection rate, in those with infertility due to ovarian abnormality, decreased from 91% on week 1 to 87%, 62%, 70%, 40%, 25% and 9% on weeks 2, 3, 4, 5, 6 and 7. On the other hand those cows that were infertile and had clinical uterine disease subsequent to day 50 postpartum the infection rate was 100% on week 1 and 85%, 50%, 62%, 25% and 44% infected on weeks 2, 3, 4, 5, 6 and 7, respectively (53).

Mycoplasma media is not routinely used in culturing endometrial samples. However, in one study in which mycoplasma were cultured for, 44% of 16 samples were positive (7). In another survey of microflora of the cervico-vaginal mucus of repeat breeder cows and normal cows, 5.5% of the normal cows and 11.4% of the repeat breeder cows yielded large colony or O mycoplasmas, all except one of which were Mycoplasma bovigenitalium. However, the significance of M. bovigenitalium in causing metritis is unknown.

Following artificial insemination of heifers with semen inoculated with Mycoplasma bovigenitalium, the organism could be isolated from 75% of the heifers three days later. None of the animals became pregnant and a granular vulvovaginitis and mucopurulent discharge was present (64). However, in another study, ureaplasmas, formerly called T strain mycoplasmas because they form tiny colonies on the media, were isolated from 15.2% of the normal cows and 27.1% of the repeat breeder cows. The role that ureaplasmas play in conception failure in cattle is unknown (47, 62). They are known to cause a granular vulvitis and purulent discharge after breeding (62). These organisms have been isolated from field cases of endometritis, salpingitis and abortion (63). A correlation between ureaplasma infection and infertility has not been demonstrated in some studies (62) but severe infertility occurred in other herds (63). The organism has very specific growth requirements on artificial media and many laboratories may not be equipped to isolate the organisms. Samples for ureaplasma culture must be submitted in transport media or as frozen tissue and should reach the laboratory within 24 hours. Ureaplasmas may not survive transport in the mail (63). Care should be taken in interpretation of positive cultures obtained from the vagina of clinically normal cows. Up to 20% of normal animals may carry ureaplasma in the vagina, making interpretation of single isolates difficult. If organisms are isolated from the uterus, they should be regarded as significant as they are seldom in this location in normal animals (63).

Initial ureaplasma and mycoplasma infection in a herd may occur due to infected semen. Addition of the antibiotics (lincomycin-spectinomycin) to extenders controls mycoplasmas but not ureaplasma which can be controlled by the addition of minocycline hydrochloride (63).

The repeat breeder syndrome has been discussed previously and a relatively small proportion of these are due to bacterial infection or endometritis. In the definition of the repeat breeder syndrome animal, a failure to become pregnant after two or more breedings, normal estrous cycles, lack of palpable abnormalities on rectal examination and freedom from abnormal discharges from the genital tract are all included (14), thereby ruling out many of the cases of moderate and severe endometritis (20). Some authors have stated that the uterus of the repeat breeder animal during the luteal phase of the estrual cycle appears to be more resistant to bacterial invasion than that of the first service animal; possibly indicating a deficiency of progesterone actions (19). In one study it was shown that bacteria were found in 11.5% of 69 repeat breeders on day 16 after breeding. This is the time when the major portion of embryonic death occurs. However, no control animals were included in this study (18).

In a study by Griffin, cows were classified into three groups. These were:

Group I. Clinically normal animals which had a nongravid period of 117 days or less and had conceived to first service.

Group II. Clinically normal animals which had a nongravid period of 117 days or less but required 2 or 3 services.

Group III. Clinically normal repeat breeder cows: animals which were free of palpable genital lesions, purulent discharge and abnormalities of the estrous cycle and had at least 3 unsuccessful services.

Endometritis was present in 45% of Group II within 14 days prior to first service and was probably partially responsible for them not becoming pregnant at that service. Incidence of endometritis in Groups I and III were 11% and 17% which was not regarded as statistically significant. The total incidence of microbial infection was lower in Group III (67% cultures sterile) than Group I (55% sterile) in cows sampled in the 14 days prior to service. Thirteen percent of the cultures yielded pathogenic organisms in Group III and 5% in Group I, whereas nonpathogens accounted for 20% of the cultures in Group III and 50% in Group I. Unfortunately, the author did not specify which organisms he considered pathogens. When cows were cultured within 10 days after the first service, 0% of Group 1 were infected, whereas 44.4% of Group III were infected, but the majority of these organisms were nonpathogens. Fourteen percent of the cows in Group III had evidence of mild, moderate or severe metritis on uterine biopsy at this time, whereas none of the cows in Group I did. The authors concluded that the very low incidence of pathogenic organisms and endometritis in Group III within 10 days after first service supported observations that in clinically normal cows, nonspecific bacterial infection after service has no effect on fertilization rates or on embryonic survival (65). A comparison between bacterial isolation from clinically normal animals and cows with clinical uterine disease subsequent to

day 50 postpartum was made. Cows were sampled at weekly intervals in this study (53) (Table 4).

A comparison of bacterial isolates from the cervico-vaginal mucus of 72 normal fertile and 70 repeat breeder cows is shown in Table 5 (47). Very few surveys of uterine cultures have included virus isolation. In one study involving 17 cows with metritis, Parainfluenza-3 was isolated from 5 of them. The authors did not comment on the significance of this finding (66). The involvement of other viruses in endometritis should probably also be considered.

Susceptibility of Uterine Pathogens to Antibiotic

Several surveys have been carried out on the antibiotic susceptibility profiles of various isolates from the uterus of cows with endometritis. However, before treatment the clinician should determine that the organism has, in fact, caused the endometritis and is not just a transient infection following the elimination of the causative organisms (53). Treatment based on the culture of nonpathogenic organisms may result in the development of drug resistance and may cause further damage to the endometrium if irritant preparations are used. Not only are unnecessary treatments an added expense but some medications may be of little value or even detrimental. Several medications alter the estrus cycle and others are secreted into the milk, making it illegal for sale (35).

One survey of bacterial isolations from cows with assisted births, retained fetal membranes, secondary metritis and primary metritis and 12 control animals showed that all isolates were most sensitive to

TABLE 4

A COMPARISON BETWEEN UTERINE BACTERIAL FLORA OF CLINICALLY NORMAL ANIMALS WHICH HAD A NONGRAVID PERIOD OF 117 DAYS OR LESS AND HAD CONCEIVED TO FIRST SERVICE (Gp 1); AND INFERTILE ANIMALS WITH CLINICAL UTERINE DISEASE SUBSEQUENT TO DAY 50 POSTPARTUM (Gp 11). COWS WERE SAMPLED AT WEEKLY INTERVALS.

Weeks Postpartum	1		2		3		4		5		6		7	
Group No.	1	11	1	11	1	11	1	11	1	11	1	11	1	11
Total Sampled	7	7	8	9	12	7	6	8	12	8	7	12	12	9
Staphylococcus coagulase positive	2	1	1	0	3	1	0	0	0	0	0	0	0	0
Staphylococcus coagulase negative	5	0	1	2	2	0	0	0	0	1	1	0	0	0
Streptococcus. spp.	2	2	1	5	8	2	1	1	3	1	2	0	3	1
Enterobacteria	1	2	1	1	1	0	0	1	0	0	0	0	0	0
Corynebacterium pyogenes	1	3	4	6	3	5	0	3	0	3	1	3	0	3
Diptheroids	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Proteus spp.	0	2	0	0	3	0	0	0	0	0	0	0	0	0
Bacillus spp.	3	1	1	5	3	0	0	0	0	0	0	0	0	0
Other Organisms	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Sterile	1	0	0	0	1	1	6	4	9	3	4	9	10	5

Source: Griffin JFT, Hartigan PJ, Nunn WR (1974) Theriogenology 1, 91-106.

TABLE 5

RELATIVE FREQUENCY OF OCCURRENCE AND TOTAL COUNTS OF MICROORGANISMS IN NORMAL FERTILE AND REPEAT BREEDER COWS

	Normal Fertile	Repeat Breeder
Micrococcus spp.	15	20
Staphylococcus aureus	0	2
Staphylococcus epidermidis	19	17
Streptococcus bovis	31	25
Streptococcus faecalis	18	18
Streptococcus faecium	5	3
Streptococcus acidominimus	7	7
Streptococcus spp.	11	13
Escherichia coli	29	23
Proteus vulgaris	5	0
Proteus inconstans	1	1
Klebsiella pneumoniae	1	2
Bacillus licheniformis	17	23
Bacillus pumilus	17	20
Bacillus subtilis	0	2
Bacillus firmus	6	7
Corynebacterium pyogenes	7	16
Kurthia spp.	6	7
Neisseria spp.	1	1
Bacteroides catarrhalis	4	3
Acinetobacter spp.	2	2
Pasteurella multocida	1	2
Gram Positive anaerobes	2	9
Gram Negative rods	1	4
Haemophilus somnus	0	1
Candida spp.	8	17

Source: Panangala, Fish & Barnum, Can. Vet. J. 19: 83-89, 1978.

chloramphenicol (96%), tetracycline (59.5%), penicillin (44%), and triple sulfas (37%) (10) (Table 6). In another survey of antibiotic sensitivity of uterine pathogens from clinical cases of endometritis, the results are as follows: Three Pseudomonas spp. were all resistant to penicillin and had 100% sensitivity to streptomycin, and 33% to tetracycline and chloramphenicol. Five Streptococcus spp. were all sensitive to chloramphenicol and streptomycin, 80% were sensitive to tetracycline, and 60% to penicillin. Of twelve isolates of Staphylococcus spp. 92% were sensitive to chloramphenicol and tetracycline, 66% were sensitive to streptomycin, and 41% to penicillin. Seven isolates of E. coli were all sensitive to chloramphenicol, 71% sensitive to tetracycline, 57% sensitive to streptomycin and all resistant to penicillin (67).

The most extensive survey of antibiotic sensitivity was made on the cervico-vaginal mucus of normal fertile and repeat breeder cows (68) (Table 7). Resistance varied from 95.8% for colymicin, to 0.4% for chloramphenicol. Resistance to other antibiotics were: 51.5% lincomycin, 40.5% bacitracin, 34.6% penicillin, 34.2% triple sulfas, 32.1% methicillin, 31.2% streptomycin, 28.7% tetracycline, 24.9% novobiocin, 23.2% erythromycin, 18.6% kanamycin, 13.1% gentamicin and 8% ampicillin (68). In this study the susceptibility profile was determined by the method of Kirby-Bauer. In another study the minimum inhibitory concentrations (MIC) of several antibiotics for bacteria isolated from the uterus was determined (31, 59) (Table 8). The value of the determination of MIC levels for uterine pathogens is in evaluating the effectiveness of any antibiotic treatment in achieving adequate levels at the site of infection.

TABLE 6

ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF BACTERIAL ISOLATES FROM THE UTERUS OF POSTPARTUM COWS

Antimicrobial	% of All Isolates Sensitive	% E. coli Sensitive
Penicillin	44.0	0.0
Tetracycline	59.5	48.8
Triple Sulfa	36.9	48.8
Chloramphenicol	96.4	97.7
Cephalothin	94.0	93.0
Furacin	86.9	93.0
Gentamicin	77.4	100.0
Ampicillin	77.4	65.1
Tribrissen	54.8	72.1
Neomycin	52.4	69.8
Streptomycin	28.6	39.6
Methicillin	27.4	0.0

Source: Bretzlaff, Whitmore, Spahr and Ott, Theriogenology 17, 527-535, 1982.

TABLE 7

PREVALENCE OF DRUG RESISTANCE AMONG 237 BACTERIA ISOLATED FROM BOVINE CERVICO-VAGINAL MUCUS TO 14 ANTIMICROBIAL DRUGS (KIRBY-BAUER TECHNIQUE)

Organism	Total Isolates Tested	Antimicrobial Drug													
		P	ME	AM	S	K	GM	NB	C	TE	L	E	B	CL	SSS
Micrococcus spp.	24	5	0	8	0	0	0	30	0	42	0	0	0	100	0
Staphylococcus epidermidis	24	67	0	54	21	0	0	0	0	41	0	0	0	100	13
Staph. aureus	2	0	0	0	0	0	0	0	0	100	0	0	0	100	0
Streptococcus bovis	12	0	0	0	91	100	83	0	0	33	8	0	0	100	75
Streptococcus faecalis + faecium	19	100	100	0	100	100	100	15	0	47	94	0	0	100	100
Streptococcus acidominimus	12	0	0	0	16	92	0	0	0	0	0	0	0	100	100
Escherichia coli	40	100	100	9	3	0	0	100	0	80	100	100	100	100	25
Proteus spp.	5	60	100	0	60	0	0	20	0	100	100	100	100	100	60
Bacillus licheniformis	17	0	0	0	0	0	0	5	0	5	100	0	94	100	0
Bacillus firmus	12	0	8	0	41	0	0	33	0	8	83	0	100	100	0
Bacillus pumilus, Bacillus subtilis	20	0	0	0	0	0	0	0	0	0	100	0	100	100	0
Corynebacterium pyogenes	23	0	0	0	96	8	8	0	4	8	4	17	0	100	100
Kurthia spp.	13	0	0	0	0	0	0	0	0	0	0	0	0	100	77
Neisseria spp.	2	0	100	0	50	0	0	0	0	0	100	0	0	0	0
Branhanella spp.	5	0	100	0	40	0	0	0	0	0	100	0	0	0	0
Acinetobacter spp.	3	0	33	0	100	0	0	0	0	0	100	100	0	0	33

KEY DRUGS: Penicillin (P), Methicillin (ME), Ampicillin (AM), Streptomycin (S), Kanamycin (K), Gentamicin (GM), Novobiocin (NB), Chloramphenicol (C), Tetracycline (TE), Lincoymcin (L), Erythromycin (E), Bacitracin (B), Colistin (CL), and Mixture of Sulfamethazine, Sulfadiazine and Sulfamerazine (SSS).

Source: Panagala VS and Barnum DA: Can. Vet. J. 19: 113-118, 1978.

TABLE 8

MINIMAL INHIBITORY CONCENTRATION OF SEVERAL ANTIBIOTICS FOR BACTERIA ISOLATED FROM THE UTERUS OF DAIRY COWS

	Bacteria									
	Corynebacterium spp.		*Pasteurella* spp.		*Streptococcus* spp.		*Escherichia coli*		*Staphylococcus* spp.	
Antibiotic	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	----Concentration (µg/ml)----									
Penicillin*	1.2	0.48-20	0.78	0.2-3.0	1.0	0.2-1.8	50	4.3-100	3.12	0.5-100
Ampicillin	3.5	0.10-35	0.15	0.05-0.62	0.45	0.05-1.0	8	1-35	3.12	0.1-100
Streptomycin	15.0	0.88-100	6.25	2.5-100	12.5	0.5-25.0	12.5	2.5-100	6.25	2.5-100
Neomycin	7.5	1.00-100	3.20	1.5-100	12.5	0.5-12.5	12.5	0.7-100	3.12	2.5-100
Oxytetracycline	12.5	0.25-50.0	25	0.5-100	25	1.5-100	60	6.25-100	1.50	0.6-12.5
Chloramphenicol	1.5	0.75-6.25	0.78	1.10-12.5	1.5	0.5-25	2.5	1.25-100	1.50	0.5-12.5
Sulfadimidine	2.5	1.25-25	1.25	0.62-100	3.1	1.2-12.5	6.25	1.25-100	1.25	0.25-50
Sulfadimidine/ Trimethoprim	0.78	0.39-3.12	0.78	0.38-12.5	1.5	0.78-25	2.5	0.78-25	1.25	0.5-12.5
Polymixin B	25.0	12.5-100	2.5	1.25-5.0	25	6.25-100	2.5	1.25-6.25	25	6.25-100
Gentamicin	1.5	0.2-4.0	1.5	0.78-4.0	12.5	0.78-25.0	2.5	0.5-6.25	1.2	0.78-6.25

*1 µg is approximately 15 i.u. of Penicillin G.

Number of isolates tested: *Corynebacterium* spp.: 23
Pasteurella spp.: 34
Streptococcus spp.: 17
Escherichia coli: 44
Staphylococcus spp.: 18

Source: Ziv G: 9th International Congress on Animal Reproduction, Madrid 1980, Vol. II, 463-473.

Cytology of the Endometrial Cells

Smears of endometrial mucosa collected by using a Folmer-Nielsen catheter are suitable for cytological examination. The staining technique, Polichrome blue or Papanicolau, is somewhat laborious and the results are not as useful in predicting the stage of the estrus cycle, as in the dog, as only slight changes occur in the cytology during the estrous cycle (32). Cells observed are mainly prismatic cells with basal nuclei and polygonal round cells with central nuclei. Granulocytic cells are also seen together with cytoplasm-free nuclei, as well as cells with phagocytosed microbes (69). The same authors have observed a correlation between the cytological and histopathological examinations of samples obtained by biopsy of the uterus (unpublished data).

Electrical Resistance

Electrical resistance may be measured between two electrodes on the tip of the probe, by inserting the probe into the vagina until the tip is against the cervix. This technique has been used in the detection of estrus as in the presence of estral mucus have a lower electrical resistance than the vaginal mucosa or sparse dry mucus present during the luteal phase of the estrous cycle (70). However, some cows with purulent endometritis have resistance levels as low as cows in estrus; and if this instrument is used as the sole indicator of estrus, false positive results may be obtained. Following effective treatment of endometritis, the electrical resistance increases significantly (71).

Correlation of Diagnostic Techniques

Histopathological findings and bacterial culture provide information that cannot be determined by clinical examination alone. Cultures of endometrial biopsy specimens yielded one or more species of bacteria from 62.7% of the cows with clinical evidence of metritis in one survey (22). Bacteria were also cultured from 65.9% of those with cervicitis and 58.6% of those without detectable clinical abnormalities. The clinical diagnosis of metritis or cervicitis, or both, was confirmed histologically in 80.4% of the cows, and 16.3% had histologically positive biopsies without detectable clinical signs. Vaginal examination of the cervix and cervical mucus may increase the number of cases detected over that of rectal palpation alone (31).

A comparison of the percentage of positive cultures found in cows diagnosed as having endometritis by rectal palpation and those diagnosed by vaginal examination showed 22% positive on rectal examination and 58% on vaginal examination. There was a high correlation between culture and biopsy results and it was concluded that the vaginal examination was a more accurate method of determining the presence of endometritis (31).

In this study the guidelines for determining the presence of endometritis on rectal examination at 30 days or more postpartum were:

Normal uterus. Uterine horns 30 mm or less in diameter, and less than 5 mm difference between the two uterine horns with a slight to moderate tone to the uterus.

In cows moderately affected with endometritis. The uterine horns were between 30 and 40 mm in diameter and there was 5 to 10 mm difference between the large and small horns. In some cases there was evidence of a small to moderate uterine discharge.

In cows with severe metritis. The uterine horn was greater than 40 mm in diameter. There was more than 5 mm difference between the two horns. The tone of the uterus was abnormal and in some cases fluid could be palpated in the uterus, but insufficient to be called pyometra. There was usually a purulent discharge from the vagina and vulva (31).

A grading system for the clinical evaluation of the reproductive tract has been suggested by Studer (20) for cows examined at 30 to 40 days postpartum:

1. Vagina:

   V1--mild inflammation.

   V2--moderate inflammation.

   V3--severe inflammation.

2. Cervix:

   C1--3.75 to 5 cm in diameter with a slightly roughened and thickened feeling with or without reddening on vaginal examination.

   C2--5 to 7.5 cm in diameter with a definite rough irregular shape, with or without reddening on vaginal examination.

   C3--Diameter greater than 7.5 cm, usually with a reddened and lacerated appearance on vaginal examination.

3. Uterus: (each uterine horn should be evaluated separately)

   U1--2.5 to 3.75 cm in diameter with a slightly thickened feeling.

   U2--3.75 to 5 cm in diameter with a definitely thickened feeling, longitudinal grooves and nodularity from enlarged caruncles. The uterus contains fluid in some instances.

   U3--Diameter greater than 5 cm, grooves and caruncles readily palpable and uterus usually contains fluid.

4. Pus:

   P1--small clumps of white exudate.

   P2--from 10-30 ml of pus.

   P3--more than 30 ml of pus (pyometra).

There was a better correlation in this study between vaginal examination for purulent exudate and biopsy rating and bacterial isolation than between rectal examination and biopsy and bacterial isolation (35). Purulent discharges were highly correlated with infection with C. pyogenes (20). In some cases, douching the uterus with sterile saline may flush out a few flakes of pus and aid in the diagnosis. The severity of endometritis cannot always be determined on clinical examination and should be confirmed by biopsy (31).

Normal Uterine Involution

The clinician should be aware of the sequence of events in the normal postpartum involution of the bovine uterus in order that endometritis is not overdiagnosed. Following expulsion of fetal membranes, uterine contractions and peristalsis continue as strong rhythmic waves

that gradually decrease until the fourth day and there are only irregular undulations of the horn on days four to eight (1). Due to these muscular contractions and vasoconstriction, there is a rapid decrease in the dimensions of the postgravid horns. The length of the horn is reduced to a half of the parturition size by day 15 and a third by 30 days. By 50 days after parturition, normal involutional processes are complete but the horn may still be slightly thicker than in the prepregnant state (60, 72).

The maternal placenta involutes by the necrosis of the caruncular stalk due to vasoconstriction, leucocytic infiltration and by dissolution of the uterine caruncle by fatty infiltration, sloughing and detachment of the entire superficial layer of the caruncle that becomes part of the uterine lochia. By 5 days after parturition, necrosis due to vasoconstriction results in complete septal disorganization. By 12 days most of the caruncular septums have sloughed, leaving a raw surface with protruding remnants of blood vessels. Sloughing is complete by 15 days and by day 25, under normal conditions the caruncular surface is again covered with epithelium (72). The maximum amount of uterine lochia--1,400 to 1,600 ml--is present during the first 48 hours after parturition. This amount decreases to 500 ml by day 8 postpartum and there is only a few milliliters present by day 18. Fluid can be detected in the uterus on rectal palpation from days 7 to 10 postpartum in almost all normal and abnormal cows. A variable amount (500-2,000 ml) of lochia is discharged from the vulva, while a few animals are able to absorb nearly all of it from the uterus (73). Vaginal exudates were

observed in 30-35% of all cows examined between days 10 and 20 postpartum (74).

On rectal palpation, the size of the uterus decreases slowly for the first 4 to 9 days postpartum. From days 10 to 14 there is a marked decrease in uterine size and increase in uterine tone. This is associated with the first postpartum estrus period that is usually a silent heat. A discharge of uterine lochia also takes place at this time. During this period, the diameter of the uterine horn decreases from 12 cm to 6 or 7 cm. The rate of involution is rapid until day 25 (75). The rate of involution between days 26 and 39 is decreased and little change can be noted between days 40 and 50 (1). The age of the animal should be considered when the uterus is examined prior to 30 days postpartum (74), as the size of the uterine horn is larger in older cows than younger cows at days 10 and 20 postpartum. The time for complete involution in the normal animal has been stated to be as low as 18 days (74) to as long as 56 days (1), with most agreeing that regression is complete by 40 days (76). Involution is more rapid in primiparous animals (76, 77) and those that are being suckled (78), although some authors state it is longer in those that are suckled (79). The involution takes longer in fall and winter than it does in spring and summer (76). Administration of hormones does not shorten the period of involution but administration of progesterone will significantly increase it (76). This may be due to the positive correlation between progesterone treatment and postpartum bacterial infection (76).

Prolonged luteal function has been demonstrated in cows with pyometra and may be due to failure of PGF 2α synthesis due to endometrial damage. It is possible that uterine infection in the immediate postpartum period may reduce PGF 2α release and consequently delay involution (78). However, when prostaglandin and progesterone levels were measured in animals that either had (a) an uncomplicated puerperal period and normal involution, or (b) presence of uterine discharges in the first 30 days postpartum. Prostaglandin levels stayed elevated for a longer period of time in group B than group A, and progesterone levels remained low in both groups until the prostaglandin release had ceased. No attempt was made to compare the role of the magnitude of the postpartum prostaglandin release for completion of uterine involution (80).

Factors that may be associated with slower rates of involution include retention of fetal membranes (1), reproductive tract infections (33, 34), milk fever (75), dystocia (75), ketosis (78), and lack of exercise (81). Production levels, environment and cystic follicles did not significantly affect the rate of uterine involution (75).

Experimental Infection

Experimental infection of the uterus has been carried out with C. pyogenes (7, 44, 49, 82), E. coli (83), Pasteurella sp. (44), a Pneumococcus sp. (7), Staphylococcus aureus (7), and bovine virus diarrhea (BVD) virus (84) in order to study the response of the endometrium, time to overcome the infection and effect of treatment.

Twelve virgin heifers were divided into four groups and inoculated by placing at the external os of the cervix either a culture of C. pyogenes, a Streptococcus sp. or a coagulase positive S. aureus culture obtained from infertile cows (7). The fourth group served as controls in this study. There was no difference between infected animals and control animals as to number of services per conception (7). The animals were again reinfected after parturition and again there was no difference in number of services per conception required between infected and control groups (7).

In another study purulent exudate from 2 cows with pyometra was infused into the uterus of 14 nonlactating cows on days 4 to 8 of the estrus cycle. The lengths of the estrus cycles of the recipient cows were shortened from 21 days to 13. Pasteurella spp., C. pyogenes and other diptheroids were isolated from 64, 57 and 86% of the cows respectively. An inflamed endometrium was associated with the presence of Pasteurella spp. on day 7 following infusion. No other associations were found when the presence of bacteria, vaginal discharge or short estrus cycles were compared (44). Infusion of a suspension of C. pyogenes into the uterus of 6 cows at day 10 of the estrus cycle induced inflammation. From 11 to 18 hours later, penicillin was infused into the uterus. The degree of absorption of penicillin was greater than previously determined in cows given penicillin alone at the same stage of the cycle (82). The reasons for increased uptake may be due to increased blood flow to the uterus, reduction in integrity of the endometrial barrier or, less likely, slight changes in the intrauterine pH associated with endometritis (82).

Pyometra has also been induced following insemination with semen infected with C. pyogenes during the luteal phase of the cycle. However, these infections could not be established during the estrus phase. Experiments on ovariectomized cows showed that exogenous progesterone promotes conditions in the uterus suitable for the growth of bacteria, whereas exogenous estrogen will render the uterus resistant to infection. The addition of antibiotics to semen at the usual rates used in artificial insemination practice should prevent the development of bacteria after insemination in the luteal phase (49).

Following experimental intrauterine inoculation with a suspension of E. coli in heifers in the luteal and estrus phase of the cycle, it was shown that ovarian hormones of luteal phase heifers delayed the leucocytic response and those of estrus heifers stimulated the response. However, eight hours after infection, leucocyte responses were similar in both groups (83). A similar response has been seen following the intrauterine inoculation of rabbits with E. coli (83).

When BVD virus was experimentally inoculated into the bovine uterus, serum antibody titers increased, anti-BVD activity was detected in uterine secretions and the virus could be isolated. However, consistent histopathologic changes were not observed (84).

Uterine Phagocytosis

It has been noted that following experimental infection the leucocytic response in the bovine uterus is less marked in the luteal phase of the cycle than in the estrus phase. This difference was not noted in the normal infiltration into the uterus in uninfected rabbits at

different stages of the cycle and an effect on the production or release of leukotactic substances may be the basis for the hormonal influence on the leukocytic response (85). It is also probably more difficult for leucocytes to pass through the endometrium of the uterus in the luteal phase after they have been mobilized from the blood stream (86). In one study no difference could be demonstrated in the phagocytic ability of neutrophils in the luteal and estrus phase of the cycle and in the production of noncellular bactericidal factors by uterine leucocytes. The increased severity of the infection during the luteal phase is probably due to the slower mobilization of the defense system allowing invading bacteria to multiply (86).

Major species differences occur in uterine leucocyte concentrations and response to estrogen (88). When ovariectomized mares inoculated in utero with Streptococcus zooepidemicus were treated with either estrogen, progesterone or left as controls, estrogen treatment significantly enhanced uterine phagocytic activity and clearing, while progesterone treatment decreased these parameters (88). However, following observations of phagocytosis in the bovine mammary gland during estrus, it was noted that there was neither an increase in numbers of circulating neutrophils or their phagocytic activity associated with the elevated estrogen levels (89). Very little research has been done on the effects of various treatments for endometritis on phagocytosis. Prostaglandins which are used frequently in the treatment of chronic metritis and pyometra have been shown to significantly increase phagocytosis in mouse macrophages but whether or not this occurs in the bovine is unknown (88).

Effect of Metritis on Cycle Length

Cows with postpartum metritis had first estrus on an average of 12 days later than control cows. Differences in rate of involution and differences in the amount of debris in the uterus is influenced, at least in part, by variable severity and duration of metritis. This could explain much of the variation noted for ovarian cycles. Cellular debris in the involuting uterus may inhibit both neural and hormonal feedback mechanisms regulating the endocrine control of normal estrus cycles (90). Distension of the uterus with a viscous gel-like substance in the early luteal phase, however, shortened the cycle length to 12.5 days (91). Infusion of purulent material into the uterus on days 4 to 8 of the estrus cycle shortened the cycle to 13 days (44). It was suggested that shortened estrus cycles were probably due to persistent endometrial irritation with possible release of prostaglandin (44). Infection with C. pyogenes has been associated with clinical anestrus. Initially, this is functional with silent estrus, but total ovarian inactivity or organic anestrus occurs when the purulent endometritis has been present for 10 weeks (33). The fact that these cows have silent estrus is not compatible with the theory of Dawson that "stasis of the ovarian cycle" during the postpartum period is the primary lesion which permits C. pyogenes to exercise its pathogenic properties within the bovine uterus (92). Some of the differing findings in the above publications are probably due to the fact that an acute inflammation induced in the endometrium stimulates the production of prostaglandin, which, if it occurs in the luteal phase of the

cycle, results in luteolysis and a shortened cycle; whereas, with a chronic degenerative endometritis there is some loss of prostaglandin secreting tissue, the corpus luteum remains functional and pyometra ensues.

Routine Postpartum Treatment to Prevent Metritis

Up to 90% of cattle develop mild to occasionally severe postpartum uterine infection. The routine postpartum treatment of all cows with antibiotic boluses was thought to reduce the incidence of metritis and, thereby, improve reproductive efficiency. When this procedure was evaluated in over 119 dairy cows, cows treated with two intrauterine boluses, containing a total of 1,000 mg neomycin sulfate 24 hours postpartum, required more services per conception (1.7 to 1.4) and were open more days before conception (100.5 to 88.5) than controls (94). The authors suggested that endometrial irritation associated with the boluses might have contributed to the lower reproductive efficiency. The tightly pressed, hard uterine boluses often dissolve very slowly over a period of days or even weeks, and by acting as foreign bodies within the uterus delay normal uterine involution (95). In another study comparing the use of intrauterine boluses containing either nitrofurazone and urea or citric acid, chloromycetin, carbacol and ethinyl estradiol administered within 24 hours of calving or untreated control animals, no differences were apparent in days to first estrus, days to first service, days open or services per conception. In another study, cows were treated with either two 1 gm boluses of oxytetracycline or two boluses composed of neomycin, urea, boric acid

and sulfathiazole, or left as untreated controls, postpartum. Conception after first service was 49%, 40% and 60% in the three groups respectively. Conception after three services was 73.3%, 72.9% and 85.3% and breedings per conception were 2.24, 2.56 and 1.67 (97). Thus it is concluded that routine postcalving intrauterine treatment is not beneficial for cows that calve without problems. However, results do not preclude the use of recommended intrauterine treatment for cows with uterine problems following calving.

In the treatment of retained placenta, it is generally agreed that manual removal increases the incidence of secondary complications and delays involution and conception.

In a comparison of 89 cows either treated by the administration of 2.5 gms of tetracycline in gelatin capsules every other day until one treatment after the placenta was expelled, or receiving a vaginal examination alone, the treated group had a first service conception of 46% compared to 33% in the controls. Services per conception were 1.8 and 2.3, and days open were 93 and 111 (98) when intrauterine boluses are administered. Careful attention should be paid to sanitation, as unsanitary insertion of boluses into the uterus by the farmer may introduce a wide variety of harmful, resistant bacteria from the environment or perineal area (95).

Levels of Antibiotics in Uterine Tissue

Very few trials have been carried out to determine if adequate levels are achieved in the uterus following intrauterine and systemic administration (31, 99). Traditionally the local (intrauterine) route of

administration has continued to be the preferred route of treatment for infections of the bovine reproductive system. One of the main reasons that intrauterine treatment is more popular, as well as the fact that local treatment empirically has seemed to yield good results, is the widely held belief that following intrauterine administration of antibiotics, detectable levels are not present in the milk (31). This was true in the past when relatively insensitive tests were used; but with more effective tests presently available, this is no longer the case (100). Systemic administration of antibiotics achieves good results and adequate levels in the tissue in other organ systems such as the respiratory system. Another advantage of systemic treatment over intrauterine is the avoidance of further contamination of the genital tract (101).

When selecting the route of administration, the clinician should be aware that genital infections frequently are not limited to the uterine cavity and the endometrium. The concentration of the drug in other parts of the tract might be equally important (103). The treatment of an infectious process in the bovine uterus should follow the pharmacological principles of treating an infectious process in any other tissue of allowing an adequate concentration of an effective antibiotic to be present for an adequate period of time at the site of infection. There is also evidence that indicates that some antibiotics have a high affinity for inflammatory exudates when administered parenterally, which might ensure high tissue levels in endometrium during uterine infections (101). This fact should be taken into account when interpreting

results of pharmacokinetic studies on normal cows. Levels of antibiotics in endometrial tissue have been compared in several studies following administration by the intrauterine and systemic routes of penicillin (31, 101-103), oxytetracycline (104-106), and dihydrostreptomycin (31).

Following intramuscular injection of 10,000 i.u./lb of penicillin in healthy cycling cows, serum levels rose rapidly to peaking at 12.93 i.u./ml at 15 minutes; whereas mean peak levels of 8.80 i.u./gm in endometrial tissue were not obtained until 60 minutes postinjection but persisted at a higher level than the serum for a longer period of time. These experiments confirm that serum levels may poorly reflect the levels of antibiotic present in the tissues (101). Intramuscular injections resulted in higher blood concentrations than intrauterine infusions and a longer duration of detectable concentrations (12 hours and 4 hours respectively). On the other hand intrauterine infusions gave a higher concentration of penicillin in the uterus during a longer time (24 hours vs 8 hours) than the intramuscular administration. A systemic treatment with penicillin would need to be repeated more frequently than a local uterine treatment to maintain adequate concentration in the endometrium or uterine lumen over the same period of time (102). Following intrauterine administration of crystalline and procaine penicillin, detectable levels of penicillin were present in the endometrium at 30 and 36 hours. No detectable levels of either antibiotic were present in the serum after 45 minutes (31).

Twenty-four hours after intramuscular administration, oxytetracycline concentrations in tissues of the genital tract (ovaries,

oviducts, myometrium, serosa, cervix and vagina) were 100% higher than concentrations in plasma. Intrauterine administration resulted in high concentration in the endometrium and uterine cavity over a 72 hour period; whereas plasma concentrations were considerably lower than after the intramuscular injection. Levels in the genital tract except for the endometrium and uterine lumen were not detectable after 24 hours. Absorption of antibiotics from the uterus was less in cows with severe endometritis. Lowered plasma concentrations indicated that in the diseased endometrium there is impaired absorption of the drug from the uterus. In this study levels of antibiotics in other tissues of the genital tract were not determined in cows with endometritis (106). In a second study following intrauterine administration, 50% of the drug was absorbed in healthy postpartum cows but only 23% for cows with metritis. Concentrations of oxytetracycline in this study were high in the caruncles and endometrium of all cows at 24 hours after intrauterine infusion. Concentrations in plasma, uterine wall and ovaries were low with mean concentrations in these tissues lower in cows with metritis than healthy cows (105). In another study after 8 hours of constant intravenous infusion of oxytetracycline to achieve a steady state of 5 μg/ml, the reproductive tract was surgically removed. The mean plasma (μg/ml) and tissue (μg/g) concentrations in healthy and infected postpartum cows were plasma 4.95 and 5.23; uterine tissue 3.65 and 4.18; and ovarian tissue 4.57 and 4.53. The mean plasma to genital tissue ratios for healthy and infected postpartum cows was 1.38 and 1.31 for uterine tissues (104).

Computer simulated uterine tissue concentrations of oxytetracycline after twice daily intravenous doses of 11 mg/kg indicated that this dosage regimen would provide postpartum uterine tissue concentrations greater than 5 μg/g during most of the dosage interval. This study indicated that the mean plasma to uterine tissue ratio was significantly higher in postpartum cows than in cycling cows. This would mean that if 11 mg/kg of oxytetracycline was administered once daily, concentrations greater than 5 μg/g would be maintained in uterine tissues for 12 hours in the cycling cows but only 9 hours for the postpartum cows (104). Thus in the treatment of uterine infections in the postpartum period higher dosage levels with shorter dosage intervals should be more appropriate.

Following intrauterine administration of 1 gm of dihydrostreptomycin, antibiotics could be detected in the uterine lumen for up to 24 hours; but could not be detected in the endometrium or serum (31), although sizeable concentrations were observed in the serum in another study (107).

Changes in Absorption During the Estrous Cycle

Absorption from the uterus of sodium benzylpenicillin occurred to a greater extent in cows in estrus than those in the luteal phase of the cycle (108). A similar finding was observed following the intrauterine infusion of sulfonamides (109).

The differences in absorption may either be due to a difference in permeability of the endometrium or could possibly be due to passage of

the antibiotic solution up the oviduct to be absorbed by the peritoneum. The oviducts have been shown to be patent to a barium sulfate solution during the estrus phase of the cycle but not the luteal phase (108). An alternative theory is that increased blood circulation to the uterus, which occurs during estrus, may be responsible for the increased absorption (82). Increased blood flow has been induced by the administration of 10 mg of estradiol benzoate either by the intrauterine or intramuscular route one hour before intrauterine administration of penicillin. The same effect was observed following the infusion of a suspension of C. pyogenes; 11 to 18 hours prior to penicillin infusion. Levels of penicillin absorption were higher in all 3 groups than that previously determined in cows given penicillin alone at the same stage of the cycle (82).

Absorption from the uterus is also affected by days postpartum. Cows less than 60 days postpartum absorbed an average of 27.4% of the dose of chloramphenicol infused into the uterus, whereas those more than 60 days postpartum absorbed 54.6% of the dose. All cows had normally involuting uteri with no evidence of metritis (110).

Another study showed low serum drug levels shortly after parturition when infusing penicillin, dihydrostreptomycin or oxytetracycline into the uterus. As the time interval from parturition increased, serum concentrations of antibiotic following intrauterine infusion increased (107). One would think that absorption from the uterus in the immediate postpartum period would be increased due to an increased surface area and vascularity. However, there may be a

natural barrier to absorption established in order to prevent the absorption of toxins, drugs and other agents from the uterus (110).

Infusion Causing Luteolysis

Intrauterine infusions have an effect on the duration of the estrous cycle. It is thought that this occurs by stimulating the synthesis of prostaglandins by the endometrium. It is not certain whether this is caused by local irritation or distension of the uterus. When a viscous gel was infused into the uterus in the early luteal phase, the cycle was shortened to 12.5 days, while when it was administered in the late luteal phase the cycle was lengthened to 25.8 days. Gel treatment produced a temporary endometritis associated with cloudy mucus and numerous leukocytes and epithelial cells in the vagina (91). A similar effect was seen following intrauterine administration of an iodine solution or oxytetracycline hydrochloride. Infusion on day 4 reduced the interestrual interval to 10.6 days, whereas infusion on day 15 did not alter the duration of the estrous cycle. Superficial endometrial necrosis was present 24 hours after infusions of both medications (111, 112). It is thought likely that this local irritant effect is responsible for luteolysis rather than the uterine distension as volumes of iodine solution too small to cause distension (1-5 ml) were equally as effective as larger volumes in altering the interestrous interval (112). Measurement of serum progesterone showed that in cows given an intrauterine infusion of iodine solution on day 4 of the cycle resulted in values that paralleled the normal curve and then decreased rapidly to baseline over a 3 day period. After intrauterine infusion on day 15,

serum progesterone remained at diestrus concentrations until day 21, approximately 4 days longer than the control values. The suggested mechanism for the delay in estrus was that the iodine solution initially destroyed the source of the luteolytic factor, prostaglandin, and that synthesis of luteolysin probably resumed during endometrial repair. The synthesis of luteolysin was followed by luteolysis and estrus (113).

Other solutions that have been infused into the uterus and cause luteolysis include 200 ml of a 20% solution of calcium borogluconate (114), and nitrofurazone (115). The infusion of a penicillin solution induces luteolysis in the horse (116), but this has not been reported in cattle. Following the experimental infusion of a purulent exudate from cows with pyometra into 14 recipient animals, it was noted that the infusion irritated the endometrium causing an early return to estrus rather than causing pyometra (44).

Antimicrobial Agents Used in the Treatment of Metritis

Ampicillin

Ampicillin has been used in several trials as an intrauterine treatment but no pharmacokinetic studies have been carried out (1, 31, 59, 66, 117-120). The formulation of ampicillin used will affect the levels present in the uterus and length of time they are present. Following intramuscular administration, sodium ampicillin produced peak serum levels that were approximately twice as high as those achieved with an equal dose of ampicillin trihydrate. Serum levels remained above

2.0 µg/ml for six hours with the trihydrate preparation but only three hours for the sodium preparation (120).

In a series of 237 bacteria isolated from bovine cervico-vaginal mucus, it was demonstrated that 92% were sensitive to ampicillin (68). Only chloramphenicol had a higher percentage of sensitive isolates. However, following a single intramuscular injection of 10 mg/kg ampicillin trihydrate, serum levels greater than the mean minimal inhibitory concentrations (MIC) against bacteria isolated from the uterus were not achieved for Corynebacterium sp., E. coli or Staphylococcus aureus (59) (Table 9). Research to determine if tissue levels or levels in the uterine lumen were greater than the MIC have not been carried out (101).

Recommended dosages for intrauterine infusions are 500 mg in 15 ml of distilled water (119) or 2 gms in 50 ml (66, 117). The aqueous suspension of ampicillin trihydrate is less soluble than the sodium salt and has been reported to persist in the equine uterus for ten days. Following intrauterine infusion of four normal cows in the estrual stage of the cycle, no detectable antibiotic levels were present in the milk. Serum levels were not determined (117). In another study a dose of 1 gm ampicillin trihydrate in 15 ml of distilled water gave detectable residues in the milk for 48-72 hours (100). The only study comparing the efficacy of intrauterine infusion of ampicillin with other antibiotics in the treatment of endometritis showed a 61% conception for ampicillin compared with 69% for streptomycin, 72% for chloramphenicol, 77% for tetracycline and 88% for furacin (119).

TABLE 9

DURATION OF FREE DRUG CONCENTRATION IN BOVINE SERUM AFTER A SINGLE INTRAMUSCULAR INJECTION OF ANTIBIOTICS IN RELATION TO THE MEAN MINIMAL INHIBITORY CONCENTRATION OF BACTERIA ISOLATED FROM THE UTERUS

Antibiotic	Dose (mg/kg)	Bacteria				
		Corynebacteria	Pasteurella	Strep.	E. coli	S. aureus
		----Hours----				
Penicillin G Sodium	10	2	2	2	0	2
Penicillin G Procaine	10	4	4	4	0	0
Ampicillin Sodium	10	2	4	2	1	1
Ampicillin Trihydrate	10	0	6	4	0	0
Ampicillin Trihydrate	20	4	12	8	0	4
Streptomycin	10	1	2	1	1	2
Streptomycin	20	2	4	1	1	4
Neomycin	10	2	4	1	1	4
Oxytetracycline	10	0	0	0	0	4
Oxytetracycline	20	0	0	0	0	6
Oxytetracycline I.V.	10	1	0.5	0.5	0	8
Chloramphenicol Base	25	2	6	2	0	2
Chloramphenicol Base	50	10	12	10	4	10
Chloramphenicol Base I.V.	25	8	10	8	6	8
Sulfadimidine I.V.	100	8	12	6	2	8
Sulfadimidine/ Trimethoprim	25	12	12	8	6	6
Polymixin B	2.5	0	4	0	4	0
Gentamicin	3	6	6	0.5	4	6

Source: Ziv G: 9th Int. Cong. Animal Reprod., Vol. II, 463-473, Madrid 1980.

Chloramphenicol

As stated before, it is now illegal to use chloramphenicol in the treatment of food producing animals; however, the drug has been used extensively in the past in the treatment of endometritis (6, 7, 59, 67, 100, 110, 120-122).

In the past recommended dosages for uterine infusions have varied from 200 mg to 5 gms of chloramphenicol either alone or in combination with furacin or neomycin in solution in water, propylene glycol or vehicle 0 (6, 7, 67, 100, 110, 121).

Chloramphenicol base at a dosage of 50 mg/kg given intramuscularly will maintain serum levels greater than the MIC for 10, 12, 10, 4 and 10 hours against Corynebacterium spp., Pasteurella spp., Streptococcus spp., E. coli, and S. aureus respectively (59). Of 237 isolates from cervico-vaginal mucus, all except four were sensitive to chloramphenicol (68). Whether adequate levels of antibiotic were obtained in the endometrium or uterine lumen following systemic administration has not been determined.

Following intrauterine administration of 5.5 mg/kg levels achieved in the serum were greater than 0.6 µg/ml for four hours (110). However, the minimum therapeutic blood level of chloramphenicol recommended for most susceptible infectious agents is 5 µg/ml. When given intravenously at a dose of 11 mg/kg, therapeutic levels of antibiotic were maintained for up to five hours. The same dose given intramuscularly resulted in blood levels of 1.4 to 2.0 µg/ml which are unlikely to be therapeutically effective. (123). The average MIC of

chloramphenicol for bacteria isolated from the uterus is 1.5 µg/ml for Corynebacterium spp., Streptococcus spp., and Staphylococcus spp. and 2.5 µg/ml for E. coli with some organisms having an MIC as high as 100 µg/ml (59).

The absorption of chloramphenicol from the uterus increases with days postpartum, 16% being absorbed into the systemic circulation on day fourteen to 80% on day seventy-seven (110).

Following intrauterine infusion of 1 gm of chloramphenicol, 61.8% of cows with metritis conceived; this was compared with 50% for 100 mg tetracycline, 77.8% for 400 mg tetracycline, 66.6% for penicillin-streptomycin and 82.5% for Polymixin-bacitracin (7). In a comparison of treatment of metritis with a mixture of 200 mg chloramphenicol and 300 mg framycetin given in three doses at weekly intervals and untreated controls, the overall clinical recovery rates estimated by 60 days after parturition were 49% in the treated animals and 33% in the controls. The mean interval from calving to conception was 131 days in the treated animals and 147 in the controls. Bacteria were isolated from the uteri of 94% of the cows and C. pyogenes were present in 51%. All bacterial isolates were sensitive to chloramphenicol and 85% to framycetin (6)

Gentamicin

Gentamicin has been used as an intrauterine infusion in several studies (100, 119, 124-127). Dosages recommended include 160 mg in 15 ml of distilled water (100), 200 mg in 20 ml (126, 127) or 100 ml of saline (66) 250 mg in 50 ml saline (124), or 500 mg in 10 ml of distilled

water (100). Of 237 bacterial isolates from cervico-vaginal mucus, 88% were sensitive to gentamicin (124) whereas 99% were sensitive to gentamicin in a second study (127).

The distribution of gentamicin in plasma and the uterine lumen has been studied both after intrauterine and intramuscular administration in normal cows (124). Following intramuscular administration of 4 mg/kg, concentrations of gentamicin in the plasma were above mean MIC levels for Corynebacterium spp., Pasteurella spp., E. coli and S. aureus for over six hours. Levels achieved in the uterine lumen after this treatment were also above the mean MIC for those organisms after fifteen minutes and gradually increased up to six hours. Levels were not measured after six hours (124). Following intrauterine administration of 225-275 mg in 50 ml of saline, antibiotic levels in the uterine lumen remained above the MIC for these organisms for six hours but mean serum levels were not above the MIC levels (124). Following intrauterine infusion of gentamicin in distilled water, 40-56% of the infused solution was absorbed during the subsequent five post-infusion hours for a total absorption of 61% of the infused dose. Following intrauterine infusion of gentamicin in saline only 18% of the infused volume was absorbed from the uterus by six hours postinfusion (125). This difference was thought to be due to the possible retention of drug in uterine tissue using the distilled water venicle (125).

The treatment of 44 cows with metritis by the intrauterine infusion of 160 mg in 15 ml of distilled water, 3 to 5 times, on alternate days, resulted in conception rates of 62%. This compared to 69% for

streptomycin, 72% for chloramphenicol, 78% for tetracycline and 88% for furacin (119). Following the intrauterine infusion of 200 mg gentamicin in saline, 91% of 44 normal cows and 91% of 46 infected cows conceived after one to three services. This compared with 95% of 40 untreated normal cows. The interval between treatment and conception was similar for treated normal and treated infected cows (127). In another study comparing gentamicin (200 mg in 20 ml of saline) with Utonex^{R} (Schering) (20 ml containing 0.1 mg of ethinyl estradiol and 1 mg nitrofurathiazide in a peanut oil vehicle per ml), 96% of normal cows treated with gentamicin conceived at the first service after treatment compared to 89% of the normal cows treated with Utonex. Mean post-treatment conception times for the two groups of treated normal cows were comparable (127). Gentamicin sulfate was administered by intrauterine infusion 10 minutes following first service insemination at the rate of 200 mg to dairy cows allotted at random to treatment and control groups to evaluate the effect on fertility. The first service pregnancy rate was 43.8% in treated cows and 51.7% in control cows. The pregnancy rate for both first and second services was 68.7% for treated and 71.1% for controls. Fertility was not enhanced by this treatment and intrauterine medication should be reserved for only those cows exhibiting clinical signs of infection or subclinical cases diagnosed by uterine culture and biopsy procedures (126).

Penicillin

Penicillin and tetracycline are probably the two most widely used antibiotics for intrauterine infusions. For penicillin the recommended

dosages vary considerably from 100,000 to 6,000,000,000 units of potassium, sodium, procaine or benzathine penicillin. These recommended dosages are either alone or in combination with sulfonamide, chloramphenicol, streptomycin, tetracycline or aureomycin hydrychloride. Diluents used include sterile water, saline or an oil in water emulsion and quantities infused varied from 10 to 150 ml (1, 7, 31, 82, 97, 102, 103, 107, 108, 120, 121, 126, 128-130). Of 237 bacterial isolates from cervico-vaginal mucus, 66% were sensitive to penicillin; however, penicillin may be quickly inactivated by penicillinases produced by some potential pathogens of the uterus (68, 120).

Septic metritis is preferably treated by intramuscular injection (59). Penicillin G sodium at a dose of 10,000 i.u./kg intramuscularly will maintain levels above the mean MIC of Corynebacterium spp., Pasteurella spp., Streptococcus spp. and S. aureus for two hours, levels are not achieved that are inhibiting to E. coli. Procaine penicillin G given at the same dose will maintain levels above the mean MIC for Corynebacterium spp., Pasteurella spp. and Streptococcus spp. for four hours, but will not achieve adequate levels against E. coli or S. aureus in the serum (59). Following intrauterine administration of 22,000 i.u. per kg of aqueous sodium benzyl penicillin, at day twelve of the estrous cycle, levels above the mean MIC for Corynebacterium spp., Pasteurella spp., and Streptococcus spp. were achieved for four hours in the serum. Levels above the mean MIC for S. aureus were achieved for 90 minutes but were not achieved for E. coli (59, 108).

Absorption from the uterus at estrus occurred to a greater extent than in the luteal phase. Intrauterine infusion of 10% Lugol's iodine solution (an irritant that causes disruption of the endometrium) thirty minutes before the administration of penicillin (112) did not significantly increase antibiotic absorption (108). Injection of 10 mg of estradiol benzoate either intramuscularly or into the uterus in cows on day 12, one hour before infusion of penicillin, increased absorption significantly as did the infusion of a suspension of C. pyogenes 18 hours prior to infusion of penicillin (82). The reasons for the increased absorption were suggested to be increased blood flow to the uterus or following infusion of C. pyogenes, a reduction in the integrity of the endometrial barrier following disruption of the tissues (82). In another study, intramuscular injection of 20,000 i.u./kg in cows in the luteal phase of the cycle resulted in higher blood concentrations than intrauterine infusions of the same dosage; and levels were detected for a longer period of time, 12 hours for intramuscular administration and four hours for intrauterine (103). Levels were detected for eight hours in the endometrial tissue following intramuscular administration and 24 hours following intrauterine.

Levels in the endometrium following intramuscular administration were higher than in serum for eight hours. Higher concentrations were achieved in the endometrium following intrauterine administration than the same dose intramuscularly (102). Antibiotic levels in the plasma and endometrium following intramuscular administration did not exceed the mean MIC for any of the common bacteria isolated from the

uterus (59). Levels in the endometrium following intrauterine treatment were above the mean MIC for Corynebacterium spp., Pasteurella spp. and Streptococcus spp. for up to 12 hours (103). The effect of days postpartum on absorption was evaluated by administering 2,000,000 units of penicillin by intrauterine infusion in 20 ml of aqueous solution on day 3, 13, 23 and 33 postpartum. Penicillin concentrations in serum were approximately 10 to 15 times higher when infusions were given at 13 days postpartum or later when compared to infusions on day 3 when penicillin concentrations were low and lasted for a longer period of time. This indicated a very low efficiency of absorption in the early postpartum uterus. Penicillin concentrations in serum were depleted at eight to ten hours postinfusion and were below the mean MIC for all common uterine bacteria by two hours (59, 107). Following intrauterine administration of 1,000,000 units of procaine penicillin G, levels higher than the mean MIC for all common uterine bacteria were present in the uterine lumen for 36 hours and were present for 30 hours with crystalline penicillin. In these studies levels achieved in the serum were not above the mean MIC for any common uterine organisms for either antibiotic (31).

The efficacy of infusions of penicillin in improving conception rates is debatable. Several authors report a positive effect while others suggest no effect or a negative effect. Following the infusion of 600,000 units of procaine penicillin G, 200,000 units penicillin G. sodium and 2 grams of dihydrostreptomycin in 20 ml of saline at the time of breeding in 130 cows that had been bred an average of 3.3

times, 50% conceived to that breeding, 24% to the following one, and 18% to subsequent breedings; 8% were finally disposed of as sterile (97). Repeat breeder cows that had returned to service for the third time were either infused with 10 ml of 0.9% saline solution or 300,000 units procaine penicillin G and 1 gm of dihydrostreptomycin sulfate in 10 cc of saline. Conception rate for the subsequent five breedings was 68.4% for the treated animals and 60.6% for the controls (16). Infusion of 200,000 units of crystalline penicillin and 1 gm of streptomycin at the time of breeding in repeat breeder cows resulted in a 66.6% subsequent conception rate, which was better than that achieved with chloramphenicol or 100 mg tetracycline, but not as good as 400 mg tetracycline (77.8%) and polymixin-bacitracin (82.5%) (7). When repeat breeder cows were treated with 500,000 units of penicillin sodium and 1 gm of streptomycin in 50 cc of sterile saline, a conception rate of 64% was achieved at the concurrent heat which compared to 33% in untreated controls. When the antibiotics were dissolved in sterile water the conception rate was 51% compared to 78% if dissolved in 12% sulfapyridine (128). In a comparison of the intrauterine treatment of repeat breeder cows with infusions of iodine, penicillin in various preparations and a preservice uterine douche with 500 ml of isotonic ringers solution and dextrose, there was no difference in subsequent conception between treatments or when compared to untreated animals. There was a slight difference that indicated it was better to administer intrauterine infusions during estrous or within 24 to 48 hours after estrus than at other times of the cycle (130).

Treatment of repeat breeder cows with a mixture of 250 mg aureomycin, 1 gm dihydrostreptomycin and 100,000 units of crystalline penicillin in 125 ml of sterile water on either day 17 of the estrous cycle or when the cow was in estrus; but 12 hours prior to breeding resulted in a 28.6% conception rate of the next two breedings in cows treated on day 17 and 50% in the controls. In cows treated at first sign of estrus there was a 37.5% conception and 57.9% in the controls (20).

Streptomycin

When streptomycin is administered in the treatment of endometritis it is generally in combination with penicillin (7, 16, 97, 107, 128, 130, 131). Of 237 bacterial isolates from bovine cervico-vaginal mucus only 30% were resistant to streptomycin; however, it should be remembered that resistance to streptomycin may develop rapidly during treatment (68). Following intramuscular administration of 10 mg/kg, levels were achieved in the serum above the average MIC for one hour for Corynebacterium spp., Streptococcus spp., E. coli and two hours for Pasteurella spp. and S. aureus. When the dose was increased to 20 mg/kg, levels were achieved for one hour for Streptococcus spp. and E. coli, two hours for Corynebacterium spp. and four hours for Pasteurella spp. and S. aureus (59).

Following intrauterine infusion of 1 gm of dihydrostreptomycin, detectable levels were present in the uterus at 24 hours after infusion but were not detected in the serum or endometrial tissue at any stage (31). This may have been due to the use of an incorrect assay (132).

A rapid disc assay, designed to determine levels of penicillin, was used as the assay to detect oxytetracycline. In another study, cows were infused with 2,000,000 units of procaine penicillin G and 2.5 gms dihydrostreptomycin sulfate in 2.0 ml of aqueous solution on days 3, 13, 23 and 35 postpartum. The amount of dihydrostreptomycin absorbed on day 3 was much lower than in the three subsequent infusions. Peak serum levels were achieved within one hour and detectable levels were present in the serum for 24 to 48 hours (107). The only clinical trial where streptomycin was used to treat cows with endometritis by uterine infusion of 1 gm in 15 ml of distilled water resulted in a 69% conception rate which was better than ampicillin and gentamicin, but not as good as chloramphenicol (72%), tetracycline (78%), or furacin (88%) (119).

Sulfonamides

With the development of newer antibiotics, sulfonamides are not used as frequently as they have been in the past. However, in the treatment of infections of the reproductive tract they are very useful. Yet, it has been suggested that since they are rather insoluble and tend to settle in the apex of the horn that they are of questionable value (59). Of 237 bacteria isolated from cervico-vaginal mucus, 66% were sensitive to sulfonamides (68). Following intravenous administration of 100 mg/kg sulfadimidine, levels higher than the mean MIC were present in the serum for two hours against E. coli, six hours against Streptococcus spp., eight hours against S. aureus and Corynebacterium spp., and 12 hours against Pasteurella spp. (59).

Intrauterine infusion of 250 ml of a 25% solution of sulfamethazine resulted in serum concentrations that reached a peak of .2 µg/ml at two hours, and detectable levels were present in the serum for over 24 hours, and were above the mean MIC levels for the common uterine bacterial isolates for over 12 hours (59, 133). Following intrauterine infusions of a 40% solution of sulfadimethoxine at a dosage of 100 mg/kg, levels greater than the mean MIC for the common organisms were present in the serum for greater than 48 hours (134). Peak sulfonamide levels following intrauterine infusion of the same dose of sodium salts of sulfamethazine, sulfapyridine, sulfathiazole, sulfanilamide and sulfacetamide varied from 23.3 mg/100 ml for sulfamerazine to 1.5 mg/100 ml for sulfacetamide. Uteri in the proestrus and estrus phase absorbed more drug than uteri in the postestrus or anestrus phase (109). In cows infused with 66 mg/kg of a 25% solution of sulfamethazine on days 3, 13, 23 or 33 postpartum, the effect of enhanced absorption with number of days postpartum was not as obvious as it was with oxytetracycline or penicillin, but peak concentrations occurred earlier and were somewhat higher after infusions on days 23 or 33 as compared with peak values after infusions on days 3 or 13. The amount of sulfamethazine in serum remained higher than 5 µg/100 ml, which is well above the mean MIC for most common uterine pathogens for 24 hours after all infusions (107). No experimental data are available for levels of the drug in the endometrium either following intrauterine or intravenous administration or levels in the uterine lumen following intravenous administration. The addition of 12% sulfapyridine

solution to 1 gm of streptomycin sulfate and 500,000 units of sodium penicillin G gave a 78% conception at the concurrent breeding when infused on the second day following service. When the antibiotics were diluted in sterile distilled water, conception rate was 51% and controls were 33% (128). Results for conception after two breedings was 89% for treatment with sulfas and 61% with sterile water (128).

Tetracycline

Tetracycline either alone or in various combinations is probably the most commonly used intrauterine infusion; however, of 237 bacterial isolates from cervico-vaginal mucus only 66% were sensitive to tetracycline (68). Dosages used vary from 100 mg to 5 grams of either the intravenous solution, soluble powder or a lyophilized-calcium-glucose-terramycin complex. This was either administered undiluted or diluted in water, saline, 0.4% furacin, Vehicle 0, or with iodochlorhydroxyquinolone, furazolidone or ethinyl estradiol (7, 8, 10, 28, 50, 56, 67, 100, 105-107, 112, 121, 129, 135-141).

Following intramuscular administration of 10 mg/kg or 20 mg/kg of oxytetracycline levels in the serum greater than mean MIC were only achieved for S. aureus; 4 hours for the lower dose and 6 hours for the higher dose. If the tetracycline was given intravenously at a dose of 10 mg/kg, levels greater than the mean MIC were achieved for 8 hours for S. aureus, one hour for Corynebacterium spp. and 30 minutes for Pasteurella spp. and Streptococcus spp. Levels greater than the mean MIC for E. coli were not achieved (59). Concentrations in the endometrium following intramuscular administration of 8 mg/kg

were considerably higher than the concentration in plasma and lasted longer. The uterine secretions also contained a substantial amount of oxytetracycline but was not quantitated (106). Levels in the endometrium were not measured prior to 12 hours post administration of the antibiotic but at this stage, they were below the mean MIC for all common bacteria isolated from the uterus except S. aureus (59, 106). As mentioned previously, it was calculated that following twice daily administration of 11 mg/kg oxytetracycline intravenously, uterine tissue concentrations greater than 5 µg/g would be maintained during the dosage interval. This is below the mean MIC for all common uterine bacteria except S. aureus (59, 106). These MIC levels may be higher than in many cases as it has been stated that plasma concentrations of oxytetracycline should not be smaller than 0.6 µg/ml for good therapeutic results, and that the MIC for many of the common mastitis pathogens would be about 1.0 µg/ml (106). However, in another survey, organisms causing endometritis were found to have higher mean MIC than in the study previously mentioned (31).

Following intrauterine infusion of 5.5 mg of oxytetracycline/kg the levels present in the endometrium at 24 hours were greater than the mean MIC for Corynebacterium spp., Pasteurella spp., Streptococcus spp., and S. aureus, but was not as high as mean MIC for E. coli (59). Levels in the endometrium were higher than those achieved following intravenous administration of 11 mg/kg b.i.d. and stayed elevated over 4 µg/ml for greater than 72 hours. Plasma concentrations were considerably lower than after intramuscular administration of the

same dose. Plasma levels were even lower in cows with endometritis indicating a decreased absorption from the uterus (106). In another study plasma oxytetracycline was present by 30 minutes following intrauterine infusion, peaks at 2 hours and remains detectable through 24 hours (112). Intrauterine infusion with oxytetracycline, either in a chelated or nonchelated form, resulted in significantly lower levels in the serum with the chelated form and undetected levels in the milk (132, 141). It was suggested that such a product may have a longer antibacterial life span in the uterus than one that rapidly passes through the uterus and into the general circulation (112).

Following infusion of 40 ml of an oxytetracycline in propylene glycol preparation (50 mg/ml) on day 4 of the estrus cycle, the mean duration of the cycle was shortened to 10.7 days as opposed to 21 days for control cows infused with normal saline at the same time. Endometrial biopsy specimens taken 24 hours after infusion showed the surface epithelium to be sloughed with marked edema and superficial necrosis. These findings were similar to endometrial biopsies taken after infusions of dilute iodine solutions (141), which have also been shown to cause shortening of the estrual cycle.

Only 50% of repeat breeder cows treated by intrauterine infusion of 100 mg of injectable tetracycline conceived, whereas 78% treated with 400 mg of a soluble powder in solution conceived. This compared to 61% for chloramphenicol, 67% for penicillin-streptomycin and 82.5% for polymyxin-bacitracin (7). In another survey of cows with metritis treated by infusion of 1 gm of tetracycline in distilled water, 78%

conceived. This conception rate was only exceeded by furacin (88%) and was better than chloramphenicol (72%), streptomycin 69%, and gentamicin and ampicillin, both 62% (119). Treatment of 39 cows with endometritis with a solution consisting of 500 mg each of oxytetracycline, iodochlorhydroxyquinoline and furazolidone with 0.5 mg ethinyl-estradiol resulted in a conception rate of 80% (137). Routine infusions of all cows 30 days postpartum with a solution of 100 mg of lyophilized-calcium-glucose-terramycin complex dissolved in 20 ml of distilled water resulted in an average of 1.46 services per conception in treated animals compared to 2.18 for untreated controls (139). A similar study using the same product reduced services per conception on the control cows of 1.7 to 1.57 for treated animals (56). In those cases associated with Ureaplasma sp., treatment with nitrofurazone, chloramphenicol, chlorhexidine, 0.2% Lugol's iodine or acriflavine solutions were of little value, but the intrauterine infusion of 1 gm tetracycline solution 24 hours postbreeding resulted in a significant improvement in conception rates (66).

Iodine

Iodine is used both for its stimulating action on the genital tract as well as its antiseptic properties (130). It has previously been mentioned that some irritating solutions can irritate the endometrium and by the stimulation of production of prostaglandin induce premature estrus. An intrauterine infusion of 5 ml of strong Lugol's iodine in 250 ml of saline can cause a sloughing of the endometrium within 24 hours (113). In light of this, infusion with iodine solutions should be

given on day 5 of the estrous cycle. Thus, by the time of estrus, which occurs 5 days after infusion, the endometrium has regenerated. Breeding on this induced estrus might improve the conception rate in repeat breeders, as a change in the uterine environment may make the endometrium more suitable for implantation, or this effect might be due to normalization of hormonal events due to changes in the synthesis of prostaglandins by the endometrium (99, 112). It has been confirmed that the mechanism of action of premature estrus is induction of prostaglandin synthesis. Levels of PGF 2α increased 50 to 75 hours after intrauterine infusions and were followed by a decline in progesterone similar to the pattern observed with spontaneous estrus (113, 142). Following infusions on day 15, the mean estrus length was increased by three to four days. This was thought to be due to destruction of the source of prostaglandins and that synthesis resumed during endometrial repair (113).

The amount of iodine infused has varied from 20 to 500 ml depending on the size of the uterus and amount of pus present. Concentrations have varied from 0.5% to 10% solution of strong Lugol's iodine (7, 28, 37, 99, 112, 130). However, as little as 0.1 ml of an iodine solution (I:KI:distilled water; 1:2:60) can cause luteolysis if infused into the ipsilateral horn (143). In some cases of pyometra, an indwelling catheter has been placed in the uterus and the farmer instructed to infuse 60-100 ml of a 2% Lugol's solution every 12 hours (8).

Iodoplex^{R} (Hydrophilics International Inc., New York), a slow release iodine preparation, has been used either in oblet form in cows

with retained placenta, or infused in cows with endometritis. Treatment was repeated weekly until the vaginal discharge was clear. A subsequent conception rate of 70.5% with an average of two services was achieved. Conception rate on first service was 50% compared to 40% for the rest of the herd, and open days were 102 compared to 120 for all cows with retained placenta (135). In another study the difference in conception rates in cows with endometritis infused with iodine, penicillin or controls (not infused) was significant (130).

Other Treatments

Other drugs used in the treatment of endometritis by intrauterine infusion have included dapsone, a sulfone used in the treatment of leprosy (120), erythromycin (59, 68, 100), furacin (8, 31, 66, 115, 119, 121, 145, 146), kanamycin sulfate (59, 100, 104), lincomycin (8, 68, 121), neomycin (8, 59, 68), nitrofurathiazide and ethinyl estradiol (127), polymyxin either alone (8, 59, 68) or in combination with bacitracin (7), spectinomycin (8, 121), tetracycline and amphotericin (58), and thiamphenicol (147).

In the immediate postpartum period, absorption of certain drugs such as penicillin, dihydrostreptomycin and oxytetracycline is usually markedly decreased (107), but absorption of dapsone in this period is very rapid.

A recommended dose of erythromycin for intrauterine infusion is 2 gms in 10 ml of sterile water (100). Seventy-nine percent of the bacteria isolated from cervico-vaginal mucus have been shown to be sensitive to erythromycin (68).

Furacin has been used extensively in the past as a uterine infusion, but it has recently been demonstrated that even though many bacterial isolates are initially sensitive to it, resistance rapidly develops (8). Furacin is used either as an 0.2% or 4.2% solution either alone or in combination with tetracycline, neomycin, chloramphenicol, isopropyl alcohol or 50% dextrose in total amounts varying from 15-500 ml. Infusion on day 3 of the estrous cycle of 40 ml of 0.2% solution of furacin caused premature estrus at day 15, compared to 20.8 days for controls (115). In a study of the treatment of pyometra, it was shown that cloprostenol was superior to estradiol cypionate. Intrauterine infusion of nitrofurazone following evacuation of the uterus appeared not to be effective in prevention of relapses and had a negative effect on conception rates (66% vs 85%) (145). However, an 88% conception rate was achieved following the treatment of endometritis with 5 g of furacin in 15 ml of distilled water. This compared to 78% for tetracycline, 72% for chloramphenicol, 69% for ampicillin or gentamicin (119).

Kanamycin sulfate has been used as an intrauterine infusion at a dose of 500 mg in 10 ml of sterile distilled water (100). Of 237 bacterial isolates from cervico-vaginal mucus, 82% were sensitive to kanamycin (68).

Lincomycin has been used either alone or in combination with spectinomycin. One recommended uterine infusion consists of mixing 1 packet of LS 50 (Upjohn) containing 16.7 gms of lincomycin and 33.3 gms of spectinomycin in 500 ml of distilled water. From this, take

90 ml and q.s to 500 ml and use 30 to 60 ml for infusion, depending on the size of the uterus (121). However, of 237 bacterial isolates of cervico-vaginal mucus, only 59% were sensitive to lincomycin but 96% of the isolates of C. pyogenes were sensitive (68).

Treatment of normal cows with a preparation containing 2 mg of ethinyl estradiol and 20 mg of nitrofurathiazide in a peanut oil vehicle did not cause uterine inflammation, prolong the estrous cycle or impair conception and there was a 93% conception rate compared to 95% for untreated normal animals. The conception rate following treatment of infected cows was 86%, and the mean parturition pregnancy interval was 123.3 days for infected cows treated with the estradiol/nitrofurathiazide preparation as compared to 141 days for those treated with gentamicin (127).

Following intramuscular administration of 2.5 mg/kg polymyxin B, levels greater than the mean MIC for Pasteurella spp. and E. coli isolated from the bovine uterus were achieved for four hours, but insufficient levels were obtained for the treatment of Corynebacterium spp., Streptococcus spp. or S. aureus (59). Intrauterine infusion of 16,000 units of polymyxin sulfate and 800 units of bacitracin dissolved in 10 ml of sterile distilled water was administered to infertile cows with a resulting conception rate of 82.5%. This was better than that following infusion of 400 mg tetracycline (77.8%), penicillin-streptomycin (67%), chloramphenicol (62%), or 100 mg tetracycline (50%) (7).

In one microbiological survey of cases of endometritis, fungi were isolated from 60% of the cows. In this study it was found that fungal

induced endometritis responded well to a douche with a 0.05% solution of copper sulfate followed by treatment four times at three day intervals with 400 mg tetracycline and 200 mg Amphotericin B infused into the uterus (58).

Treatment of endometritis with infusions of a combination of a broad spectrum antibiotic, thiamphenicol with an estrogen and microlytic substance, N-acetyl-L-cysteine resulted in a 94% conception rate. N-acetyl-L-cysteine is used in human medicine for its mucolytic action on the secretion of the respiratory tract. Unlike proteolytic enzymes, it does not damage living tissue and its mucolytic effect is greatest at pH 7 or slightly higher, therefore, conditions for its effectiveness are optimal in the uterus affected with endometritis (147).

Because of their effect on tissue debris, a number of enzyme preparations containing urea, yeast, extract papain and pepsin have also been used in treating endometritis. However, their efficacy is questionable (1, 120).

The majority of the other preparations that have been used, if they are effective it is probably due to the stimulation of a mild irritating action on the endometrium similar to the effect achieved with iodine solutions. Preparations that have been used in the past include solutions of aromatic soap, mild quaternary ammonium, chlorine, dilute potassium permanganate, sodium bicarbonate, weak "Lysol," mild acriflavine, chlorhexidine, charcoal, boric acid, bismuth subnitrite or silver oxide. Various chlorine, iodine, iodoform and perborate preparations have been used. These compounds release much gas on contact

with tissue debris which results in foaming similar to that resulting from the use of hydrogen peroxide. Products causing a severe inflammatory reaction in the uterus should be avoided (1, 120). When large numbers of different treatments are reported it probably indicates that any treatment would be just as effective. Unfortunately, in most of the above experiments, very few contained control animals and when they did the controls were few in number (1).

Hormone Administration

Pyometra is characterized by the failure of estrum and retention of pus or mucopurulent material in the uterus. The amount varies from 60 cc to several gallons. Pyometra is almost always a sequelae to chronic metritis and is thought to be due to destruction of the endometrium at the level where prostaglandin is synthesized, thus acting as a luteolytic agent that lyses the corpus luteum and induces estrus in the normal cycle. Because of this lack of prostaglandin production, it is usually necessary to treat cows that have pyometra or chronic endometritis with exogenous hormones in order to induce estrus.

Metritis has been associated with an increased percentage of anestrous first ovulations, an increased interval to first estrus and longer intervals for the first ovarian cycle. In one study plasma luteinizing hormone (LH) was consistently lower and plasma corticosterone was consistently higher in the early postpartum period than in normal cows. The reason for this persistently lower plasma LH was not understood (148). Serum progesterone was 9.7 ng/ml for the pyometric cows and 5.7 ng/ml for the control cows. Progesterone

concentration was greater in both groups of cows with a palpable corpora lutea (CL).

Ninety-six of the pyometric cows had palpable corpus luteum (CL), as compared to 57% of the control group. Comparing serum progesterone only in cows with a palpable CL, the mean concentration was still greater in the pyometric group (10.6 ng/ml) than the control group (6.6 ng/ml) (149). This prolonged luteal function in cows with pyometra might result from failure of PGF 2α synthesis due to endometrial damage (78). It is also possible that uterine infection in the immediate postpartum period may reduce PGF 2α release and consequently delay involution (78). A group of 22 heifers with postpartum metritis varying from 7 to 160 days after calving had lower progesterone and higher 17 β estradiol levels than normal cycling cows; 73% were under 0.5 ng/ml progesterone. This emphasizes the need for the practitioner to carefully evaluate any abnormal postpartum condition before using prostaglandins, as without an active CL very little or no therapeutic effect would be anticipated (150).

Prostaglandin (PGF 2α) levels undergo a dramatic increase shortly before parturition and early in the postpartum period. This increase in PGF 2α concentration appears to be extremely important for normal uterine involution. The PGF 2α levels parallel the rate of uterine involution with a peak at day 4 postpartum and remain elevated for up to 20 days (78, 80). It has been demonstrated that cows with prolonged uterine involution times also had relatively extended periods of elevated plasma PGF 2α levels in comparison to cows with normal uterine involutional times (78, 80).

Administration of hormones, including estrogens, oxytocin and prostaglandins, have been used in the treatment of metritis and pyometra. Estrogens aid the uterus in reducing and overcoming infection by dilating the cervix, increasing uterine muscle tone and increasing uterine blood supply and capillary permeability. This allows increased absorption of fluids from the uterus and greater influx of phagocytic cells (112).

Cows in the luteal phase of the estrual cycle were experimentally infected with a culture of C. pyogenes and were then given intramuscular injections of 25,50,75 or 100 mg of stilboestrol. Three days later the cows were slaughtered, the uterus removed and cultured. The uteri of cows given 25 mg and 50 mg contained obvious pus but those given 75 mg showed no clinical signs of infection but bacteria could be isolated from the uteri. Cows given 100 mg showed no signs of infection and were bacteriologically sterile (49).

In a group of 102 cows with endometritis, treatments consisted of: (1) 20 mg stilboestrol diproprionate intramuscularly, (2) 500 µg Cloprostenol (PGF 2α) i/m, (3) an intrauterine infusion consisting of 500 mg oxytetracycline, 500 mg furazolidone, 500 mg iodochlorhydroxyquinoline and 0.5 mg ethinylestradiol and (4) a control group with no treatment. The length of time between calving and conception was 185, 105, 142 and 141 days for the four groups. Treatment with prostaglandins appeared to be beneficial but parenteral treatment with stilboestrol was contraindicated (12).

Several trials of intrauterine treatments with antibiotics have included estrogens in the infusion. It is difficult to determine what

effect the hormone has on the resolution of metritis as the treatments were not compared with antibiotics without estrogens (12, 127, 137, 147, 151). In one series of experiments in the treatment of postpartum metritis, it was demonstrated that the addition of diethyl stilboestrol alone or combined with proserine to an antibiotic intrauterine preparation decreased the length of time until purulent discharge was no longer present, decreased the time to conception and increased the percentage conception. Proserine alone shortened the period of recovery but prolonged the interval from recovery to conception (152). Proserine added to intrauterine infusions or administered by subcutaneous injection gave tone to the uterine muscles and increased the secretory function of the endometrium (152).

Under the influence of estrogens, the uterus possesses a high degree of bactericidal activity, it is less efficient when levels of progesterone are high. Maximal absorption of penicillin is also seen during estrus (59, 82). It has been shown that the simultaneous uterine infusion of estradiol benzoate and penicillin aid the intramuscular injection of estradiol benzoate followed by intrauterine administration of penicillin increased the degree of absorption of penicillin from the uterine lumen (108).

The induction of estrus with prostaglandins enhances the bactericidal environment in the uterus and enhances the action of antibiotics administered by intrauterine infusion. Prostaglandins also have a stimulating effect on the endometrium which might be of importance when they are administered in subluteolytic doses (153).

In a study involving 43 cows with pyometra, some with pyometra for as long as five months postpartum, 91% showed estrus within seven days after the administration of 500 μg cloprostenol. In another group of 204 cows with pyometra that was treated with prostaglandins, there was a conception rate of 88.2% (153).

Twenty-two of 26 cows treated with various doses of prostaglandins came in heat 3 days after treatment. Twenty of these cows were bred at subsequent heats and 65% of these became pregnant, but this was only 50% of the initial treatment group. Some differences in conception rate were noticed between dosage groups, 73% conception with 12.5 mg i/v and 50% with 12.5 mg i/m. (73). Response to the treatment of pyometra with prostaglandins was better when the condition was diagnosed early (154).

Fifty-one out of 56 cows with postpartum chronic endometritis treated by intramuscular injection of 500 ug of cloprostenol (CLP) (ICI: Alderley Park, Macclesfield, Cheshire, England) in the presence of a corpus luteum showed estrus within 14 days with resolution of the condition, five others did not respond. Of three cases without a palpable CL, no response was detected. This lack of response was not unexpected and supports the view that the mode of action of cloprostenol in this treatment regime is to induce luteolysis (155). Treatment of 55 cows with postpartum metritis with 25 mg dinoprost (PGF 2α dinoprost THAM Salt, Upjohn) resulted in a 76.3% recovery rate (156). In another trial investigating the use of prostaglandin in the treatment of purulent endometritis, an initial conception rate of 43.4% at the first insemination and 79.5% overall was achieved. The average interval

between parturition and treatment was 60.9 days and between treatment and first insemination or conception 36.5 and 60.5 days respectively (157). In the treatment of pyometra, the uterus emptied after one administration in 93.2% and a second treatment was necessary in the remaining animals. Initial conception at the first breeding, which was an average of 49.7 days after treatment, was 38.4%. The average between treatment and conception was 83 days, and an overall conception rate of 87.7% was achieved (157).

One hundred and fifty-three cows with metritis diagnosed thirty days postpartum were treated either with (1) intrauterine antibiotics (chloramphenicol and framycetin) given in three doses, one week between doses; (2) prostaglandin (PGF 2α) given i/m in two doses, 14 days between doses; or (3) a placebo. Clinical recovery at 60 days postpartum was 49% in the treated groups and 33% in controls. The mean interval from calving to conception was 147 days for controls, 131 days for antibiotic treatment and 123 days for prostaglandin treatment. On the average, anestrous cows recovered in 14 days and conceived 34 days later than did cycling cows (6).

Before the development of prostaglandins, luteolysis could be induced by estrogens, infusions of irritant substances into the uterus, or by manual expression of the corpus luteum per rectum. Administration of estrogens often resulted in the development of cystic ovaries. Uterine infusions were seldom successful in the presence of a large volume of pus and enucleation of the CL is usually accompanied by hemorrhage which may be severe and give rise to ovaro-bursal adhesions, infertility and even death (155).

One hundred and fifty-two dairy cows affected with pyometra were either treated with CLP or estradiol cypionate (ECP). After two treatments, 95% of CLP treated cows had evacuated the purulent exudate from their uteri, whereas only 78% of ECP treated animals had responded. Treatment by breeding, treatments to conception intervals and services per conception were not significantly different in those cows responding to treatment. The incidence of relapse 9.3% vs 5.1% and follicular cysts within 30 days of treatment, 16.4% vs 7.0% was greater in the CLP treated group (145).

Postbreeding Infusions

In repeat breeder cows or when a small amount of purulent material is present in the estrual mucus, a postbreeding infusion may be indicated. These should not be given more than 72 hours after breeding, should be in water in neutral pH and no more than 30 ml in volume. In some cases, infusions may be indicated three times, once at first signs of estrus followed by a second infusion one to two hours after breeding and a third 24 hours after breeding (8). Suggested treatments include penicillin in saline (8), chloramphenicol in dimethylsulfoxide (DMSO) (8), neomycin-polymyxin (8), lincomycin-spectinomycin (8), penicillin-streptomycin (158), gentamicin (126), nitrofurazone (159), or sulfonamides (141). The results of postbreeding infusions have been discussed under the sections on the various antibiotics.

The time after insemination has an effect on conception rates. When cows were infused at the time of insemination, conception to

concurrent service was 52%, whereas 15 minutes later it was 50%, and 24 hours after breeding it was 64.5% (141). Infusion at 24 hours postbreeding of repeat breeder cows will allow palpation of the ovaries to determine if delayed ovulation has occurred. In cows that have not ovulated by 24 hours after insemination, a second insemination will probably increase the chances for conception (141).

Presence of Antibiotics in Milk

Following systemic or intramammary treatment with antibiotics, antibiotic residues may be present in the milk for periods up to seven days. This necessitates disposal of the milk and makes it illegal for sale. One of the supposed advantages of intrauterine treatment of metritis is that residues are not present in milk. As has been seen previously, the absorption of antibiotics from the uterus is affected by the stage of the cycle, concurrent administration of hormones and endometrial damage caused by metritis. Also, if levels of antibiotics are not present in the milk, it may be that effective levels of the antibiotic are not present at the site of the infection which in severe metritis is in the endometrium and not just in the uterine lumen.

The current screening test used for detection of antibiotic residues is the Bacillus stearothermophilus disk assay (Delvo-test P, G.B. Fermentation Industries). This is much more sensitive than the Bacillus subtilus assay (100) which was previously used in the test to detect anitibotic residues in food products. Eighteen normal cows were infused with either 1×10^6 i.u., 1.5×10^6 i.u. or 2×10^6 i.u. potassium penicillin G in sterile diluent, 12 to 48 hours postestrus.

Detectable residues were determined by B. stearothermophilus assay and were present within 12 hours of infusion in 15 of 18 cows. After 12 hours, no samples were positive in the two lower treatment groups and only three of thirty samples were positive in the high treatment group representing two of six cows (129). When 1.5×10^6 i.u. of procaine penicillin G were infused into 13 cows with metritis, residues were detectable for 60 to 84 hours (100); and in six normal cows given 1.5×10^6 i.u. penicillin and 1.5 g dihydrostreptomycin, residues of penicillin were detected for up to four subsequent milkings for penicillin and up to the second subsequent milking for dihydrostreptomycin (158). Detectable levels of antibiotic were not present in milk after two hours following intrauterine treatment with 1×10^6 i.u. of procaine penicillin or crystalline potassium penicillin. However, the less sensitive Penny cylinder assay was used (31).

In four normal cows infused with two grams of ampicillin trihydrate in standing estrus or diestrus, no residues were detected in any samples (117); but in five cows with metritis infused with 1 gram of ampicillin, residues could be detected for 48-72 hours (100).

Several sulfonamides including sulfamerazine, sulfacetamide or sulfapyridine infused i.u. at the rate of 107 and 214 mg/kg were detectable in blood and milk for 24 to 48 hours after infusion. Concentrations were greater when infusion occurred during estrus. Nitrofurazone can also be detected in milk after intrauterine administration (112).

Following the placement of four 1 g oxytetracycline pessaries in the uterus of 50 cows with retained placenta, detectable levels of

tetracycline were present in the milk of 33 cows and were present in four cows at the fifth milking after treatment. Following intrauterine infusion of 4 mg/kg in normal cycling cows, residues were detected in the milk for up to 24 hours (106, 132), but no detectable levels were present at the same dosage of a chelated oxytetracycline preparation (132). In cows with metritis infused with either one infusion of 3 gms or three infusions of 500 mg of oxytetracycline, detectable residues may be present for up to 84 hours (100).

Following one infusion of 1 gm of chloramphenicol, residues were present in the milk for up to 96 hours; and following three infusions of 1 gm for up to 108 hours (100). Following one infusion of 500 mg of Kanamycin sulfate, residues were present for over 72 hours; and three infusions at the same dose gave residues for over 120 hours (100). One infusion of 2 gms of erythromycin resulted in residues in the milk for up to 84 hours (100).

As new tests are developed for detecting antibiotic residues, smaller amounts will be detected in the milk and this advantage of intrauterine infusion over systemic treatment will no longer be present. The same criteria that are applied to the treatment of infectious diseases in other parts of the body should be applied to the treatment of metritis. The organisms causing the infection should be identified, their antibiotic susceptibility profile determined and then it should be ensured that an effective antibiotic is present at the site of infection for an adequate period of time at the correct level. When these criteria are fulfilled a significant improvement over the results obtained with no treatment may be observed.

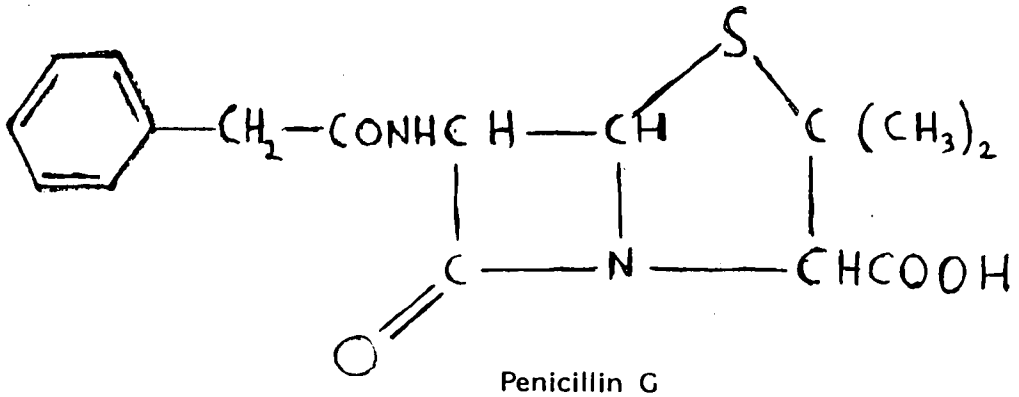
Pharmacokinetics of Drugs Used in the Treatment of Endometritis

Penicillin

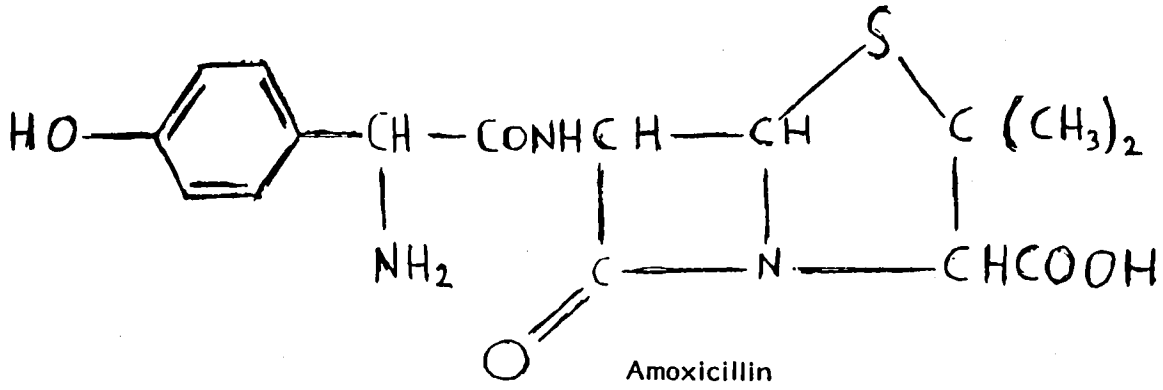
Penicillin has been used both systemically and by intrauterine infusion in the treatment of endometritis (160). The chemical formula of penicillin consists of what is known as the beta-lactam thiazolidine with various side chains depending on the particular penicillin product (Figure 1). The physical and chemical properties, particularly the solubilities of penicillins are related to the structure of the acyl side chain and the ability of cations to form salts (160).

Hydrolysis of the beta-lactam ring is the main cause of penicillin degradation. The optimal pH for the stability of penicillin is 6-6.5 with a practical range of 5.5 to 7.5. An alkaline pH may occur in the uterus in association with metritis and may decrease the antibiotic activity (160). The pH may also influence the bonding of penicillin to serum protein. Above pH 9.0 there was marked reduction in the binding of penicillin G to bovine serum protein (161). Prolonged exposure of crystalline penicillin G to water promotes hydrolysis. Penicillin is incompatible with the ions of heavy metals, oxidizing agents and strong concentrations of alcohol (160).

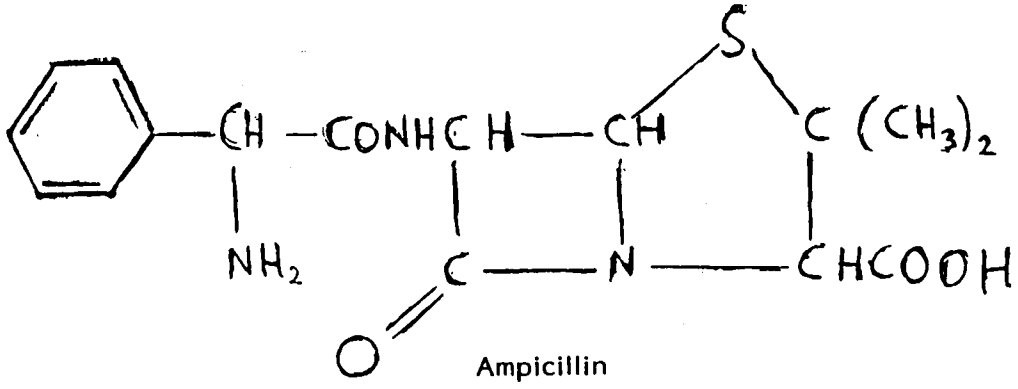
Other factors that can affect the efficacy of penicillin include the amount of necrotic tissue and exudate at the site of infection (161). The mean percentage of penicillin penetration into an uninfected site (an intraperitoneally implanted plastic reservoir) was 19.9%, whereas it was only 1.5% in a similar site infected with Bacteroides fragilis (162).



Penicillin G



Amoxicillin



Ampicillin

Figure 1. The Molecular Structure of Penicillin, Amoxicillin and Ampicillin.

The density of the bacterial population and age of the infection also influenced activity. The intensity and duration of penicillin therapy required to abort or cure experimental infection in animals increases with the number of bacteria inoculated and with the duration of infection (163).

Host defenses are important in the ultimate disposition of certain penicillin damaged but viable bacteria and at times outweigh the direct bactericidal activity of the antibiotic. In most individuals with agranulocytosis who are infected with microorganisms highly sensitive to penicillin, failure to respond to drug doses far in excess of those indicated for the in vitro susceptibility of the offending bacteria occurs (163). Impaired defense mechanisms may be a major factor in the development of bovine uterine infections and in the failure of therapeutic agents to eliminate such infections. Ovarian hormones affect uterine defense mechanisms; under the influence of estrogens, the uterus possesses a higher degree of bactericidal activity than when levels of progesterone are high (1, 59).

Penicillinase is present as a natural substance in many microorganisms. This enzyme can produce resistance if present in sufficient quantities (160). Penicillinases hydrolyse the antibiotic molecules by opening the beta-lactam ring and rendering the molecules bacteriologically inactive (164). Penicillinases produced by gram positive bacteria such as S. aureus and Bacillus subtilis are plasmid mediated whereas those produced by gram negative bacteria are part of the normal, chromosomal structure of the microorganism (165).

In gram-positive bacteria, such as staphylococci, penicillinases are extracellular enzymes. When liberated into the surrounding environment, they destroy penicillins even before the antibiotic contacts the bacterial cell (165). The beta-lactamases produced by gram-negative bacteria are cell bound and are made in lesser amounts than are the gram-positive enzymes. Most of the plasmid-mediated beta-lactamases are constitutive enzymes and are not induced by the presence of substrate, whereas the chromosomal enzymes can be induced. Beta-lactamase resistance has been a problem in treating infections caused by S. aureus, E. coli, Klebsiella spp., and Salmonella spp. (165).

The precise role of the beta-lactamases in resistance is unclear. The ability of a beta-lactam antibiotic to inhibit gram-negative organisms depends on many factors. Among these are the concentration of antibiotic in contact with the bacteria, the diffusion characteristics of the antibiotic through the outer cell layers, the affinity of the antibiotic for the receptor site, the affinity of the antibiotic for a beta-lactamase, the rate of hydrolysis by the beta-lactamase, the location of the beta-lactamase in the bacterial cell, and the amount of enzyme produced (165). It is important to recognize that the presence of a beta-lactamase does not necessarily mean that a microorganism will be resistant (165). The converse has also been noted, that protection of a penicillin susceptible pathogen on mucosal surfaces can occur due to production of beta-lactamase by other organisms (158).

Many of the newer semisynthetic penicillins have been structured to resist inactivation by penicillinases. This resistance is due to a ring structure attached to the carbonyl carbon of the amide side chain sterically protecting the lactam ring from beta-lactamases (160).

Broad spectrum penicillins, such as amoxicillin, have antibacterial activity against gram-positive and gram-negative bacteria. This increase in antibacterial spectrum has been accomplished by structural changes in the acyl portion of the amide side chain (160).

Most penicillins are rapidly absorbed when injected in aqueous suspension intramuscularly. Procaine penicillin G in a buffered aqueous suspension containing 300,000 units/ml is available for intramuscular injection. Absorption of penicillin from this preparation is prolonged over that for sodium penicillin G. Procaine penicillin G contains one molecule of procaine combined with one molecule of penicillin, to give a compound with 41.5% procaine and a penicillin potency of 940 units/mg (160).

Levels present in the serum following intramuscular administration have been measured in several surveys (Table 10). Determination of serum levels of penicillin is by means of a bioassay. Sensitivities of the various organisms used in bioassay procedure vary. For bioassays used to determine penicillin concentrations Bacillus stearothermophilus is used. This assay is able to detect concentrations as low as 0.01 i.u. of penicillin/ml and is the standard assay for determination of antibiotic residues in milk (129). These levels are well below the MIC for most pathogenic organisms and therefore an organism as sensitive

TABLE 10

BLOOD SERUM CONCENTRATIONS OF PROCAINE PENICILLIN G FOLLOWING INTRAMUSCULAR ADMINISTRATION IN AN AQUEOUS VEHICLE

	Dose (1000 i.u./kg)	Time (hrs)								
		2	4	8	12	24	36	48	60	72
		Serum (i.u./ml)								
A	6.6	0.135	0.20	0.146	0.09	0.033	0.009	0	0	0
B	12.9	0.389	0.496	0.461	0.226	0.057	0.270	0.006	0	0
C	18.0	0.318	0.51	0.343	0.223	0.094	0.026	0.009	0.008	0.002
D	9.3	1.5	1.2	0.75	0.5	0.1	0.04	ND	ND	ND
E	6.9	1.8	1.2	0.4	0.3	ND	ND	ND	ND	ND
F	6.5	2.0	1.9	1.0	0.5	0.05	ND	ND	ND	ND

ND = Not detectable.

A-C = Procaine penicillin G.

Source A-C: Schipper, Filipous, Ebeltoft, and Schermeister, Javma 158: 494-500, 1971.

D = Neopen®, Intervet, Boxmeer, The Netherlands (Procaine penicillin G and neomycin sulfate).

E = Pendipan Forte®, Leo, Denmark (Procaine penicillin G and dihydrostreptomycin).

F = Depomycin®, Mycofarm, Delft, The Netherlands (Procaine penicillin G and dihydrostreptomycin).

Source D-F: Nouws and Ziv, Zbl Bl. Vet. Med 25A: 312-326, 1978.

as this does not need to be used in pharmokinetic studies to determine elevation of therapeutic levels of antibiotics. Other organisms that are used in the bioassay method are B. subtilis which can detect levels as low as 0.05 i.u./ml and Sarcinia lutea also known as Micrococcus lutea which can detect levels as low as 0.1 i.u./ml (166), although others have stated that levels as low as 0.006 i.u./ml can be detected with this bacterium (167).

Metabolism

Approximately 90% of a given dose of penicillin is excreted unchanged primarily in the urine. Most of the penicillin in blood is in the plasma and less than 10% is found in the erythrocytes. Penicillin G is partially metabolized in the body by unknown mechanisms (160).

The distribution of penicillin is rapid but not uniform in various fluids and tissues. The apparent volume of distribution is a relationship between total antibiotic present in the body and serum levels. Distribution volumes equivalent to 30% or less occur with penicillin G. The distribution volume for amoxicillin is 30 to 60% and tetracycline is greater than 60% (120). Experiments with ^{35}S-labelled penicillin demonstrate that the concentration is consistently higher in the kidneys than any other body tissues, although in the liver it is occasionally as high. Diffusion of penicillin into the tissues and fluids occurs as long as the unbound plasma concentration exceeds that of tissues and fluids (160). Other factors that affect extravascular tissue levels are the degree of protein binding in serum and tissues, the diffusibility,

which is determined by molecular size and pk' value, and the lipid solubility (120). Although there is a parallel decline of tissue and blood levels of antibiotics, these do not occur at the same time (120).

It has been suggested that milk may pharmacokinetically represent extravascular and extracellular fluid as the majority of drugs pass from blood into milk by nonionic passive diffusion. Moreover this assumption seems justified since milk is essentially devoid of serum proteins, has a pH almost equivalent to intracellular body pH and is excreted by a highly vascularized organ (168). The calculated blood to milk "permeability index" for ampicillin was significantly higher than that for penicillin G. Ampicillin was detected in the milk 0.25 hours after administration and 0.08% of the dose was recovered in the milk whereas penicillin G was not present in the milk until 2.5 hours after administration and only 0.0004% of the dose was present (168).

The pka for penicillin G is 2.7 for the carboxyl ion whereas ampicillin has a pka of 2.7 for the carboxyl ion and 7.2 for the amino ion. The percentage of unionized antibiotic in serum at a pH of 7.4 was 0.001% for penicillin G and 0.4% for ampicillin. The percentage of unbound drug in the serum was 66% for penicillin G and 80% for ampicillin. The oil to water partition coefficient was 58 for penicillin G and 150 for ampicillin. The permeability index, which is the product of percentage unionized x percentage unbound x log of oil to water partition coefficient was 0.116 for penicillin and 70.648 for ampicillin (168). All these factors are involved in the greater penetration of tissues of ampicillin compared to penicillin.

A high permeability index is associated with a high percentage of drug recovery in milk and a shorter appearance time in the weakly acidic antibiotics. The permeability indexes for these antibiotics are strongly influenced by differences in the percentage of nonionized drug in serum, i.e., by the pka. The indexes were influenced to a much lesser extent by differences in the oil to water partition coefficients and even less so by differences in serum binding (168).

It is essential in antibacterial therapy that the concentration of the unbound drug at the site of infection be adequate. As determination of drug concentration at the site of infection is very difficult, if not altogether impossible, the kinetic behavior of the drug in blood is usually taken to indicate its distribution in the body. Yet, from the chemotherapeutic point of view it is in the extravascular space where the drug bacteria interaction usually takes place. Results of these studies by Ziv indicate that the blood milk-barrier can possibly be used as a useful tool in pharmacokinetic evaluation of drugs in cows (168).

The degree of lipid solubility and extent of ionization of penicillin in various body fluids determine the absorption, distribution and fate of the antibiotic in the body. Both absorption and distribution take place by passive diffusion, a process which is characterized by the movement of drug molecules down a concentration gradient without the expenditure of energy. Only lipid soluble molecules can diffuse through mucosal membranes, penetrate cell membranes and enter transcellular fluids (169). Ampicillin which is more lipid soluble, less

ionized in the blood plasma, and less protein bound than penicillin, achieved endometrial values 10-15% higher than serum concentrations whereas sodium penicillin G achieved levels 20-80% lower in the endometrium of the horse than serum concentrations (164).

Extensive binding to plasma albumen restricts the distribution of the drug, giving higher total concentrations in the plasma than are available for tissue activity whereas lightly bound drugs may diffuse more rapidly into the interstitial fluid (189). Protein binding is reversible so that it provides a reservoir of potentially active drug within the circulating blood. Binding also has the effect of delaying excretion by glomerular filtration but does not affect tubular secretion which is an active carrier mediated process. Enterohepatic circulation may account for the slow excretion of ampicillin compared with penicillin G (169). All penicillins are organic acids and are predominantly ionized (99%) in the plasma. Binding of residues to plasma albumen ranges from 16-22% for ampicillin (169) to 18% for amoxicillin (170), 50-65% for penicillin G (169) and over 80% for cloxacillin (169). Other authors have stated that penicillin G is only 30% bound to albumen in the cow (120, 170).

Variations between extent of binding occur between sera of individual cows. In one study the percentage of penicillin G bound to serum protein varied from 24.3 to 32% (170). Factors that may affect level of protein binding include pH, protein concentrations and the presence of other agents during determination (120). The concentration of the drug in serum also affects the percentage that is bound.

Novobiocin at a concentration of 16 μ/ml is 93.6% bound to protein while at 1 μg/ml it is 96.8% bound. This represents a 16 fold decrease in total concentration but a 31 fold decrease in active drug (170).

The affinity constants of an antibiotic for albumen will determine the degree of release of the agent. Only free antibiotic can exert its antibacterial action and level of free drug in the tissues cannot exceed the levels of free drug in the serum. This is an important consideration as it might be expected that leakage of serum into an area of the body would provide a higher concentration than would be expected based on diffusion of unbound drug. It is unlikely that inhibitory concentration of antibiotic will be achieved in tissue if the concentration of free drug in serum is below inhibitory levels (120). Data are not available on the extent of binding of antibiotics by the uterus and its lochial discharge (120).

Several studies have shown that among a homologous series of drugs, such as penicillins, an increase in lipid solubility of the compound is accompanied by an increase in serum protein binding (170). The effect of serum binding in drug distribution in the body depends on the strength of association and on the dose of the drug. A strongly bound drug at low drug concentrations is concentrated primarily in the plasma compartment. However, at higher dosages the fraction of bound drug in serum will be markedly reduced. Another characteristic attributed to serum binding of drugs with high affinities for proteins is that there is a dose range within which small increases in the dose result in relatively large increases in the amount of unbound drug in the body (170).

Antibiotics must reach the bacterial receptor sites, all of which lie below the outer cell wall layers. If the antibiotic is bound to a large molecule such as albumen, the entry of the drug is prevented (120). Protein binding will affect the duration of drug concentration in the blood and the rate of elimination of drugs from the body. Maintenance of high serum levels for long periods is an important practical consideration for antibiotic therapy. Even if the level of free drug needed to inhibit a pathogen is low, adequate levels in the tissues need to be present for several hours. The duration of antibiotic residues in the body is longer for drugs that are extensively protein bound to serum and tissue in comparison with antibiotics that possess minimal protein binding properties (120).

The plasma concentration of penicillin required to cure experimental streptococcal infection in laboratory animals is two to five times higher than those necessary to kill the same organisms in vitro, probably because the antibiotic is partially protein bound and diffusion into areas of infection is difficult. The tissue concentration of penicillin effective at a focus of infection is considered the same as the inhibitory concentration in vitro (163).

A specific blood concentration of penicillin that permits diffusion of penicillin to lung, liver or kidney tissue may not allow bactericidal concentrations to diffuse to other tissues. Efforts to overcome rapid excretion of penicillin have been made by prolonging absorption from the site of injection by the use of sparingly soluble salts of penicillin and slowly absorbed vehicles (160). The serum half life of penicillin G is 0.7 hours while that of amoxicillin is 1.25 hours (160).

Following the administration of 3 million i.u. of procaine penicillin G, peak serum concentrations of 0.3 i.u./ml were achieved. This dosage maintained the serum concentration above 0.05 i.u./ml for 10 hrs and above 0.10 i.u./ml for four hours. At a dose of 6 million units, a peak serum concentration of 0.5 i.u./ml was achieved and over 0.05 i.u./ml for 24 hours and above 0.10 i.u./ml for 12 hours. At a dose of 9 million i.u. a peak serum concentration of 0.6 i.u./ml was achieved, again levels above 0.05 i.u./ml were achieved for 24 hrs and above 0.10 i.u./ml for 12 hours. Even at this high dose levels above 0.50 i.u./ml were not achieved for a sufficient length of time (167). Serum levels of penicillin following intramuscular administration of procaine penicillin G in several experiments are summarized in Table 10 (p. 93).

When sodium penicillin G was administered at a dose of 6 million i.u. intramuscularly a peak serum concentration of 1.0 i.u./ml was achieved and levels above 0.05 i.u./ml were maintained for six hours, four hours above 0.10 i.u./ml and one hour above 0.50 i.u./ml. At a dose of a million i.u. peak serum concentrations of 1.4 i.u./ml were achieved and levels were above 0.05 i.u./ml for ten hrs, six hours above 0.10 i.u./ml and two hours above 0.50 i.u./ml (167).

It has been stated by Roberts that the parenteral administration of antibiotics does very little to enhance topical treatments of metritis and is probably indicated only in cases in which there is septicemia or other extension of the infection (1). Following the intramuscular injection of a single dose of 22,000 i.u./kg of an aqueous solution of

sodium penicillin G the mean serum drug levels rose rapidly, reaching a peak of approximately 13.0 i.u./ml at fifteen minutes, whereas the peak levels in the endometrium did not occur until sixty minutes after the injection but persisted at a higher level than the serum for a longer period of time. The ratios of mean tissue to mean serum levels were 0.18 at ten minutes and 0.48, 0.76, 1.36, 1.29 and 1.30 at 15, 30, 60, 90 and 120 minutes respectively. These results suggest that serum drug levels may be a poor indicator of the level of chemotherapeutic agent present within the tissues (101).

The blood level of 0.03 to 0.05 i.u./ml is bacteriostatic, whereas bactericidal levels are above 0.5 i.u./ml. These levels in themselves are not important except that they are an indication of levels in the tissues where the bacteria are multiplying. Once the tissue levels are adequate they may be retained long after the blood levels have dropped below 0.03 units/ml (171). If bacteriostatic concentrations are present in the blood and tissue the affected bacteria become very susceptible to attack by the body defenses, both cellular and humoral and remain so for several hours after the disappearance of effective blood levels (171). Controlled studies of penicillin G in man indicate that it is not necessary to maintain continuously bactericidal drug concentrations in blood or tissue fluids. Investigation of several treatment schedules in scarlet fever has led to the conclusion that, so long as penicillin G is detectable in the blood for a total of 6 hours per day, clinical as well as bacteriological cure results (163).

The route of administration of the antibiotic also affects levels achieved in the serum and duration of these levels. Higher levels are

obtained by intravenous administration than by intramuscular and subcutaneous and significant differences in drug absorption occur between various muscle locations (169). Delayed absorption from certain parenteral formulations may be attributable to the effect of the vehicle on dissolution of the drug substance. The aim of sustained release dosage forms such as procaine penicillin G is to maintain therapeutic levels of the drug for an extended period of time. Even though the half life of penicillin G is 0.7 hours in the cow, a single dose (30,000 units/kg) of procaine penicillin G in aqueous suspension given by intramuscular injection was shown to maintain the serum penicillin concentration above 0.05 units/ml for 24 hours (169).

Increased dose size of repository forms such as benzathine penicillin G may not elevate plasma concentrations because its low solubility (0.02% in water) makes the surface area of the intramuscularly injected volume a critical determinant of its absorption (163).

Pharmacodynamics

Penicillins interfere with the development of the bacterial cell wall. The enzyme transpeptidase is inhibited and rendered inactive, thus removing its ability to form cross linkages between two linear peptidoglycan strands which are cross linked with techoic acid and form a thick center part of the wall (160). This results in an inability to produce sufficient cell wall for their needs, so that they outgrow their walls and become lysed as the protoplasm flows through the gaps, under the influence of osmotic pressures (172).

The arrangement of the cell wall is more complex in gram negative organisms than it is in gram positive organisms. The peptidoglycan layer is thin but between this and the outer coat there are three extra layers which are absent in the gram-positive bacterial cell wall. Starting from the peptidoglycan layer outwards, there is a layer of lipoprotein bundles, an outer membrane with composition similar to the cytoplasmic membrane of phospholipids and a third lipopolysaccharide layer. Thus, in gram negative bacterial cell walls, the antibiotic has to travel a more difficult route to reach the site of action, the peptidoglycan network (173). Broad spectrum penicillins such as ampicillin, amoxicillin, betacillin and carbenicillin have antibacterial activity against gram negative bacteria (160). Their efficacy varies due to the variation in the rate at which they penetrate the gram negative cell wall, and reach the site of the drugs action, the peptidoglycan complex. Amoxicillin appears to travel much more rapidly than does ampicillin (173).

Penicillins also interfere with the assimilation and concentration of glutamic acid (172). They affect growing cells and have little effect on dormant bacteria. The antibacterial action of penicillin is highest during the period of greatest bacterial multiplication. Penicillin is inert to mammalian tissues because mammalian cells do not possess the rigid cell wall characteristics of bacteria (160).

Amoxicillin

Amoxicillin differs from procaine penicillin G in that it has antibacterial activity against gram positive and gram negative bacteria.

The antibacterial spectrum has been accomplished by structural changes in the acyl portion of the amide side chain and the addition of the parahydroxy group (Figure 1, p. 89). Ampicillin has the same structure as amoxicillin except that the hydroxyl group is not present. Despite this similarity the amoxicillin molecules, unlike some ampicillin pro-drugs like hetacillin, pivampicillin and metampicillin is not converted to ampicillin in the body (174). The biologic half life of amoxicillin is 17 hours whereas the half life for ampicillin is twelve hours (160). The biological half life should be differentiated from the serum half life which is much shorter, 1.25 hours for amoxicillin and is defined as the time taken for the serum concentration to be decreased by 50% whereas the biological half life is the time taken for 50% of the administered dose to be excreted from the body.

In experimental infections in mice the activity of amoxicillin is much greater than that of ampicillin. It has also been shown in vivo, that amoxicillin has a more rapid and complete bactericidal effect than ampicillin against certain gram negative bacilli. This was demonstrated when the blood levels of the two compounds were similar (172). This is thought to be due to the fact that amoxicillin can diffuse through the cell wall of the gram negative bacteria faster than ampicillin leading to direct cell lysis in a much shorter time (173). Oral amoxicillin results in greater serum concentration than oral ampicillin and ampicillin is also absorbed more rapidly and completely from the bovine udder than is ampicillin (160). When given orally in a number of species, approximately twice as much of the dose is absorbed

compared with ampicillin (i.e., about 70% and 35% respectively) and double or even higher serum levels are attained (175).

It is interesting to speculate that all three of the major advantages of amoxicillin over ampicillin--its greater absorption from the alimentary tract, its apparent capacity to attain more effective concentrations in certain sites such as the intrabronchial site in bronchitis and its more rapid penetration of the gram negative bacterial cell wall--all derive from a basic capacity for greater mobility in biologic tissues (173). Orally administered amoxicillin has a relative bioavailability 6.4 times greater than that of ampicillin (161). In comparison with ampicillin, amoxicillin penetrates the bronchial secretions as efficiently when the mucosa is uninflamed as when it is inflamed (176), whereas levels of ampicillin achieved in the noninflamed bronchial mucosa were less than those in inflamed mucosa.

Beta-lactam antibiotics have relatively different morphologic effects on the cell (177). Amoxicillin was not included in these studies but the difference between the effects of amoxicillin and ampicillin might be differences in affinity for protein or enzyme binding sites as well as the capacity of amoxicillin to achieve earlier higher concentrations at these binding sites (173).

When amoxicillin or ampicillin were added to a multiplying culture of E. coli at a concentration of 10 μg/ml, twice that of the MIC for both drugs, ampicillin reduced the viable cell count by one order of magnitude within 90 minutes. During the same time period, amoxicillin reduced the count by four orders of magnitude, nearly eliminating the

population (173). It has been suggested that in the terms of MICs what is relevant is the action at 1 to 6 hours and not after the 12-24 hours period in conventional MIC determination. If in the above example MIC were determined at 90 minutes it would be greatly in favor of amoxicillin because of its rapid bactericidal activity. However, after the 18-24 hour period used in standard sensitivity testing with constant antibiotic levels ampicillin would have caught up with amoxicillin (173).

The therapeutic activity of amoxicillin and ampicillin were compared in an experimental disease model by infecting groups of mice intraperitoneally with various organisms. The size of the infecting dose was predetermined to produce 100% mortality within 24-48 hours. The antibiotics were injected subcutaneously resulting in essentially equal serum and tissue levels. The dose of amoxicillin required for survival of 50% at four days posttreatment (CD50) was significantly less for the majority of the organisms tested. In fact, all but two organisms showed a lower CD50 value for amoxicillin. Thus, amoxicillin was more active than ampicillin when administered by subcutaneous injection to mice experimentally infected with a range of gram-positive and gram-negative bacteria (175).

If comparatively low levels of an antibiotic can inflict reversible damage on bacterial cells during brief periods, that antibiotic will be more effective than one that tends to leave a proportion of cells damaged but still capable of resuming normal replication when the level of inhibiting substance falls sufficiently low (173).

As the 24 hour dosage interval seems to be satisfactory in the field we must assume that satisfactory bactericidal activity does not depend on uninterrupted maintenance of plasma concentrations of amoxicillin greater than 2 μg/ml. This may simply reflect the long held belief that intermittent exposure is the better way to use bactericidal antibiotics. It may mean however, that bactericidal levels persist in the tissue fluids for a longer period than that suggested by plasma concentrates, or that the bactericidal action of amoxicillin quickly reduces bacterial counts to a level where the body defense mechanisms again become effective (171).

Amoxicillin trihydrate, aqueous suspension given at a dose of 12.5 mg/kg intramuscularly achieved peak serum concentrations of 3.5 μg/ml and achieved levels above 1 μg for 12 hours and 2 μg for six hours (178). A dose of 20-25 mg/kg has been recommended for satisfactory therapeutic levels (174). When the drug was given at a dose of 14 mg/kg to preruminant calves peak levels of 6.5 μg/ml were achieved at two hours and were above 4 μg/ml for six hours (173). When it was given at a dosage of 7 mg/kg peak serum levels of 3 μg/ml were achieved at two hours and were above 1 μg/ml for ten hours (173). Levels in kidney, liver, upper small intestinal contents, lower small intestinal contents, bile and urine were greater than serum but levels in joint fluid, mediastinal lymph node, tonsils, spleen, skin, fat, lung and muscle were lower (179).

The concentration and volume of injection affects the peak blood levels. The site of injection also has an effect on the bioavailability,

at least for amoxicillin. Amoxicillin trihydrate aqueous suspension - 20% was administered at a dose of 4 mg/kg (1) subcutaneously in the dewlap, (2) intramuscularly in the lateral neck one hands breadth anterior to the scapula and the same distance below the dorsal margin of the neck, (3) intramuscularly in the M. tricepsbrachii and (4) intramuscularly in the M. semitendineus (buttock) respectively. Great differences in the bioavailabilities of amoxicillin for the four routes of administration in the first 12 hours were observed. In this period only 24.2% of a dose administered subcutaneously was absorbed, whilst the proportions after intramuscular injection into the buttock, M. triceps and lateral neck were 49.2, 76.2 and 79.2%. Peak serum levels achieved for the four routes were 0.38, 1.10, 1.39 and 0.65 µg/ml for the same dose level of 4 mg/kg (180).

The differences in absorption may be related to the differences in muscle blood flow and/or to the extent of absorption area. The dewlap region is relatively poorly vascularized, resulting in a slower absorption rate and a longer persistence of amoxicillin trihydrate at the injection site; in the plasma the half life is three to seven times longer than that obtained with the other injection sites studied. Similarly after subcutaneous injection in the dewlap of sodium penicillin G, the half life was three times longer than that after intramuscular injection in the lateral neck of cows (180).

Following injections in the triceps and neck, serum levels above 1 µg/ml were achieved for four hours and three hours but not after injection in the dewlap or buttock. Levels above 0.5 µg/ml were not

achieved following subcutaneous injection in the dewlap but were present for ten, four and nine hours following injection in the M. triceps, buttock and neck. Levels greater than 0.1 μg/ml were present for 31, 24, 24 and 24 hours following subcutaneous injection in the dewlap and intramuscular injection in the M. triceps, buttock and neck (180).

After parenteral administration, the amoxicillin concentration in mucous secretions, saliva, muscles, cerebrospinal fluid, prostatic fluid, and in milk was five to ten times lower than in plasma during steady-state level. For extravascular infections it would seem that plasma amoxicillin concentrations should be five to ten fold higher than in vitro MIC on theoretical grounds, therefore, at the dose levels administered in these experiments, therapeutic blood concentrations are obtained only for those extravascular infections caused by highly sensitive bacteria such as Corynebacterium spp. (MIC: 0.01-0.04 μg/ml) and Streptococcus spp. (MIC 0.01-0.1 μg/ml). However, the therapeutic effect will vary, and according to the site of infection other infectious agents may be susceptible (180).

It has been shown that the elimination half life of ampicillin and amoxicillin is very short (171). Probenecid is a drug that inhibits the system which actively transports weak organic acids such as the beta-lactam antibiotics into the renal ultrafiltrate. In man, the concomitant administration of probenecid and ampicillin or amoxicillin resulted in higher serum concentrations of these drugs being maintained for longer periods due to diminished rate of antibiotic elimination (181).

The parenteral administration of probenecid with amoxicillin appears to be more effective in elevating and prolonging serum antibiotic concentrations than the combined parenteral injection of probenecid and ampicillin. This difference can perhaps be attributed to the greater proportion of amoxicillin actively secreted by the renal tubules (181).

Concentration of ampicillin and amoxicillin ranging between 2.5 and 5 μg/ml are considered sufficient to inhibit many gram-negative pathogenic bacteria. The duration of antibiotic in serum at concentrations above 5 μg/ml was extended from 2-3 hours when the antibiotics were administered alone to five and six hours by the coadministration of probenecid (181).

It has been suggested that the effect of probenecid in increasing the concentrations of penicillins and cephalosporins in the serum might be at the expense of their concentration in tissues (182). This would mean that the coadministration of probenecid with beta-lactam antibiotics could result in a relatively lower antibacterial efficacy if a reduction in the distribution of an antibiotic were associated with less active drug available at the site of the infection. It has been reported that probenecid does not restrict penicillin distribution into the interstitial fluid (183) and in experimentally infected mice probenecid enhanced both serum concentrations and the bacteriological cure rate (184). Probenecid appears to be a safe drug. When administered at three consecutive milk feeding times at 1.5 g/calf, changes were not found in the renal function of the kidney as reflected by serum urea and creatinine concentrations (181).

Many of the bacterial organisms commonly involved in bovine endometritis are sensitive to amoxicillin. Corynebacterium pyogenes has an MIC of 0.01 µg/ml, E. coli of 5 µg/ml (173). Of 166 strains of E. coli tested, the MIC varied from 1.25 to over 100 µg/ml with the majority under 5 µg/ml (185). The MIC for P. haemolytica was 0.08-0.32 µg/ml, S. faecalis 0.5-1.25 µg/ml, S. aureus (nonpenicillinase producers) 0.07-0.3 µg/ml (173).

Levels of amoxicillin in serum and tissues are determined by means of a bioassay. The common organisms used and their minimal inhibitory concentrations are B. stearothermophilus ATCC 10149, 0.005 µg/ml, B. subtilis ATCC 6633, 0.15 µg/ml and S. lutea NCTC 8340, 0.005 µg/ml (173). Other studies suggest the MIC for B. subtilis is 0.05 µg/ml and S. lutea 0.1 µg/ml (166).

Tetracyclines

Tetracyclines are amphoteric compounds forming salts with acids or bases. Tetracyclines inhibit bacterial cellular metabolism by blocking attachment of aminoacyl transfer ribonucleic acid ribosomes, which interferes with protein synthesis of rapidly growing and reproducing bacteria. Only a small portion of the drug is irreversibly bound and it appears that the reversibly bound antibiotic is responsible for antibacterial action. Most of this binding is to the 30S ribosomal subunit (186).

The uptake of tetracycline by bacteria appears to be dependent upon an active transport process. Resistance to tetracyclines seems to

result from decreased uptake of drug in strains of bacteria carrying an R factor. It has been shown that R factor mediated resistance is accompanied by a decreased uptake of labeled tetracycline. Present evidence suggests that R factor modified resistance to tetracyclines results from the induced synthesis of an inhibitor of transport of these antibiotics (187).

The three commonly used forms of tetracycline in the United States are the conventional form (OTC-C) (Liquamycin-100®-Pfizer) which contains 100 mg oxytetracycline base as oxytetracycline hydrochloride and on a weight per volume basis 0.96% magnesium oxide; 0.43% magnesium formaldehyde sulfoxylate (as a preservative); 19.0% povidone; monoethanolamine to adjust pH; up to 0.05% simethicone emulsion and water for injection per ml (188). Liquamycin-50®-Pfizer is a similar preparation containing 50 mg of oxytetracycline hydrochloride per ml. The long acting preparation (OTC-LA) (Liquamycin-LA 200®-Pfizer) contains, per ml, 200 mg of oxytetracycline base as amphoteric oxytetracycline and on a weight per volume basis, 40% 2-pyrrolidone, 5% povidone, 1.8% magnesium oxide and 0.2% sodium formaldehyde sulfoxylate (as a preservative), monoethanolamine and/or hydrochloric acid as required to adjust pH (189).

The suggested dose for intravenous or intramuscular injection of conventional tetracyclines is 4.4-11 mg/kg daily. An initial intravenous dose of 10 mg/kg followed by maintenance doses of 7.5 mg/kg at twelve hour intervals has been projected for oxytetracycline. These doses should provide serum concentrations of oxytetracycline that

would be effective against most systemic infections caused by sensitive bacteria (186).

From examination of the pharmacokinetics and plasma protein binding it was determined that in order to maintain an MIC of 0.13 µg/ml, intravenous dosage of 2.1 mg/kg b.i.d. or 7.3 mg/kg s.i.d. would be required. In order to maintain the level above an MIC of 1 µg/ml, dosages would be 6.1 mg/kg q.i.d., 15.8 mg/kg b.i.d. or 55.1 mg/kg s.i.d. To maintain a level above 2.44 µg/ml doses would be 14.5 mg/kg q.i.d., 37.3 mg/kg b.i.d. or 130.2 mg/kg s.i.d. (190). It is very important in the treatment of bacterial infections with bacteriostatic antibiotics to keep the level of antibiotic at the site of infection above the MIC at all times. This is not as important with bactericidal drugs (190).

Other studies have shown that, depending on the number of sites of injection and the organic solvent used, 20 mg/kg I/M achieved peak serum levels of 0.8-6.2 µg/ml and maintained levels above 0.5 µg/ml for four to twelve hours, above 1.0 µg/ml for two to six hours and above 2.0 µg/ml for one to four hours. At a dose of 10 mg/kg peak levels of 0.8-5.2 µg/ml were achieved and levels above 0.5 µg/ml were maintained for one to six hours. Levels above 1.0 µg/ml and 2.0 µg/ml were achieved for zero to four hours and zero to two hours respectively. At a dose of 5 mg/kg peak levels achieved were 0.8-1.3 µg/ml and levels were maintained above 0.5 µg for zero to six hours (120).

Persistence of the tetracyclines in blood following absorption is in contrast to other antibiotics that are eliminated more rapidly. Plasma concentrations of oxytetracycline administered intramuscularly are detectable within 15 minutes, reach a peak within one hour and maintain significant blood levels for about 12 hours declining to trace amounts approximately 24 hours after injection. For maximum blood concentrations in food producing animals not more than 10 ml should be injected in a single intramuscular site (186). Serum levels were considerably lower when given in one injection site than when administered at multiple sites. When oxytetracycline was injected intramuscularly at a dose of 11 mg/kg, peak serum levels of 5.2 μg/ml were attained three hours after administration when given in multiple sites. When the injection was given at a single site, peak levels of 3.8 μg/ml were not achieved until six hours after injection (191).

The recommended dose for the long acting tetracycline (OTC-LA) is 6.6-11 mg/kg per day which is the same dose recommended for the shorter acting preparation, OTC-C. A single dosage of 20 mg/kg is recommended when retreatment is impractical due to husbandry conditions (189). When OTC-LA is injected intramuscularly at a dose of 6.6-11 mg/kg peak blood levels are not as high as they are with the same dose of OTC-C. However, levels persist for a longer period of time (189). In cattle treated with OTC-LA at a dose of 20 mg/kg a serum concentration of 2.5 μg/ml was obtained within 60 minutes. A concentration of 4 μg/ml was maintained over 12 hours. Serum concentrations then decreased slowly but still exceeded 0.5 μg/ml for 72

hours after injection (192). When given at a dose of 40 mg/kg, levels peaked at 13.4 µg/ml and were above 0.5 µg for 120 hours and 0.2 µg for 108 hours following a single intramuscular injection (193).

The half life for the long acting preparation is 23 hours and 12 hours for the shorter acting preparation (189). This prolonged effect is claimed to be due to the aqueous 2-pyrrolidone formulation which should lead to a controlled precipitation of oxytetracycline at the injection site without significant tissue damage (194).

Clinical trials evaluating the treatment of anaplasmosis and bacterial pneumonia have shown that a single intramuscular treatment of the long acting preparation at 20 mg/kg is as effective as two or three repeated daily treatments of the shorter acting preparation at 6.6-11 mg/kg (189, 194). The long acting preparation is particularly efficacious in the treatment of acute anaplasmosis (194). Clinical trials have also indicated that a single intramuscular injection of the long acting formulation was equivalent in efficacy to two daily administrations of a conventional formulation in the treatment of pasteurella pneumonia (195), bovine dermatophilosis (196), and undifferentiated bovine respiratory tract disease occurring in feedlots (197).

Tetracyclines undergo metabolism to various degrees. The most frequently identified substance in urine, feces and tissue is the parent tetracycline. Tetracyclines are reversibly bound to plasma proteins and are widely distributed. Oxytetracycline is 18.8 to 22.4 percent protein bound (120, 170) while other studies show only 7.9% is bound (187). Tetracyclines are secreted into bile in high concentrations due

to active transport processes (187). Bile concentration may be thirty times that of blood, however, enterohepatic recirculation limits bile secretion and prolongs maintenance of therapeutic concentrations (186).

Tetracyclines diffuse throughout the body and are found in highest concentrations in kidney, lever, spleen and lung (186). Levels in lung were greater than in serum and levels greater than 0.5 µg/ml were maintained for 65 hours after a single intramuscular injection of 20 mg/kg. Oxytetracycline concentrations in liver and kidneys were up to seven times higher than in serum (192).

Some studies have suggested that oxytetracycline levels in endometrium are greater than that in plasma (106). However, others have shown that an eight hour intravenous infusion, designed to maintain plasma concentration of OTC at 5 µg/ml in cycling cows, the mean plasma concentration was 4.86 and the mean uterine tissue concentration of OTC was 4.50 (198). In both healthy and infected postpartum cows, the mean plasma to uterine tissue ratio of OTC was significantly higher than in normal cycling cows (1.36 vs 1.08). This study indicated that concentrations could be maintained above 5 µg/ml following twice daily intravenous administration of 11 mg/kg in both groups of cows (104).

Although tetracyclines may be excreted in the bile, the major route of excretion is through the kidney with approximately 25-30% of a single dose being excreted in the urine (186). Since glomerular filtration is the major mechanism of excretion, probenecid does not alter the free drug clearance (186). The serum half life of oxytetracycline is approximately 9.1-9.5 hours (186, 189). However

other studies have estimated serum half lives for the intravenous administration at 6.1 hours (199), 6.5 hours (198) and 4.1 hours (200).

Fecal elimination of oxytetracycline occurs regardless of the route of administration and elimination in the milk also occurs. The concentration in the milk is about one-half that in the serum. Oxytetracycline administered parenterally produces peak milk concentration in six hours and traces can be detected in the milk 48 hours post administration (186).

Intramuscular injection of OTC-C resulted in peak plasma concentrations of 7 µg/ml at six to eight hours post administration of the drug, with a half life of thirteen hours. This half life was considerably longer than that observed following intravenous administration. This was probably due to continual absorption from the injection site during the elimination phase. The absorption rate thus becomes the limiting factor for clearing of the drug from plasma. Absorption of oxytetracycline from the injection site after an intramuscular injection of OTC-LA was faster than absorption of OTC-C. However, the resulting plasma concentrations were not significantly different from each other (199). The maximum plasma concentration of OTC-LA was also seen at six to eight hours after administration. The bioavailability for both preparations was about 100% (199).

It has been suggested that the lowest therapeutic serum concentration OTC-LA is 0.5 µg/ml. This may be obtained by administering doses of 20 mg/kg every 48 hours. This was stated to be better than

the response for OTC-C given at 10 mg/kg every 26-32 hours (201). However, when both preparations were administered at a dose of 20 mg/kg, levels remained above 0.5 µg/ml for 60 hours for OTC-C and 66 hours for OTC-LA (199). As OTC-LA has been shown to cause far more tissue damage in cows than OTC-C it is suggested that the long acting preparation has no advantage over the conventional preparation (199).

Bacteria with MIC values less than 4 µg/ml for tetracycline are considered to be sensitive. This suggests that many bacteria which are classified as sensitive in vitro may be eliminated after treatment with the recommended doses of OTC (201).

Following intrauterine administration of tetracyclines the level of antibiotic absorbed from the uterus is affected by the condition of the endometrium with less being absorbed from cows with endometritis (105, 106). The form of tetracycline infused into the uterus also influences the rate at which it is absorbed from the uterus. When nonchelated oxytetracycline preparations were infused into the uterus, at a dose level of 4 mg/kg, levels of antibiotic were detected in the serum at 30 minutes with peak levels of 0.39 µg/ml being present in the serum at two hours. These levels persisted for 24 hours post infusion. Following the administration of the chelated form, antibiotic levels were not detected in the serum until two hours post administration with peak levels of 0.16 µg/ml being present at four hours. By 12 hours post administration antibiotic levels were no longer detectable. Antibiotic levels could be detected in the milk of cows infused

with the nonchelated form for six to 24 hours but no detectable levels were present in the milk following infusion with the chelated preparation (132).

At a dose level of 8 mg/kg by intrauterine infusion peak serum levels of 0.5 µg/ml were achieved at one hour postinfusion and serum levels greater than 0.3 µg/ml were maintained for 12 hours. Following intramuscular administration of the same dose, levels peaked at 1.01 µg/ml at four hours and were greater than 0.25 µg/ml for 48 hours. Levels achieved in the endometrium were 4, 2.1, 1.05 and 0.4 µg/ml at 12, 24, 48 and 72 hours post administration. Following intrauterine infusion, levels present in the endometrium were greater than 4 µg/ml for 72 hours after infusion but levels could not be detected in other parts of the reproductive tract. Following intramuscular treatment, levels exceeded serum levels in all parts of the reproductive tract and were greater than 1.3 µg/ml (106). The intrauterine treatment with OTC seems to yield a good concentration of the drug in the uterine cavity and in the endometrium for a long time. Undoubtedly this would be an advantage if the need was to disinfect the uterine cavity, but it should be remembered that many infections are not restricted to the cavity or to the endometrium (106).

In performing pharmacokinetic studies, the majority of the work is performed in calves, primarily because they cost less than adult animals. However, it is important that data obtained is the same as in adult animals. Following intramuscular administration, similar plasma OTC concentration-time curves, serum half lives and bioavailabilities

were observed for fourteen-week-old calves and nonlactating cows, although the dose given to the calves was twice the dose administered to the cows (202). Similar serum half lives were observed for OTC in both calves and cows. This indicates that to obtain the same therapeutic effect twice the dose per unit body weight should be given to calves as to adult animals. In mammals, oxytetracycline is predominantly excreted from the kidney by glomerular filtration. Thus, it may be assumed that three weeks after birth the glomerular filtration of OTC is at the same rate in preruminant calves and in adult cattle (202).

A possible reason for the differing pharmacokinetics with increasing age in calves may be linked to the corresponding decrease in extracellular and total body water, and the progressive increase in the volume of the gastrointestinal tract, particularly the rumen, following weaning (202).

Several newer forms of tetracyclines are being developed that have shorter serum half lives and can attain greater serum levels. Pyrrolidinomethyltetracycline has a serum half life significantly shorter than that of tetracycline and reaches peak serum levels almost twice that of oxytetracycline at the same dose level. Serum levels at twelve hours are just lower than oxytetracycline but were significantly below those levels achieved by oxytetracycline at twenty-four hours (203) and well below levels of 0.65 μg/ml suggested as therapeutic levels (190). Following intramuscular administration, pyrrolidinomethyltetracycline was detectable in the serum very rapidly indicating rapid

absorption from the injection site. The very rapid absorption may be due to the fact that pyrrolidinomethyltetracycline is a very water soluble drug, formulated in an oil suspension, from which it separates very rapidly when given an opportunity to move to a more polar environment. The very rapid absorption after intramuscular injection, as well as the high serum levels, would appear to have therapeutically beneficial consequences (203).

Two other new tetracyclines are minocycline and doxycycline. These tetracyclines are more highly bound to serum proteins than oxytetracycline and therefore have a longer serum half life. Also an inverse relationship exists between lipid solubility and urinary excretion, presumably because of greater tubular reabsorption of doxycycline and minocycline than oxytetracycline. These antibiotics are both far more lipid soluble than oxytetracycline and thus achieve a greater degree of penetration into tissues. A direct relationship exists between lipid solubility and biliary excretion as well as in the penetration of antibiotics across the blood brain barrier. Following administration, the concentration of minocycline and doxycycline were markedly higher than were those of oxytetracycline in cerebrospinal, brain, ocular fluids, aqueous and vitreous humor. Other studies have also been reported on the ability of doxycycline and minocycline in achieving greater tissue and tissue fluid concentrations than do other tetracyclines in the prostate gland, saliva and tears, lungs, bronchial secretions and milk (187). Levels in the uterine tissues have not been measured but these antibiotic might be more effective in the treatment of endometritis than oxytetracycline.

Absorption rates of several tetracyclines from the mammary gland have been studied. These studies demonstrated that minocycline and doxycycline are absorbed much more rapidly than was oxytetracycline. The persistence of doxycycline in milk was found to be considerably greater than that of oxytetracycline after the intramuscular injection of these antibiotics (204). Thus, it is unlikely that doxycycline would be approved for use in lactating cattle in the United States. The potential advantages of minocycline over the other tetracyclines would seem to reside in its greater activity against Staphylococcus spp., Nocardia spp., and anaerobes and in its superior distribution throughout the body (187).

Levels of tetracycline in tissue and serum may be determined by means of a bioassay. Bacterial organisms used and their minimal inhibitory concentrations are Bacillus cereus var mycoides ATCC 11778, 0.05 µg/g (106), or 0.1 µg/ml (192), and Bacillus subtilis BGA 0.3 µg/ml (166, 202). Other assay procedures used have included a chromatographic assay (104, 105, 205); spectrofluorometric method (190, 199); radioenzyme assay (205, 206); radioimmunoassay (205, 206) and turbidometric procedures (205, 206).

Serum Progesterone Assay

It has been shown that phagocytosis in the uterus is enhanced during the estrual stage of the cycle (85-88) and antibiotics may be absorbed from and secreted into the uterus in greater concentrations in the estrual phase of the cycle (82, 101, 109). Treatment during the estrual phase of the cycle may be of more benefit to the cow with

endometritis. The stage of the cycle can be determined by rectal palpation (207) and confirmed by means of a serum progesterone assay. Peripheral blood levels of progesterone are a good indicator of luteal function (208, 209). In one study progesterone levels in the peripheral plasma and in the CL were highly correlated (210). Progesterone has a biological half life of 22.4 minutes. Following enucleation of the corpus between the time required for progesterone to decrease to one half of its original concentration ranged from 15 to 30 minutes (211). In one study, however, the presence or absence of a CL was not related to levels of progesterone in the plasma in the postpartum period in cows with cystic ovaries and metritis (147).

Assays for progesterone consist either of a radioimmunoassay (113, 148, 149, 212-215), gas liquid chromatography with electron capture detection and liquid scintillation spectrometry (216-218), radiometric assay based on thin layer chromatography (219) or competitive protein binding assay (147, 208, 220-224).

Serum progesterone levels of less than 0.5 ng/ml indicate that no functional CL is present to produce progesterone. This may be due to the cow being in the estrual phase of the cycle or to cyclic activity not having resumed after parturition (149, 224, 225).

Progesterone levels are low (0.6 ng/ml) for two days before to two days after estrus, they then rise sharply from the third to seventh day of the cycle and then tend to rise very slowly or maintain a plateau fluctuating about a mean of 5.4 ng/ml until the sixth day before estrous. The level then declines gradually until a steep decline

occurs between the third and second day before estrus, although in some cows the most abrupt fall occurs between the fourth and third days before estrus (219).

Some early results indicating levels during the estrual stage of the cycle greater than 1 ng/ml (217, 219), were probably due to the insensitivity of the test method (213, 219). Mean levels during the luteal phase of the cycle were 6.6-7 ng/ml with a range of 6.1 to 10.2 ng (216), whereas cows with pyometra had a mean level of 10.6 ng/ml (148). Levels in the first few weeks postpartum period are low (0.6 ng/ml) (224). These basal plasma progesterone levels indicate that the CL of pregnancy is no longer functional (78). In the postpartum period it was noted that normal CL were formed during only 62.5% of postpartum cycles (78). Follicular cysts with plasma progesterone levels of less than 1 ng/ml were observed following none, one or two normal ovulations (78). In other studies, average plasma progesterone was almost constant (7-8 ng/ml) at 0.5, 3, 8 and 13 to 60 days after calving (147). The CL of pregnancy appears to be a major source of progesterone during pregnancy. However, after calving progesterone is very low in ovarian venous plasma and in the regressing CL. A nonluteal source of progesterone is indicated for at least two weeks after calving because palpable new CL are absent and peripheral levels, although variable, average 7 to 10 ng/ml. The adrenal is a probable source as the progesterone level in this gland is substantially higher seven to thirty days after calving (147). By five to seven days after calving the ovarian follicles could be a significant source of

progesterone especially if these imitate the biosynthetic activity indicated for cystic follicles. Ovarian follicles in all stages of regressive changes have been described for the postpartum cow (226).

The average of 4 ng/ml for cows with cystic ovaries and metritis was significantly lower than normal cows (147). Three days before estrus, progesterone levels were significantly higher (10.4 ng/ml) in those cows showing silent estrus than cows with normal estrus (5.6 ng/ml) (147). Cows with metritis had significantly lower progesterone during the nonestrous stage of the cycle than control cows but cows that had cystic ovaries and metritis had significantly higher levels at the same stage than control cows (147). Follicles in the absence of CL on the ovary may synthesize and secrete progesterone in quantities sufficiently large to markedly elevate plasma levels since progesterone was often present in greater amounts than other steroids including estradiol in fluids from follicular cysts of cows. In normal follicles the level of progesterone was relatively low when compared to estradiol (226).

CHAPTER 3

MATERIALS AND METHODS

Experimental Animals

Four Hereford-Angus crossbred cows were used in the experiments. All cows were at least four weeks postpartum and had no clinical evidence of metritis. It was initially decided to only use cows in the luteal phase of the cycle and estrus was synchronized by means of two intramuscular injections of prostaglandin F_2-alpha[a] at 11 day intervals. However, intrauterine infusion induced luteolysis in several cows and therefore experiments were carried out with the cows in all stages of the estrous cycle.

Determination of the Stage of the Cycle

Progesterone levels were determined from a blood sample drawn prior to infusion and the stage of the cycle was determined to be luteal if the progesterone level was above 1 ng/ml. A commercially available radioimmunoassay kit (215)[b] was used in determination of progesterone levels. The RSL progesterone kit is a highly sensitive progesterone assay which allows for the direct determination of progesterone in unextracted plasma. Radioimmunoassay is a method of

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[a]Lutalyse--The Upjohn Company, Kalamazoo, Michigan.

[b]RSL ^{125}I Progesterone Assay--Radioassay Systems Laboratories, Inc., Carson, California.

measuring the concentration of antigen molecules using a radioactive label that quantitates the amount of hormone by determination of the extent to which it combines with its antibody. In the assay a limited amount of specific antibody is reacted with the corresponding hormone labelled with a radioisotope (^{125}I). Upon addition of an increasing amount of the unlabeled hormone in a serum sample, a correspondingly decreasing fraction of the radioisotope tagged hormone is bound to the antibody. After separation of the bound from the free radioisotope tagged hormone by various means, the amount of radioactivity in one or both of these two fractions is evaluated and used to construct a standard curve against which the unknown samples are measured (215). In performing the assay it is important to use glass test tubes and not plastic as some compounds may adhere to the plastic wall. The assay is set up in duplicate. The procedure following the manufacturer's instructions. This procedure is outlined below:

1. Reagents were brought to room temperature prior to use.
2. Added 0.1 ml of the 0.0 ng/ml Standard (Progesterone free serum) to tubes number 1, 2, 3 and 4.
3. Added 0.1 ml (in duplicate) of each Progesterone Standard (0.2 ng/ml-40 ng/ml) to tubes 5-16.
4. Added 0.1 ml (in duplicate) of control serum or patient serum or plasma to tubes numbers 17 to end of assay.
5. Added 0.5 ml of Assay Buffer, consisting of 0.005% rabbit gamma globulins in 0.1 M phosphosaline-gelatin buffer pH 7.0 to tubes numbers 1 and 2.

6. Added 0.5 ml of Anti-Progesterone to tubes number 3 to end of assay. Anti-Progesterone was prepared by using 11 α-Hydroxyprogesterone-11-α-hemisuccinate-HSA as the antigen to generate antiserum in rabbits. The antiserum binds 40-50% of the radioisotope bound progesterone in the absence of non-radioactive progesterone. The antiserum was diluted with the Assay Buffer. The entire rack of tubes was shaken for thirty seconds.
7. Added 0.2 ml of radioisotope labeled progesterone to all the tubes. Vortex mix and incubate at 37°C for sixty minutes.
8. After sixty minutes of incubation 0.1 ml of a precipitating antibody was added consisting of goat antirabbit gamma globulins in 0.01 M phosphosaline buffer pH 7.5. All the antibody bound antigen was precipitated by 0.1 ml of this antibody at room temperature in a minimum of sixty minutes. Vortex mix and incubate at 37°C for sixty minutes.
9. After sixty minute incubation, all assays were centrifuged at 2300-2500 rpm (1000 x g) for 15 minutes and the supernatant decanted.
10. The precipitate was counted in a gamma counter.

The readings of all duplicate tubes were averaged and the reading of the blank value subtracted from these. This value divided by the zero standard value was the percent bound.

A standard curve was made by plotting the concentration of the progesterone standards (0.2 to 40 ng/ml) against the percentage bound. Sample values were read directly off the standard curve.

Cross reaction to the antiserum was minimal. Reaction with progesterone was 100%. 20-alpha-dihydroprogesterone had a 6.25% cross reaction, desoxycorticosterone was 3.2% and corticosterone 0.42%. The cross reaction percentages for 17-alpha-hydroxyprogesterone, pregnenolone, androstenedione and testosterone were 0.15, 0.06, 0.04 and 0.03 respectively. All other substances were under 0.01% cross reactivity.

On average 93% of exogenous progesterone added to the assay was recovered and the assay specificity and correlation with an extraction method was good. Interassay and intraassay variation was less than 10% (213).

Administration of Antibiotics

Four different antibiotics were used. Procaine penicillin G,[c] amoxicillin trihydrate,[d] oxytetracycline hydrochloride (OTC-C),[c] and long acting oxytetracycline (OTC-LA).[c] Procaine Penicillin G contained 300,000 units per ml. Amoxicillin trihydrate contained 250 mg/ml. Each vial contained 25 gms amoxicillin activity as the trihydrate; sodium citrate 1.05 gms; sodium chloride 210 mg; lecithin 105 mg; povidone 525 mg; methyl paraben (as preservative) 93.7 mg; propyl paraben (as preservative) 10.5 mg.

Oxytetracycline hydrochloride contains 100 mg oxytetracycline base as oxytetracycline hydrochloride per ml and on a weight per

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[c]Pfizer Inc., New York.

[d]Beecham Laboratories, Bristol, Tennessee.

volume basis 0.96% magnesium oxide; 0.43% magnesium formaldehyde sulfoxylate (as a preservative); 19.00% povidone; monoethanolamine to adjust pH, up to 0.05% simethicone emulsion and water for injection q.s. Long acting oxytetracycline contains 200 mg of oxytetracycline base as amphoteric oxytetracycline per ml and on a weight/volume basis, 40.0% 2-pyrrolidone, 5.0% povidone, 1.8% magnesium oxide, 0.2% sodium formaldehyde sulfoxylate (as a preservative) and monoethanolamine and/or hydrochloric acid as required to adjust pH.

Antibiotics were either administered by deep intramuscular injection in the gluteal muscles or infused into the uterus. Each antibiotic treatment was given a total of six times by both routes for a total of forty-eight sets of experimental data.

Doses of antibiotics administered were as recommended in the literature for intrauterine and systemic administration in the treatment of bovine endometritis:

Expt. 1--20,000 i.u./kg Procaine penicillin G intramuscularly.

Expt. 2--Intrauterine infusion of 2,000,000 in Procaine penicillin G.

Expt. 3--10 mg/kg amoxicillin trihydrate intramuscularly.

Expt. 4--Intrauterine infusion of 1 gram of amoxicillin trihydrate.

Expt. 5--8 mg/kg of conventional oxytetracycline intramuscularly.

Expt. 6--Intrauterine infusion of 3 gms of conventional oxytetracycline.

Expt. 7--8 mg/kg of long acting oxytetracycline intramuscularly.

Expt. 8--Intrauterine infusion of 3 gms of long acting oxytetracycline.

Collection of Uterine Fluid Samples

Cows were restrained in a stanchion headgate and caudal epidural anesthesia induced. The area over the sacral and cranial coccygeal vertebrae was clipped with a surgical clippers and surgically prepped with betadine scrub,[e] betadine solution[e] and an alcohol spray. An 18 gauge, 6 cm long hypodermic needle was inserted through the first intercoccygeal space into the epidural space. Eight ml of 2% lidocaine hydrochloride[f] was injected. It was determined that the needle was in the correct position by an absence of resistance while injecting the solution. Induction of complete analgesia took approximately ten minutes. During this time the perineal area was washed and disinfected with betadine scrub and betadine solution. The cervix was located by rectal palpation and grasped by the hand. The cervix was moved anteriorally to straighten the folds in the anterior vagina and a 16 ga Foley catheter[g] with a metal stillette inserted through the vulva and vagina until it was at the external os of the cervix. By careful manipulation of the cervix the catheter was introduced into the body of the uterus and into one of the horns. Once the tip of the catheter was into the horn, the balloon of the catheter was inflated with 30 cc of 0.9% sterile saline. The antibiotic solution was infused into the uterine horn through the Foley catheter and the end of the catheter

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[e]Purdue Frederick Company, Norwalk, Connecticut.

[f]American Veterinary Products, Fort Collins, Colorado.

[g]Bard Urological Division, C. R. Bard, Inc., Murray Hill, New Jersey.

protruding through the vulva clamped off with a plastic tube clamp.[h] Antibiotics were administered in a volume q.s. to 50 ml with phosphate buffered saline to determine the extent of absorption of the infusion volume (125). The composition of the infused solution in cows administered the antibiotics by intramuscular injection consisted of 50 ml of 0.9% sterile phosphate buffered saline. One ml aliquots of the infused solution were removed at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 hours and stored at 4°C until assayed.

Collection of Blood Samples

Blood samples were collected at the same time intervals, as the uterine fluid samples, from the caudal vein except in one cow where samples were collected from the jugular vein due to thrombosis of the tail vein. Samples were collected using vacutainer tubes and needles.[i] Following collection samples were allowed to clot for several hours, centrifuged and the serum removed. The serum was stored at 4°C until assayed. Both serum and uterine fluids were assayed for antibiotic concentration on the day they were collected.

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[h]American Scientific Products, Stone Mountain, Georgia.

[i]B-D Division of Becton, Dickinson and Company, Rutherford, New Jersey.

Preparation of Plates

Antibiotic levels in serum and uterine fluids were determined by means of a bioassay. Petri dishes[j] (150 x 25 mm) were poured with 30 mls of base E agar[k] which was allowed to solidify. This layer was overlayed with 15 mls of a seed agar containing either S. lutea or B. subtilis. Sarcinia lutea was used in the bioassay for penicillin and amoxicillin. Bacillus subtilis was used in the bioassay for both conventional and long acting oxytetracycline.

Sarcinia lutea Plates

Sarcinia lutea (ATCC 9341) was grown up overnight on a BHI plate[l] at 35-37°C. The growth was washed from the surface of the plate using sterile phosphate buffered saline and a sterile cotton swab and placed in a sterile test tube. The concentration of bacteria in this suspension was calibrated to 50% transmission at 550 nm wavelength using a Spectronic 20 spectrophotometer.[m] Antibiotic assay medium A was prepared and inoculated with 3 ml of the above suspension per liter. Caution was exercised to ensure that the temperature of agar A was below 45°C to prevent a heat kill of the organism.

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[j]Miles Scientific, Division of Miles Laboratories, Inc., Napierville, Illinois.

[k]BBL Microbiology System, Becton, Dickinson and Company, Cockeysville, Maryland.

[l]Gibco Laboratories, Madison, Wisconsin.

[m]Bausch and Lomb, Fisher Scientific, Inc., Fort Lawn, New Jersey.

After the addition of the organism the seed agar was mixed thoroughly using a magnetic stirrer. The previously prepared plates containing Base E agar were prewarmed to 37°C and were overlayed with 15 ml of the seed agar containing S. lutea. Plates were placed on a level surface to ensure even distribution of the agar over the plate and special care was taken to make sure no air bubbles became incorporated in the agar. The plates were used immediately or stored at 4°C until used (less than two weeks).

Bacillus subtilis Plates

Bacillus subtilis (ATCC 6633) was inoculated on a BHI plate and incubated at 35-37°C for eighteen hours. The organism was then transferred into 10 ml of sterile phosphate buffered saline using a cotton swab and mixed well with a vortex. Ten plates of AK agar[n] were inoculated with 2 ml of the B. subtilis solution. Plates were inverted and incubated at 35-37°C for 72 hours. The growth was washed from the surface of the plates into 50 ml sterile phosphate buffered saline using a cotton swab. This saline solution was incubated in a 80°C waterbath for one hour, centrifuged and the supernatant decanted and discarded. The pellet was resuspended in 30 ml of sterile phosphate buffered saline and washed two additional times. The suspension can be stored at 4°C for up to 1 year. The solution was added to sterile phosphate buffered saline, in a sterile test tube, until the concentration was sufficient to give a 50% transmission, at 550 nm

[n]Gibco Laboratories, Madison, Wisconsin.

wavelength, using a Spectronic 20 spectrophotometer. The amount of the B. subtilis spore suspension added to Antibiotic Assay Medicine A was determined by titrating the organisms in the plates until the desired growth was attained. In these experiments it was found that 3 ml of the above spore suspension per liter of Assay Medium A was adequate. Plates were prepared as for S. lutea plates. The plates were again either used immediately or stored at 4°C until used (less than two weeks).

Preparation of the Standard Curve

Sarcinea lutea Plates

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 or amoxicillin present in the samples. The standard concentrations used for penicillin were 0.5, 1, 2.5, 5 and 10 units/ml with the concentration of 1 unit used for the midpoint. The standard concentrations used for amoxicillin were 0.039, 0.078, 0.15, 0.3 and 0.6 µg/ml with the concentration of 0.15 µg used for the midpoint. The preparations were absorbed onto .25 inch paper discs[k] at the level of 25 lambda (.025 ml). The arrangement of discs on the agar plates consisted of six discs placed in circular fashion approximately 2.5 cm from the outer edge of the agar. Each plate held three discs containing the midpoint standard alternating with three discs of one of the concentrations used as a standard.

Plates were incubated overnight at 37°C in a humidified incubator. Each plate was run in triplicate for a total of twelve plates, nine 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. All midpoint values were averaged. The values obtained for each standard concentration were averaged. The average of the midpoint concentration, on the three plates, was compared to the average midpoint concentration from all twelve plates. This correction factor, of the difference between the two averages of the midpoint, was either added to or subtracted from the average of the inhibition zone for the standard concentration to produce a corrected average zone size for each concentration of standard. The next step was to obtain a low point (LP) and high point (HP) to plot a linear curve on semilogarithmic paper. Each of the corrected average zone sizes for the standard concentrations was assigned a letter A, B, C, D or E from the lowest to the 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 with phosphate buffered saline (PBS) as the diluent. As the samples to be run would be in serum and

uterine fluids, additional curves were run using these as diluents. As there was no appreciable difference between the diluents, PBS diluent was used.

Bacillus subtilis Plates

A standard curve was prepared following the above procedure. Concentrations of oxytetracycline used were 0.625, 1.25, 2.50, 5 and 10 µg/ml with 2.5 µg/ml as the internal standard. Plates were incubated for fifteen hours in a humidified incubator at 37°C.

Preparation of Samples

Serum samples and uterine fluid samples from cows treated by intramuscular injection of antibiotics 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 on each plate. Three plates were used for each sample for a total of nine observations that were averaged and corrected by the same procedure used to correct zone sizes in the standard curve. When the corrected zone sizes were compared to the linear standard curve concentrations, the concentration of antibiotic in the sample was determined. Samples obtained from the uterus of cows administered antibiotics by intrauterine infusion had too high a concentration of antibiotics to determine by the above method. Tenfold dilutions of the samples for the different time intervals from one cow in each experiment were made and plated out on the bioassay plates and incubated overnight. The dilution that gave a zone of inhibition closest to the midpoint was used in the remaining samples which were run as above.

CHAPTER 4

RESULTS

In each antibiotic trial there were six separate sets of data. These were obtained by using two of the four cows twice. By using the same four cows for all experiments it was felt that each cow would act as its own control and thus minimize the biological variance.

Serum and uterine fluid concentrations of procaine penicillin G, following intramuscular administration of 20,000 iμ/kg, are shown in Table 11. Serum levels of penicillin were detectable within 30 minutes of the intramuscular injection. These levels varied from 1.5 to 5.0 i.u./ml of serum and except for Cow D represented the peak serum concentration. For Cow D peak serum concentrations of penicillin were not obtained until four hours post inoculation.

Detectable penicillin concentrations were present in the uterine lumen in only two of the six treated animals, and in one of these was present for only one of the sampling periods. The cow that demonstrated the greatest serum concentrations also had detectable levels in the uterus from 2 to 8 hours following intramuscular administration.

The serum and uterine fluid concentrations of procaine penicillin G following the intrauterine administration are shown in Table 12. At zero time the concentration of penicillin within the uterine lumen was much lower for one cow than for the other five. However, by 30 minutes post inoculation this difference was no longer observed. The concentration of procaine penicillin with the uterine lumen declined

TABLE 11

SERUM AND UTERINE FLUID CONCENTRATIONS OF PROCAINE PENICILLIN G FOLLOWING THE INTRAMUSCULAR ADMINISTRATION OF 20,000 i.u./kg

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
							Serum (i.u./ml)					
A	2.2	0	3.0	2.5	2.0	2.0	1.5	1.4	0.4	0	0	0
B	1.9	0	3.0	2.9	3.3	1.7	1.3	1.4	0.3	0	0	0
C	<.1	0	2.4	1.9	1.5	1.5	1.0	0.9	0.4	0	0	0
D	.18	0	1.9	2.8	2.5	3.9	2.0	1.3	0.3	0	0	0
A	<.1	0	1.5	1.3	0.8	1.5	1.1	2.2	0.3	0	0	0
B	2.7	0	5.0	4.0	2.4	3.0	2.6	1.0	0	0	0	0
Mean		0	2.8	2.2	2.1	2.3	1.6	1.4	0.3	0	0	0
S.D.		0	1.2	1.2	0.7	1.0	0.6	0.5	0.1	0	0	0
							Uterine Fluid (i.u./ml)					
A	2.2	0	0	0	0	0	0	0	ND**	ND	ND	ND
B	1.9	0	0	0	0	0	0	0	ND	ND	ND	ND
C	<.1	0	0	0	0	0	0.9	0	0	ND	ND	ND
D	.18	0	0	0	0	0	0	0	ND	ND	ND	ND
A	<.1	0	0	0	0	0	0	0	ND	ND	ND	ND
B	2.7	0	0	0	1.6	1.1	0.3	0	ND	ND	ND	ND

*Measured at time 0.

**ND = Not determined due to dislodgement of Foley catheter.

TABLE 12

SERUM AND UTERINE FLUID CONCENTRATIONS OF PROCAINE PENICILLIN G FOLLOWING THE INTRAUTERINE ADMINISTRATION*

Cow No.	Progesterone (ng/ml)**	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
						Serum (i.u./ml)						
A	<.1	0	13.4	15.2	16.9	15.7	0	0	0	0	0	0
B	<.1	0	0	0	0	0	0	0	0	0	0	0
C	<.1	0	0	0	0	0.5	0.3	0	0	0	0	0
D	2.7	0	0	0	0	0	0	0	0	0	0	0
A	ND	0	0	0	0.4	0.3	0	0	0	0	0	0
D	ND	0	0	0	0.3	0	0	0	0	0	0	0
						Uterine Fluid (i.u./ml x 10^3)						
A	<.1	140	52	21	23	6	23	1	ND+	ND	ND	ND
B	<.1	150	57	38	33	13	12	9	2	2	ND	ND
C	<.1	230	88	88	27	40	24	6	ND	ND	ND	ND
D	2.7	100	34	27	24	17	16	18	4	1	ND	ND
A	ND	39	38	36	37	20	18	3	2	3	ND	ND
D	ND	170	45	30	30	25	21	22	ND	ND	ND	ND
Mean		138	51	40	29	20	16	10	3	2		
S.D.		65	19	24	5	11	8	8	1	1		

*2 million i.u. of Procaine Penicillin G was administered by the intrauterine route with a Foley catheter.

**Determined at time 0.

+ND = Not determined due to dislodgement of Foley catheter.

gradually over the first 12 hours but was still above the mean MIC for the common uterine pathogens for all cows at 12 hours post inoculation. At 24 and 36 hours samples were only obtained from 3 cows as the catheters had become dislodged from the other. However, the penicillin concentrations were still above the MIC's of the common uterine pathogens.

Serum levels of penicillin following intrauterine administration were detected in only 4 cows. Two of these were in the nonluteal phase of their cycle as determined by progesterone levels of less than 0.1 ng/ml. Cow B did not show detectable concentrations of penicillin in the serum whereas serum concentrations in Cow A were greater than those achieved in the same cow, in a previous experiment, following intramuscular administration of a larger dose of procaine penicillin G. One of the four other cows in this experiment had no detectable concentrations of penicillin G present in the serum, two had low detectable concentrations at two sampling periods (0.3 to 0.5 i.u.) and the fourth cow only had a detectable concentration at one sampling period (0.3).

The distribution of penicillin in the serum, following intramuscular inoculation, and uterine lumen, following intrauterine infusion in relation to time are shown in Figures 2 and 3, the log of the serum and uterine antibiotic concentrations was plotted against time. The metabolic clearance of the drug followed a logarithmic pattern. Absorption of antibiotics from the uterus following intrauterine infusion of penicillin and secretion into the uterus following intramuscular

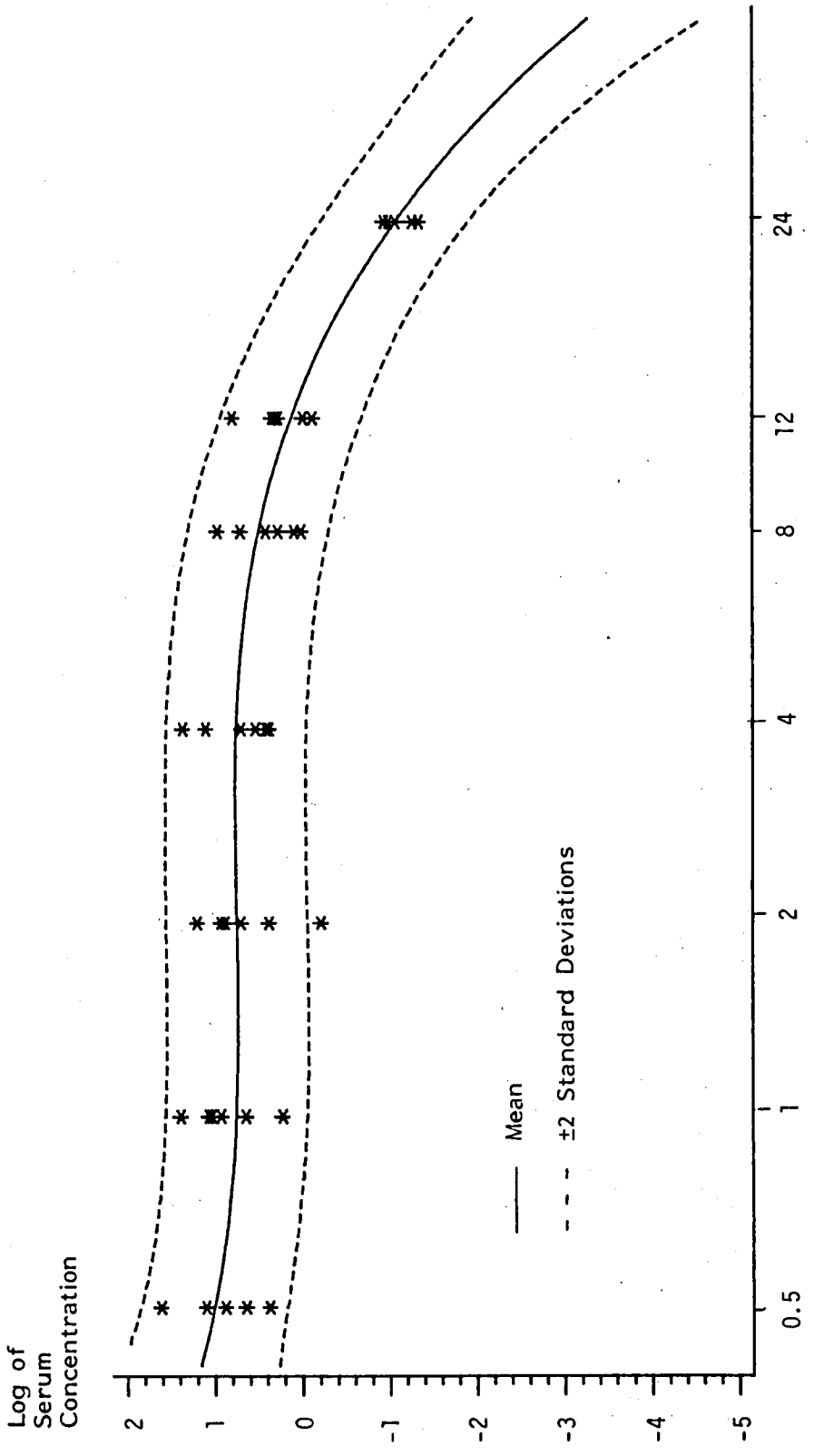


Figure 2. Serum Concentrations of Procaine Penicillin G Following Intramuscular Administration at 20,000 i.u./kg.

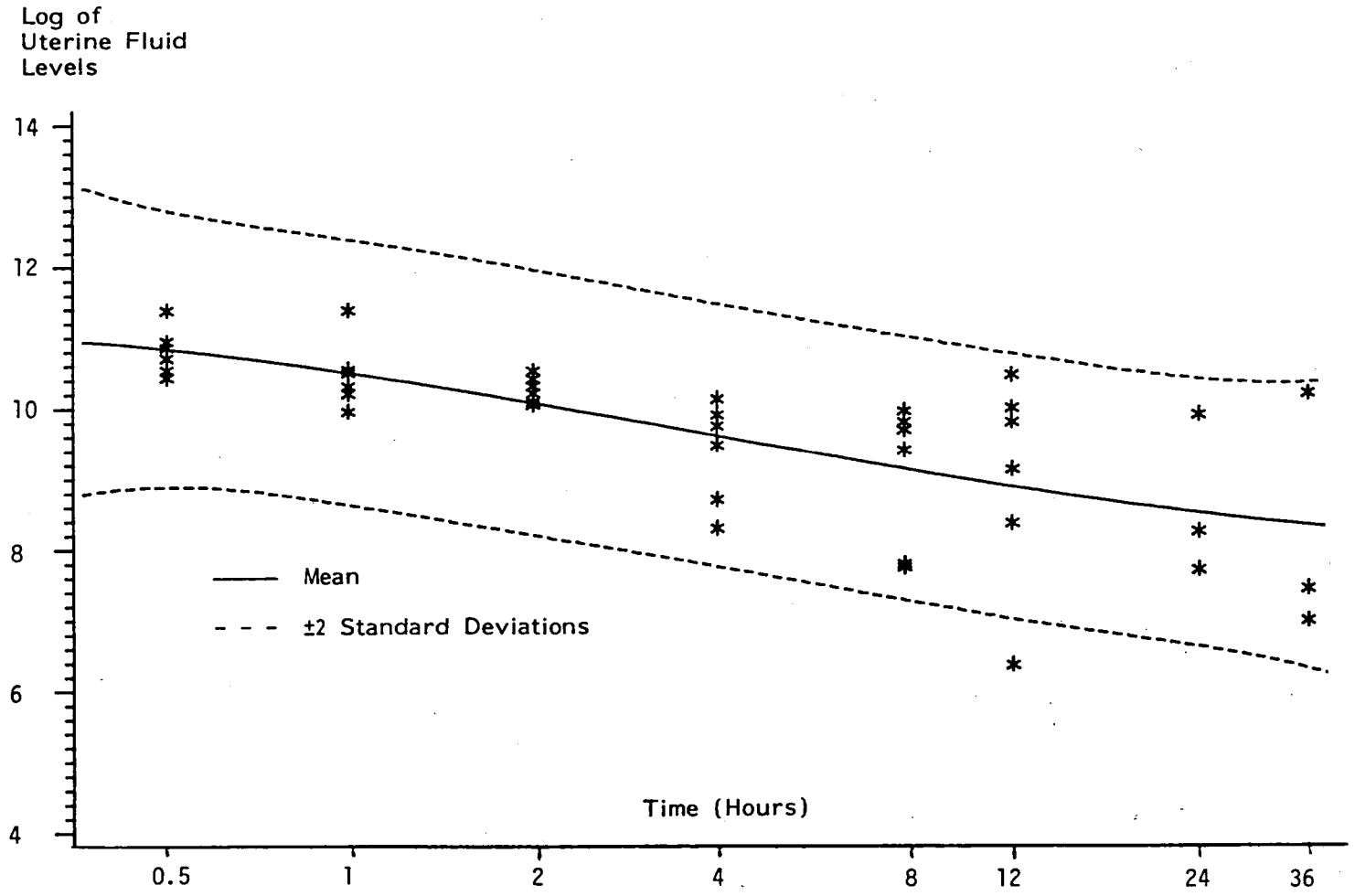


Figure 3. Uterine Fluid Concentrations of Procaine Penicillin G Following Intrauterine Administration of 2 Million Units.

injection was quite variable, and the data did not lend itself to presentation in graphic form.

The serum and uterine fluid concentrations of amoxicillin following the intramuscular administration of 10 mg/kg of the trihydrate salt, are shown in Table 13. At 30 minutes post inoculation, serum concentrations were consistent. However, at one hour post inoculation the results were quite variable and remained variable for up to 8 hours post inoculation ranging from 0.4 to 4.1 μg/ml. By 8 hours post inoculation the serum concentrations were relatively consistent (mean 3.4 with a standard deviation of 1.1). Serum levels of amoxicillin were still detectable by 48 hours post inoculation for all cows. Peak serum level were achieved between 8 and 12 hours (.29 to .54 μg/ml), in the majority of animals, whereas with penicillin, peak serum levels were achieved at 0.5 hrs (Figure 2). Detectable concentrations of amoxicillin were also present in the uterine lumen in all animals treated for at least two sampling periods, but in only four of the seven animals were detectable concentrations present for a period of time greater than 2 hours. However, there did not appear to be any correlation between the serum concentrations and detectable concentrations of antibiotics in the uterus as Cow B had the highest serum levels of amoxicillin but amoxicillin could only be detected in uterine samples taken at 30 minutes and 1 hour past injection. Seven cows were used in this group rather than six. This was due to some difficulty occurring in the initial stages of these studies with dislodged catheters and three of the four cows were used twice in this experiment in order to safeguard

TABLE 13

SERUM AND UTERINE FLUID CONCENTRATIONS OF AMOXICILLIN TRIHYDRATE FOLLOWING THE INTRAMUSCULAR ADMINISTRATION OF 10 mg/kg

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
						Serum (µg/ml x 10^{-1})						
A	0.66	0	0.6	0.8	0.4	0.5	3.1	4.5	4.1	2.7	1.1	0
C	0.16	0	0.4	0.4	0.3	0.4	2.9	1.6	1.7	1.4	0.4	0
D	0.21	0	0.8	0.9	0.4	0.5	3.8	4.1	2.6	1.5	0.7	0
A	1.05	0	0.6	4.4	4.4	4.1	2.8	3.5	1.2	0.8	1.2	0
B	1.65	0	0.6	5.0	4.8	4.0	5.4	4.0	1.5	1.0	0.9	0
C	0.4	0.	0.6	3.2	2.6	2.4	1.9	1.8	1.5	1.3	1.3	0
D	4.4	0	0.6	10.5	7.8	3.5	4.5	5.8	2.4	1.0	0.6	0
Mean			0.6	3.6	2.9	2.2	3.4	3.6	2.1	1.3	0.8	
S.D.			0.1	3.6	2.80	1.6	1.1	1.4	0.9	0.6	0.3	
						Uterine Fluid (µg/ml x 10^{-1})						
A	0.66	0	0.5	1.0	0.8	0.8	0.5	0.4	0	ND+	ND	ND
C	0.16	0	0	0	0.7	0.6	0.8	2.6	0.6	0.9	0	ND
D	0.21	0	0	0.4	0.6	1.1	1.2	0.3	ND	ND	ND	ND
A	1.05	0	0.5	1.0	0	0	0	0	ND	ND	ND	ND
B	1.65	0	0.6	0.4	0	0	0	0	ND	ND	ND	ND
C	0.4	0	0.5	0.4	0.6	0	0	0	0	0	0	0
D	4.4	0	0.0	0.4	0	0	0	0	4.5	ND	ND	ND
Mean			0.5	0.5	0.5							
S.D.			0.1	0.3	0.6							

*Determined at time 0.

+ND = Not determined due to dislodgement of Foley catheter.

against one cow dislodging the catheters very early in the experiment. As all cows retained the catheters it was decided to utilize the seven sets of data.

Serum and uterine fluid concentrations of amoxicillin trihydrate following the intrauterine infusion of 1 gram of amoxicillin trihydrate are shown in Table 14. Concentrations of amoxicillin in the uterine lumens declined at different rates. For example, Cow C was used twice in this experiment. Following the first infusion, a level of 10,500 μg/ml was present in the uterus; whereas following the second infusion, a level of 780 μg/ml was present. Due to the dislodgement of the catheters, samples were not obtained on six of the seven cows beyond 8 hours post infusion. Detectable serum concentrations were present in all but one cow in this experiment but were only present for greater than 2 hours in two cows.

The distributions of amoxicillin in the serum following intramuscular inoculation, and the uterine lumen, following intrauterine infusion, in relation to time are shown in Figures 4 and 5. The log of the serum and uterine antibiotic concentrations was plotted against time. The metabolic clearance of the drug following intramuscular injection was relatively constant throughout the sampling times but followed a logarithmic pattern following intrauterine infusion. Absorption of antibiotics from the uterus following intrauterine infusion and secretion of amoxicillin into the uterus following intramuscular inoculation was quite variable and did not lend itself to graphic illustration.

TABLE 14

SERUM AND UTERINE FLUID CONCENTRATIONS OF AMOXICILLIN TRIHYDRATE FOLLOWING THE INTRAUTERINE ADMINISTRATION OF 1 GRAM

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
						Serum (µg/ml)						
A	1	0	0	0	0	0.1	0.1	0	0	0	0	0
C	0.12	0	0	0.11	0.1	0.1	0	0	0	0	0	0
D	0.29	0	0.1	0.4	0.3	0	0	0	0	0	0	0
A	<.1	0	0	0	0	0	0	0	0	0	0	0
B	<.1	0	0.1	0.1	0.1	0	0	0	0	0	0	0
C	0.21	0	0	0	0	0	0	0	0	0	0	0
D	0.12	0	0.1	0	0.3	0	0	0	0	0	0	0
						Uterine Fluid (µg/ml x 10^2)						
A	1	230	170	125	88	82	8	2	ND**	ND	ND	ND
C	0.12	180	110	140	105	44	14	2	2	2	ND	ND
D	0.29	320	70	91	43	43	28	ND	ND	ND	ND	ND
A	<.1	150	82	60	49	48	44	ND	ND	ND	ND	ND
B	<.1	200	135	105	45	45	38	ND	ND	ND	ND	ND
C	.21	240	90	50	8	4	3	ND	ND	ND	ND	ND
D	.12	320	70	82	43	6	3	ND	ND	ND	ND	ND
Mean		234	104	93	54	39	20					
S.D.		66	37	33	32	27	17					

*Determined at time 0.

**ND = Not determined due to dislodgement of Foley catheter.

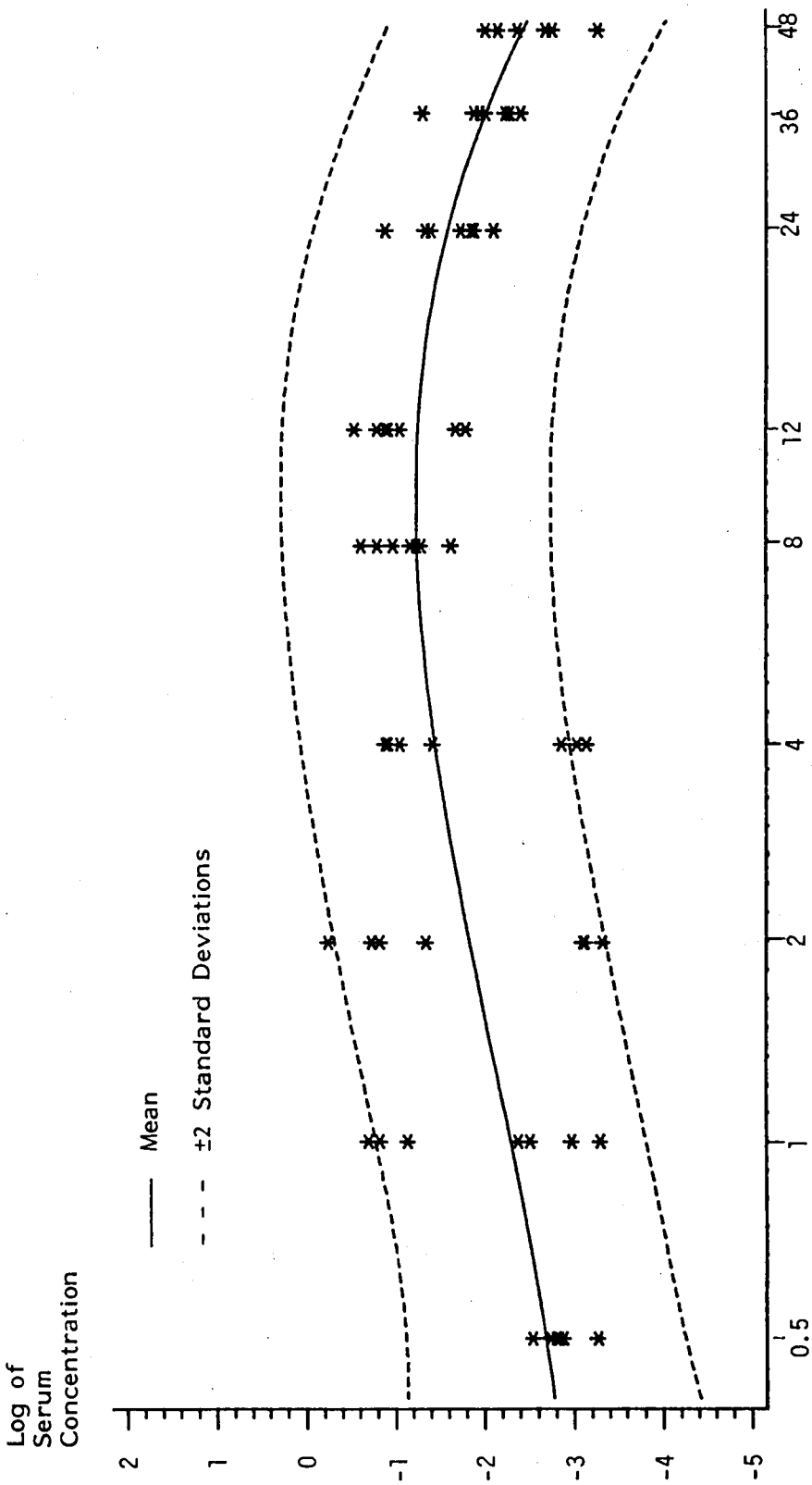


Figure 4. Serum Concentrations of Amoxicillin Trihydrate Following Intramuscular Administration of 10 mg/kg.

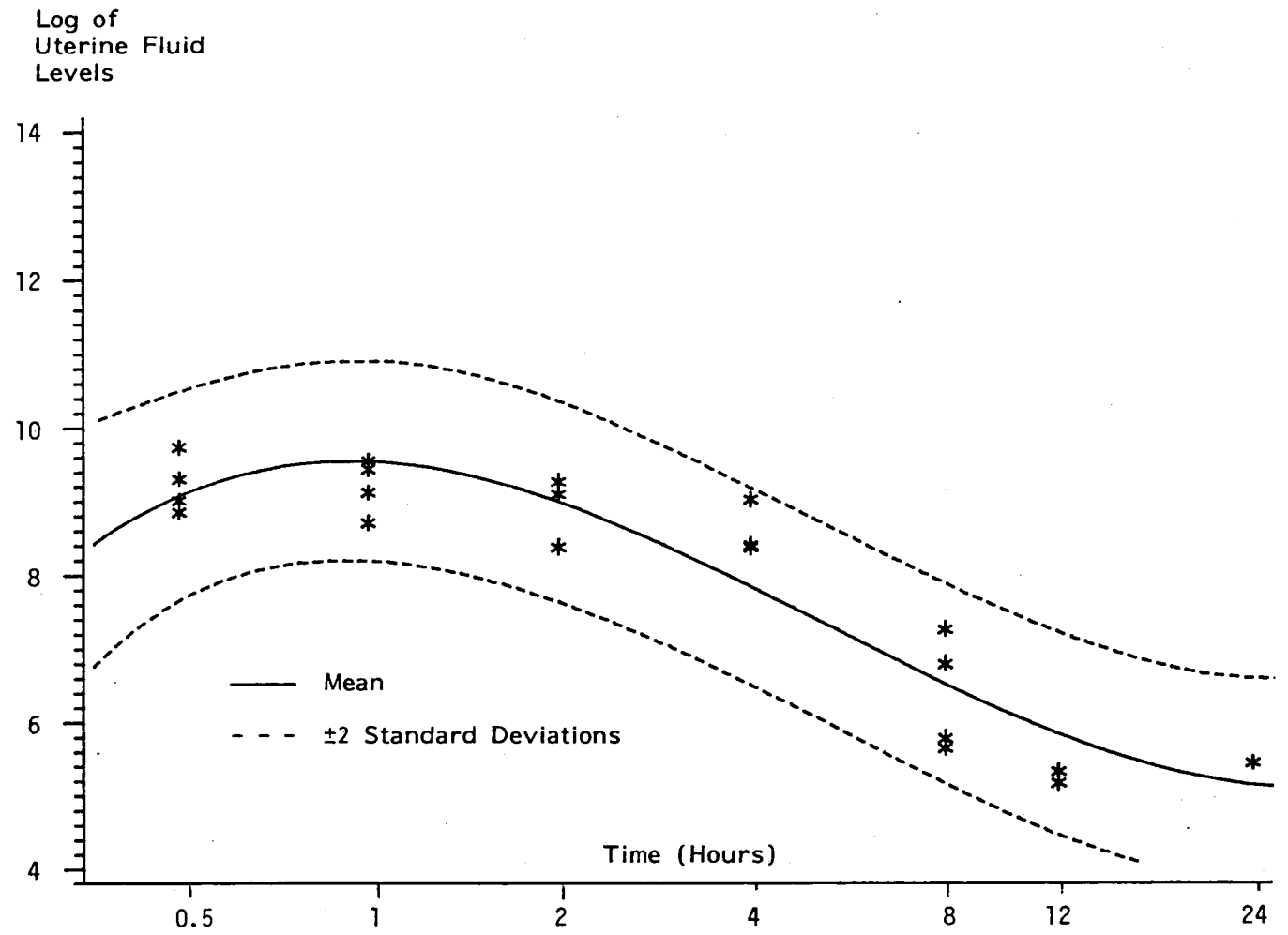


Figure 5. Uterine Fluid Concentrations of Amoxicillin Trihydrate Following Intrauterine Administration of 1 Gram.

Serum and uterine fluid concentrations of oxytetracycline following intramuscular administration of 8 mg/kg of OTC-C are shown in Table 15. Serum concentrations of oxytetracycline achieved following intramuscular administration of OTC-C were more consistent than serum concentrations of penicillin or amoxicillin administered by intramuscular injection, with peak serum concentrations achieved rapidly (2-4 hours). Detectable concentrations of oxytetracycline were present within the uterine lumen of all treated animals, but there was a marked degree of variability in concentrations present.

Serum and uterine fluid concentrations of oxytetracycline following intrauterine administration of 3 grams of OTC-C are shown in Table 16. Detectable serum were not present in two of six animals treated via intrauterine infusion, and were present at only one sampling period in two other animals. Serum concentrations of oxytetracycline were present for up to 8 hours in the remaining two animals.

The distribution of oxytetracycline hydrochloride (OTC-C) in the serum, following intramuscular inoculation; and the uterine lumen, following intrauterine infusion in relation to time are shown in Figures 6 and 7. As with the previous antibiotics the log of the serum and uterine antibiotic concentrations was plotted against time. The concentration OTC-C in the serum showed a slight peaked at 4 hours post inoculation but remained relatively constant throughout 8 hours sampling time. On the other hand, the clearance of OTC-C from the uterus was fairly constant in all animals following intrauterine

TABLE 15

SERUM AND UTERINE FLUID CONCENTRATIONS OF OXYTETRACYCLINE FOLLOWING INTRAMUSCULAR ADMINISTRATION OF 8 mg/kg OF OTC-C

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
							Serum (µg/ml)					
A	0.52	0	2.3	2.3	3.4	3.9	4.7	2.2	2.3	2.0	0.9	0
B	0.98	0	1.0	1.9	3.6	3.5	3.9	2.8	2.8	2.0	1.2	0
C	2.2	0	1.2	2.3	3.9	4.4	3.9	2.3	2.6	2.1	1.6	0
D	0.37	0	1.8	3.6	3.9	4.6	4.2	2.7	1.7	3.2	1.1	0
A	1.55	0	1.8	3.7	4.3	5.6	3.0	3.2	1.2	0.8	0	0
B	<.1	0	1.1	1.1	1.2	1.6	2.6	2.4	2.7	1.6	0	0
Mean			1.5	2.5	3.4	3.9	3.7	2.6	2.2	1.9	0.8	
S.D.			0.5	1.0	1.1	1.4	0.8	0.4	0.6	0.8	0.6	
							Uterine Fluid (µg/ml)					
A	0.52	0	5.5	0	0	2.9	9.2	ND**	ND	ND	ND	ND
B	0.98	0	1.2	5.0	5.8	3.5	3.5	3.5	2.7	ND	ND	ND
C	2.2	0	34.5	14.5	18	11.5	24	15.5	24	17.5	11.8	ND
D	0.37	0	2.2	1.9	3.1	5.3	14	6.4	ND	ND	ND	ND
A	1.55	0	0	0	1.6	5.7	31	15	12.2	35	48	ND
B	<.1	0	1.6	8.1	3.1	1.8	6.6	1.2	3.6	13.5	0	0
Mean			9.3	4.9	5.3	5.1	14.7	8.3	16.7			
S.D.			13.0	5.6	6.5	3.5	10.7	6.5	10.8			

*Determined at time 0.

**ND = Not determined due to dislodgement of Foley catheter.

TABLE 16

SERUM AND UTERINE FLUID CONCENTRATIONS OF OXYTETRACYCLINE FOLLOWING INTRAUTERINE ADMINISTRATION OF 3 GRAMS OF OTC-C

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
		Serum (mg/ml)										
A	2.0	0	0	0	0	0	0	0	0	0	0	0
B	2.7	0	0	0	0	0	0	0	0	0	0	0
C	0.13	0	0	0	0	2.5	0	0	0	0	0	0
D	3.1	0	0.6	0.5	0.6	1.0	0.9	0	0	0	0	0
C	1.05	0	0	0	0	0	0.3	0	0	0	0	0
D	21	0	0.6	1.2	0.87	0.4	0	0	0	0	0	0
		Uterine Fluid ($\mu g/ml \times 10^3$)										
A	2.0	150	97	86	48	29	59	12.8	ND**	ND	ND	ND
B	2.7	170	80	62	31	36	16	20	2.4	1.1	ND	ND
C	0.13	140	96	84	48	28	9.3	19	ND	ND	ND	ND
D	3.1	135	80	84	69	38	28	4.9	1.2	0.6	ND	ND
C	1.05	90	90	88	32	21	29	31	2.9	0.7	0.9	0
D	21	90	90	68	13	31	20	11.2	4.5	1.6	1.2	ND
Mean		129	88	78.6	40.2	32	26.9	16.5	2.8	1.0		
S.D.		32	7.4	10.9	19	6.9	17.4	9	1.4	0.5		

*Determined at time 0.

**ND = Not determined due to dislodgement of Foley catheter.

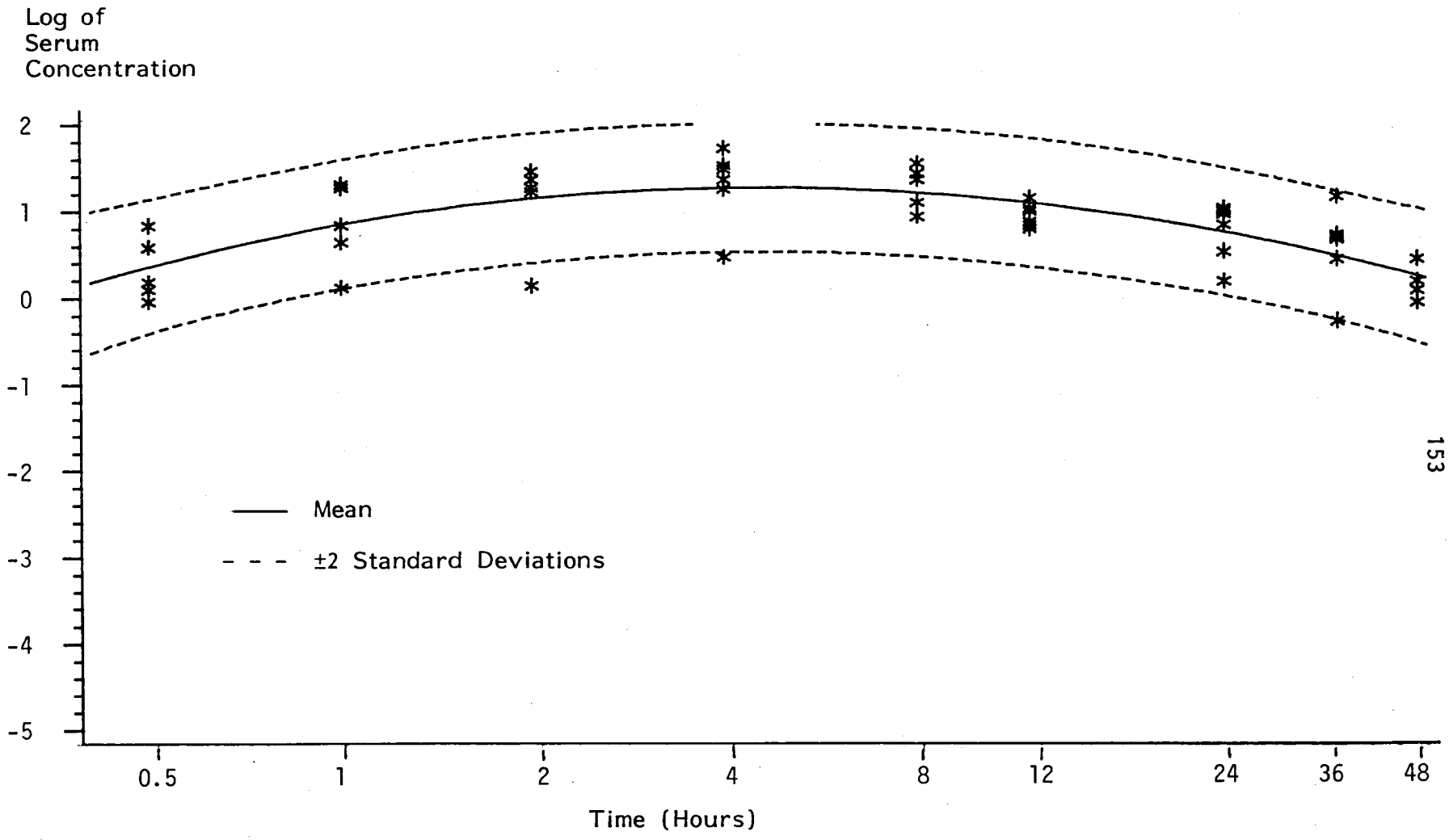


Figure 6. Serum Concentrations of Oxytetracycline (OTC-C) Following Intramuscular Administration at 8 mg/kg.

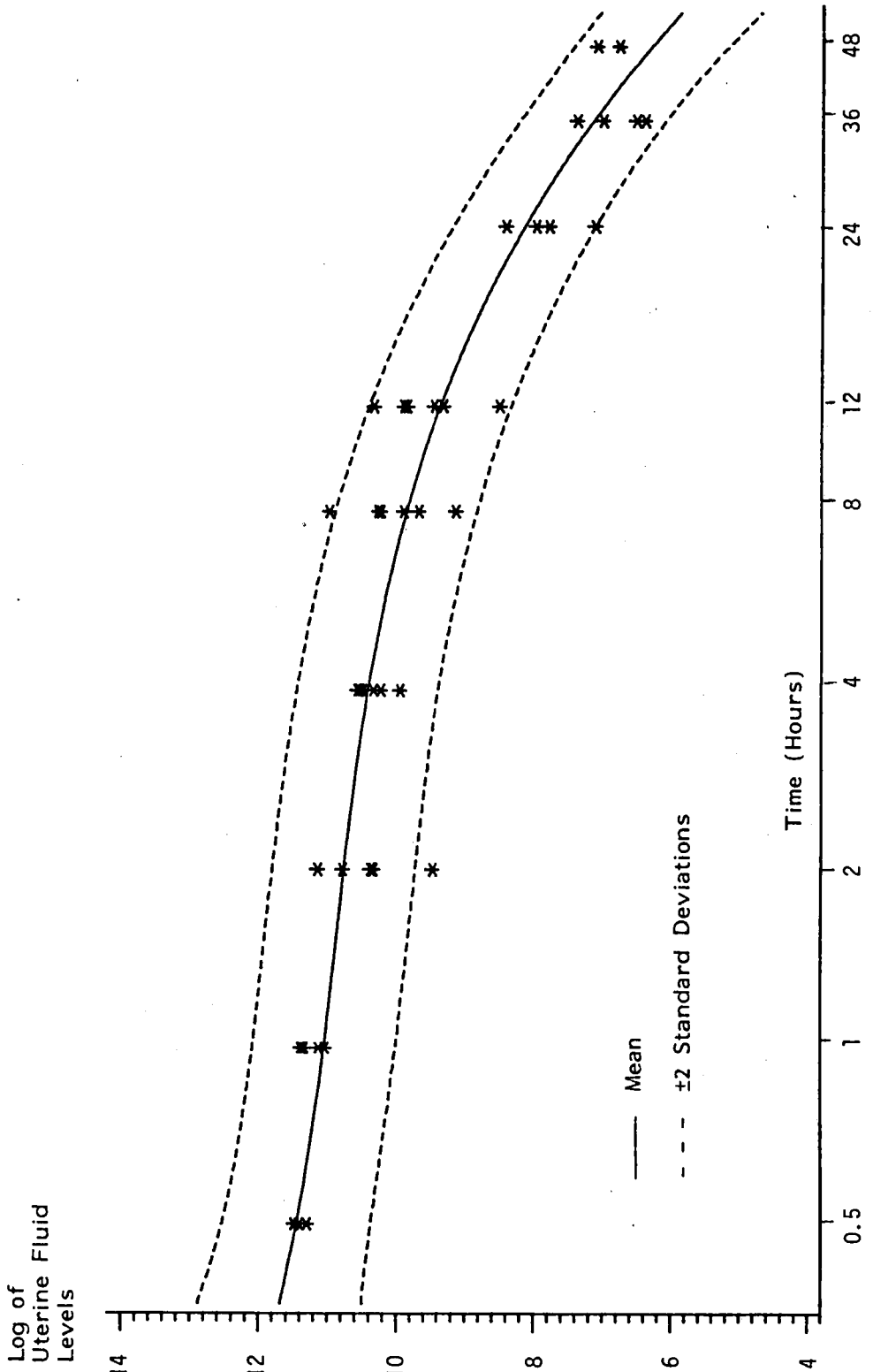


Figure 7. Uterine Fluid Concentrations of Oxytetracycline Following Intrauterine Administration of 3 Grams of OTC-C.

infusion and followed a logarithmic relationship between concentration time. Absorption of antibiotics from the uterus following intrauterine infusion and secretion of OTC-C into the uterus following intramuscular inoculation was quite variable and did not lend itself to graphic illustration.

Serum and uterine fluid concentrations of oxytetracycline following intramuscular administration of 8 mg/kg of Liquamycin OTC-LA are shown in Table 17. In comparing Tables 15 and 17, it is noted that following administration of the same dose of OTC-LA, that concentrations achieved in both the serum and uterus were lower than those achieved following the administration of OTC-C. Serum concentrations of oxytetracycline were fairly consistent, but there was a marked variation in uterine fluid, concentrations, although differences were not as great as those observed following the intramuscular administration of OTC-C.

Serum and uterine fluid concentrations of oxytetracycline following intrauterine administration of 3 grams of OTC-LA are shown in Table 18. Detectable serum concentrations were present in all but one cow, and persisted for a longer period of time (for up to 12 hours in three of the six cows) following infusion of OTC-C. The decrease in serum and uterine fluid concentrations of oxytetracycline OTC-LA are shown in Figures 8 and 9.

Due in part to the luteolytic effect of intrauterine infusions it was noted that the majority of cows were within the nonluteal phase of the cycle. All cows were cycling during the period of the experiment as

TABLE 17

SERUM AND UTERINE FLUID CONCENTRATIONS OF OXYTETRACYCLINE FOLLOWING INTRAMUSCULAR ADMINISTRATION OF 8 mg/kg OF OTC-LA

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
							Serum (µg/ml)					
A	0.56	0	0	1.0	1.0	1.2	2.4	2.3	1.6	1.1	0.7	0
B	1.55	0	1.1	1.3	2.6	3.0	3.1	3.0	1.5	0.8	0.7	0
C	0.92	0	2.3	2.2	3.0	2.4	2.6	2.4	1.2	0.8	0.7	0
D	10.6	0	2.5	2.4	2.6	2.6	2.6	2.0	1.1	0.6	0	0
C	0.26	0	1.6	1.6	1.3	1.0	1.0	1.0	0.7	0.6	0	0
D	3.25	0	2.0	1.8	1.6	1.6	1.6	1.2	0.7	0.5	0	0
Mean			1.6	1.7	2.0	2.0	2.2	2.0	1.1	0.7	0.3	
S.D.			0.9	0.5	0.8	0.8	0.8	0.8	0.4	0.2	0.4	
							Uterine Fluid (µg/ml)					
A	0.56	0	0	3.1	5.0	5.0	5.7	8.2	3.5	3.0	2.6	4.1
B	1.55	0	8.1	14.5	15.5	4.2	3.9	4.5	4.5	2.7	1.9	ND**
C	0.92	0	2.8	2.3	3.2	4.4	4.6	4.5	0.6	0	0	0
D	10.6	0	2.5	2.5	4.1	6.9	5.0	5.3	4.7	6.8	6.8	6.0
C	0.26	0	18	17	0.8	1.8	0.4	ND	ND	ND	ND	ND
D	3.25	0	1.5	0.4	1.8	2.1	1.9	2.0	ND	ND	ND	ND
Mean			5.5	6.6	5.1	6.1	3.6	4.9	3.3	3.1	2.8	3.4
S.D.			6.7	7.1	5.4	1.9	2.0	2.2	1.9	2.8	2.9	3.1

*Determined at time 0.

**ND = Not determined due to dislodgement of Foley catheter.

TABLE 18

SERUM AND UTERINE FLUID CONCENTRATIONS OF OXYTETRACYCLINE FOLLOWING INTRAUTERINE ADMINISTRATION OF 3 GRAMS OF OTC-LA

Cow No.	Progesterone (ng/ml)*	Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
						Serum (µg/ml)						
A	0.68	0	1.1	0.7	0.8	0.7	0.5	0.5	0	0	0	0
B	3.0	0	0	0	0	0	0	0	0	0	0	0
A	0.15	0	0.8	1.0	0.7	0.6	0.5	0	0	0	0	0
B	<.1	0	0.8	0.8	0.6	0.3	0	0	0	0	0	0
C	<.1	0	1.2	1.5	1.2	1.4	0.8	0.4	0	0	0	0
D	3.4	0	1.0	0.7	0.8	0.8	0.5	0.3	0	0	0	0
Mean			0.8	0.8	0.7	0.7	0.7	0.2				
S.D.			0.4	0.5	0.4	0.5	0.3	0.2				
						Uterine FLuid (µg/ml x 10^3)						
A	0.68	23	31	20	14	7	4	ND**	ND	ND	ND	ND
B	3.0	22	29	19	13	11	10	8	1	1	ND	ND
A	0.15	44	31	15	14	4	0	0	0	ND	ND	ND
B	<.1	60	32	18	12	18	ND	ND	ND	ND	ND	ND
C	<.1	39	20	15	16	22	10	4	1	1	ND	ND
D	3.4	39	29	25	18	11	ND	ND	ND	ND	ND	ND
Mean		38	28	19	14	12	6	4				
S.D.		14	4	4	2	1	5	4				

*Determined at time 0.

**ND = Not determined due to dislodgement of Foley catheter.

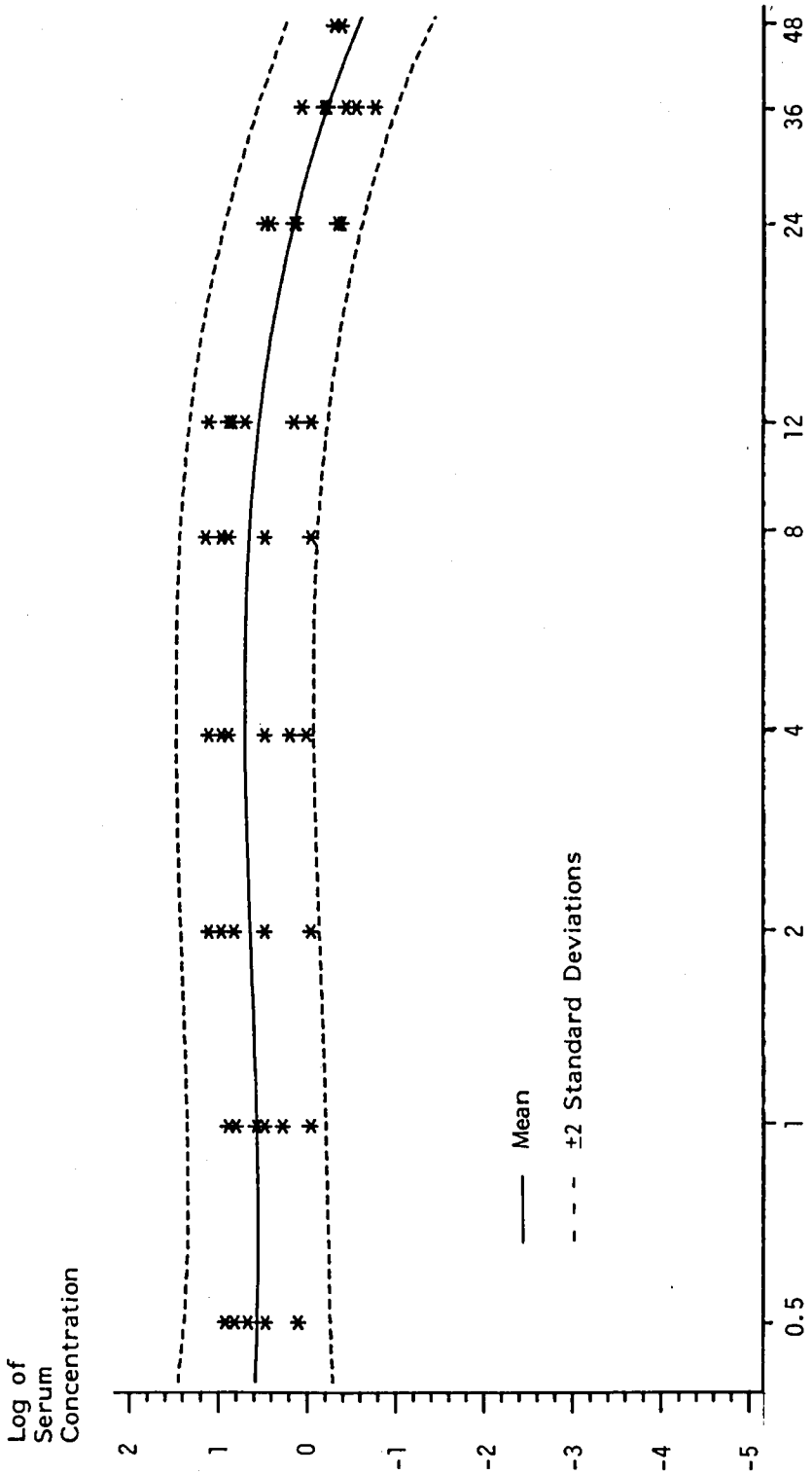


Figure 8. Serum Concentrations of Oxytetracycline Following Intramuscular Administration at 8 mg/kg of OTC-LA.

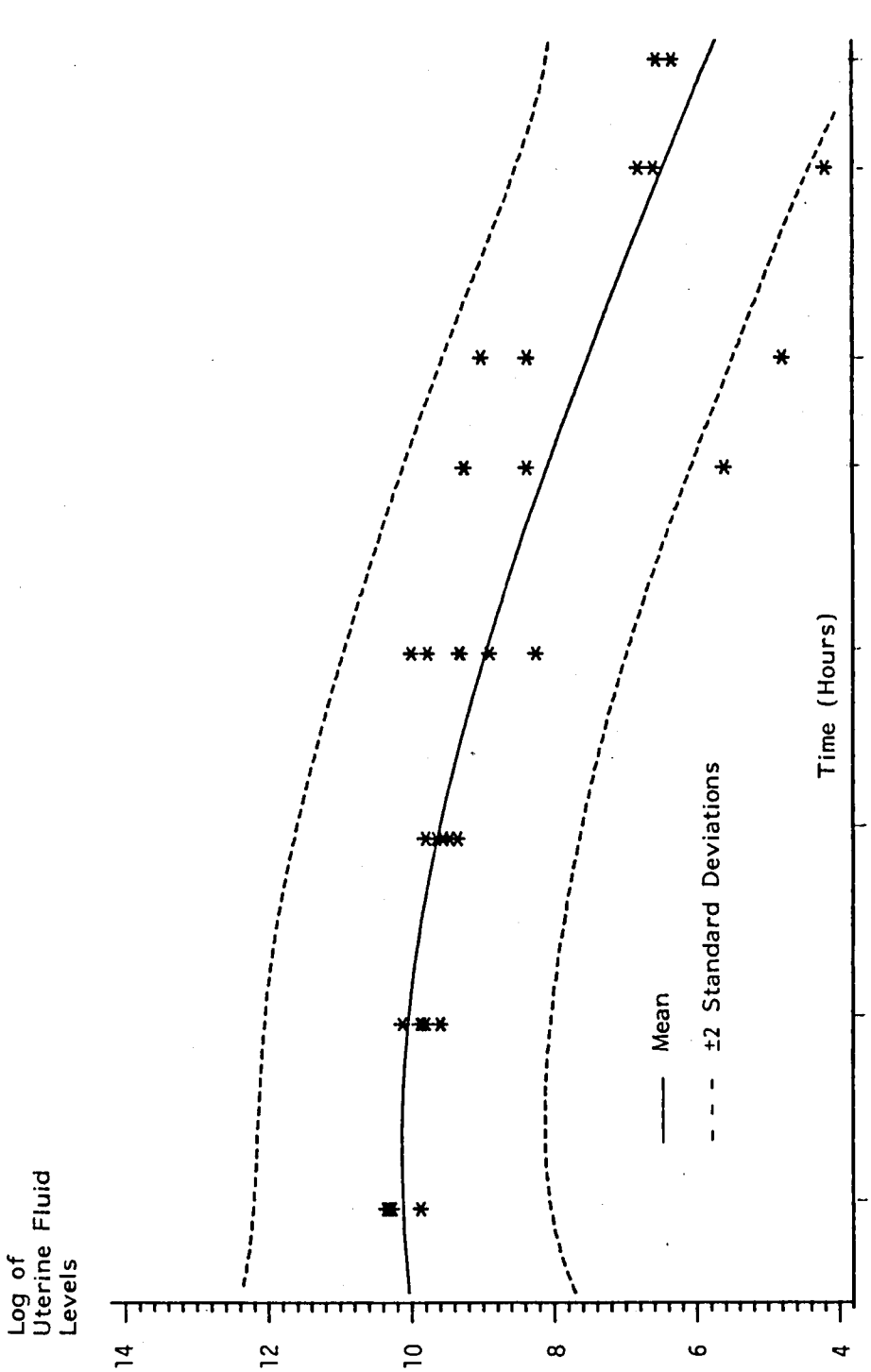


Figure 9. Uterine Fluid Antibiotic Concentrations Following Intrauterine Administration of 3 Grams of OTC-LA.

demonstrated by at least one progesterone level above 1 ng/ml and one below for each cow. It had been hoped to compare results of absorption in cows in the luteal phase with those in the nonluteal phase. Only in the group of cows given OTC-LA by intrauterine infusion were the number of cows evenly divided between luteal and nonluteal stages of the cycle. This enabled a valid comparison to be made. The results, Table 19, indicated that absorption of antibiotics from the uterine lumen after the administration of 3 grams of OTC-LA was greater in the nonluteal phase of the cycle. Despite the large standard deviations seen in those samples obtained from cows in the luteal phase.

The secretion of antibiotics into the uterine lumen following intramuscular administration is compared between cows in the luteal and nonluteal stages of the cycle in Table 20. Following intramuscular administration, very little penicillin was secreted into the uterine lumen. Only one cow in the luteal stage and one in the nonluteal stage showed detectable concentrations of antibiotics in the uterine lumen so no valid comparisons can be made from these data. Levels achieved in the uterus following the administration of amoxicillin trihydrate were higher and persisted for a longer period of time in those cows in the nonluteal stage of the cycle. However, following the administration of either oxytetracycline preparation, concentrations in the uterine fluid were higher in the luteal stage of the cycle than in the nonluteal stage. Levels achieved in the uterine lumen were higher in both stages of the cycle following the administration of the

TABLE 19

COMPARISON OF SERUM CONCENTRATIONS OF OXYTETRACYCLINE FOLLOWING INTRAMUSCULAR ADMINISTRATION OF 3 GRAMS OF OTC-LA TO COWS IN THE LUTEAL AND NONLUTEAL STAGE OF THE ESTROUS CYCLE

		Time (hrs)										
Stage of Cycle		0	0.5	1	2	4	8	12	24	36	48	72
		Serum (µg/ml)										
Luteal	Mean	0	0.48	0.34	0.39	0.41	0.24	0.16	0	0	0	0
	S.D.	0	0.68	0.48	0.55	0.57	0.35	0.22	0	0	0	0
Nonluteal	Mean	0	0.95	1.00	0.79	0.76	0.45	0.21	0	0	0	0
	S.D.	0	0.20	0.32	0.25	0.46	0.34	0.30	0	0	0	0

TABLE 20

COMPARISON OF UTERINE FLUID CONCENTRATIONS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF PROCAINE PENICILLIN G, AMOXICILLIN TRIHYDRATE, OTC-C OR OTC-LA IN COWS IN THE LUTEAL AND NONLUTEAL STAGE OF THE ESTROUS CYCLE

Antibiotic	Stage of Cycle		Time (hrs) 0	0.5	1	2	4	8	12	24	36	48	72
			Uterine Fluid (i.u. or µg/ml)										
Penicillin	Luteal	Mean	0	0	0	1.6	1.12	0.25	0	ND*	ND	ND	ND
		S.D.	0	0	0	0	0	0	0	ND	ND	ND	ND
Penicillin G	Nonluteal	Mean	0	0	0	0	0	0.85	0	0	ND	ND	ND
		S.D.	0	0	0	0	0	0	0	0	ND	ND	ND
Amoxicillin	Luteal	Mean	0	0.05	0.06	0	0	0	0	0.45	ND	ND	ND
		S.D.	0	0.01	0.03	0	0	0	0	0	ND	ND	ND
Amoxicillin	Nonluteal	Mean	0	0.03	0.05	0.09	0.06	0.06	0.08	0.03	0.05	0	0
		S.D.	0	0.03	0.04	0.06	0.05	0.05	0.12	0.05	0.07	0	0
Oxytetracycline	Luteal	Mean	0	17.25	7.25	9.77	8.6	27.5	15.25	18.1	26.25	29.87	ND
		S.D.	0	24.39	10.25	11.63	4.1	4.9	0.35	8.3	12.37	25.63	ND
Oxytetracycline	Nonluteal	Mean	0	5.31	3.75	2.99	3.36	8.32	3.7	15.3	13.5	0	0
		S.D.	0	4.78	3.55	2.37	1.48	4.44	2.6	16.54	0	0	0
Oxytetracycline-LA	Luteal	Mean	0	4.03	5.79	7.11	4.4	3.6	3.93	4.6	4.75	4.35	6.0
		S.D.	0	3.55	7.61	7.35	2.4	1.57	1.72	0.14	2.89	3.46	0
Oxytetracycline-LA	Nonluteal	Mean	0	6.93	7.46	2.98	3.72	3.55	6.35	2.03	1.5	1.3	2.05
		S.D.	0	9.68	8.26	2.13	1.73	2.83	2.61	2.08	2.12	1.83	2.89

*ND = Not determined.

conventional oxytetracycline preparation than they were following administration of the long acting preparation.

Following intrauterine administration of penicillin, absorption sufficient to cause detectable serum concentrations only occurred in four of the six treated animals. Progesterone levels above 1 ng/ml were only present in one cow, which did not absorb any penicillin so valid comparisons could not be made between absorption in animals in the luteal stage and the nonluteal stage of the estrous cycle.

All cows treated with amoxicillin had a progesterone level of 1 ng/ml or less and were judged to be in the nonluteal stage of the cycle. Only one cow did not absorb sufficient antibiotic to give detectable concentrations in the serum. Detectable concentrations were present in the serum of one cow for up to twelve hours but were present for four or less hours in the remaining animals.

Absorption into the serum was greater following the intrauterine administration of the long acting tetracycline than the conventional preparation. Of the six cows infused with the conventional tetracycline, all but one cow were in the luteal phase of the cycle. The long acting oxytetracycline absorption was better during the nonluteal stage of the cycle. With the conventional tetracycline two cows did not absorb any tetracycline into the serum and two others only had detectable concentrations for one sampling period. The concentrations in the other two cows were not detectable after eight hours.

Following the intrauterine administration of long acting tetracycline, concentrations were detected in the serum for up to 12 hours in 50% of the animals.

The decline in concentrations of antibiotics in the uterus was similar for all four antibiotics. Concentrations declined more rapidly for penicillin and amoxicillin than they did for the two tetracyclines, although at thirty-six hours, 1 percent of each antibiotic was still present in the uterine lumen (Table 21). At the dosage levels used in these experiments the concentration present in the uterine lumen was above the MIC for sensitive organisms at this time.

Although some of the differences shown in the previous tables may appear to be significant, the numbers of animals used in the experiments, six in each, was too small to attach any statistical significance to differences when this group of six cows were further divided, in comparing absorption and secretion in the luteal and nonluteal stages of the cycle. Therefore, rather than showing any statistically significant differences between absorption or secretion, these findings only indicate trends that would need to be either confirmed or refuted in additional experiments with larger numbers of animals. The differences in concentrations achieved with OTC-C and OTC-LA are significant and would tend to confirm the findings of Xia, (199), who also noted that OTC-LA did not produce higher serum concentrations or concentration that persisted for a longer period of time, following intramuscular administration, than OTC-C.

TABLE 21

PERCENTAGE OF INFUSED DOSE REMAINING IN THE UTERUS FOLLOWING INTRAUTERINE INFUSION OF PENICILLIN, AMOXICILLIN, OTC-C, OTC-LA

Antibiotic	Time (hrs)										
	0	0.5	1	2	4	8	12	24	36	48	72
Procaine Penicillin G	100	37.8	28.9	20.9	14.5	11.2	7.1	1.9	1.3	ND	ND
Amoxicillin Trihydrate	100	44.3	39.8	23.2	16.6	8.4	0.8	0.9	0.8	ND	ND
Oxytetracycline--Conventional	100	68.2	60.9	31.1	24.8	20.8	12.7	2.1	0.7	0.8	ND
Oxytetracycline--Long-Acting	100	74.0	48.9	37.8	32.0	16.6	11.1	1.4	1.6	ND	ND

ND = Not determined.

CHAPTER 5

DISCUSSION

Antimicrobial therapy for the treatment of bovine metritis may consist of either intrauterine infusion, systemic therapy or both. The use of intrauterine infusion in the treatment of metritis originated before the era of antibiotics when antimicrobial agents, being too toxic to administer systemically, were applied directly to the site of the infection. Despite the fact that most antibiotics on the market today are relatively nontoxic, this method of treatment has continued, partially due to expense and partially due to the age-old tradition that it is best to treat an infection at the site of the infection. The objectives of this study were to determine: (1) if intrauterine infusion of a few selected antibiotics resulted in therapeutic levels of the antibiotics at the site of infection and if so for how long; (2) if local therapy could result in therapeutic levels systemically in the event the metritis developed into an endometritis or septicemia; and (3) if systemic administration provided therapeutic levels of the antibiotics in uterine fluids.

In determining which antibiotics would be used in this experiment, it was determined that penicillin and oxytetracycline should be used as they are among the most commonly used antibiotics in the clinical treatment of bovine endometritis. Gentamicin is also commonly used as a intrauterine infusion but serum and intrauterine concentrations of gentamicin following intrauterine and intramuscular administration have recently been determined (124) using a radioimmunoassay.

Spectinomycin was considered as it has been used as an intrauterine infusion, has chemical and biological properties similar to gentamicin and a similar antibacterial spectrum. However, after testing various acceptable microorganism using a bioassay, we were unable to find any bacterium that was sensitive to spectinomycin below 10 μg/ml and did not consider that concentration of spectinomycin to be sensitive enough. Chloramphenicol has also been used extensively, both systemically and by intrauterine infusion, in treating bovine metritis, however, this drug has recently been declared illegal to use in food animals, and is no longer available for such use. Other antibiotics were considered such as ticarcillin, but it was felt that this drug was too expensive for practical systemic use in the treatment of endometritis.

Although several studies have been carried out on serum concentrations of amoxicillin following systemic administration, it has not been reported to have been used in the clinical treatment of endometritis. Furthermore, pharmacodynamic studies to determine serum and uterine fluid concentrations of amoxicillin following intrauterine infusion have also not been performed. Since the tissue penetration of amoxicillin is seventy times greater than that of penicillin (173), it was considered probable that concentrations achieved in the serum following intrauterine infusion would be greater than those achieved following intrauterine infusion of penicillin. Also it was thought probable that following systemic administration of both drugs that concentrations achieved in

the uterus would be greater following administration of amoxicillin than penicillin.

Several studies have been carried out comparing serum concentrations achieved following the administration of OTC-C versus OTC-LA, but there have been no studies showing uterine fluid concentrations of oxytetracycline following the systemic administration of OTC-LA or concentrations in the serum following the intrauterine infusion of the same drug. It has been claimed by the manufacturers that serum concentrations are maintained for a longer period of time following intramuscular administration of the long acting preparation of tetracycline than the conventional preparation. If this is true the administration of OTC-LA might result in concentrations greater than the mean MIC for common uterine pathogens, and persist above these levels for a longer period of time, both in the uterine lumen, following intramuscular administration and in the serum following intrauterine administration.

Dosages of antibiotics used by the systemic route were those recommended by the manufacturers for the treatment of infectious diseases in the bovine. The doses used by the intrauterine route were obtained from the literature, as doses used in the practical treatment of bovine endometritis. These doses were determined, not be therapeutic trials or by determining concentrations of antibiotic present in the serum, endometrium or uterine lumen but rather were doses that did not result in detectable concentrations of antibiotics in the milk when the S. lutea bioassay was used to detect antibiotic

residues by milk processors. The recent development of a newer bioassay, using B. stearothermophilus as the test organism, which can detect much lower concentrations of antibiotics in the milk, has shown that the previous recommended doses can result in antibiotic residues in milk (100, 129). Based on data using this newer bioassay it may be beneficial to markedly increase the intrauterine dose and discard the milk for several days following treatment, as the findings in several of the experiments reported in the present study, using recommended doses, serum concentrations were well below the mean MIC for the common uterine pathogens, following intrauterine infusion of antibiotics.

On the other hand, concentrations of antibiotics present in the uterine lumen for all four antibiotics administered by intrauterine infusion were above the mean MIC for the common bacteria involved in endometritis for up to 36 hours. However, this did not imply that these concentrations were present except in the superficial endometrium. The number of cows available for this study did not enable us to carry out concurrent estimations of antibiotic concentrations in endometrial tissue. The use of biopsy samples in determining antibiotic concentrations has to be restricted to two or three samples per experimental period, as otherwise the defect in endometrium caused by the biopsy instrument may cause exudation of serum into the uterine lumen or changes in the absorption from the uterus.

In several experiments concentrations in the uterine lumen were not determined for the full experimental period. This was due to the

Foley catheter being expelled from the uterine horn. This occurred more frequently when cows were in the estrus stage of the cycle. The duration of epidural anesthetic was approximately two hours. It was not felt practical to maintain continued anesthesia, which would have prevented the cows from straining to dislodge the catheter, for the duration of the experiment. Some alternatives to administering lidocaine every two hours would be insertion of a catheter into the epidural space and a very slow continuous infusion of a solution containing a local anesthetic. A technique used by some practitioners for the treatment of chronic tenesmus is the use of an alcohol epidural. This will often be effective for two to three weeks. It has the disadvantage of being irreversible in some animals, causing constant soiling of the tail and inability to remove flies. In some cases it may even cause sloughing of the tail.

In the preliminary stages of this investigation the balloon on the Foley catheter was inflated with air. However, it was found that when the balloons were inflated with air the catheters were found to be more likely to be displaced than when normal saline was used to inflate the balloons.

Other reasons that might cause the Foley catheter to dislodge are the increased mucous secretions present in cows in the estrual stage of the cycle and also the increased muscular tone of the uterus. It may be observed from the results in Table 14, p. 147, in which all the cows but one were in the nonluteal phase of the cycle, that all of the cows except two had dislodged their catheters by 12 hours post

infusion. However, in Table 16, p. 152 where all cows but one were in the luteal phase of the cycle, only two of the six cows had dislodged their catheters by 36 hours post infusion.

It is possible that if there is an increase in mucous production in the nonluteal phase of the cycle that this mucus causes a dilution of the infused antibiotic and falsely low concentrations following antibiotic determination. Al Guedawy et al. (124) in their study on concentrations of gentamicin in the uterine lumen and serum following intramuscular and intrauterine administration, incorporated into the infusion solution a 1/1600 dilution of phenolsulphonphthalein (PSP). The source of this material was not noted in their publication. In our initial experiments we incorporated PSP (Fisher Scientific Company, Fort Lawn, New Jersey) in the infusion solution. At the concentration infused, PSP gave a standard curve that had a logarithmic relationship between concentration and absorbance of light at 600 nm. However, following infusions calculated concentrations did not appear to follow any trend and we could not obtain the results published by Al Guedawy. We presumed that this was possibly due to our using a laboratory grade reagent, whereas Al Guedawy might have used the intravenous preparation, that is used in a diagnostic test of kidney function. The purpose of including this reagent in the intrauterine infusion is that it is a large molecule that is not absorbed from the uterus. If the concentration increases, this can be interpreted as meaning that absorption of the infused solution is occurring. If the concentration decreases, secretion into the uterine lumen is taking

place. Also the concentration of PSP can be used to determine the volume still present in the occluded uterine horn. This may be important as absorption may be affected by the volume of solution in which the antibiotic is diluted. Removing an aliquot of infused solution for determination of antibiotic level, as well as removing some of the antibiotic dose, would also lower the infused volume and might increase of decrease the rate of absorption. The times and quantities of sample removal were constant for all cows, and all antibiotic treatments, and therefore this effect should have been evenly applied over all the experiments.

In another paper, Al Guedawy showed that the use of a distilled water vehicle resulted in greater absorption of gentamicin from the uterus than if the antibiotic was administered in a saline vehicle. In the present experiments all cows were infused with antibiotics in a phosphate buffered saline vehicle. It may be that in future experiments, increased absorption of these antibiotics may be noted if they are infused in a distilled water vehicle.

Serum antibiotic concentrations after intramuscular administration, and antibiotic concentrations in uterine fluid following intrauterine administration were similar in all animals treated. This would indicate that factors such as variable absorption rates were not affecting the data.

Concentrations of antibiotics achieved in the uterine lumen following intramuscular administration of antibiotics were, as stated previously, not predictable. Concentrations achieved in the uterine

lumen following intramuscular administration of penicillin and amoxicillin were below the MIC for all the common uterine pathogens (59, 173, 185) (Table 8, p. 35) except that amoxicillin did achieve concentrations in the uterine lumen greater than the mean MIC for C. pyogenes for up to 36 hours (173).

It is possible that in these experiments, and in several others, when one cow had a detectable concentration present either in uterine fluid following intramuscular administration or in the serum following intrauterine infusion, at one time period only, that laboratory error might be involved. However, this is unlikely because the inhibition zone that developed following plating of the samples was determined nine times and the zone sizes averaged. It is unlikely that an antibacterial substance other than the injected antibiotic was present in these samples because the environment, handling and feeding of animals was closely monitored. Possible methods of ruling out this unlikely antibacterial substance contamination would be the inclusion of negative controls. The negative controls in the assays with penicillin and amoxycillin would consist of treating the sample with a beta-lactamase prior to determining the zone of inhibition. The negative control for the tetracycline assay would consist of simultaneously determining zones of inhibition on a plate inoculated with a tetracycline resistant organism.

Concentrations achieved in the uterine lumen following intramuscular administration of conventional tetracycline (OTC-C) were higher than following the administration of the same dose of the long acting

preparation (OTC-LA). This was probably due to the lower serum concentrations achieved with OTC-LA. Concentrations achieved in the uterine lumen were above the mean MIC for Staphylococcus spp. for over 48 hours for both preparations and OTC-C achieved concentrations above the mean MIC for Corynebacterium spp. after 4 hours and maintained them until 48 hours. Neither preparation gave concentrations exceeding the mean MIC for Pasteurella spp., Streptococcus spp. or E. coli (59) (Table 8, p. 35).

Concentrations achieved in the serum following intramuscular administration of penicillin were similar to results reported previously (178) but higher than those achieved in another study (167). This would indicate that the method used to determine penicillin concentrations in the samples in the present study were accurate. Detectable concentrations of penicillin were present in the serum for 24 hours. However, serum concentrations were below the mean MIC for all the common pathogens isolated from endometritis. This would indicate that a higher dose of penicillin should be used in the treatment of endometritis than that of 20,000 i.u./kg used in this experiment, which is the dosage recommended by the manufacturer.

Serum concentrations of amoxicillin were lower than those reported in previous studies (178, 180) and did not exceed the mean MIC for E. coli or S. faecalis. The MIC for P. haemolytica was exceeded for 24 hours and the mean MIC for C. pyogenes for 48 hours (173).

Concentrations achieved in serum following the intramuscular administration of OTC-C were higher than those achieved following the

same dose of the long acting preparation. This has previously been observed (189), but in that study, levels persisted for a longer period of time after administration of OTC-LA which was not the case in the present study. These findings would tend to agree with those of Xia who suggested that the long acting preparation has no advantage over the conventional preparation (199). Serum concentrations achieved following intramuscular administration of OTC-C were similar to those reported in another study (120) but levels above 2 μg/ml were maintained for 24 hours. In the previous study a dose of 10 mg/kg was used compared to 8 mg/kg used in this study where levels of 2 μg/ml were detectable for only 4 hours. This may have been due to a different site of intramuscular administration (180). Serum concentrations were above the mean MIC for Staphylococcus spp. for 36 hours but did not approach the mean MIC for Corynebacterium spp., Pasteurella spp., Streptococcus spp. or E. coli (Table 8, p. 35) (59).

Serum concentrations achieved after intramuscular administration of OTC-LA were less than those achieved with OTC-C. Concentrations were above the mean MIC for Staphylococcus spp. for 12 hours but did not approach the mean MIC for the other organisms commonly associated with endometritis. The fact that concentrations achieved both in the uterus and serum following intramuscular administration of OTC-LA were lower than following the same dose of OTC-C would indicate that there was no advantage in using the long acting preparation, especially since it has been shown to cause more tissue damage when injected intramuscularly.

Serum and intrauterine drug levels for penicillin and the two forms of tetracycline following i/m administration were below the mean MIC for almost all the common pathogens involved in the etiology of endometritis. This would indicate that if these drugs are to be used in the treatment of endometritis by the systemic route a higher dose of the drug should be used, if this is feasible in regard to toxicity, expense and dosage volume. Although amoxicillin only achieved levels in the serum and intrauterine fluid greater than the MIC for C. pyogenes, this organism is the major cause of endometritis and the use of amoxicillin at the systemic dose used in this experiment should be considered satisfactory in the treatment of bovine endometritis when C. pyogenes is the causative agent. As stated previously antibiotic levels in the uterine lumen were above the mean MIC for the common bacterial pathogens for up to 36 hours following intrauterine administration of all four antibiotics. The decrease in uterine fluid concentrations of the four antibiotics administered by intrauterine infusion are shown in Figures 6 though 9, pp. 153, 154, 158, 159. As has been stated previously, these figures are expressed as the log of concentration against time. Due to the wide individual animal variation, the data lends itself better to graphic depiction on the log scale than if the actual concentrations were plotted against time. Thus, following intrauterine administration of penicillin serum levels were only present in four of the six animals treated and levels above the mean MIC for any of the major pathogens were only seen in one cow. Following intrauterine administration of amoxicillin, serum levels

were obtained greater than the mean MIC for C. pyogenes for up to 8 hours but did not exceed the MIC for other common uterine pathogens. Serum levels following intrauterine administration of OTC-C were only present in 50% of cows treated and were not above the mean MIC for any of the common uterine pathogens. Following intrauterine infusion of OTC-LA serum concentrations were present in all but one cow but did not exceed the mean MIC for any of the common uterine pathogens.

As noted in the discussion of Table 11, p. 139, serum penicillin concentrations following the intramuscular administration of procaine penicillin G were quite variable. This has been shown in several other studies (101, 166, 167). The highest concentration achieved in this experiment, following systemic administration of 20,000 i.u./kg was 500 i.u./ml, whereas, in other studies peak levels achieved at the same dosage as used in the present study were 1293 i.u./ml (101) and 0.51 i.u./ml (167). Similar findings were noted when 6,000 i.u./kg were administered, levels at 2 hours post administration were 0.135, 1.8 and 2.0 i.u./ml in three experiments (166, 167). This individual variation in serum concentrations following the administration of the same dose of antibiotic may be due to the site of administration. It has been shown that absorption from the site of injection resulted in a bio-availability that varied from 24.2% when amoxicillin was administered subcutaneously to 49.2, 76.2 and 79.2% when administered I.M. in the buttock, triceps and lateral neck muscles respectively (180). These findings probably also apply to other antibiotics. In the present study intramuscular injections were all given in the gluteal muscles.

The level of tissue and serum proteins can vary with individual animals and have an effect on the serum antibiotic concentration. The presence of foci of inflammation in the body may also affect serum levels as may the rate of metabolism and excretion.

It has been noted previously, that following the infusion of antibiotics into the uterus, in the present study, a premature onset of estrus occurred in several cases. This could have been due to the local irritant effect of tetracycline causing a superficial endometrial necrosis (111, 112). This results in destruction of the source of the luteolytic factor, prostaglandins, and resumed synthesis of prostaglandin during endometrial repair (113). This luteolytic effect may also be caused by distension of the uterus (91). In the present experiment the premature estrus could have been due to either of these factors. To have proved that prostaglandin production was initiated by this treatment, we would have needed to measure prostaglandin levels, which was not performed.

Progesterone assay was carried out by the RSC procedure. This procedure has been validated for use in the bovine. However, the levels obtained from our samples were lower than those reported in the literature which included mean levels in the luteal phase of the cycle of 5.4 ng/ml (219), 6.6-7 ng/ml (216) and 7-8 ng/ml (147). If progesterone levels are examined in the present experimental data it will be noted that there are a large number of levels between 0.5 and 4 ng/ml. Cows are classified as being in the luteal phase if they have progesterone levels greater than 0.5 ng/ml, but if a functional corpus

luteum is present levels of approximately 6 ng/ml should be present. The reason for progesterone levels being lower than normal in this study was not determined. It was thought unlikely that they were caused by a laboratory error as a standard curve is run every time the test is carried out. It has been reported that cows with endometritis have significantly lower levels than normal cows (147) and it may have been possible, that by infusing these cows at weekly intervals, that a sterile endometritis may have been induced.

It will be noted from Table 20, p. 162 that on increase in absorption of amoxycillin occurred during the nonluteal stage of the estrual cycle whereas a decrease in absorption occurred with tetracycline at this stage. Two possible explanations for this would be an increase in blood supply to the uterus during the nonluteal phase of the cycle and an increase in binding of antibiotic to uterine secretions occurring during the nonluteal phase of the cycle. It has previously been noted that the greater the blood supply to a muscle, the greater is the absorption following intramuscular injection of amoxicillin (180). It is possible that differences in absorption between amoxicillin and tetracycline could be due to tetracycline being more extensively found than amoxycillin in uterine secretions during the nonluteal phase and therefore the absorption of tetracycline being interfered with. It will be noted from Table 20 that in several cases standard deviations are larger than the mean of results. This would indicate that greater numbers of animals should be used before significance is attached to these differences.

Our supposition when we commenced these experiments was that if an endometritis involved any more than the superficial cells of the endometrium, that it would be necessary to achieve detectable levels of antibiotic in the serum, for an adequate period of time, in order to treat it.

As with infectious processes in any body system, it is important that antibiotic concentrations, greater than the MIC of the infecting organism, are present at the site of infection for three days or more. If the antibiotic is administered by the systemic route, then levels should be attained in the uterine lumen above the MIC. If the organism causing the endometritis is tissue invasive, the route of choice is probably systemic, particularly if there is some likelihood that the infection may go systemic. Another advantage of the systemic route of treatment is that it may lessen the danger of introducing resistant bacteria into the uterus during the treatment process.

It has been shown from the results of the present study that penicillin by the intramuscular route at the dosage used did not produce adequate serum or uterine lumen concentrations of antibiotic to be effective against any of the common pathogens associated with endometritis. Penicillin by the intrauterine route produces levels above the MIC for all organisms for up to 36 hours in the uterine lumen, but absorption into the systemic circulation was inconsistent and did not reach the MIC of any of the common pathogens. This would indicate that at the dose used in this study penicillin would be adequate for the treatment of a noninvasive bacteria, but that

treatment should be repeated by the intrauterine route at 24 hour intervals.

Serum concentrations were above the mean MIC for Staphylococcus spp., Pasteurella spp. and C. pyogenes for 24 hours and uterine concentrations greater than the mean MIC for C. pyogenes for 12 hours in half the animals treated, following the intramuscular administration of amoxicillin. A larger dose, than that used in the present study, may be necessary to achieve concentrations in the uterus in the other cows for a longer period of time, and to achieve concentrations in the uterus greater than the mean MIC for the other uterine pathogens.

Adequate concentrations were present in the uterine lumen for up to 8 hours following intrauterine infusion of amoxicillin. Concentrations were not determined after this time due to catheters becoming dislodged. Detectable concentrations were only present in the serum for up to 2 hours in the majority of animals. It may be, that by increasing the dose infused into the uterus, that higher levels are achieved in the serum, which persist for a longer period of time.

Following systemic administration of both tetracycline preparations at the dosages used in these experiments, serum and uterine fluid concentrations of tetracyclines were only above the mean MIC for S. aureus. Serum and uterine fluid concentrations were lower following the administration of OTC-LA than OTC-C and we could find no advantage in using the long acting preparation over the conventional one. Following intrauterine infusion of both preparations satisfactory

concentrations were present in the uterine lumen. Absorption was more consistent with the long acting preparation but at the dosages used serum concentrations were not achieved greater than the mean MIC for any of the common uterine pathogens with either tetracycline preparation.

The objectives of this study were to determine: (1) if intrauterine infusion of a few selected antibiotics resulted in therapeutic levels of the antibiotics at the site of infection and if so for how long; (2) if local therapy could result in therapeutic levels systemically in the event the metritic developed into and endometritis or septicemia; and (3) if systemic administration provided therapeutic levels of the antibiotics in uterine fluids.

Corynebacterium pyogenes is the major cause of endometritis in the bovine. At dosages used in this study therapeutic levels were achieved in the uterine lumen against this organism for up to 36 hours for all four antibiotics when administered by intrauterine infusion. Systemic therapeutic levels against C. pyogenes were achieved for up to 8 hours following amoxycillin but were not achieved consistently following intrauterine infusion of any of the other antibiotics.

Therapeutic levels were achieved in the uterine lumen for up to 36 hours following the intramuscular administration of amoxycillin but levels achieved in the uterine lumen following intramuscular administration of procaine penicillin G and both conventional and long-acting oxytetracycline were not consistently above the MIC for C. pyogenes at any time interval.

In the treatment of endometritic, it may be necessary to administer antibiotics by both the intrauterine and intramuscular route, in order to achieve adequate levels of antibiotic, both within the uterine lumen and throughout the tissues of the uterus.

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VITA

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The following year was spent as an intern in medicine and surgery at the University of Bristol, School of Veterinary Science, followed by a residency in Large Animal Medicine at Cornell University. The following four years were spent in large animal practice in St. Albans and Middlebury, Vermont.

In 1980 he came to The University of Tennessee, Knoxville, as an Assistant Professor in the Department of Rural Practice and enrolled in graduate school in 1981. In 1983 he became a diplomate of the American Board of Veterinary Practitioners with a speciality in Food Animal Practice. In 1984 he was awarded the Norden Distinguished Teacher Award and the Large Animal Clinician of the Year Award. He graduated in December 1984 with a Master of Science degree.

He is a member of Phi Zeta, American Veterinary Medical Association, Irish Veterinary Association, American Association of Bovine Practitioners, American Association of Swine Practitioners and American Association of Sheep and Goat Practitioners.

In 1976 he married Una M. Logan. They have two sons, Joe and Eoin. He is currently engaged in private veterinary practice in Newport, Co. Mayo, Ireland.