OXYTETRACYCLINE THERAPY IN BOVINE MASTITIS

by

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INTRODUCTION

Infectious bovine mastitis is one of the most important disease problems of dairy cattle. There is a tremendous economic loss each year to the dairymen of the United States through reduced milk production, lowered quality of product and permanent damage to the udder necessitating the culling of young cows. Oxytetracycline, introduced in 1951, is one of the more recent of various agents which has been found useful as a therapeutic aid in the control of bovine mastitis. The treatment of field mastitis cases with oxytetracycline generally has been satisfactory; however, little controlled work has been published. The purpose of this work is to evaluate the efficacy of oxytetracycline treatment in infectious bovine mastitis in controlled clinical trials and to study the milk levels of the drug following local udder infusion and parenteral administration.

LITERATURE REVIEW

Finlay et al. (5) reported in 1950 the isolation of oxytetracycline (terramycin®) from Streptomyces rimosus. The antibiotic was found to be inhibitory to the growth of both gram positive and gram negative microorganisms as well as certain rickettsiae and large viruses. The first reports on the use of the drug in treatment of infectious bovine mastitis appeared in 1951. Tableman (23) reported on eight cases treated with oxytetracycline udder infusion ointment. All became clinically normal within 72 hours but he did not identify the etiologic agents. Cromley and Hagley (2) discussed the effectiveness of oxytetracycline in several clinical conditions in large and small animals including two cases of acute bovine mastitis. They found the drug to be effective in controlling the general symptoms which accompanied the mastitis cases but made no mention as to whether the staphylococcus had been eliminated from the milk of the infected quarters. In 1953 Simon and Schmidt (22) treated streptococcal udder infections in six herds and reported that treatment with 200 mg. of oxytetracycline infusion ointment eliminated the infection from 60.0% of the treated quarters and treatment with 400 mg. of oxytetracycline in an infusion ointment eliminated the infection from 59.4% of the treated quarters. They also reported that there was no inflammatory response by the udder to the infusion of oxytetracycline ointment.

Tünsmeyer (26) used oxytetracycline infusion ointment for the treatment of mastitis caused by various etiologic agents. He reported that 80.0% of the coliform mastitis which was treated was clinically improved or cured and that 66.0% was cured bacteriologically. He also found that

100.0% of 15 streptococcal and 80.0% of five staphylococcal mastitis infections were eliminated by oxytetracycline infusions. Most of the treatments consisted of three 400 mg. doses administered at 24 hour intervals.

Gratzl and Jaksch (7) working to determine the blood levels of oxytetracycline in the horse, cow and dog found definite evidence of irritation following parenteral administration. Many times the injection of 10 mg. per kg. of body weight in the horse resulted in general weakness which caused the animal to lie down either after a few hours or the next day. They also observed irritation at the site of injection in all three species. The inflammatory response was much more severe with the subcutaneous injections, but many times, following intramuscular injection, an edematous swelling developed for a few days and then was followed by a hard painful nodule which persisted for several weeks. The reaction was seen especially in the horse but also was observed in the cow and dog.

Barnes (1) treated four cases of bovine mastitis caused by Kleb. pneumoniae with oxytetracycline. He found that systemic symptoms were relieved in all cases by intramuscular administration, but that local treatment did not always eliminate the organism from the milk of the infected quarters.

Schipper and Petersen (21) made a study of the routes and rates of elimination of oxytetracycline when administered intravenously, intramammarily and intramuscularly in dairy cows. They reported that therapeutic levels were detected in the milk as long as 72 hours following local administration with oxytetracycline ointment. They also noted that great

variation in concentrations occurred between different cows and between quarter samples of the same cow.

Several investigators working independently to establish milk levels of various antibiotics reported an inverse correlation between the volume milk production of the cow and the concentrations of antibiotic found. Kanegis et al. (11), McCulloch et al. (12), Edwards and Haskins (4) and Schipper and Petersen (20) all reported finding this relationship in milk levels of chlortetracycline. Edwards and Haskins (4), Jackson and Bryan (9), Hoerlein and Schalm (8), Schalm (19), Jasper and Weirether (10), Packer (16) and Thorp, Uhrik and Straley (24) found the same correlation with penicillin milk levels. Trussell and Stevenson (25) and Edwards and Haskins (4) showed the same with streptomycin milk levels. Morse, Spencer and Simon (14) determined the in vitro sensitivity to oxytetracycline of a number of bacteria isolated from animals. They did not state the specific origin of the strains tested but presumably the strains of Strep. agalactiae, Strep. uberis and some of the strains of Staph. aureus were isolated from the bovine mammary gland. They found that 15 strains of streptococci and 14 strains of staphylococci were inhibited by one mcg. per ml., while a minimum of three mcg. per ml. was necessary to inhibit three strains of staphylococci. One mcg. per ml. was lethal to all of the strains of Strep. agalactiae and three strains of Staph. aureus, but five strains of Strep. uberis were killed only by concentrations greater than 100 mcg. per ml., two strains of Staph. aureus were killed by seven mcg. per ml. and one strain of Staph. aureus was killed only by a concentration greater than 100 mcg. per ml. Two strains of Ps. aeruginosa of animal origin were inhibited in vitro by 50 mcg. per ml. for 24 hours and 100

mcg. per ml. for 48 hours. Two other strains were inhibited by 100 mcg. per ml. but were killed only by concentrations greater than 100 mcg. per ml.

Richou, Gerbeaux and Schlaepfer (18) studied the sensitivity of 21 strains of Staph. aureus to oxytetracycline and determined the pathogenicity of those strains on the basis of plasma coagulation. They found that the members of the two groups displayed little difference in their sensitivity to the drug. Sixteen strains of 21 tested were inhibited by 1.25 mcg. per ml. or less and five strains were inhibited by concentrations of between 1.25 and 5.0 mcg. per ml.

Edwards (3) reported that strains of Strep. agalactiae, Strep. uberis and Staph. aureus were inhibited by 1.0 mcg. per ml. of oxytetracycline for 24 hours at 37°C in broth. He did not mention the numbers of strains tested or their origins.

PART I. TREATMENT OF MASTITIS

DESCRIPTION OF THE HERD

All clinical trials of the treatment of mastitis with oxytetracycline* were conducted in the Iowa State College Dairy Herd. The herd was almost entirely self-contained and was composed of approximately 120 milking cows throughout the year. The five major dairy breeds were represented with Holsteins predominating. Sanitation measures in the herd were above average. All cows in the main barn were milked in one room where four cows could be milked simultaneously by two attendants. All grossly visible dirt and manure were washed from the udder and teats with clear water before the cows were allowed to enter the milking stalls. The base of the udder and the teats were then wiped with an individual clean damp cloth immediately prior to milking. The cows were not fed during milking. Milking was done mechanically by use of a combine milking machine with a vacuum of approximately 15 to 16 inches of mercury. Each milking unit had two claw type heads so that one could be sanitized while the other was in use. Sanitization was accomplished by immersion of the teat cups in water at 170°F for approximately four minutes. The cups were cooled with running tap water before being placed on the teats. Between milkings the teat cups were filled with a one percent solution of commercial lye. Milk from each cow was weighed and recorded during each milking. A strip cup was available to each attendant and he was encouraged to use it on each cow before applying the milking machine. Any abnormalities which were observed during milking were reported to the author.

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*Furnished by Chas. A. Pfizer Co., Brooklyn, New York.

The entire herd was tested annually for brucellosis and tuberculosis. Only a few brucellosis reactors had been found in the herd for 15 years prior to this work and in all instances the reactor animal was immediately shipped to slaughter. No tuberculosis reactors had been found in the herd for the same length of time with the exception of one reactor in 1953 and two reactors in 1954. No gross lesions of tuberculosis were found on post mortem examination of any of these three animals.

No cases of mastitis caused by Streptococcus agalactiae had been found in the herd since 1944 (17). The incidence of mastitis as determined by examination of 124 milking cows during July and August of 1952 was as follows: 44 cows (35.88%) were infected in one or more quarters, and 81 (18.33%) of 491 quarters examined were infected. The incidence of infected quarter by etiologic agent was as follows:

Staphylococcus aureus	45.78%
Streptococcus uberis	42.16%
Pseudomonas aeruginosa	7.22%
Streptococcus dysgalactiae	2.41%
Escherichia coli	2.41%
	99.98%

METHODS OF PROCEDURE

Diagnostic Methods

The following tests were used in the diagnosis of mastitis: strip cup test, Hotis test, pH determination, catalase test, Whiteside test and bacteriologic culturing. This combination of tests was used to establish a diagnosis because no one test was completely accurate nor did it furnish all the information which was desired.

The udder was prepared for the collection of milk samples by cleansing the teats and the base of the udder with a clean cloth which had been soaked in a warm aqueous solution of calcium hypochlorite containing approximately 200 ppm available chlorine. Particular effort was made to clean the external orifice of the streak canal and then the extremity of the teat and the orifice were swabbed with tincture of iodine, USP. One or two streams of milk from each quarter were then drawn onto the screen of a strip cup. Following this, approximately seven milliliters of milk were drawn into a sterile vial containing 0.3 ml. of a 0.5% aqueous solution of bromcresol purple to be used for pH determination, Hotis test and bacteriologic culturing. A second sample was then collected from each quarter to be used for the measure of catalase activity and to conduct the Whiteside test.

The catalase test followed the procedure of Monlux (13). The Whiteside test was conducted according to the modification of Murphy and Hanson (15) except that any detectable coagulation of the milk elements was considered as a positive test without further classification. The bromcresol purple tubes were examined within one hour for any evidence of

pH change and then incubated overnight at 37°C. The tubes were subsequently examined for the Hotis reaction after which each sample was streaked out on a quarter of a five percent bovine blood agar plate. The plates were incubated overnight at 37°C and then examined for growth. Small colonies which resembled those of streptococci were picked into infusion broth. The next day smears were made and stained. Those cultures which showed the typical morphology and staining of streptococci were further differentiated by biochemical fermentations. Typical staphylococcal colonies showing double-zone hemolysis were identified as Staph. aureus. If the hemolysis was weak or atypical, a colony was transferred onto 7.5% sodium chloride, mannite agar. If growth occurred with fermentation of the mannite, a loopful was transferred into tubes for the coagulase test. Coagulation of the dilute rabbit plasma within 24 hours was considered as confirmative of Staph. aureus. Ps. aeruginosa was identified directly from the blood agar plate on the basis of beta hemolysis, green pigment formation and the odor of trimethylamine. Colonies resembling those produced by coliform organisms were transferred into infusion broth and then tested for biochemical reactions. Many such organisms were also further identified by means of their antigenic structure.

Whenever Staph. aureus or Strep. uberis was identified from a milk sample, a diagnosis of mastitis caused by that organism was made. Coliform organisms and Ps. aeruginosa were not considered to be of pathogenic importance unless they were isolated from two or more successive milk samples, there were definite signs of inflammation in the quarter and no other organism was isolated which could be considered as a pathogen.

Treatment Procedure

Treatment of clinical cases of mastitis was initiated just after milk samples were collected during the first examination. All quarters were lactating. The usual treatment consisted of the intramammary infusion of one 400 mg. commercial ointment infusion tube (7.1 gm., 60 mg. per gm.) of oxytetracycline every 24 hours for a total of three treatments. If the condition was unusually severe but local, 800 mg. (two commercial tubes) were infused into the quarter once every 24 hours for a total of three treatments. Treatment in either instance was sometimes extended to a total of five or six days when the symptoms were not alleviated in three days. Acute mastitis cases accompanied by systemic symptoms were treated by the intramuscular administration of one or two grams of oxytetracycline in addition to the local treatment. If the generalized symptoms were especially severe, the initial dose was followed by a second one gram dose 12 hours later. Otherwise, the first dose was repeated every 24 hours until improvement of the animal was observed. No other medication was used.

Routine treatment of cows having mastitis was usually performed approximately three hours following the morning milking. The quarters were not milked out preceding this therapy. Treatment conducted at any other time during the day was preceded by complete milking of the quarter. Therapy with oxytetracycline was begun in all clinical cases of mastitis without knowledge of the etiologic agent. If it became obvious during therapy that oxytetracycline was ineffective in overcoming the infection, some other therapeutic agent was employed.

The results of therapy were determined by periodic examination of milk samples from the treated quarters with the same procedure as was described for diagnosis. The treatment was considered successful if the causal organism could not be isolated from the milk during any of three weekly examinations conducted following treatment and the indirect mastitis tests indicated that the inflammation of the quarter was subsiding. If the infective organism was isolated during this three week period, the treatment was considered unsuccessful. The number of examinations that were conducted on various quarters following treatment were variable. When the examinations could not be conducted during the three week period as planned, the milk samples were collected several times within the following three months. The treatment was considered as successful if all samples were negative to bacteriologic and indirect mastitis tests. Any data were discarded for use in this report if the post treatment examinations were not sufficient to give a clear picture of the results of therapy.

Procedure for Determining Irritant Properties

Certain clinical and laboratory observations seemed to indicate that oxytetracycline was irritating to the mammary gland. So it was decided to watch the clinical reaction of the udder as well as the catalase and Whiteside reaction of the milk of a few quarters during and following local treatment. Six quarters, three normal and three infected, were selected to be treated with oxytetracycline infusion ointment using various dosage schedules. Milk samples were collected once daily from each quarter for seven days preceding treatment, during treatment and for five

days following the last infusion. A physical examination including palpation and the strip cup test was conducted each day to detect gross evidence of inflammation. The samples were examined by the Whiteside and catalase tests conducted in the manner previously described.

Methods for Determining Sensitivity of Mastitis Organisms to Oxytetracycline

It was decided to determine the *in vitro* sensitivity of a few strains of *Staph. aureus* and *Strep. uberis* to oxytetracycline. Strains of these two organisms were selected from cows that had recovered and from those that had not recovered with oxytetracycline therapy. A total of 21 strains was used, 12 strains of *Staph. aureus* and nine strains of *Strep. uberis*. All strains were subjected to experiments to determine both the bacteriostatic and the bactericidal levels for each.

The bacteriostatic levels were determined by inoculating the organisms onto blood agar plates containing varying amounts of oxytetracycline per ml. Ten ml. of whole citrated bovine blood were added to 185 ml. of melted tryptose agar and then five ml. of brain-heart infusion broth (Difco) containing the calculated amount of oxytetracycline were added and the components mixed thoroughly. The medium was then poured into a sterile Pyrex baking dish measuring 7.5 x 12 x 1.75 inches which was used as the culture plate. The plate was covered with aluminum wrapping foil. A loopful of a 24 hour brain-heart infusion broth culture of the organism to be tested was then streaked onto a small area of the hardened agar. The plate was incubated at 37°C and was examined for growth at 24 and 48 hours after inoculation. Plates were prepared with the following amounts

of oxytetracycline per ml. of agar: 2.5, 1.25, 0.63, 0.32 and 0.16 mcg. All 21 strains were tested simultaneously on five different plates containing the antibiotic concentrations.

The bactericidal concentration of oxytetracycline for each strain of Staph. aureus was determined by using serial doubling dilutions of antibiotic in infusion broth dispensed in 2.0 ml. amounts. The concentrations used were: 200, 100, 50 and 25 mcg. per ml. Each dilution tube was inoculated with one drop of a 10^{-1} dilution of a 24 hour infusion broth culture of the staphylococcus. At intervals of 24 and 48 hours following inoculation, a loopful of material from each dilution tube was streaked onto a quarter of a five percent bovine blood agar plate. This plate was then incubated at 37°C for 24 hours and examined for growth. If no growth appeared, the concentration of oxytetracycline represented by the sample was considered to be lethal. If there was any question as to the presence of growth on the blood agar plate, it was further incubated for another 24 hours and re-examined.

RESULTS

Treatment of Mastitis

Table 1 shows the results of treatment of 115 infected quarters with oxytetracycline. Of these, 59 infected quarters or 51.3% recovered and 56 or 48.69% did not recover. Infections caused by Strep. uberis showed the best response to treatment with 82.69% recovery. Three infections caused by Strep. dysgalactiae recovered in 33.3% of those treated. The over-all rate of recovery of streptococcic mastitis cases was 79.59%. Of 44 Staph. aureus infections treated, only 25.0% recovered. Organisms of the coliform group were eliminated more successfully. Thirteen infections caused by members of this group were treated, including those of the genera Escherichia, Klebsiella, Aerobacter and Paracolobactrum, and nine, or 69.23%, recovered. No udder infections caused by Ps. aeruginosa were eliminated by the use of oxytetracycline.

Table 1. Results of Treatment of Infectious Mastitis with Oxytetracycline

Infective Agent	Number Treated	Number which Recovered	Percent of Recovery	Number which did not Recover	Percent of Nonrecovery
Strep. uberis	46	38	82.69	8	17.39
Strep. dysgalactiae	3	1	33.30	2	66.60
Staph. aureus	44	11	25.00	33	75.00
Ps. aeruginosa	9	0	0.00	9	100.00
Coliform group	13	9	69.23	4	30.76
Total	115	59	51.30	56	48.69

Table 2 compares the results of treatment of clinical and subclinical mastitis with oxytetracycline. It will be noted that the drug was more effective in treatment of subclinical infections. This difference was especially noticeable in cases caused by Staph. aureus where 31.03% of the subclinical cases recovered but only 13.33% of the clinical cases recovered. In mastitis caused by Strep. uberis there was a difference of 10.29% in the percentage recovery of the treated cows in the subclinical over the clinical group. An evaluation of the totals for all cases treated showed that treatment of subclinical infections was 18.20% more effective than treatment of clinical infections.

Table 2. Comparison of the Results of Treatment of Clinical and Subclinical Mastitis Cases with Oxytetracycline

Etiologic Agent	Type of Case	Number Treated	Number Recovered	Percent Recovered
Strep. uberis	clinical	12	9	75.00
	subclinical	34	29	85.29
Strep. dysgalactiae	clinical	3	1	33.30
	subclinical	-	-	-
Staph. aureus	clinical	15	2	13.33
	subclinical	29	9	31.03
Coliform group	clinical	13	9	69.23
	subclinical	-	-	-
Ps. aeruginosa	clinical	8	0	0.00
	subclinical	1	0	0.00
Total	clinical	51	21	41.17
	subclinical	64	38	59.37

Table 3 compares the results of treatment of recently acquired and long standing subclinical infections of the bovine mammary gland with oxytetracycline. An arbitrary time of one month was used as the point of division of the two types. It will be noted that recently acquired cases of staphylococcal mastitis were successfully treated at a rate approximately twice that of long standing staphylococcic infections. Streptococcal mastitis cases were treated with about the same efficacy in both recently acquired and long standing cases.

Table 3. Correlation of Results of Treatment in Subclinical Cases with Duration of Infection

	Recently Acquired Infections			Long Standing Infections		
	Number Treated	Number Cured	Percent	Number Treated	Number Cured	Percent
Streptococcal	16	12	75.0%	18	14	77.7%
Staphylococcal	11	4	36.4%	27	5	18.5%

Irritant Properties of Oxytetracycline

Table 4 compares the amount of inflammatory response by various mammary glands following intramammary infusion of oxytetracycline ointment, as indicated by the catalase and Whiteside tests. The results show that both normal and abnormal quarters demonstrated an inflammatory reaction following infusion and that the amount of inflammation varied with the individual quarter. The presence of a chronic inflammation did not appear to predispose the udder to irritation by oxytetracycline. Quarter 2392 LR retained the same catalase values following treatment even though it was chronically infected with Staph. aureus, while 3157 RR, a normal quarter, showed a marked increase in the catalase value with the same dose of the drug. A third quarter receiving the same treatment, 2643 RF, had a chronic infection and yet it showed only a moderate reaction to the drug. The quarters receiving one infusion showed less reaction to the catalase test than those receiving three treatments, indicating that the inflammatory reaction of the mammary gland to the drug may be quantitative. Most of the quarters returned to the same range of catalase values within

five days after the last administration. The inflammation caused by the oxytetracycline ointment was also demonstrated by the Whiteside test. Five of the six previously negative quarters showed a positive reaction to this test following treatment. The sixth quarter showed positive reaction to all examinations previous to the experiment and did not become any more pronounced with treatment. Those quarters which received only one tube of oxytetracycline became negative to the Whiteside test within two or three days after the last administration. The quarters receiving three treatments were positive to the Whiteside test for three or four days after the last infusion.

Palpation conducted on the udders during this experiment showed that some quarters became inflamed to the extent that mild clinical swelling and edema could be detected. This was seen only in a few of the quarters that showed a marked reaction to the laboratory tests. Other quarters showed only a mild sensitivity to moderate pressure or showed no change on palpation.

Table 4. Results of the Examination of Milk Samples Using Catalase and Whiteside Tests before, during and after Treatment with Oxytetracycline

		Seven Days Preceding Treatment			During Treatment			Five Days Following Last Treatment			
			Catalase*			Catalase			Catalase		
		Whiteside	Range	Avg.	Whiteside	Range	Avg.	Whiteside	Range	Avg.	
One Dose 400 mg.	3157 LF	+ −	3-8	4.7	+	13-17	15.6	−	3-12	6.6	Normal quarter
	2392 LF	−	4-6	5.0	+	6-8	7.0	−	4-6	5	Normal quarter old cow
	2643 RR	+ −	3-8	5.3	+	5-7	6.0	−	2-4	3	Chronic Strep. uberis and Staph. aureus infection
400 mg./ 24 Hrs./ 3 days	3157 RR	−	1-5	3.0	+	20.0	20.0	+ −	7-16	10.2	Normal quarter
	2392 LR	+	11-17	13.3	+	12-14	13.3	+	10-14	12.6	Chronic infection Staph. aureus
	2643 RF	−	6-8	6.7	+	12-15	14	−	6-12	9.2	Chronic infection Staph. aureus

*All catalase values expressed as mls. free gas.

Sensitivity of Certain Mastitis Organisms to Oxytetracycline

Examination of Table 5 shows that all 21 strains of Staph. aureus and Strep. uberis were inhibited for 24 hours by less than 2.0 mcg. of oxytetracycline per ml. Twenty of the strains were inhibited by 1.0 mcg. per ml. or less. The strains of Staph. aureus were only slightly more resistant to oxytetracycline than the strains of Strep. uberis. In most instances, a higher concentration of drug was required to inhibit the organism for 48 hours than that needed for 24 hours, although the increase was apparently never more than one or two mcg. per ml. There is a striking difference in the concentrations of antibiotic necessary to kill the strains of the two organisms, Staph. aureus being resistant to concentrations 100 times or more those which were lethal to Strep. uberis.

Table 5. Bacteriostatic and Bactericidal Levels of Oxytetracycline *in vitro* for 21 Strains of Mastitis Staphylococci and Streptococci

Concentration in mcg. per ml.	Bacteriostatic				Bactericidal			
	Strep. uberis		Staph. aureus		Strep. uberis		Staph. aureus	
	24 Hrs.	48 Hrs.	24 Hrs.	48 Hrs.	24 Hrs.	48 Hrs.	24 Hrs.	48 Hrs.
<1.0	9	3	11	0	-	-	-	-
1.0-2.0	0	5	1	5	-	-	-	-
2.0-3.0	0	1	0	7	-	-	-	-
<10	-	-	-	-	7	8	0	0
10-20	-	-	-	-	1	1	0	0
20-100	-	-	-	-	1	0	1	1
100-200	-	-	-	-	0	0	2	2
>200	-	-	-	-	0	0	9	9

DISCUSSION OF RESULTS

Oxytetracycline is of definite value in the treatment of Strep. uberis mastitis with a recovery rate of 82.69% of the treated quarters. This drug was also effective against coliform mastitis and it eliminated the infective agent from the gland in 69.23% of the treated cases. It was somewhat less successful in eliminating Staph. aureus infections, since only 25.0% recovered, and it was entirely unsuccessful in the treatment of Ps. aeruginosa infections. However, local symptoms were relieved in all quarters whether complete recovery occurred or not. Those cases in which the infective agent was not eliminated frequently showed clinical symptoms again soon after oxytetracycline therapy was discontinued. Intramuscular administration of oxytetracycline was found to be routinely successful in relieving the systemic symptoms which accompanied an acute mastitis attack, irrespective of the etiologic agent.

There is little published work with which to compare the results of treatment in this investigation, particularly in the case of Staph. aureus and Ps. aeruginosa mastitis. Some comparison might be made, however, with the results obtained by Simon and Schmidt (22) in the treatment of streptococcal mastitis. They treated cases of Strep. agalactiae mastitis with one oxytetracycline infusion and obtained results which were approximately 23.0% lower than those observed in this work when Strep. uberis mastitis was treated with three infusions. The in vitro sensitivity of these two streptococci to oxytetracycline is very similar (3) (14) and, although Strep. uberis seems to be more resistant to the lethal effect, the improved results in this investigation could possibly be attributed to the use of repeated doses.

Failure of oxytetracycline to eliminate any of the Ps. aeruginosa infections demonstrates in vivo the refractiveness which this organism exhibits to the drug in vitro (14). It also coincides with the relative lack of susceptibility which this organism has to most antibiotics.

Two factors may influence the rate of recovery of Staph. aureus mastitis cases. They are the relative resistance of the organism to the lethal effect of oxytetracycline and the duration of the infection at the time of treatment. Both Staph. aureus and Strep. uberis are inhibited in vitro by approximately one mcg. per ml. of the antibiotic. However, Staph. aureus is by far the more resistant to the lethal effect and the results of treatment correspond much closer to the necessary lethal concentrations than to the inhibitory levels. If the staphylococcus infections cannot be eliminated from the gland unless they are killed, the refractiveness of Staph. aureus would influence the recovery rate a great deal. This may indicate that large doses of oxytetracycline should be used when attempting to eliminate udder staphylococcic infections. The treatment of recently acquired subclinical Staph. aureus mammary gland infections was approximately twice as successful as that of infections of long standing. No information was obtained as to why this difference occurred; however, it points out the importance of the early treatment of mastitis cases for a better rate of recovery.

Although the number reported was small, a significant percentage of the coliform mastitis cases recovered with oxytetracycline therapy. These cases were considered as a group because there were not sufficient numbers of any one genus to be representative. This report concurs with that of Tünsmeyer (26) that oxytetracycline is an effective therapeutic agent for

this type of mastitis. Intramuscular oxytetracycline was used along with intramammary infusions to treat coliform mastitis cases which were accompanied by systemic symptoms, and the simultaneous administration was routinely effective in alleviating those symptoms. In some instances the combined therapy relieved the systemic symptoms and temporarily relieved the local symptoms in the udder but did not eliminate the etiologic agent from the milk. Therapy with another drug was necessary in those cases to eliminate the infective agent (1).

Although it was not the main objective of this paper to compare various dosage schedules, there were several observations made. As is shown in Table 2, the results of therapy of subclinical cases were better than those of clinical cases. This indicates that the infective agent may be protected by exudate. Acute cases which improve slowly should possibly be treated for a longer period of time in order to assure elimination of the infective organism. The results of treatment of chronic subclinical cases with either increased doses or for a time longer than three days were not any better as far as elimination of the infective agent was concerned. A few attempts were made to cure chronic local mastitis by the combined administration of intramuscular and intramammary oxytetracycline, but they did not prove to be any more effective in eliminating the infection than local administration alone.

As is shown in the results of the irritation study, there is little doubt that the infusion of oxytetracycline ointment into certain bovine mammary glands produces a measureable amount of inflammation. This is contrary to the work of Simon and Schmidt (22) who reported that they had

found no evidence of irritation to the mammary gland following local infusion of oxytetracycline.

In most instances there were no gross changes detected in the milk or the mammary gland following the infusion of oxytetracycline ointment in subclinical cases. In some cases, however, the strip cup test became positive within 24 to 48 hours after therapy was begun, as though the inflammation had become more acute. The catalase and Whiteside tests on milk samples from these quarters also indicated increased inflammation. Other clinical observations were made which showed oxytetracycline to be irritating. One quarter with a chronic staphylococcic infection was treated with two 2 gram doses of oxytetracycline infusion ointment at 24 hour intervals. On examination 24 hours after the first treatment, the secretions from the quarter were blood tinged and the quarter was sensitive to mild pressure. Twenty-four hours after the second treatment, the secretions were obviously bloody and the quarter was edematous and inflamed. The oxytetracycline infusions were stopped at this point and no other therapy was given. During the next few days the quarter and its secretions gradually returned to the same condition as before the therapy. The infection was not eliminated by the treatment.

Intramuscular administration of one or two grams of oxytetracycline did not cause distress to the patient except temporary pain at the site of injection. However, one undesirable reaction was seen following two large intramuscular administrations in one animal. A 1000 pound cow with a chronic staphylococcic mastitis in one quarter was treated simultaneously with a 400 mg. dose of oxytetracycline udder infusion ointment

and five grams of oxytetracycline intramuscularly. No untoward response was noted in the quarter at any time and nothing unusual was noted when the cow was examined 24 hours following the first five gram dose. However, 24 hours following the second five gram dose, there were marked general symptoms of anxiety, restlessness, anorexia and weakness. There was also definite edema and soreness at the points of injection. Treatment was stopped after the second administration but the cow did not return to normal until approximately five days after the last treatment. No further symptoms were seen. These observations were similar to those of Gratzl and Jaksch (7) in 1953 in which they described an inflammatory response following intramuscular and subcutaneous administration of oxytetracycline in the cow, horse and dog. The weakness which they described in horses was similar to the reaction in the case mentioned above.

PART II. MILK LEVELS FOLLOWING TREATMENT WITH OXYTETRACYCLINE

METHODS OF PROCEDURE

Assay Materials

Pyrex baking dishes 7.5 x 12 x 1.75 inches were used as the culture plates. Covers for these plates were made with aluminum wrapping foil. Filter paper pads number 740-E available from Carl Schleicher and Scheull Company, Keene, New Hampshire, were used to hold the milk sample for assay. The zones of inhibition were measured by projecting the image of the plate onto a screen with a Master Vu-graph projector, Charles Beseler Company, Newark, New Jersey.

The same medium was used for both the base layer and the seed layer of the culture plate. The formula for that medium is found in Table 7. The prepared media was measured into flasks in 100 and 200 ml. amounts and autoclaved at 121°C for 12 minutes. The sterile media was then held at room temperature until needed.

The inoculum for the seed agar was an aqueous suspension of spores of the test organism, Bacillus cereus var. mycoides (A.T.C.C. 9634, P.C.I 213). The spores were obtained by growing the cultures of the organism on the surface of agar slants at 30°C for one week. The formula for this medium is given in Table 6. The bacterial growth containing the spores was harvested from the surface of the agar with sterile distilled water and placed in sterile vials containing a few glass beads. The vials were shaken vigorously to break up clumps of the organism and then immersed in a water bath at 65°C for 30 minutes to kill the vegetative cells. The solution of spores was centrifuged three times and each time the supernatant fluid decanted off and sterile distilled water added. Following

this the spore suspension was again heated at 65°C in the same manner. By a process of centrifuging and decanting, the spore suspension was concentrated to obtain a solution of approximately 2.2×10^5 spores per ml. This was the stock suspension of inoculum for the assay plates and was kept under refrigeration at 7°C. Care was taken that no culture of the stock organism had any contact with oxytetracycline before being used in the assay procedure.

Sterile skim milk was used to dilute milk samples containing unknown amounts of oxytetracycline. A stock solution was dispensed into glass tubes in approximately 12 to 15 ml. amounts and autoclaved at 121°C for 10 minutes. These were held at room temperature until needed.

With the exception of the filter paper pads, all laboratory equipment and material used was sterile. It was reasoned that autoclaving the filter paper pads would cause them to absorb moisture so they might not be equally absorbent when used with the milk samples so they were used directly from the commercial package. Very few contaminants were encountered when the pads were used on the agar without autoclaving.

Table 6. Medium for Growth of Spores

Peptone	6.0 gm.
Pancreatic Digest of Casein	4.0 "
Yeast Extract	3.0 "
Beef Extract	1.5 "
Glucose	1.0 "
Agar	15.0 "
Distilled Water q.s.	1000 ml.
pH of 6.5 before autoclaving.	

Table 7. Medium for Assay Plate Culture

Peptone	1.2 gm.
Trypticase BBL	0.8 "
Yeast Extract	0.6 "
Beef Extract	0.3 "
Agar	3.0 "
Distilled Water q.s.	200 ml.
pH 6.5 before autoclaving.	

Collection of Milk Samples and Treatment of Quarters

All cows used for determination of milk levels of oxytetracycline were in the Iowa State College Dairy Herd. Animals were chosen as representatives of the following production groups: 10 to 20 pounds per day milk production, 20 to 40 pounds per day milk production and 40 to 60 pounds per day milk production. All quarters were clinically normal.

Milk samples for assay were collected in the milking room just prior to the regular twice-daily milking. Collection was performed so as to minimize the chance of contamination of the sample. The udder and teats were cleaned in the same manner as has been previously described in Part I. After discarding two streams of milk, samples were collected into sterile rubber stoppered vials. Only strict fore-milk samples were collected. The samples were identified by number and immediately returned to the laboratory where they were either refrigerated at 7°C until they could be assayed (usually within six hours) or frozen for use at a later time.

Oxytetracycline ointment infusions were administered after removal of the milking machine and before the cow left the milking room. A treatment

consisted of the administration of the entire contents of one or more commercial ointment infusion tubes (7.1 gm.), each containing 60 mg. of oxytetracycline per gram of vehicle. The contents of one tube were considered as a 400 mg. dose and the contents of two tubes were considered as an 800 mg. dose. Following administration, the teat and udder were briefly massaged with an upward motion to encourage dispersion of the ointment through the quarter.

Quarters which received only one 400 mg. treatment were sampled at milking time every 12 hours for 72 hours following treatment. The quarters receiving three 400 mg. doses at 24 hour intervals were sampled every 12 hours for either 60 or 72 hours following the last treatment. The quarters which were treated with three 800 mg. doses were sampled for 72 hours following the last infusion.

Preparation of Oxytetracycline Standard

The standard solution of 100 mcg. per ml. of oxytetracycline was prepared by dissolving the contents of one vial of 20,000 mcg. of assay quality oxytetracycline in 200 ml. of sterile skim milk. After thorough mixing this was dispensed into sterile glass vials in two ml. amounts and immediately frozen. Three different batches of oxytetracycline standard were prepared during the assay trials. The lot number on all three vials used was WGD 527087. The zones of inhibition produced by various concentrations of drug from each vial were checked against the same concentrations from the preceding vial. All were found to have the same inhibitory activity against the test organism.

Serial dilutions of oxytetracycline were used to establish a standard curve and to serve as a control for each assay plate. These dilutions were obtained by serial dilution of the 100 mcg. per ml. standard solution to concentrations of 80, 40 and 20 mcgs. per ml.

Technique of Assay

The method of assay which was employed followed the technique of Vincent and Vincent (27) as modified by Grady and Williams (6) to use large Pyrex baking dishes for culture plates. This is ordinarily called the pad-plate method and involves the principle that a given concentration of an antibiotic will always produce the same size zone of inhibition in agar against a test organism under standard conditions. It was found in the present work that oxytetracycline could be measured in whole milk with this method in amounts as small as 1.25 mcg. per ml.

Two layers of culture medium (Table 7) were poured in the assay culture plates so as to assure uniform thickness of the top layer of agar. Two hundred ml. of melted medium were poured into a sterile plate with the plate in a level position. After this layer had hardened, 100 ml. of melted medium was inoculated with 0.1 ml. of the suspension of washed spores of B. cereus var. mycoides and layered over the top. All plates were poured so that no more than 30 minutes elapsed before the filter paper pads were placed on the agar. The plates were covered with aluminum wrapping foil to prevent both air-borne contamination and dehydration of the agar during the period of incubation. It was found that prevention of dehydration was the most important consideration.

It was determined by repeated trials that there was little difference in results obtained from assay of oxytetracycline in whole milk and skim milk samples taken from the same original material, so all unknown specimens were diluted with sterile skim milk to reach the range of most accurate readings. This range was found to be between 10 and 30 mcg. of oxytetracycline per ml. If it was found that the dilution was not in the correct range for accurate interpretation, the sample was reassayed with the dilutions adjusted to bring the readings closer to that range. All samples were assayed in duplicate on each of two plates, using a separate dilution procedure for each plate. A separate series of standard dilutions of oxytetracycline in sterile skim milk was prepared for each assay plate.

During early assay trials the filter paper pads were impregnated with the milk to be tested by placing them on a clean glass plate, pipetting 0.1 ml. of the sample onto the pad and allowing it to air dry at room temperature for 30 minutes. The pads were then placed on the surface of the seeded agar with thumb forceps. Later it was found that there was less variation in the zone diameters if the pads were immersed in the sample for 30 seconds and then shaken to remove the excess fluid before placing them on the agar. The plates were incubated at 30°C for times varying from 16 to 22 hours. No significant variation in the results could be found when the incubation times were varied but the zones were sharper and easier to read if incubation was terminated at 16 hours.

The zones of inhibition were measured by projecting the image of the plate and the zones on a screen with a Master Vu-graph projector. The upper mirror of the projector was placed nine feet six inches from the

screen. The zone diameters were measured by holding a centimeter scale against the screen in the image of the inhibition zone. It was estimated that the image was magnified 75 times by such projection.

Data for the standard curve were prepared by averaging the diameters of the zones of inhibition produced on all plates by the various standard concentrations. These averages were plotted on the logarithmic scale of the abscissa against the zone diameters in centimeters on the ordinant of semilogarithmic graph paper and gave a straight line curve. The measurement of the diameter of the zone formed by unknown material was applied to the scale to find the micrograms per milliliter of oxytetracycline in the diluted sample. This number was multiplied by the dilution factor to give the final reading of micrograms per milliliter of oxytetracycline in the original sample. All of the original samples which contained less than 30 mcg. per ml. of the drug were assayed in duplicate without dilution.

Samples of milk and blood collected from cows which were injected intravenously and intramuscularly with oxytetracycline were assayed by the same method as described above. The blood samples were allowed to clot and the serum withdrawn for assay. All samples were assayed in duplicate on each of two plates using undiluted serum.

RESULTS OF ASSAY

Table 8 and Figure 1 show that all quarters had a detectable level of oxytetracycline for 24 hours or longer following one 400 mg. infusion. Oxytetracycline was detected in the milk of nine treated quarters for periods of time ranging from 24 to 72 hours, the average being 37.3 hours. The first samples collected following treatment showed the highest levels and each succeeding sample showed a sharp decrease in concentration. There was much variation in the concentrations found in different cows and in different quarters of the same cow. However, there was not much variation in the length of time that detectable levels were present in different quarters of the same cow or in different cows within the same milk production group. The quarter of the cow with the lowest daily milk production showed the highest levels of oxytetracycline at various sampling times and held a detectable level of the drug for the longest time. The quarters of cows which had the highest daily production showed the lowest levels of drug at various sampling times and retained a level of the drug for the shortest time.

Figure 1. Milk Levels of Oxytetracycline Following Intramammary Infusion of One 400 mg. Dose

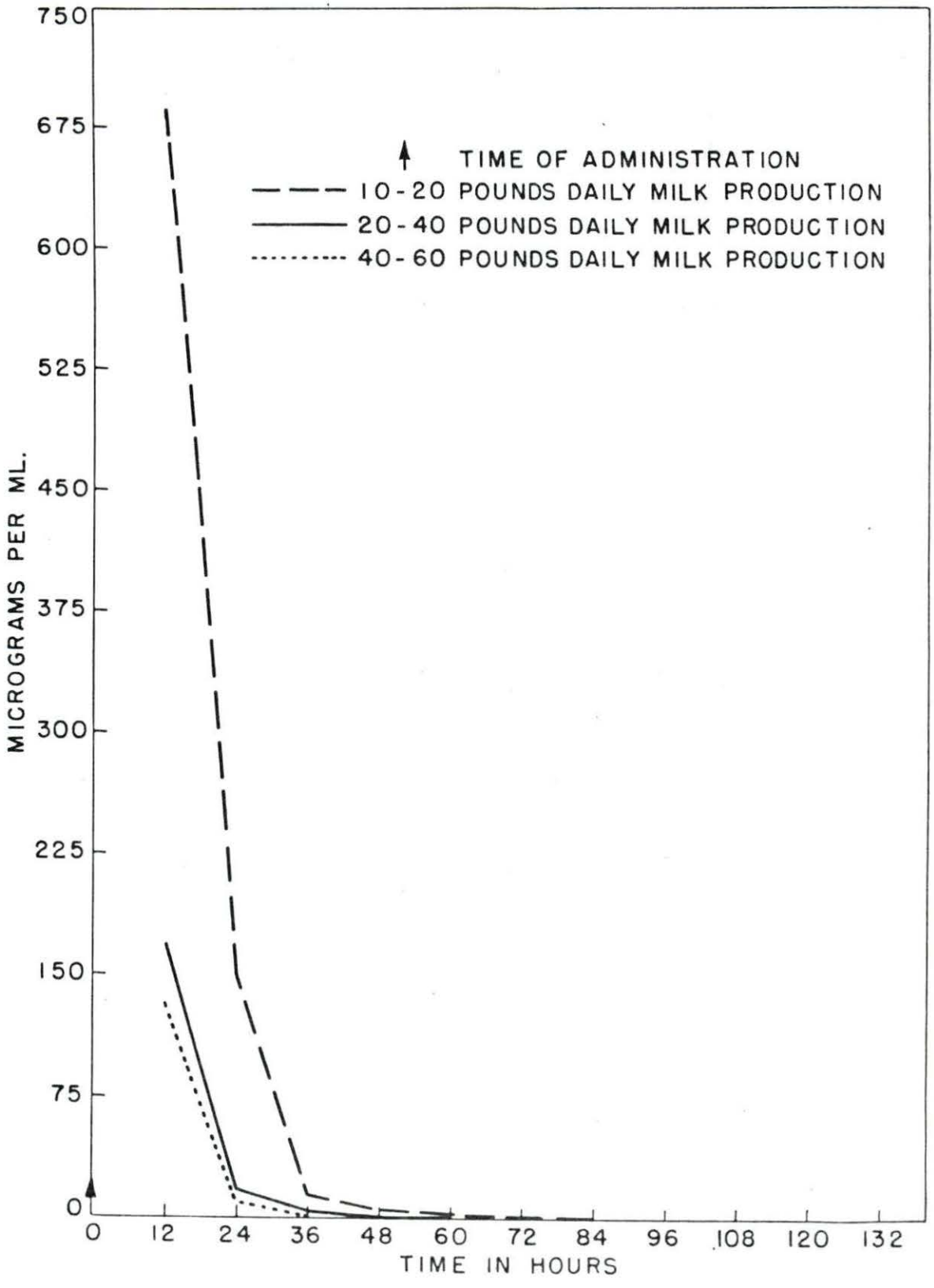

750
675
600
525
450
375
300
225
150
75
0
MICROGRAMS PER ML.
TIME OF ADMINISTRATION
10-20 POUNDS DAILY MILK PRODUCTION
20-40 POUNDS DAILY MILK PRODUCTION
40-60 POUNDS DAILY MILK PRODUCTION
0
12
24
36
48
60
72
84
96
108
120
132
TIME IN HOURS

Table 8. Milk Levels of Oxytetracycline Following Intramammary Infusion of One 400 mg. Dose

Range of Milk Production		10-20 Pounds	20-40 Pounds					40-60 Pounds					
Actual Production		11.2	39.3	39.3	39.8	39.8	39.6	50.8	50.8	48.6	48.6	49.7	40.9
Time in Hrs.	Treatment	Quar. 3291 RR	Quar. 2159 RR	Quar. 2159 LR	Quar. 3432 LR	Quar. 3432 RF	Avg.	Quar. 3550 RF	Quar. 3550 LR	Quar. 3078 RR	Quar. 3078 LF	Avg.	Overall Avg.
0	400 mg.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	-	686.2	128.4	226.7	157.1	167.7	170.0	120.3	159.3	141.1	113.5	133.5	211.1
24	-	149.5	16.8	27.4	14.5	6.8	16.4	8.8	7.5	10.1	12.1	9.6	28.2
36	-	14.3	5.2	4.6	trace	0.0	2.4	0.0	0.0	0.0	0.0	0.0	2.7
48	-	4.0	trace	trace	trace	0.0	trace	0.0	0.0	0.0	0.0	0.0	0.4
60	-	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
72	-	trace	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84	-	0.0	0.0										0.0
96	-	0.0											

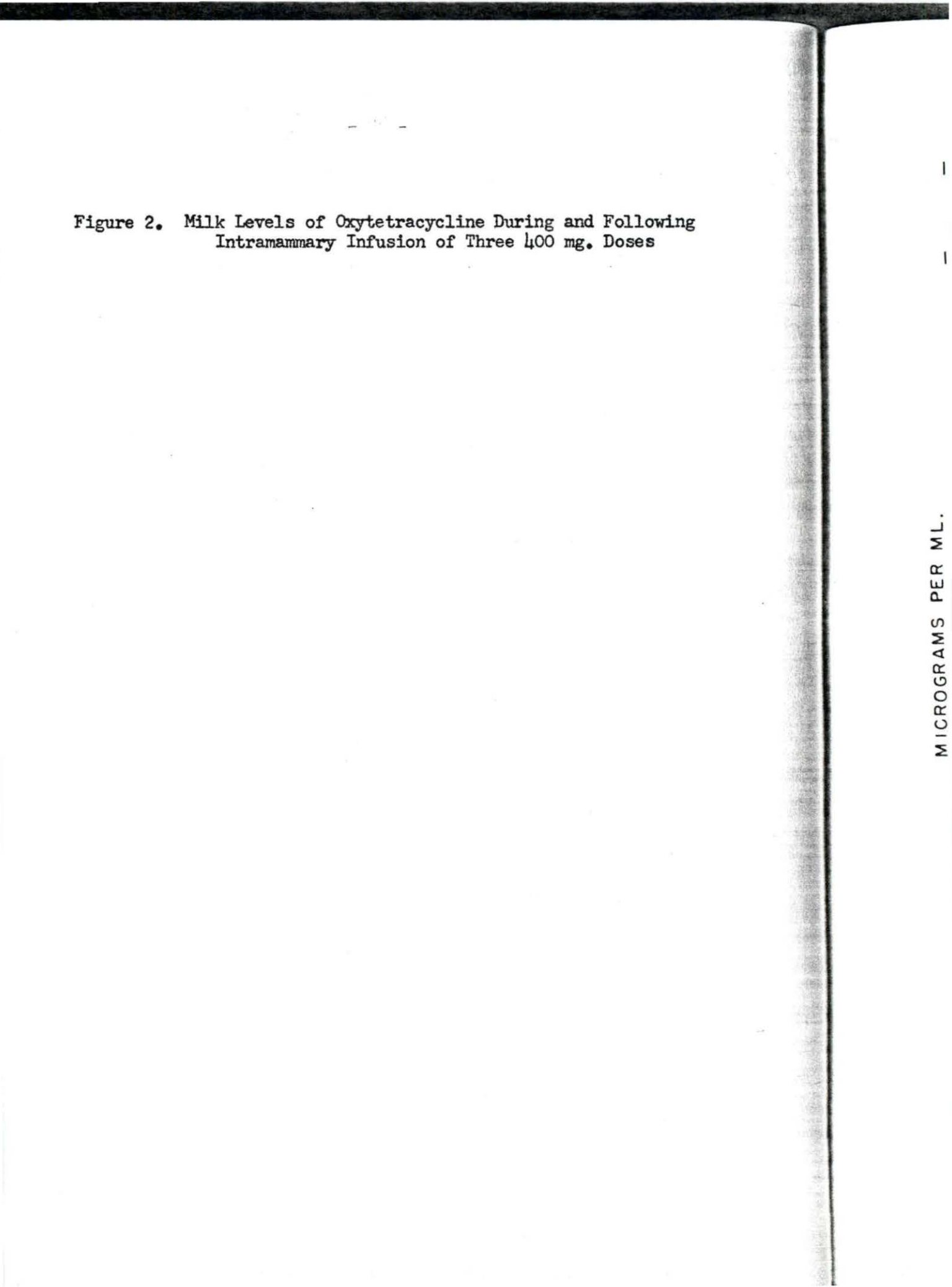
Figure 2 and Table 9 show that the highest concentrations of oxytetracycline were found 12 hours following each infusion. The levels of oxytetracycline found in the samples collected 12 hours following the first dose are comparable with those collected at the same time following the single 400 mg. dose. Following the high level attained after each treatment, the level of oxytetracycline dropped precipitously. One quarter, 2553 RF, alters this pattern somewhat in the sample collected at 60 and 72 hours, but the sharp drop is seen in the next sample. Table 9 shows the variation which occurred in levels of oxytetracycline between different cows and between different quarters of the same cow with the same dosage. The highest levels of oxytetracycline were found in samples from the cow having the lowest daily milk production and the lowest levels of drug were found in samples from a cow in the group of highest daily milk production. In general, the detectable levels were found for the longest times in the cows with a low daily milk production and the shortest duration occurred in cows with a high daily milk production.

There did not appear to be a consistent cumulative effect in the milk levels corresponding to repeated doses. It will be noted that individual levels found following the third dose were not necessarily greater than the levels found following the second administration, and the levels seen following the second treatment were not necessarily greater than those found following the first infusion. Sometimes the increase with each infusion was very evident, especially in quarters of low producing animals, but in several instances the concentration was nearly the same or even lower in the sample collected 12 hours following the second or

third treatment, as compared to the 12 hour sample following the first dose.

All treated quarters showed a detectable level of oxytetracycline for 24 hours and most of them for at least 36 hours following the last infusion. The longest time that oxytetracycline could be detected in the milk of a treated quarter was 84 hours after the last treatment or a total of 132 hours from the beginning of therapy. The shortest time that it could be found in the milk was 24 hours after the last treatment. The average total time that the drug could be detected in the milk of all quarters was 93.3 hours and the average time detectable levels were found following the last infusion was 45.3 hours.

Figure 2. Milk Levels of Oxytetracycline During and Following Intramammary Infusion of Three 400 mg. Doses



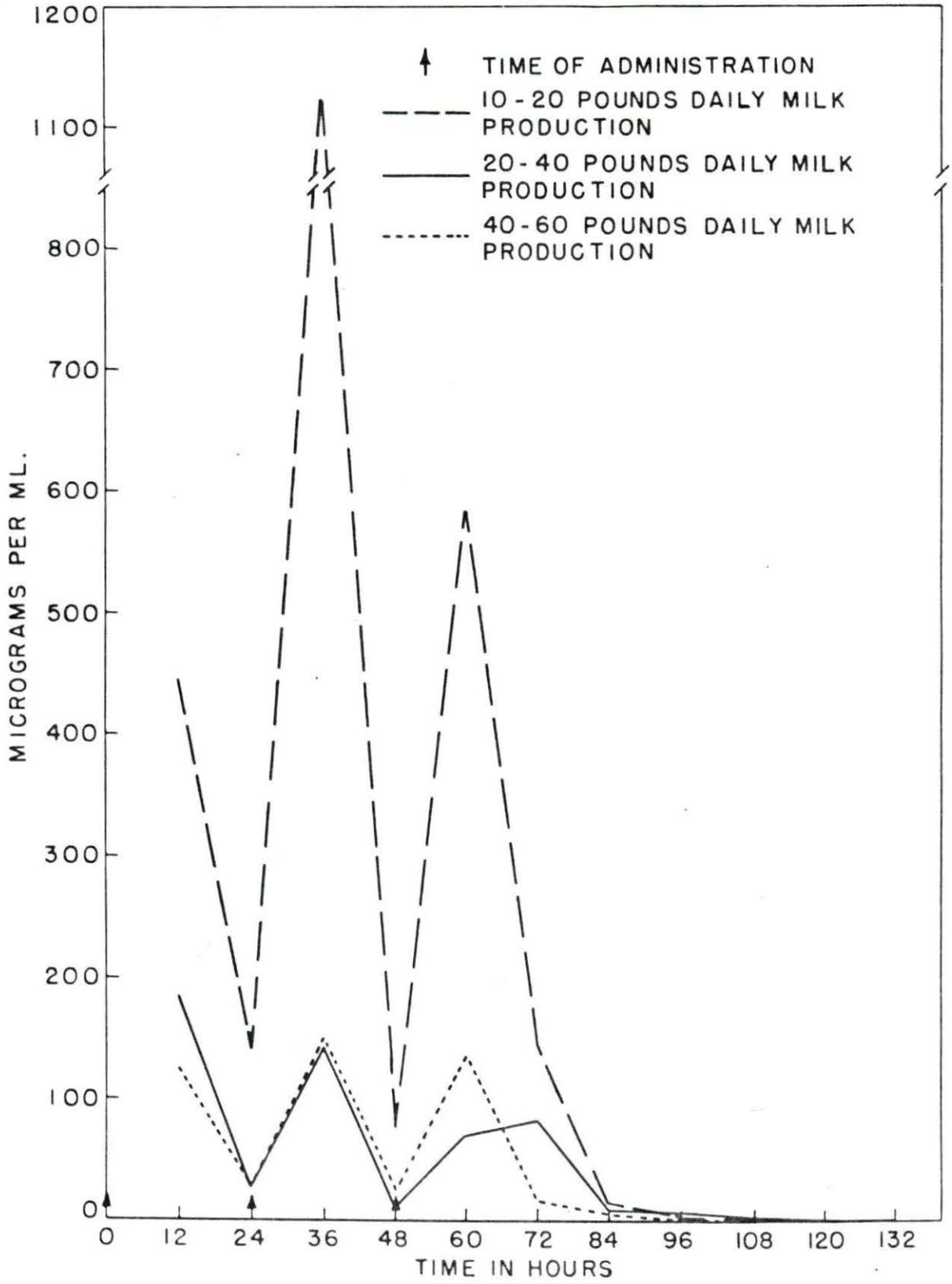

TIME OF ADMINISTRATION
10 - 20 POUNDS DAILY MILK PRODUCTION
20 - 40 POUNDS DAILY MILK PRODUCTION
40 - 60 POUNDS DAILY MILK PRODUCTION
MICROGRAMS PER ML.
1200
1100
800
700
600
500
400
300
200
100
0
0
12
24
36
48
60
72
84
96
108
120
132
TIME IN HOURS

Table 9. Milk Levels of Oxytetracycline during and Following Intramammary Infusion of Three 400 mg. Doses

Range of Milk Production		10-20 Pounds			20-40 Pounds			40-60 Pounds						
Actual Production		11.3	14.6	13.0	24.5	39.8	32.2	50.8	54.0	49.0	48.6	48.6	50.2	31.8
Time in Hrs.	Treatment	Quar. 3291 RF	Quar. 3294 LR	Avg.	Quar. 2553 RF	Quar. 3432 RR	Avg.	Quar. 3550 RR	Quar. 3128 LF	Quar. 2541 RF	Quar. 3078 LR	Quar. 3078 RF	Avg.	Overall Avg.
0	400 mg.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	-	990.4	In-complete	445.2	292.3	77.7	185.0	276.8	97.0	67.8	56.5	138.7	127.4	249.7
24	400 mg.	254.9	26.5	140.7	47.8	6.9	27.3	17.0	95.1	14.3	4.1	10.0	28.8	52.9
36	-	2088.4	155.2	1121.8	177.8	105.8	141.8	216.8	336.6	64.4	27.0	105.8	150.1	364.2
48	400 mg.	148.3	7.4	77.8	18.1	3.9	11.0	2.1	118.2	4.8	1.7	4.6	26.3	34.3
60	-	982.7	180.9	581.8	44.9	96.2	70.5	249.8	223.5	76.2	56.6	77.9	136.8	221.0
72	-	275.3	9.8	142.6	161.0	4.0	82.5	9.5	59.2	10.4	5.8	7.4	18.4	60.3
84	-	24.0	1.6	12.8	14.3	trace	7.1	0.0	15.6	1.8	trace	1.5	3.8	6.5
96	-	3.6	0.0	1.8	12.5	0.0	6.2	0.0	6.5	0.0	0.0	0.0	1.3	2.5
108	-	1.3	0.0	0.7	2.1	0.0	1.1	0.0	trace	0.0	0.0	0.0	0.0	0.4
120	-	0.0		0.0	2.0		1.0		0.0	0.0			0.0	0.2
132	-				0.5		0.3							0.1
144	-				0.0		0.0							0.0

Table 10 and Figure 3 show the milk levels of oxytetracycline which were found in two quarters following successive 800 mg. infusions at 24 hour intervals. It appears that the levels found were approximately double those found in the quarters treated with repeated 400 mg. doses. There was no obvious tendency for a cumulative effect with the second and third doses. Following all three treatments there was a marked drop in the concentrations between the 12th and the 24th hour following the infusion. It should be noted however, that the level at the 24th hour following each 800 mg. infusion was consistently higher than that found at the same hour following each succeeding 400 mg. dose. Detectable levels of oxytetracycline were found for an average of 54 hours following the last administration and for an average total of 102 hours following the initiation of therapy. There is some correlation between the volume of milk production, the height of drug concentration attained with the dosage and the length of time it was maintained.

Figure 3. Milk Levels of Oxytetracycline Following Intramammary Infusion of Three 800 mg. Doses

*Assay incomplete; concentration at least 1200 mcg. per ml.

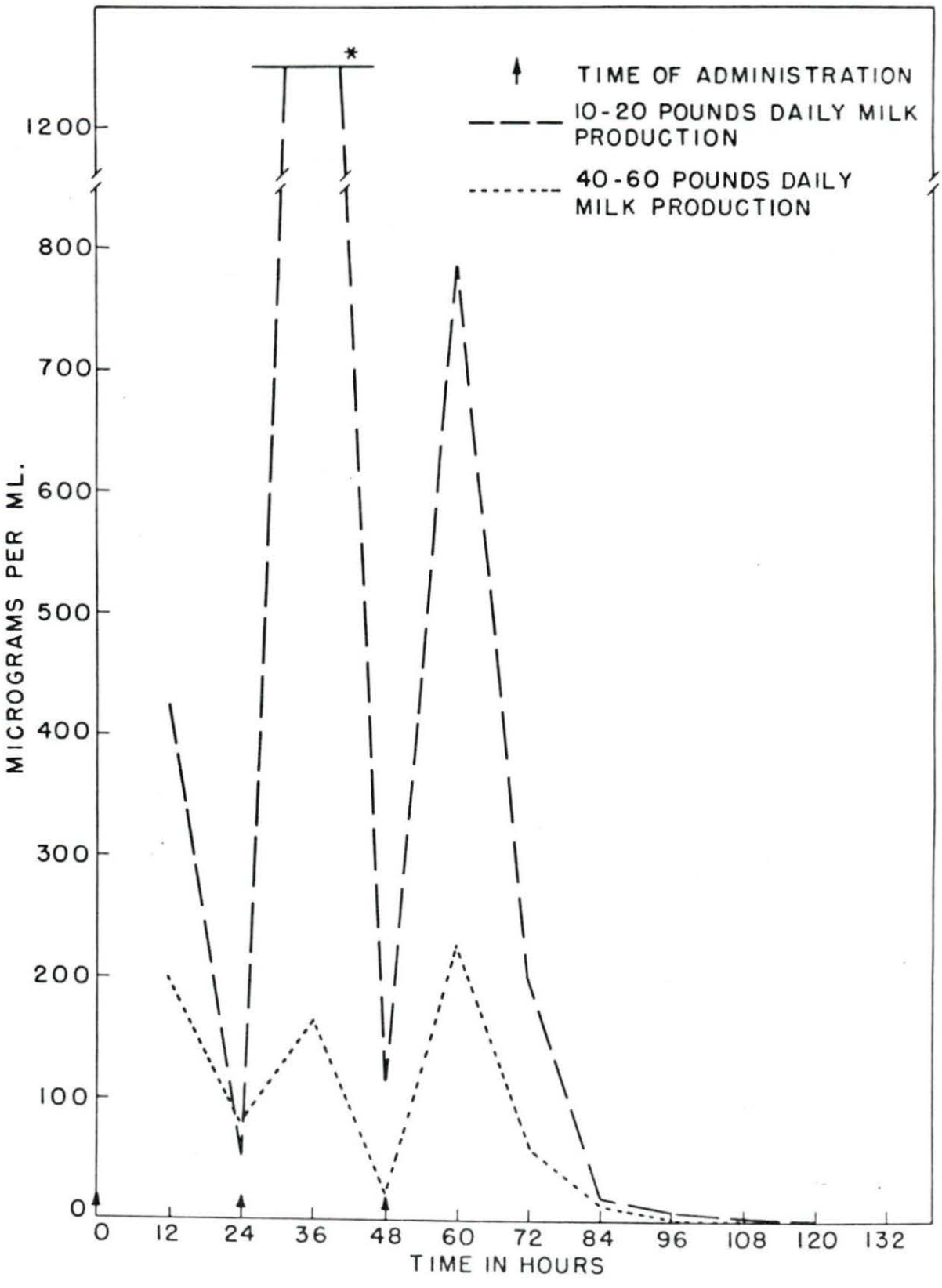

TIME OF ADMINISTRATION
10-20 POUNDS DAILY MILK PRODUCTION
40-60 POUNDS DAILY MILK PRODUCTION
*
1200
800
700
600
500
400
300
200
100
0
MICROGRAMS PER ML.
0
12
24
36
48
60
72
84
96
108
120
132
TIME IN HOURS

Table 10. Milk Levels of Oxytetracycline Following Intramammary Infusion of Three 800 mg. Doses

Range of Milk Production		10-20 Pounds	40-60 Pounds	
Actual Production		14.6	49.0	31.8
Time in Hrs.	Treatment	3294 RR	2541 RR	Over-all Avg.
0	800 mg.	0.0	0.0	0.0
12	-	423.5	200.3	311.2
24	800 mg.	51.6	81.7	66.7
36	-	>1200.0	165.2	165.2
48	800 mg.	116.8	21.6	69.2
60	-	786.3	226.8	506.5
72	-	199.0	61.0	130.0
84	-	17.0	13.7	15.4
96	-	6.3	2.8	4.5
108	-	2.0	0.0	1.0
120	-	0.0	0.0	0.0
132	-	0.0	0.0	0.0
144	-	0.0	0.0	0.0
156	-	0.0	0.0	0.0

Table 11 demonstrates that there was no transfer of detectable amounts of oxytetracycline from treated to untreated quarters following intramammary infusion, even when amounts as high as 1200 mg. were administered simultaneously into the other quarters. The amount of daily milk production appeared to have no influence on possible transfer of the drug from treated to untreated quarters.

Table 11. Milk Levels of Oxytetracycline in Untreated Quarters of Cows Receiving Intramammary Infusions in Other Quarters

Range of Milk Production	10-20 Pounds		20-40 Pounds				40-60 Pounds	
Actual Production		14.6		39.2		24.5		54.0
Time in Hrs.	Total Treatment in Other Quarters	3294 RF	Total Treatment in Other Quarters	2159 RF	Total Treatment in Other Quarters	2553 LR	Total Treatment in Other Quarters	3128 RF
0	1200 mg.	0.0	800 mg.	0.0	800 mg.	0.0	400 mg.	0.0
12	-	0.0	-	0.0	1200 mg.	0.0	-	0.0
24	1200 mg.	0.0	-	0.0	-	0.0	400 mg.	0.0
36	-	0.0	-	0.0	1200 mg.	0.0	-	0.0
48	1200 mg.	0.0	-	0.0	-	0.0	400 mg.	0.0
60	-	0.0	-	0.0	1200 mg.	0.0	-	0.0
72	-	0.0	-	0.0	-	0.0	-	0.0
84	-	0.0			800 mg.	0.0	-	0.0
96					-	0.0	-	0.0
108					-	0.0	-	0.0
120					-	0.0	-	0.0
132					-	0.0		

In Table 12 are the results of the assay of blood and milk samples from two cows following the intramuscular and intravenous administration of one gram of oxytetracycline. Blood levels were measurable in both cows but neither animal showed any detectable amount of oxytetracycline in the milk.

Table 12. Blood and Milk Levels Following Intravenous and Intramuscular Administration of One Gram of Oxytetracycline

	Cow	Daily Milk Production	Material Assayed	Time in Hours Post Treatment (mcg. per ml.) 1	2	4	8	12	24	36	48
Intra-muscular	3681	30.3 lbs.	blood	-	1.25	1.25	-	-	-	-	-
			milk	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Intra-venous	3554	45.0 lbs.	blood	8.65	-	trace	-	-	-	-	-
			milk	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DISCUSSION OF RESULTS

As might be expected, the highest concentrations of oxytetracycline were found in the first milk samples collected following administration. From this point the levels progressively diminished at a rapid rate until the drug could no longer be detected. Following one 400 mg. infusion, all quarters examined demonstrated oxytetracycline for at least 24 hours, with the average time being 37.3 hours. Repeated 400 mg. doses resulted in generally higher levels of the drug than the single dose, but the assay did not show that there was a consistent cumulative effect. Detectable levels were found for an average of 45.3 hours following the last dose and were seen in some instances as long as 96 hours after the last dose. Repeated doses of 800 mg. of oxytetracycline resulted in milk levels which were approximately double those following repeated 400 mg. doses, but there was no consistent cumulative effect. Levels were found for an average of 54 hours following the last dose of this schedule. These results are, in general, similar to those obtained by Schipper and Petersen (21), even though the present assay method does not appear to be as sensitive as theirs.

The results of this investigation show that intramammary infusions of oxytetracycline readily yield concentrations which are inhibitory to the common mastitis organisms with the possible exception of Ps. aeruginosa. In many instances, as long as oxytetracycline could be found, it was present in amounts which were inhibitory to the common mastitis organisms. The concentrations found immediately following repeated 400 mg. doses were usually high enough to be lethal to the streptococci but

perhaps not to the staphylococci. This may in part explain the relatively poor results obtained in the treatment of staphylococcic mastitis. The lowest levels seen between treatments were always sufficient to inhibit but seldom sufficient to kill the staphylococci. This is possibly borne out by the clinical improvement of mastitis cases, whether bacteriologic recovery occurred or not.

The levels of oxytetracycline attained and the length of time that they could be detected in the milk appeared to be inversely correlated with the volume milk production of that quarter. Invariably it was found that the milk of the lowest producer showed the highest levels and usually for the longest time following infusion. This information could be used in the field as a guide for the treatment of mastitis cases. Animals in high production probably should be treated more frequently and with dosages higher than 400 mg. to assure adequate therapy, while non-lactating animals or those in late lactation could be treated less frequently with a 400 mg. dose. This same inverse correlation between milk production and the levels of antibiotic has been shown by other investigators working with various antibiotics (4), (8), (9), (10), (11), (12), (16), (19), (20), (24), (25). It appears that if a constant high level were desired in a quarter, it would be necessary to repeat the dosage more often than every 24 hours. However, it probably could not be expected that increased frequency or amount of dosage would result in greatly prolonged levels of antibiotic. The expression of a cumulative effect appears to be an increase in concentration rather than a marked increase in the length of time that the drug is present.

Rather extreme variations were found in the oxytetracycline levels of different quarters of the same cow given the same treatment. Schipper and Petersen (21) pointed out similar variations following ointment infusions. No study was made to explain this variation, but possibly it would have been less if the assay samples had been collected midway in the milking instead of using foremilk samples. However, there may also have been other factors involved.

Results of the assay of milk samples collected following intravenous and intramuscular injections of one gram of oxytetracycline showed that, if present, the levels were less than 1.25 mcg. per ml. with the method used. Schipper and Petersen (21) used two gram doses of oxytetracycline intramuscularly and intravenously and were able to show that detectable milk levels could be found following the intravenous dose, but not following the intramuscular administration.

It is of significance to note that there was no transfer of detectable amounts of oxytetracycline with the assay method used from treated to untreated quarters in the same cow. No matter what the amount of infusion into the other quarters or the amount of daily milk production, each quarter appeared to be independent in this regard.

CONCLUSIONS

1. During treatment of 115 controlled clinical cases of bovine mastitis, oxytetracycline was found to be effective against various etiologic agents in the following percentages: streptococci, 76.92%, Staph. aureus, 25.0%, coliform organisms, 69.23% and Pseudomonas aeruginosa, 0.0%.

2. Nearly all acute cases showed clinical improvement during local therapy whether the treatment was completely successful or not.

3. The results of therapy of subclinical cases were better than those of clinical cases.

4. The results of therapy were better in recently acquired mastitis than in cases of long standing, especially in those caused by Staph. aureus.

5. Use of oxytetracycline intramuscularly aided in the relief of systemic symptoms in all cases, irrespective of the etiologic agent.

6. Evidence was presented which showed oxytetracycline to be irritating in certain instances when administered both intramammarily and intramuscularly.

7. Assay of milk samples following 400 mg. intramammary infusions of oxytetracycline showed that detectable levels were consistently present for 24 hours or longer.

8. Milk samples collected following one gram doses of oxytetracycline intramuscularly and intravenously showed no detectable amounts of drug with the method used.

9. No detectable amounts of oxytetracycline could be found with the assay method used in milk from untreated quarters when the other quarters were treated.

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