PROPHYLAXIS AND CONTROL OF VECTOR-BORNE ANAPLASMOSIS WITH SUSTAINED-RELEASE BOLUSES

By

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INTRODUCTION

Successful control of arthropods and the diseases they vector are often difficult due to the treatment regimen required and the severity of the infection or disease epidemiology. Current recommendations for prophylaxis and control of arthropods and diseases of cattle are based on the application of an insecticide or acaricide to the target organism and the administration of biologically active compounds via parenteral dosages, feed throughs, water additives and the use of salt or mineral blocks. These methods provide effective control only when strict and periodic programs are implemented and intensely managed. The effectiveness of these treatments are generally short-lived and require repeated applications. With an increasing emphasis on a complete herd health program commercial application of these methods have limitations under rangeland and some feed-lot conditions. Daily treatments prove laborious, time-consuming and expensive. Also, when agents are introduced via rations, water and other vehicles, intake is variable and inadequately monitored. The continual trend towards increased production in the cattle industry, arthropod and disease control procedures as currently used, may provide protection for livestock but the producer absorbs the increasing cost of maintaining such a program. From this standpoint, there is an obvious need for a new and

improved economical method for arthropod and disease prophylaxis and control.

One approach to minimize daily management costs through disease and parasite prophylaxis would be to administer therapeutics through a sustained-release system employing ear tags, implants, boluses, etc.

Sustained-release Systems

Interest in controlled-release devices has resulted in new procedures for animal health care and production. Growth stimulants have been administered via implantation of a biologically active pellet beneath the skin of the ear with sufficient levels being offered for a 100-120 day period. Insecticide-impregnated ear tags have been developed for ectoparasite control on livestock (Gladney 1976; Ahrens et al. 1977). Veterinary boluses have been designed to provide small amounts of vitamins, trace elements, and pesticides to ruminants. As a result of successful controlled-release devices for animal health and production, new procedures by which administration of acaricides and chemotherapeutic agents can be offered to ruminants over a prolonged period of time have resulted.

Early observations on the bolus technique using crude associated technology by Dewey et al. (1958) provided cobalt in small amounts to grazing sheep on cobalt deficient pastures in Australia via a "cobalt pellet" delivering therapeutic levels for 100 days. Pellets prepared by compressing trace elements with various binders such as china clay and polyvinyl acetate have also been developed and retained within the rumen in excess of 1 year (Marston 1962). In subsequent testing, Rednick and Tucker (1970) developed a bolus utilizing a sulfonamide

drug mixed with a binder and dense filler for sulfonamide administration to beef animals.

Siegrist and Katz (1970) utilized a bolus to syncronize estrus in cattle for artificial insemination. Their composition of an insoluble wax, a high-density metal derivative, a lubricant, and the therapeutic agent, provided a reliable controlled-release of the agent with a finite end point. Formulation was accomplished by preparing the composition in a liquid melt. More recently, Teel et al. (1978) investigated the feasibility of incorporating systemic pesticides for ectoparasite control in a sustained-release system and demonstrated that a 50 percent famphur systemic acaracide bolus offered effective control against two 3-host tick species for up to 60 days. Likewise, Hair et al. (1979) demonstrated the effectiveness of the same system against Boophilus ticks. The principal on which these devices operate was based on the retention of the boluses in the rumeno-reticular sac of the ruminant. Through normal peristaltic movements during rumination, the active ingredient was released through erosion, leaching or other means and was utilized systemically.

Density has been found to be a primary factor in the success of these devices since bolus retention within the rumen was related to the specific gravity. Siegrist and Katz (1970) in conducting experiments concerning minimum critical densities, found that while a density of 1.9 g/cm^3 is preferred, a density of 1.5 g/cm^3 was minimal. Teel et al. (1979) demonstrated a direct relationship between bolus density and rumen retention. They found that the degradation rate was inversely proportional to the amount of pressure used to compress the boluses. They also found that hydrophobic binders produced the longest lasting

boluses and that mixtures of candidate components could constitute the matrix base necessary to achieve the desired release rate. These authors provided insight into means of formulating a sustained-release system that has the potential to be effective in offering biologically active compounds to ruminants over an extended period of time. Effective administration was offered as long as blood levels were maintained without becoming toxic to the host.

A prolonged-release formulation has several potential advantages compared to non-prolonged forms: better animal compliance, more constant blood levels resulting in shorter and fewer treatment periods and therefore, reduced cost. The usual parenteral or oral treatment regimen results in high peak blood levels which fall well below therapeutic concentrations before administration of the next dose. Studies have indicated that therapeutic levels must be above a minimum inhibitory concentration (MIC) for a specified period to eradicate an infection or disease and this action was relatively independent of whether the level was achieved by constant infusion or intermittent bolus dosing (Schneider et al. 1978). Since therapeutic levels may be below the MIC for significant periods after parenteral dosages, there may be unnecessary prolongation of treatment and consequently, excessive drug consumption.

Vector-borne Anaplasmosis

In certain circumstances a prolonged-release antibiotic treatment regimen may allow successful treatment of vector-borne bovine diseases such as anaplasmosis where continuous therapy is needed. Anaplasmosis is an infectious, parasitic disease of cattle caused by the microorganism

Anaplasma marginale (Theiler). The disease is transmitted by arthropods either mechanically or biologically. Ticks have been established as efficient vectors of anaplasmosis since they are resistant to environmental changes, are long-lived and can overwinter. Disease transmission occurs during the ingestion of the blood meal when the disease agents are passed via the bite. Although mechanical transmission has been shown to occur by bites of horseflies (Tabanus sp.), stable flies (Stomoxy sp.), deer flies (Chrysop sp.) and horn flies (Haematobia sp.), ticks appear to be primarily responsible for the continuation and biological transmission of anaplasmosis (Howell 1957, Dickmans 1950). A number of ticks have been found associated with cattle during outbreaks of anaplasmosis. Dermacentor variabilis (Say) and D. andersoni Stiles have been shown by laboratory and field studies to be important vectors of anaplasmosis in the United States (Anthony et al. 1964, Anthony and Roby 1962, Boynton et al. 1936, Kocan 1979).

Although animals of all ages may be susceptible to anaplasmosis the severity of the disease is directly related to age. The syndrome in yearling cattle is usually sub-clinical and severe (frequently fatal) in older cattle (Blood and Henderson 1960; Ristic 1960). The disease may result in poor reproductive ability, abortion, severe weight loss, decreased milk production, fever, constipation, anemia, and sometimes death. These are accompanied by icterus, inappetence, depression, dehydration, and labored respiration (Carricaburu 1957).

It has been estimated that 50-100 thousand animals die of anaplasmosis each year with an annual economic loss of 100 million dollars (McCallon 1973). Although a vaccine is available and effective chemotherapeutic agents are in use, treatment of anaplasmosis continues to be an important tool in the control of losses due to this disease.

Christensen (1956) reported on several non-specific compounds such as sodium cacadylate, neoarspenamine, paludrine, aralen, aricyl, and other drugs were judged by the percent recovery of treated animals. In a report by Dykstra et al. (1948) most of the compounds were found to be of no value or of questionable value.

Tetracyclines are recognized as effective in prophylaxis and control of anaplasmosis and their action in suppressing the growth and multiplication of the causal agent has been described (Brock 1959). Miller et al. (1952) reported that the use of chlortetracycline and oxytetracyline halted the increase in anaplasma bodies when administered in the early phase of anaplasmosis infectivity. Foote and Wulf (1952) demonstrated that relatively large doses (27.5-47.5 g) of chlortetracycline destroyed the infection in 3 cows. Later, it was reported by Pearson and Brock (1953) that dosages of 47.5 g only temporarily prevented transmission ability (up to 67 days) and did not destroy the infection. Scales et al. (1962) found that low-level feeding of tetracyclines slowly reduced the blood titers of anaplasmosis reactors and thereby reduced their infectivity. Carrier status has been eliminated utilizing intravenous (I.V.) and intramuscular (I.M.) injections of tetracyclines for 10 consecutive days (Pearson et al. 1957).

In addition to the previous references there have been a number of reports indicating the value of tetracyclines used in the treatment of anaplasmosis. More recently, Magonigle et al. (1975) found that I.V. injections of oxytetracycline at 22 mg/kg for 5 consecutive days could eliminate the infectivity of anaplasma bodies. Results from Roby et al. (1978) showed that 2 I.M. injections of a new long-acting

oxytetracycline formulation (L-200) 7 days apart would eliminate the carrier state from recently infected yearling cattle.

Evidence suggest that the tetracyclines are capable of exerting an inhibitory effect on the anaplasma organism. Anaplasmosis varies greatly in severity, mortality, and symptoms and the treatment effectiveness depends on the following conditions: (1) age and condition of the animal infected; (2) animal resistance to the disease; (3) animal ability to regenerate red blood cells; and (4) virulence of the specific agent. A common error in evaluation of therapeutic agents is the number of spontaneous recoveries which misled observers to believe that a specific drug or procedure was effective. Another problem is that the animal infected may go undetected until the terminal phase of infection occurs. Early treatment is recognized as being most effective during early infectivity phases.

With an increasing incidence of anaplasmosis in most of the plains and west coast states, the potential of a sustained-release system involves a need to develop chemotherapeutic formulations which are applicable throughout vector seasons and in difficult management situations. Current methods previously described have limitations. Antibiotic therapy when administered parenterally in its present formulation, maintains effective blood concentrations for a reasonably short period of time. As in most cases, multiple injections are required to achieve the desired control, especially when the disease is in varying stages of development. These prolonged treatment periods are in conflict with most management practices. This, along with the expense of the drugs and labor necessary to implement such a program leads researchers to search for improved formulations which would enhance

the effect of treatment and possibly reduce the length and number of treatments. Therefore, it is the scope of this manuscript to describe the investigations into the research and development of a sustained-release oxytetracycline bolus for prophylaxis and control of anaplasmosis in bovine. The approach was 2-fold, including screening of candidate matrixes and compositions for compatibility with oxytetracycline and bio-assay determinations for efficacy against vector-borne analplasmosis.

CHAPTER II

SCREENING OF CANDIDATE MATRIXES COMPATIBLE WITH OXYTETRACYCLINE

Methods and Materials

General Procedures

Based on previous studies at the Oklahoma State University laboratory in the development of sustained-release boluses, considerations in matrix formulation were based on a number of factors which included bolus size and density, utilization of non-toxic matrix ingredients, desired release rates, and bolus longevity. In initial testing of candidate matrixes to ascertain the feasibility of formulating a long-termed sustained-release oxytetracycline bolus for use in bovine, grade hereford and angus heifers having an average weight of 364 kgs were fistulated for rumenal cannulas (Bar Diamond, Inc., Parma, Indiana). The aperture allowed for entry to the rumen on a bi-weekly basis for bolus retrieval and evaluation. After 10 days post-operative antibiotic therapy, the heifers were maintained on a cotton seed-hull based ration (Williams et al. 1977) under dry-lot conditions in order to minimize gain during the test period. Prior to administration, boluses in each animal were coded to monitor degradation of individual boluses. Bolus erosion and thus oxytetracycline released, was monitored by removing the boluses from the rumen, drying

them with a paper towel, weighing them on a digital electronic balance, and calculating release rates from bolus weight loss. Boluses were then reinserted to respective hosts.

Oxytetracycline boluses were prepared using a matrix containing a highly-hydrophobic binder (carnauba wax), a high-density non-toxic metal derivative (barium sulfate), a polymer (polyethyleneglycol) to facilitate bolus degradation, and the active ingredient oxytetracycline. The matrix was prepared as described by Teel and Hair (1978), by melting the carnauba wax in a double boiler heated above 86^{o}C for the purpose of combining the high-density barium sulfate and the polyethyleneglycol (PEG). The liquid melt was poured into aluminum trays to a thickness of 4 mm and allowed to cool to room temperature before being ground to a powder using a standard kitchen model blender. The matrix powder was screened through a U.S. standard 40 mesh sieve and then blended with the active ingredient, oxytetracycline. Appropriate aliquots of the final formulation (45 g) were hydraulically pressed(Soil Test, Inc.) to form a conventional veterinary bolus 7.6 by 2.25 cm with a mean density of 1.89 g/cm^{3}. Since testing conducted by Siegrist and Katz (1970) reported that the rate of release of the therapeutic agent is inversely proportional, within limits, to the compression rates, bolus formulations were pressed between 492-632 kg/cm^{2} for proper cohesion and binding of particles.

Varying Matrix Compositions

Results from early pilot studies indicated that in order to achieve a bolus with a high-specific gravity that would effectively deliver a desired therapeutic dose over a 45-60 day period, the proper

components, rates of each, and sufficient compression were needed to accomplish such an objective. During these preliminary trials in search of proper bolus compositions and rates, observations on the characteristics of several potential matrixes were noted.

In an effort to ascertain the feasibility of formulating a high-density long-termed sustained-release oxytetracycline bolus for use in bovine that would effectively deliver a desired therapeutic dose over a 45-60 day period, 45 g boluses were prepared of 9 g oxytetracycline blended in 36 g matrix (i.e. 20 percent oxytetracycline bolus). Five boluses were made of each of the matrix formulations I-III in Table I. The boluses were pressed at ca 562.46 kg/cm^2 and administered to 5 fistulated heifers. Bolus degradation was monitored post-administration as described in general procedures.

Increasing Binding Properties

Based upon the results of varying the matrix compositions, it was felt that the bolus release rates could be improved to provide a less variable and more consistent delivery of oxytetracycline. Also, there was an obvious need to improve on the longevity of the boluses since those in the previous study did not last for a sufficient length of time. From previous testing dealing with bolus formulation, it was noted that variability and inconsistency in release rates could be kept to a minimum by 2 methods. First of all, an increase in pressure will enhance particle binding and give a more controlled release of drug. Secondly, a decrease in the amount of PEG was thought to aid in the binding of particles within the bolus thereby, providing a longer lasting and more controlled-release of drug. A second experiment was

TABLE I

MATRIX COMPOSITIONS TESTED FOR DEVELOPMENT OF SUSTAINED-RELEASE OXYTETRACYCLINE BOLUSES

Matrix Number	Percentage Matrix Composition		
	Carnauba	Barium Sulfate	Polyethyleneglycol
I	23.6	68.0	8.2
II (A)	27.3	63.6	9.1
II (B)	27.3	68.2	4.5
III	20.0	60.0	20.0
IV	28.3	70.1	0.9
V	28.0	70.1	1.9
VI	27.8	69.4	2.8
VII	27.5	68.8	3.7

begun to alleviate the problems of excessive variability in release rates and the short life of the boluses. In this study, the matrix showing the most promising potential for sustained-release formulation was utilized (Matrix II). Four 20 percent oxytetracycline boluses were prepared using candidate Matrix II and administered to 4 fistulated heifers. Pressure applied to these boluses was increased to ca 632.77 kg/cm^2 compared with that used in previous studies. This should provide an increase in the binding of the components and give a more consistent release of oxytetracycline. Consequently, these boluses were labeled as Matrix II (A). Four 20 percent boluses were also prepared from Matrix II (B) which was a slight modification in Matrix

II (A). In this trial a lesser amount of polymer was used. Based upon results of the previous study, it was felt that this decrease in PEG would provide a stronger binding effect in the boluses thereby giving a more uniform and less variable release of oxytetracycline. These boluses were pressed at ca 681.98 kg/cm^2 and administered to 4 fistulated heifers. Bolus degradation was evaluated post-administration as described in general procedures.

Varying PEG Concentrations

The successful ability of Matrix II to handle oxytetracycline prompted the design of a study to determine the effect of varying the amount of PEG loaded into Matrix II. It was believed that the amount of PEG present affects the binding of the particles. The lesser the amount of polymer the stronger the binding effect and therefore, a more controlled-realse of drug. Twenty percent oxytetracycline boluses formulated with Matrix II and varying amounts of PEG were prepared and administered to the fistulated heifers. In this test, increasing increments of PEG ranged from 0.9-3.7 percent and 4 boluses of each formulation (Matrix IV-VII; Table I) were produced and administered to 5 fistulated heifers. Post-administration evaluation was monitored as described in general procedures.

Therapeutic Drug Quantities

Evaluation of data from earlier experiments illustrated that oxytetracycline could be formulated in a sustained-release system and that therapeutic dosages could be maintained. In order to develop sustained-release formulations for use in regimens which are applicable

in difficult management situations, it was necessary to determine the maximum amount of oxytetracycline that could be utilized in Matrix II. By knowing this, it would provide the greatest amount of drug to the animal, thereby enhancing the effect of treatment and possibly reducing the length and number of treatments needed. An experiment was designed using 45 g boluses formulated with 5 concentrations of oxytetracycline (14, 15, 17, 20 and 25 percent). Five boluses of each concentration were prepared and administered to 8 fistulated heifers. Post-administration bolus degradation was monitored as described in general procedures.

Commercial Preparations

In ascertaining feasibility of formulating a sustained-release oxytetracycline bolus by utilizing candidate matrixes, various compositions, and pressures, the potential of the phenomena of controlled-release via bolus formulations prompted several leading pharmaceutical companies to express an interest in this technology. Commercial production requires certain refinements in order to attain compositions suitable for commercial tableting machines. After reviewing descriptions of industrial punches and dies, and evaluating the circumstances under which these boluses would be produced, additional studies were designed. Since difficulty was anticipated in force pressing the boluses from industrial single punch presses, recommendations call for the addition of a lubricant to enhance the flow properties of the matrixes to prevent restrictions in flow rates. With this in mind, an experiment was designed to incorporate varying amounts of the lubricant, magnesium stearate, into the final formulation. In this study, 20

percent oxytetracycline boluses were formulated with Matrix II and 3 concentrations of magnesium stearate (0.5, 0.75, and 1.0 percent) was blended into the final formulation. Five boluses of each concentration were prepared and administered to 5 fistulated heifers. Bolus erosion was monitored as described in general procedures.

It was also recommended that a study be designed to utilize 4 different pharmaceutical grades of carnauba wax to determine which grade closely represented that used in previous trials at Oklahoma State University. This study involved formulation of 20 percent oxytetracycline boluses with Matrix II's composition but substituting the carnauba with those of the pharmaceutical companies. Previous trials at Oklahoma State University utilized the least expensive grade, Type 4, filtered, supplied by S. C. Johnson and Son, Inc. (Racine, Wisconsin). The 4 grades listed in Table II represent those commercially available and used. These are of a more refined nature and represent a higher quality of carnauba. In testing these grades of carnauba, four 20 percent boluses of each grade were prepared. Magnesium stearate was added to the final formulation at 0.5 percent and pressed at ca 681.98 kg/cm^2. Boluses were then administered to 6 fistulated heifers to determine the effects. Following administration, boluses were evaluated as described in general procedures.

Results and Discussion

Varying Matrix Compositions

Data recorded during initial efforts to ascertain the feasibility of formulating a sustained-release osytetracycline bolus indicated that

TABLE II

PHARMACEUTICAL GRADE CARNAUBA WAXES TESTED FOR COMMERCIAL APPLICATION

Carnauba Grade
-- Carnauba Wax N.C. #3 Brazilian Refined Flakes (Light) Ross
-- Carnauba Wax N.C. #3 Brazilian Refined Flakes (Dark)
-- Carnauba Wax N.C. #3 Pure American Refined Flakes
-- Carnauba Wax Ross Refined #3 N.C. Light Flakes

the controlled-release of oxytetracycline from an inert matrix within the bovine rumen could be achieved. Figure 1 shows the estimated daily release rates of the 20 percent oxytetracycline boluses from Matrix I. These boluses were discovered broken in half on day 15. They were found to be short-lived (i.e. 21 days) and therefore, considered inferior as a potential binder with oxytetracycline. The 20 percent oxytetracycline boluses prepared from Matrix II (Figure 2) produced a durable composition. Boluses of this nature had a density of ca 1.85 g/cm^3 which was sufficient to prevent passage or regurgitation from the rumen. The release rates climbed steadily for the first 25 days with a mean release of 137 mg/bolus/day. After this period, the release rates dropped sharply. Between days 25 and 39 the release rates deviated from bolus to bolus by ca 20-30 mg/bolus. It appeared that the proper components and rates of each

Figure 1. Release of Oxytetracycline Through In-vivo Degradation of 20 Percent Oxytetracycline Boluses Prepared From Matrix I

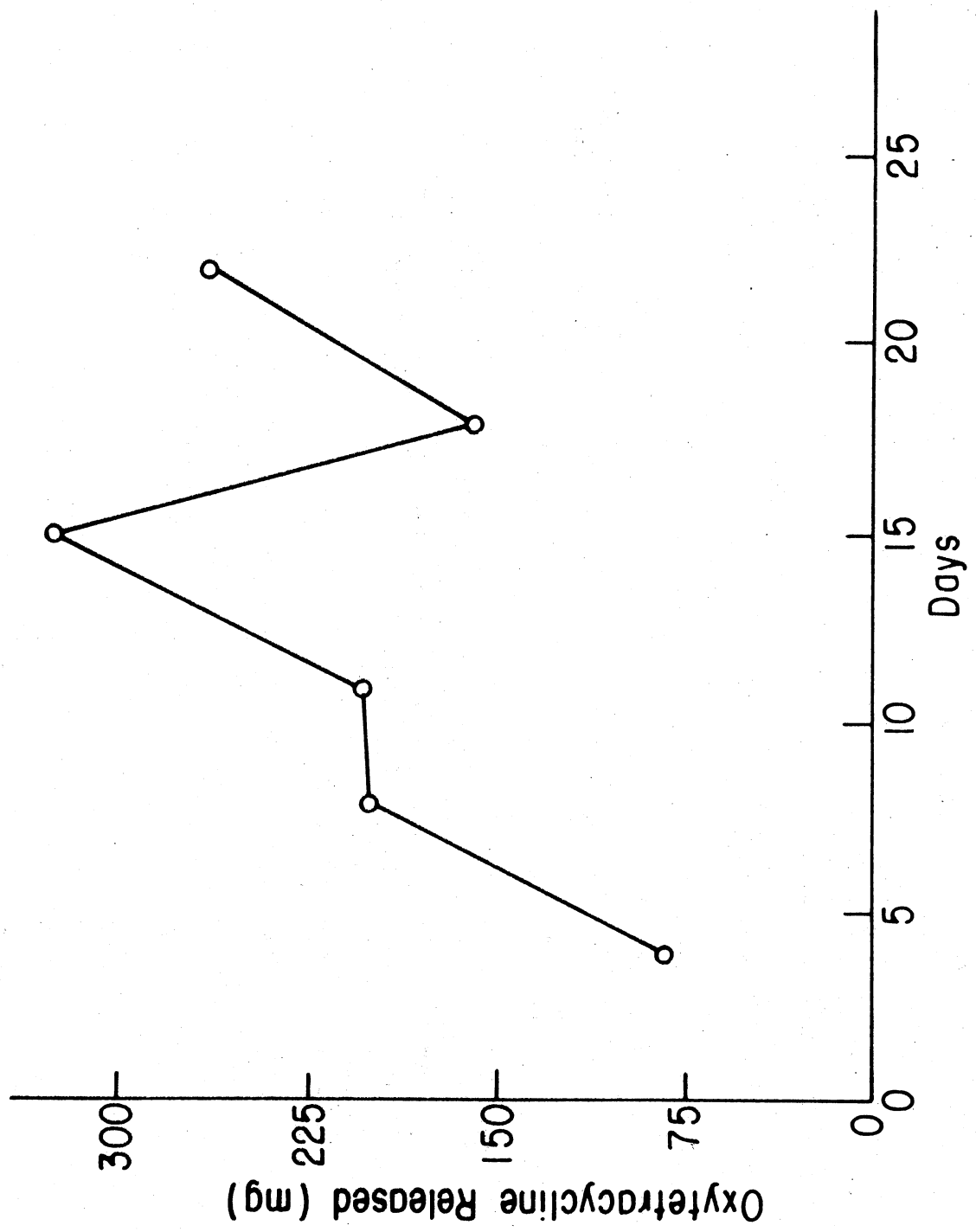
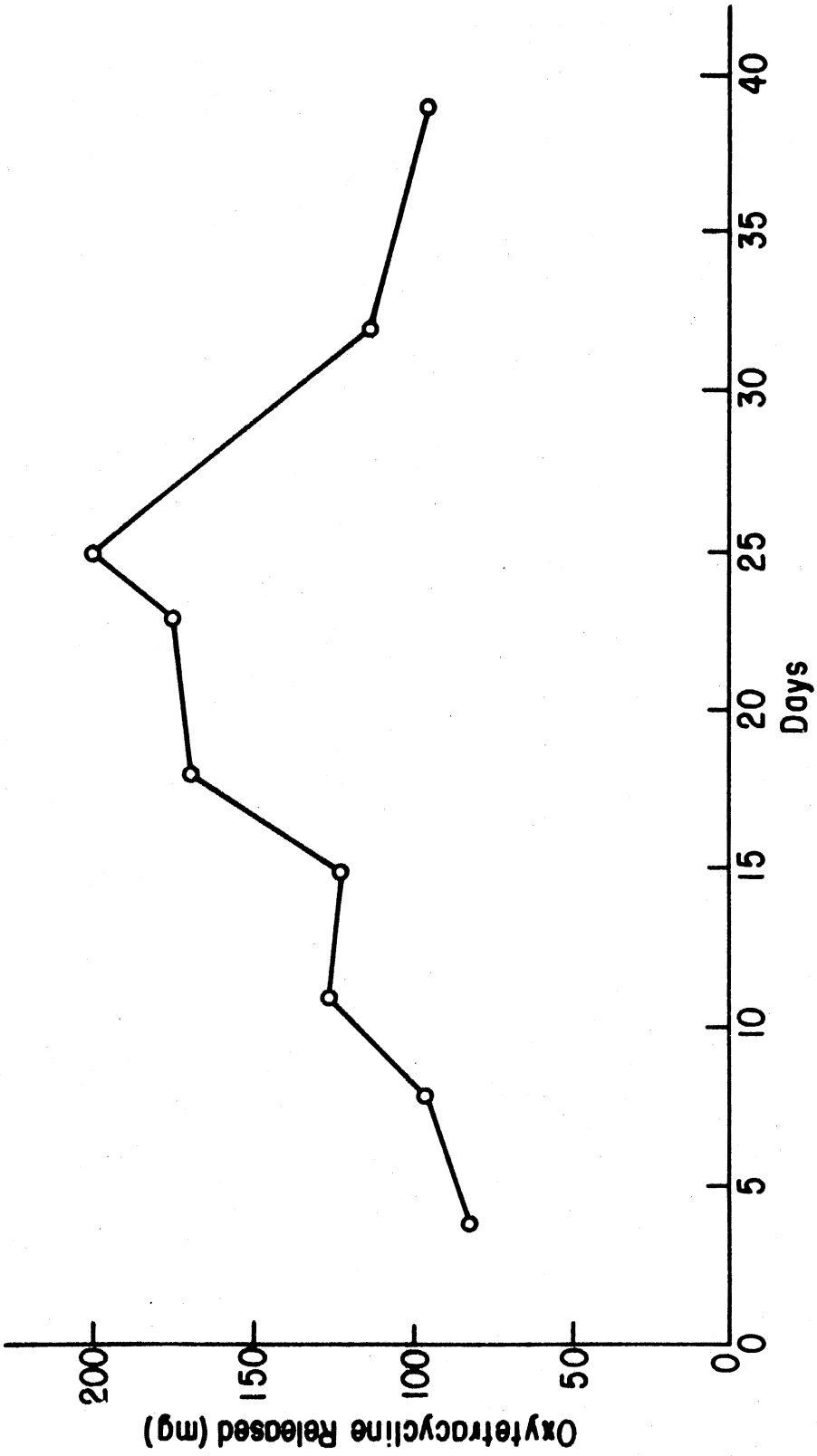

Oxytetracycline Released (mg)
300
225
150
75
0
0
5
10
15
20
25
Days

Figure 2. Release of Oxytetracycline Through In-vivo Degradation of 20 Percent Oxytetracycline Boluses Prepared From Matrix II


Oxytetracycline Released (mg)
200
150
100
50
0
0
5
10
15
20
25
30
35
40
Days

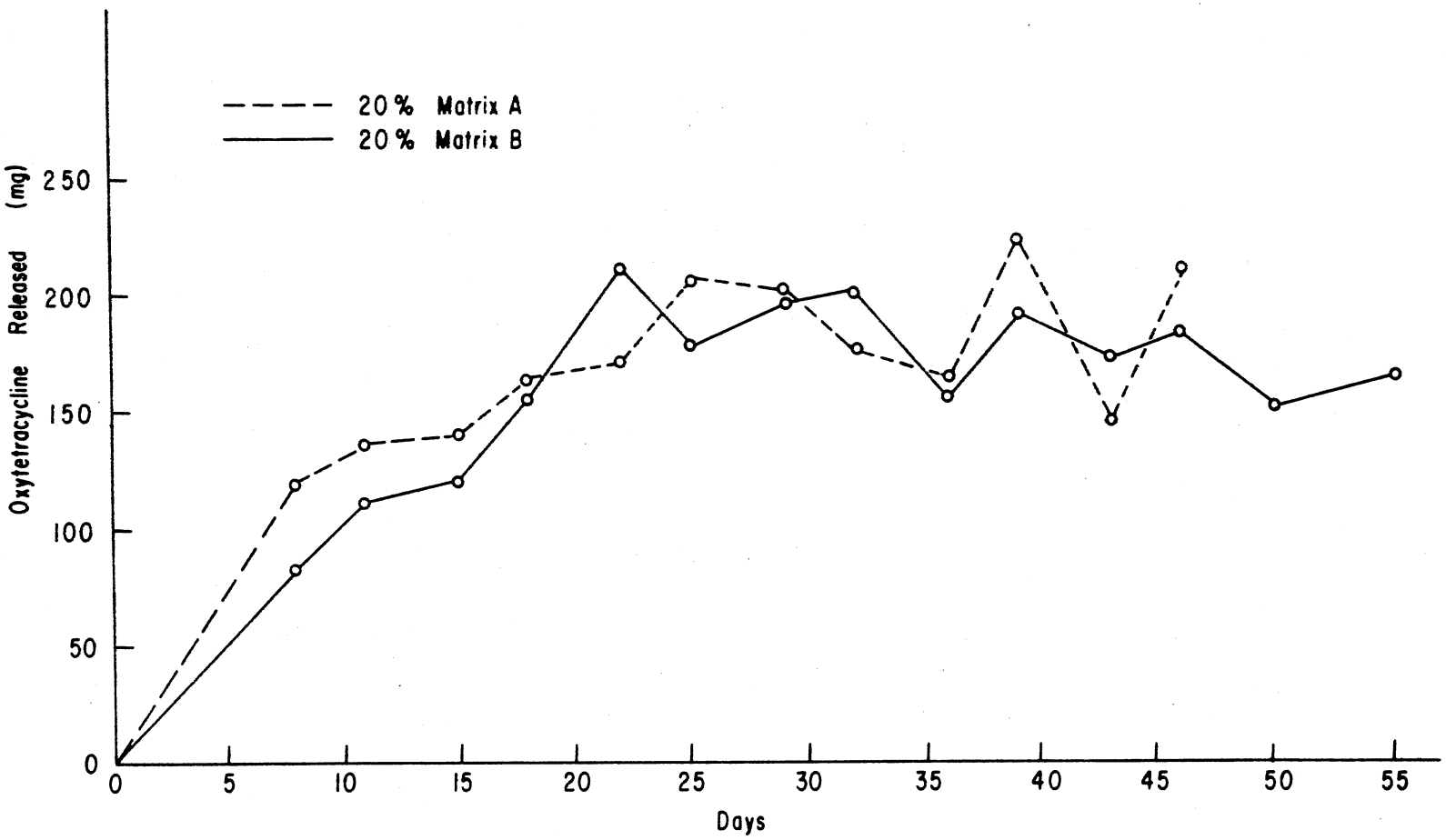
were present, but the formulation was lacking sufficient pressure to provide a consistent release rate of oxytetracycline. The test lasted for 39 days and had a mean release rate of 129 mg/bolus/day. Boluses of Matrix III developed a surface that was eroding in large layers. These boluses were found to be weaker in structure due to insufficient amounts of binder to hold the particles together. On the 4th day of inspection all boluses had broken into 6 pieces and were also considered inferior as a candidate matrix.

Increasing Binding Properties

The suitability of the 20 percent oxytetracycline boluses from Matrix II received further in-vivo testing to provide a stronger binding effect. Figure 3 shows the estimated daily release rates of the 20 percent boluses of Matrix II (A) and Matrix II (B). Boluses of Matrix II (A) were consistent in eroding at a rate of 162 mg/bolus/day for the first 35 days. After this period, release rates became irregular due to an incidence of bolus breakage within the rumen. This problem was due to a level of PEG that was not conducive for adequate bolus tensile strength. Consequently, this breakage of boluses caused a substantial increase in surface area of the boluses and thus, the release rates were influenced. Although these boluses broke in half, between days 8-46 the average daily release remained between 120 and 220 mg/bolus.

The 20 percent oxytetracycline boluses formulated from Matrix II (B) followed the same general erosion pattern as those previously described. These boluses maintained a mean release of over 150 mg/bolus/day between days 11-55. A sandstone-like bolus surface developed and all boluses eroded evenly throughout the test. The average daily

Figure 3. Degradation of 20 Percent Oxytetracycline, Matrix II Boluses


20 % Matrix A
20 % Matrix B
Oxytetracycline Released (mg)
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
50
55
Days

release rate up to the termination of the study on day 55 was 162 mg/bolus/day. In comparing the amount of PEG and the increased pressure used in the matrix, it was noted that the lesser amount of PEG provided the uniformity necessary for a consistent release of oxytetracycline, while the 226.8 kg increase in pressure appeared to provide the proper binding of particles within the bolus. Resulting properties due to this decreased amount of PEG and the increased pressure included a more consistent and uniform release of drug, an increase in bolus lengevity by ca 10 days and most important, the arrestment of bolus breakage within the rumen.

Varying PEG Concentrations

In this study of varying the percent PEG, PEG added at rates of 0.9, 1.9, 2.8, and 3.7 percent illustrated a direct relationship between the polymer and its concentration in the bolus. PEG added at 0.9 percent (Figure 4) provided a relatively stable release of oxytetracycline for the life of the bolus (67 days) with an average of 126.3 mg/bolus/day. At 1.9 percent (Figure 5) bolus degradation increased to 143 mg/bolus/day, a difference of ca 17 mg/bolus/day over the 0.9 percent concentration. In addition, this higher release rate caused a shortening in bolus longevity by 17 days. These boluses terminated at day 50. In Figure 6, the 2.8 percent PEG composition provided an average daily release of 173.5 mg/bolus for 50 days. This bolus eroded with the same general pattern as those of the 1.9 percent composition but a difference of ca 30.5 mg/bolus/day was observed. Boluses of the 3.7 percent PEG composition appeared to be eroding at a much lower level than normally seen (i.e. 75 mg/bolus/day) during the

Figure 4. Degradation Analysis of 20 Percent Matrix IV Boluses With 0.9 Percent PEG

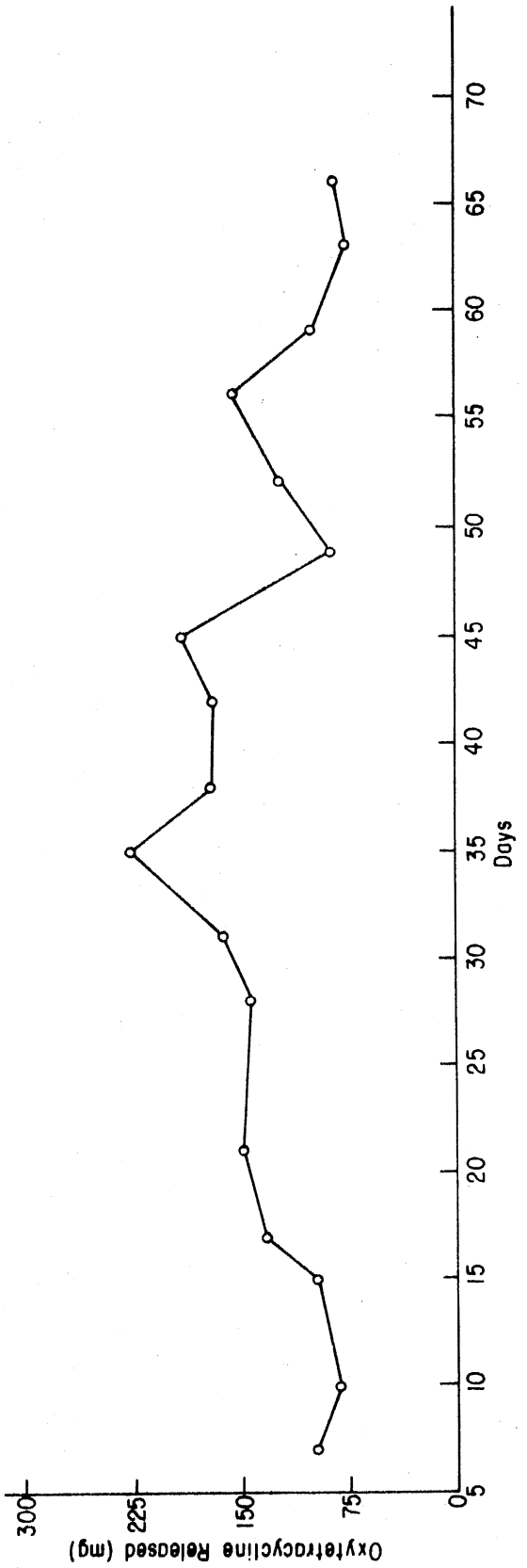

Oxytetracycline Released (mg)
300
225
150
75
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Days

Figure 5. Degradation Analysis of 20 Percent Matrix V Boluses With 1.9 Percent PEG

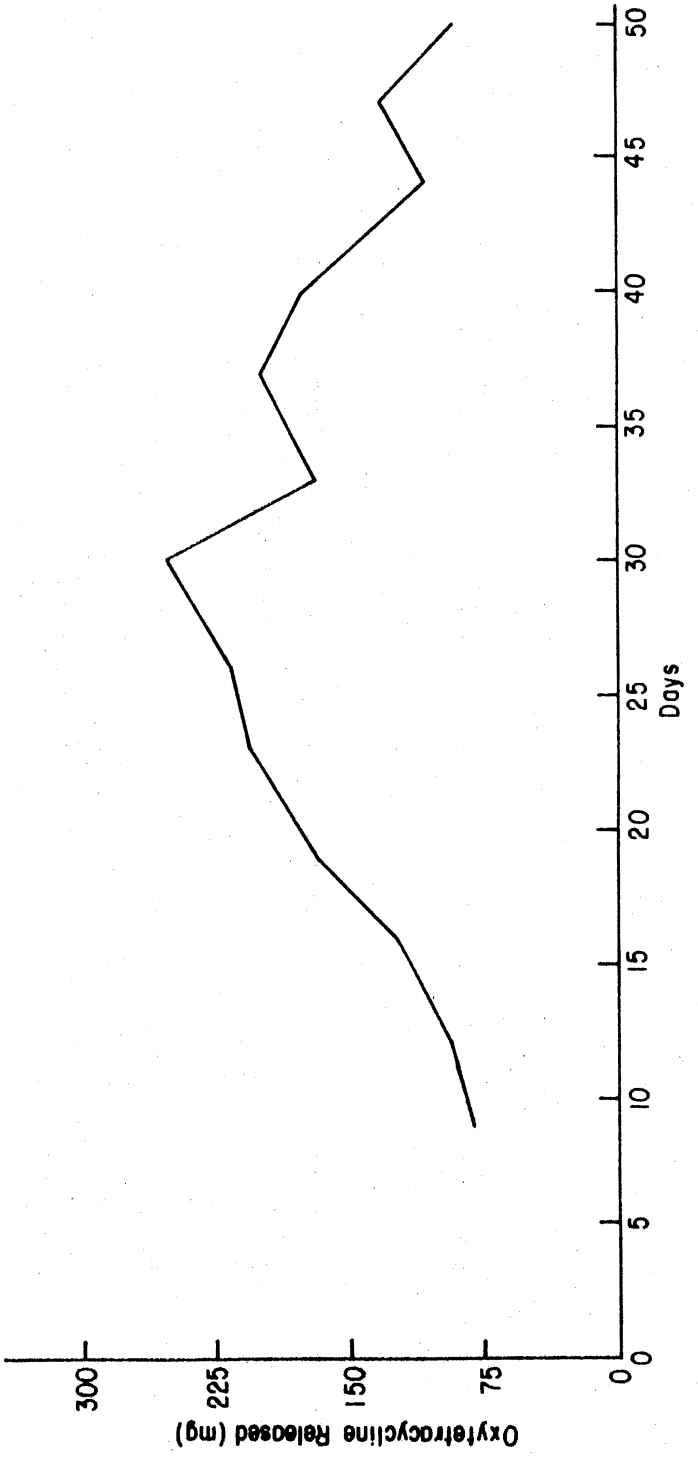
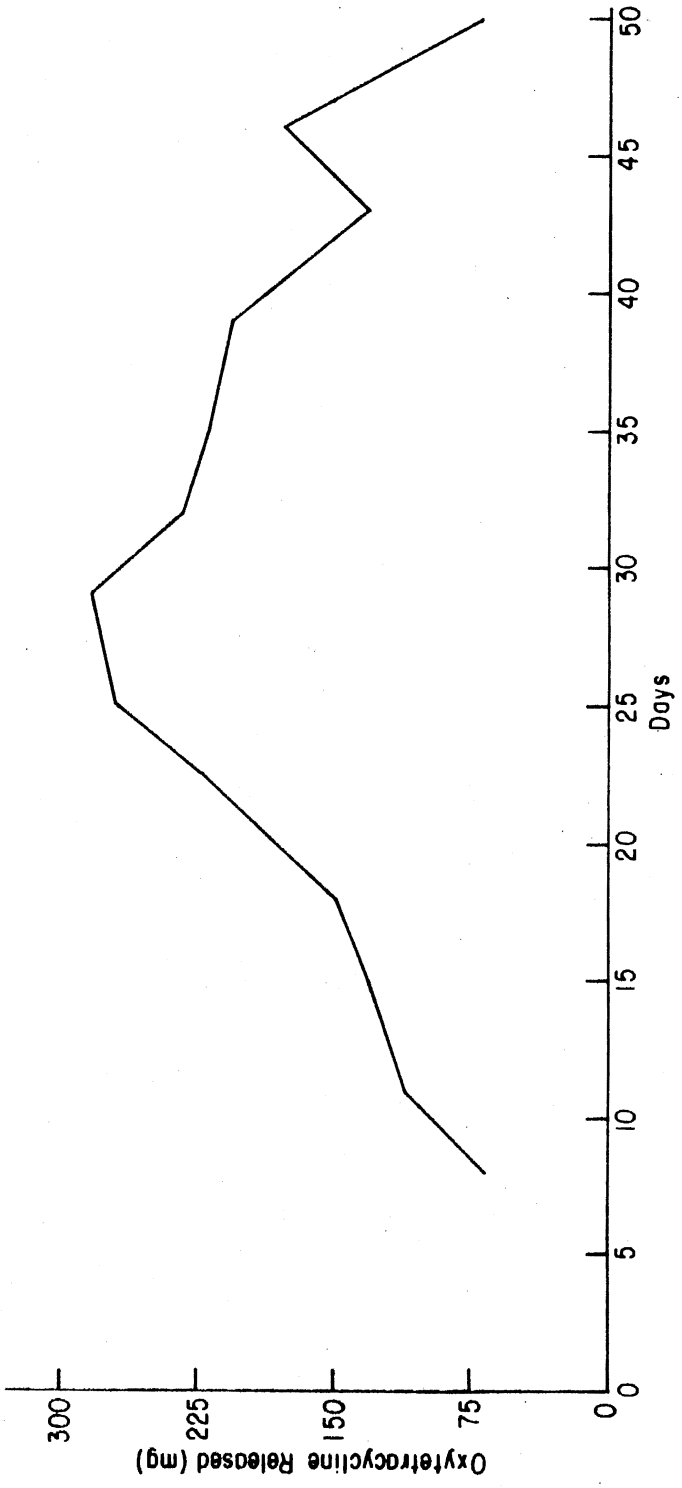

Oxytetracycline Released (mg)
300
225
150
75
0
0
5
10
15
20
25
30
35
40
45
50
Days

Figure 6. Degradation Analysis of 20 Percent Matrix VI Boluses With 2.8 Percent PEG


Oxytetracycline Released (mg)
300
225
150
75
0
0
5
10
15
20
25
30
35
40
45
50
Days

first 18 days (Figure 7). After this period, release rates increased to 178 mg/bolus/day. Also, the bolus surfaces were sluffing minute chips suggesting incompatibility and consequently, contributing to the daily variability noted during this test period.

As a result of varying the PEG concentration, PEG was considered to be the most advantageous additive to facilitate bolus erosion. The release rates were found to be proportional to the amount of PEG present in the bolus. Also, it was noted that the day to day variation increased as the amount of PEG increased, and bolus longevity decreased as the PEG concentration increased.

Therapeutic Drug Quantities

Up to this point, data has indicated that oxytetracycline can be formulated for delivery in a sustained-release system and that therapeutic dosages can be maintained. Further in-vivo testing to determine the highest therapeutic drug level that could be offered to the animal and still remain compatible with the present composition illustrated that oxytetracycline blended at 14, 15, 17, 20 and 25 percent rates yielded several important findings. The release rates given in Figure 8 illustrated the significant trend in differences between the 14, 5, 17, 20 and 25 percent oxytetracycline boluses. As the amount of active ingredient increased, the bolus release rates also increased but were followed by a shortening in longevity. The 14 percent boluses eroded uniformly throughout the test period. During the first 20 days, bolus release rates never rose above 45 mg/bolus/day. Only the last 3 inspection days did release rates exceed 40 mg/bolus/day. Consequently, the trial was terminated on day 42 although there

Figure 7. Degradation Analysis of 20 Percent Matrix VII Boluses With 3.7 Percent PEG

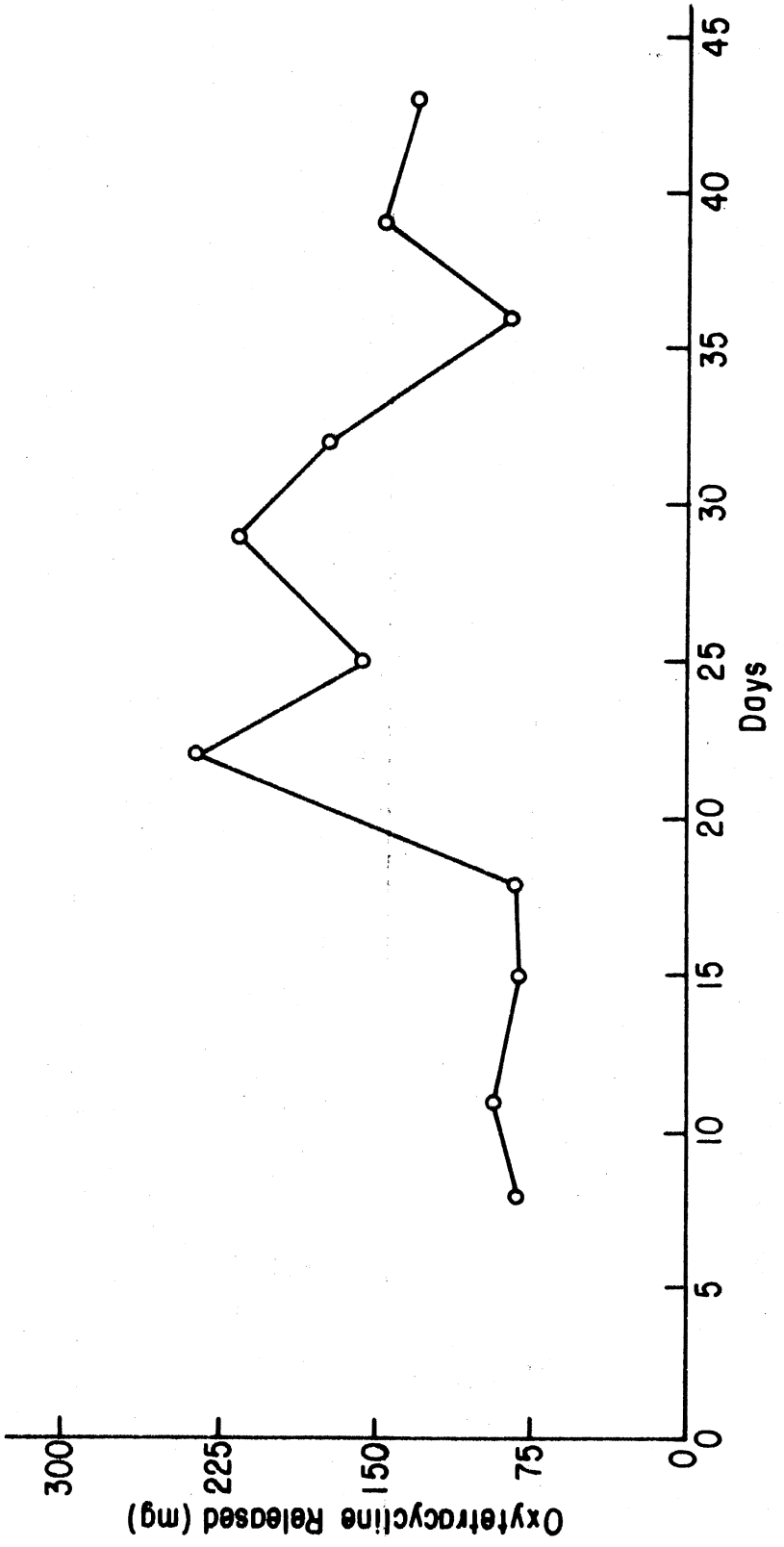
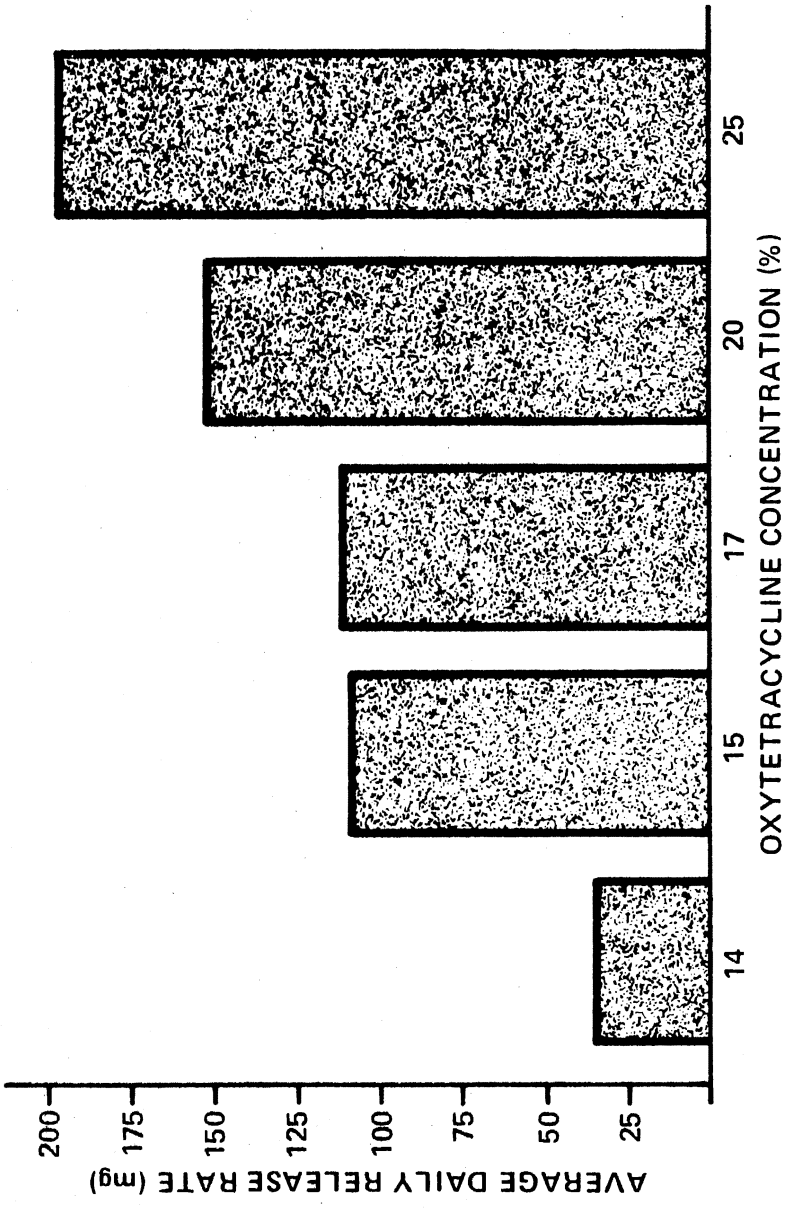

Oxytetracycline Released (mg)
300
225
150
75
0
0
5
10
15
20
25
30
35
40
45
Days

Figure 8. Comparison of Various Oxytetracycline Concentrations in a 45 g Bolus


AVERAGE DAILY RELEASE RATE (mg)
200
175
150
125
100
75
50
25
14
15
17
20
25
OXYTETRACYCLINE CONCENTRATION (%)

was sufficient material to last 25-35 additional days. The 15 percent boluses for the first 19 days had a mean release of 54 mg/bolus/day. During days 19-58 the mean release rate increased to 110 mg/bolus/day. As the boluses approached the weight of 5 g, the boluses bagan to chip and break in half. It was believed that bolus tensile strength was greatly decreased as the boluses became smaller in both size and weight. After such occurrences, broken boluses were found to influence the release rates greatly. The 17 percent boluses responded to the increased amount of oxytetracycline as was seen by a mean release of 123 mg/bolus/day for the first 51 days. After this period, release rates slowed down to yield a mean release of 110 mg/bolus/day for the 72 day study. Release rates recorded from the 20 percent oxytetracycline boluses had a mean release of 163 mg/bolus/day for the first 34 days. This increase in oxytetracycline delivery supported the assumption that an increase in the amount of active ingredient results in higher degradation and release rates since less binder is present in the formulation to hold the active ingredient (Table III). This study continued for 62 days with a mean release of 152 mg/bolus/day, a difference of 42 mg from the 15 and 17 percent boluses. The highest release rates were recorded from the 25 percent oxytetracycline boluses which had a mean release of 185 mg/bolus/day for the 42 day test period. Throughout the trial, release rates exceeded normal variability. This was due to a high level of oxytetracycline which was not conducive for proper particle binding. Also, the excessive variability noted from bolus to bolus was thought to be due to the fact that the boluses began to break in half at day 21. As they broke, the surface area of the bolus was increased and thereby caused an increase in bolus degradation.

TABLE III

RELEASE RATES OF INCREASING PERCENTAGES OF OXYTETRACYCLINE IN A 45 g BOLUS PREPARED FROM MATRIX II

Percentage Oxytetracycline in Bolus Composition	Mean Daily Release (mg) Over Test Period
14	31.3
15	110.0
17	110.5
20	152.6
25	184.5

In summarizing the data collected in this study, it was noted that the sustained-release of therapeutic drug levels could be achieved and theoretical bolus release rates were proportional to the amount of oxytetracycline present. The average daily release rates in this study were slightly above normal variability. Such variation could be attributed to animal variation in rumen content. Occasionally, fistulated animals would retain a more fluid filled rumen while others maintained the stratified layers (Smith et al. 1955) with various percentages of dry matter feed. Another factor contributing to the variability was the fact that location of individual boluses within the rumen influenced release rates. Those located in the reticulum had slightly higher release rates than those found in the ventral sac.

Commercial Preparation

The most promising ingredients providing the greatest compatibility and longevity were selected for commercial production testing. This study involved the addition of a lubricant (magnesium stearate) at rates of 0.5, 0.75, and 1.0 percent (Figures 9 and 10). Boluses formulated without any magnesium stearate proved to be more durable than those utilizing magnesium stearate. These bolus release rates remained between 105 and 130 mg/bolus/day during days 7 through 55. For the 60 day test period the mean oxytetracycline release rate was 112.5 mg/bolus/day. Oxytetracycline boluses with 0.5 percent magnesium stearate were less durable than those without magnesium stearate. These boluses followed a general pattern similar to the 0.0 percent boluses. Although the mean daily release of oxytetracycline remained at or above 100 mg/bolus during days 7 through 60, these release rates had a greater variability throughout the study. A mean release rate of 110.4 mg/bolus/day was recorded during the 60 day test period. The bolus with 0.75 percent magnesium stearate also remained between 100-121 mg/bolus/day for the entire 50 day study. Only the first inspection day had a release rate below 100 mg (95 mg). This formulation had a very consistent and uniform oxytetracycline delivery. Boluses eroded evenly during the test period with the exception that longitudinal cracks began to develop at the latter part of the test. For this reason, boluses were removed although they did not break apart. A mean release rate of 111.6 mg/bolus/day was observed. Those boluses containing the highest percent of magnesium stearate (1 percent) had a less durable composition than those just mentioned. A mean release

Figure 9. Analysis of a 0.5 Percent Magnesium Stearate Concentration in 20 Percent Oxytetracycline Boluses Prepared From Matrix II

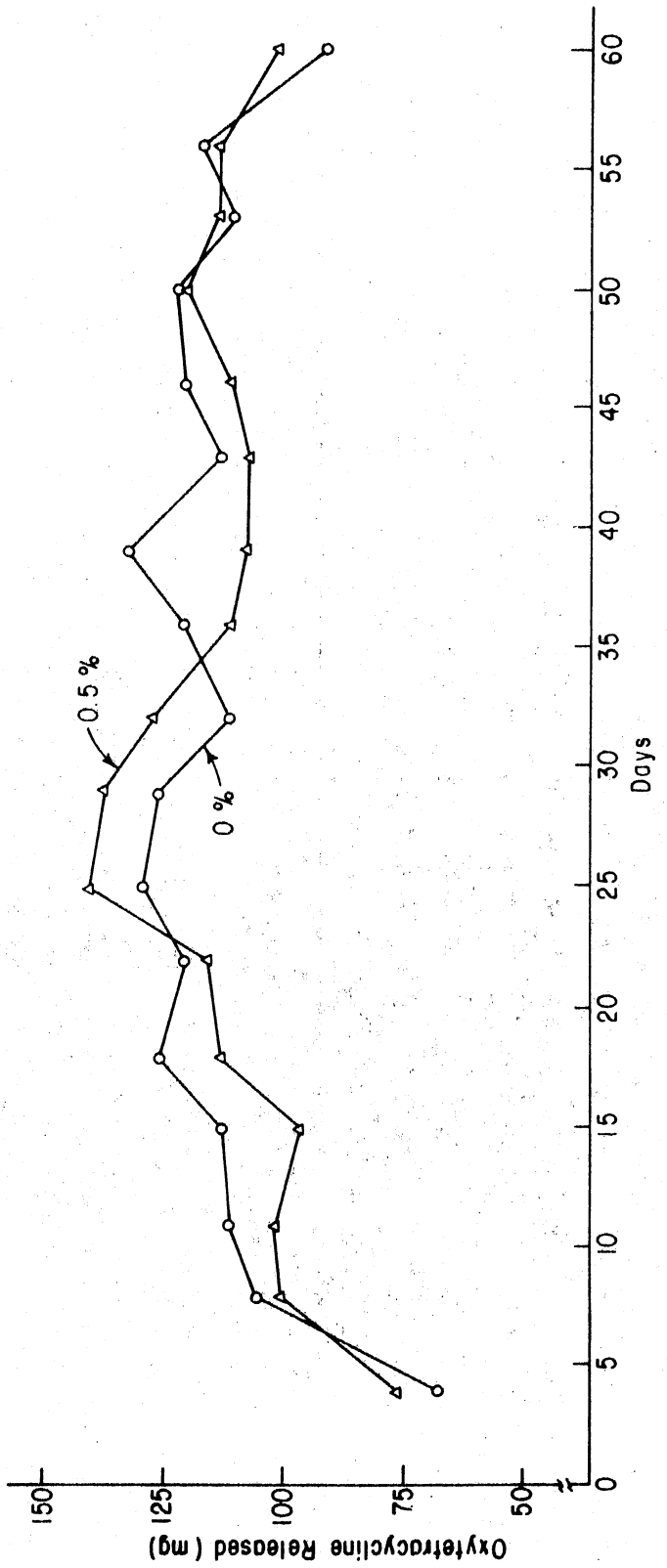
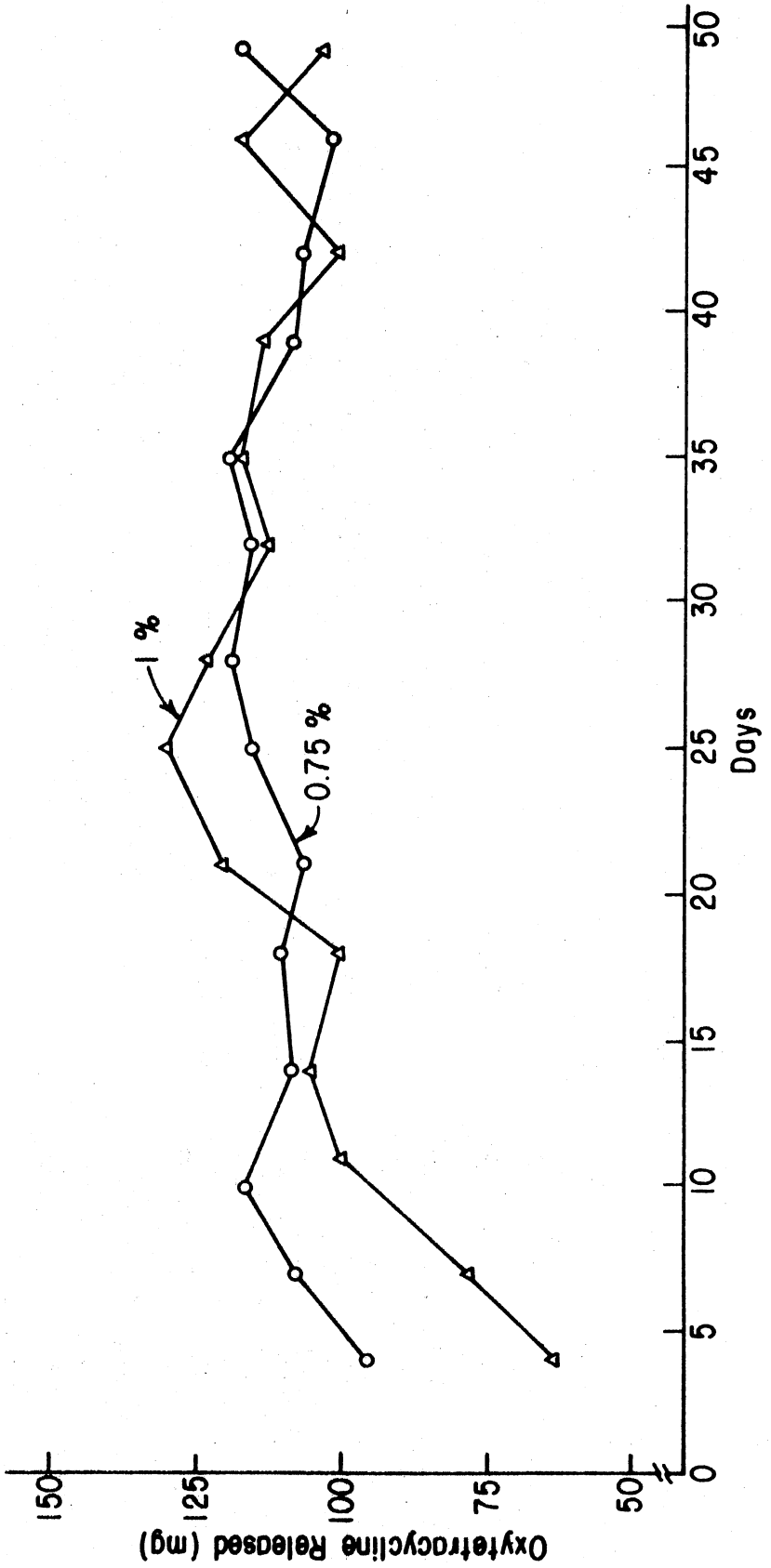

Oxytetracycline Released (mg)
150
125
100
75
50
0
5
10
15
20
25
30
35
40
45
50
55
60
Days
0.5 %
0 %

Figure 10. Analysis of a 0.75 and 1.0 Percent Magnesium Stearate Concentration in 20 Percent Oxytetracycline Boluses Prepared From Matrix II


Oxytetracycline Released (mg)
150
125
100
75
50
0
5
10
15
20
25
30
35
40
45
50
Days
1 %
0.75 %

of oxytetracycline at 104 mg/bolus/day was recorded for the 50 day test period but, release rates exceeded normal variability. The magnesium stearate appeared to influence tensile strength at the 1 percent level. Longitudinal cracks developed on day 27 and continued to expand even though boluses did not break in half.

Data from this study indicated that the lubricant magnesium stearate influenced oxytetracycline delivery. It was noted that the higher the concentration of magnesium stearate, the more variation seen in average daily release rates. Also theoretical release rates were found to decrease slightly as the magnesium stearate concentration increased. Based upon these results, the level of magnesium stearate most compatible with this formulation appeared to be the 0.75 percent level.

The 4 pharmaceutical grade carnauba waxes listed in Table II were screened for compatibility with oxytetracycline. During this study, all boluses were found to be eroding uniformly throughout the study. Termination of the study was initiated after 45 days although boluses were not completely degraded. This was done due to additional needs for these animals. Figure 11 shows that the 20 percent oxytetracycline boluses of the Brazilian Refined Flakes (light) Ross, eroded evenly with a mean daily release of 113.0 mg/bolus for the first 32 days. After this period, the release rates rose to 116.5 mg/bolus/day. For the entire 45 day test period, a mean release of 113.4 mg/bolus/day was recorded.

Boluses of the Brazilian Refined Flakes (dark) (Figure 12) were found to have a mean release of 103.3 mg/bolus/day for the first 32 days. This difference observed from the previous boluses was due to

Figure 11. Release of 20 Percent Oxytetracycline Boluses Utilizing Brazilian Refined Flakes (Light) Ross Carnauba Wax

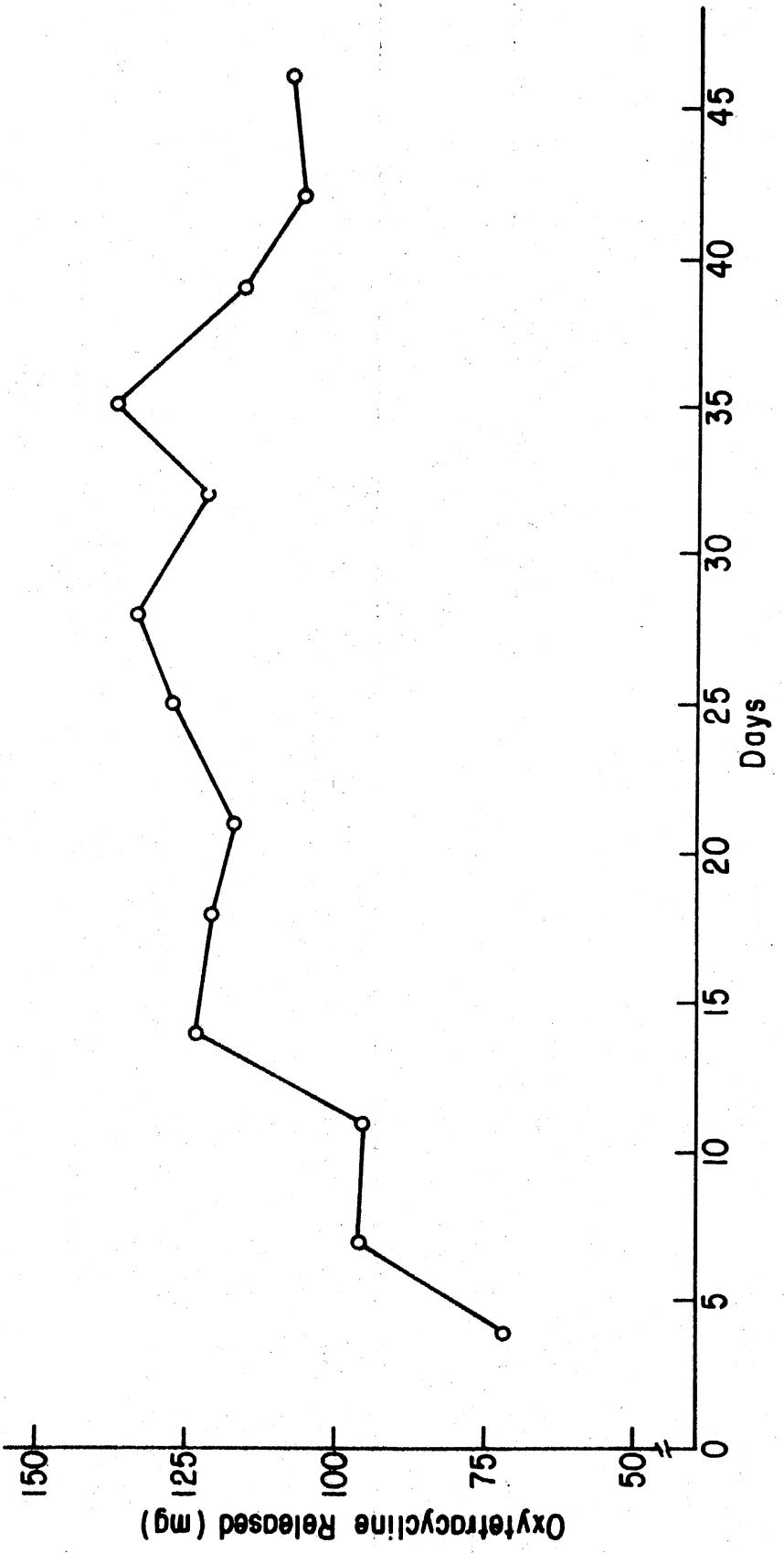
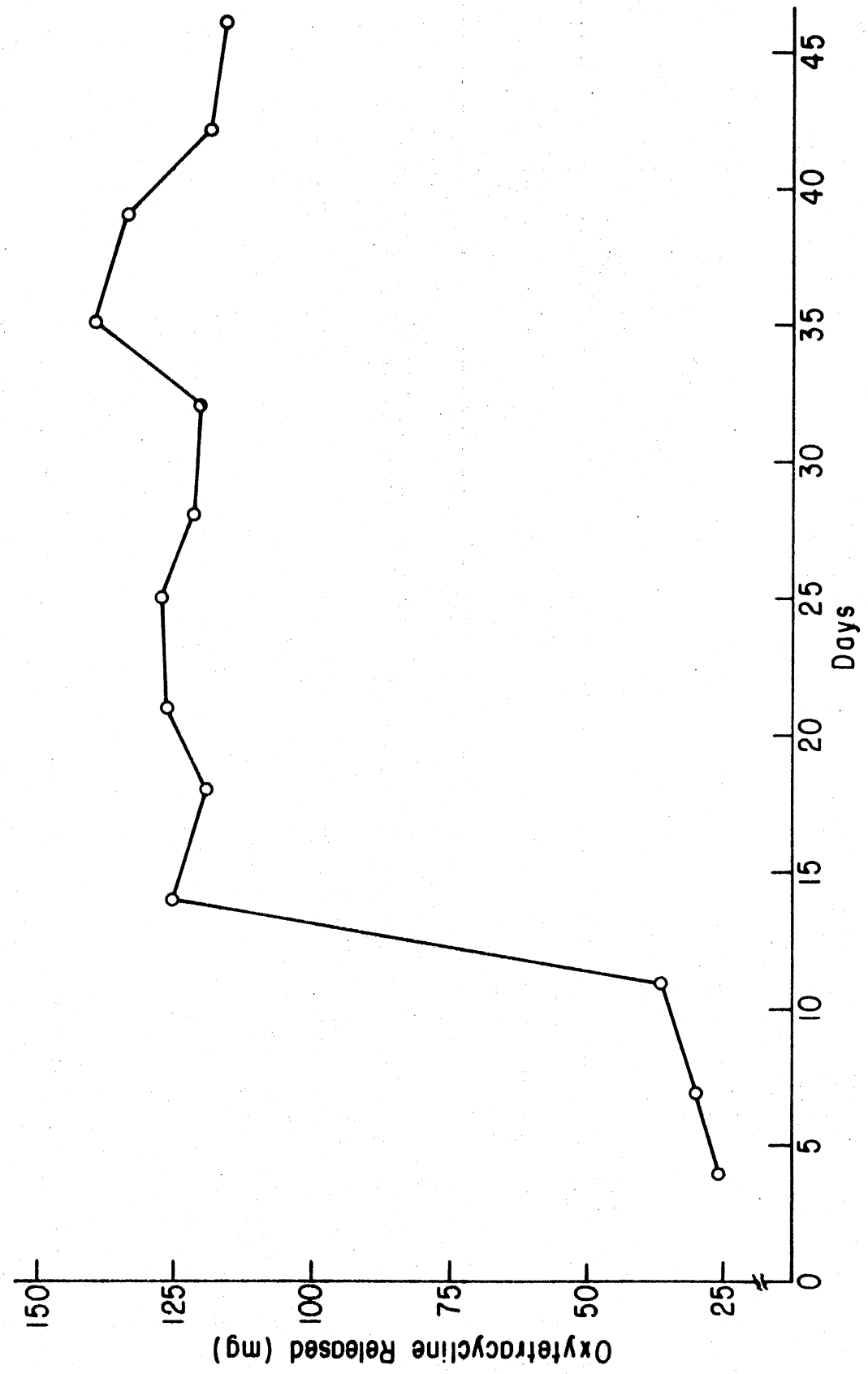

Oxytetracycline Released (mg)
150
125
100
75
50
0
5
10
15
20
25
30
35
40
45
Days

Figure 12. Release of 20 Percent Oxytetracycline Boluses Utilizing Brazilian Refined Flakes (Dark) Carnauba Wax


Oxytetracycline Released (mg)
150
125
100
75
50
25
0
5
10
15
20
25
30
35
40
45
Days

the lower release of drug for the first 3 inspection days. After this acclimation period to the rumen, boluses began to erode quicker with a mean release of 127.6 mg/bolus/day during days 14-46. The mean release rate for the test period was 111.0 mg/bolus/day. The highest release rates were recorded by the 20 percent oxytetracycline boluses from the Pure American Refined Flakes. Only on the first day of inspection were the mean release rates below 100 mg/bolus. Figure 13 shows that between days 7-46, a mean daily release of 130 mg/bolus/day was observed. These boluses exhibited a greater variation in bolus to bolus release rate than those afore mentioned. The 45 day test period had a mean release of 123.4 mg/bolus/day. Those boluses produced from the Ross Refined #3 light flakes (Figure 14) closely resembled those of the Brazilian Refined Flakes. A slow eroding bolus was noted for the first 3 inspection days. After this period, the release rates were extremely consistent. From days 14.46, a mean release of 124.6 mg/bolus/day was recorded. This trial had a mean release of 103 mg/bolus/day for the 46 day test period.

In summarizing this study, it was noted that mean release rates of 103.0-123.4 mg/bolus were recorded. These 4 pharmaceutical grades of carnauba wax proved to be similar to each other since only 20-23 mg difference was observed when comparing them. The significance seen in this study was that 2 of the grades had a slower release rate for the first 14 days, after which all carnauba grades appeared equal.

In summary, the data recorded during the development of a sustained-release oxytetracycline bolus indicated several important findings. Initial efforts of development indicated that the proper bolus components and rates of each were essential for successful

Figure 13. Release of 20 Percent Oxytetracycline Boluses Utilizing Pure American Refined Flakes Carauba Wax

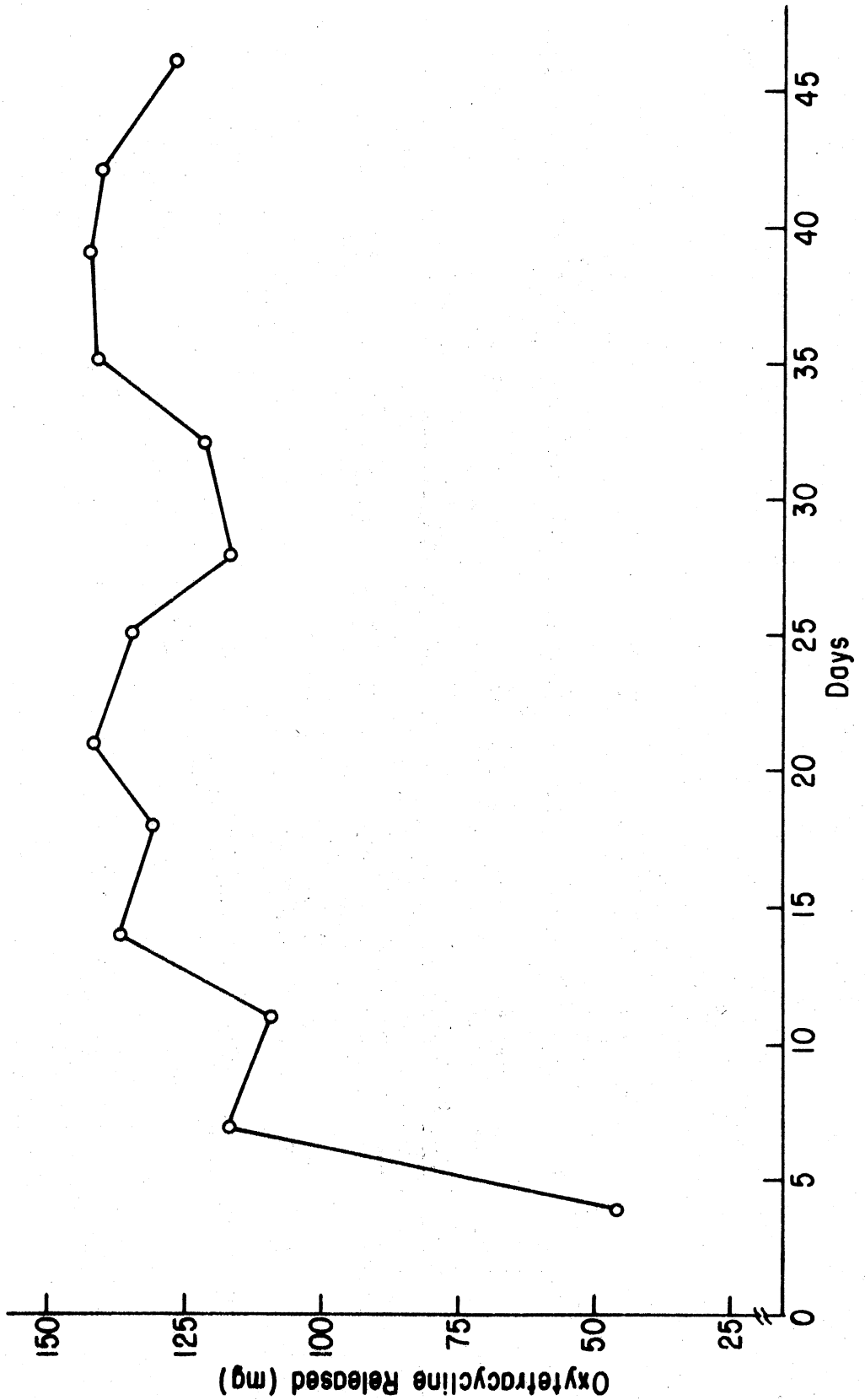
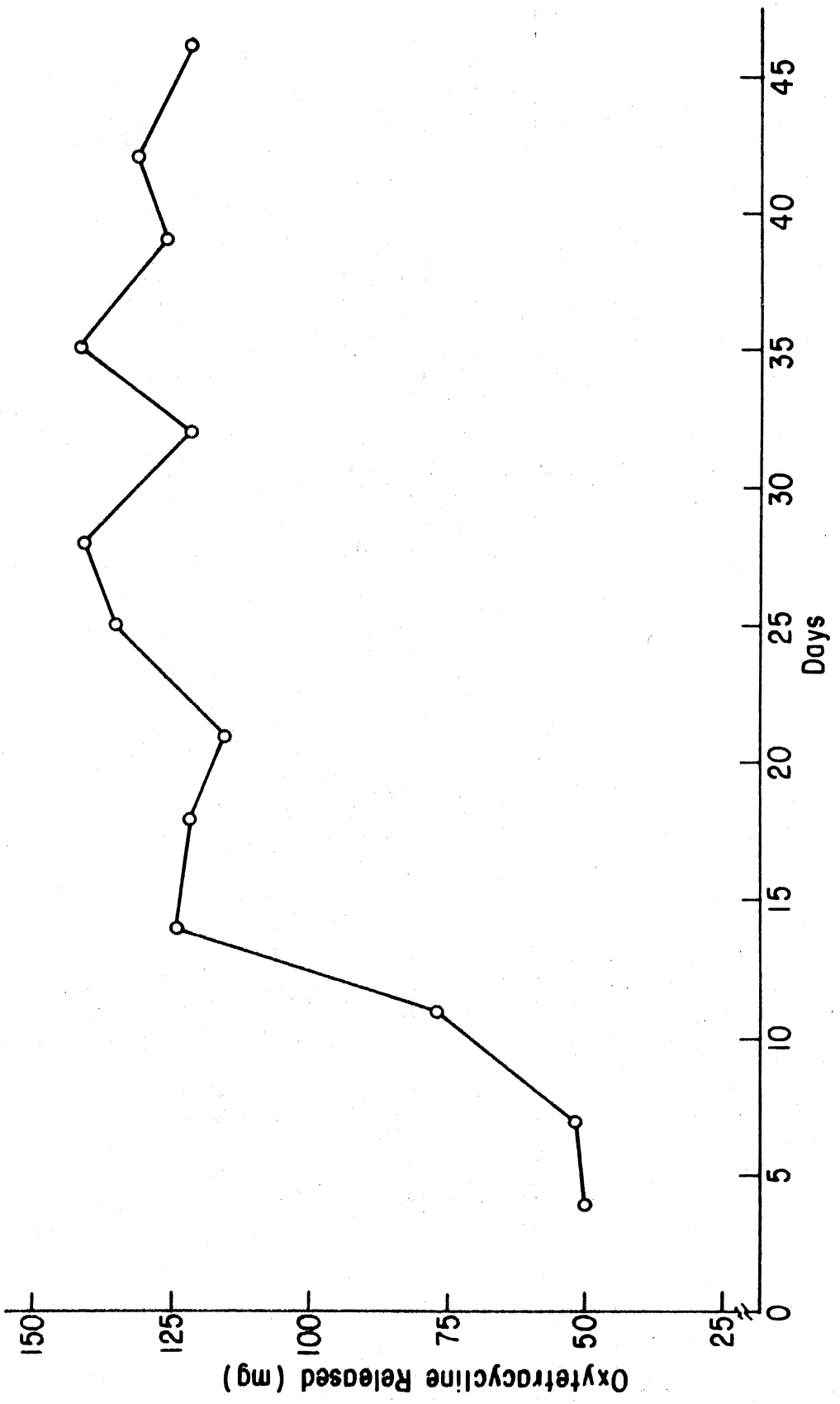

Oxytetracycline Released (mg)
150
125
100
75
50
25
0
5
10
15
20
25
30
35
40
45
Days

Figure 14. Release of 20 Percent Oxytetracycline Boluses Utilizing Ross Refined #3 Light Flakes Carnauba Wax


Oxytetracycline Released (mg)
150
125
100
75
50
25
0
5
10
15
20
25
30
35
40
45
Days

controlled-release systems. Sufficient amounts of binder, compression and PEG were needed to achieve a consistent and uniform release of oxytetracycline. It was also found that the most advantageous component to facilitate bolus degradation was PEG. As the percentage of PEG increased, the oxytetracycline release rates also increased. Along with the PEG, the theoretical release rates were found to be proportional to the amount of oxytetracycline present. In order to attain a product for commercial consideration, it was noted that the lubricant magnesium stearate at the 0.5-0.75 percent level was the optimum level necessary for commercial production.

Also, the 4 pharmaceutical grades of carnauba were found to be similar in comparison. Since commercial production would require the utilization of one of these waxes, minor refinements may be necessary to achieve a slightly higher release of oxytetracycline.

Based upon these results, it was noted that oxytetracycline could be offered to ruminants at a controlled release for an extended period of time. Also, data illustrated that therapeutic drug levels could be achieved and maintained. Although minor refinements may be necessary to produce a commercially available oxytetracycline bolus, the potential of the sustained-release oxytetracycline bolus has been demonstrated and therefore, encourages further testing to determine its efficacy against vector-borne anaplasmosis.

CHAPTER III

BIO-ASSAY FOR EFFICACY AGAINST VECTOR-BORNE ANAPLASMOSIS

Previous data collected from 20 percent oxytetracycline boluses prepared from an inert matrix containing carnauba wax, barium sulfate and PEG (Chapter II) indicated the provision of oxytetracycline at ca 160 mg/bolus/day for 50-60 days. Previous workers reporting on tetracycline treatments administered orally and via injections have indicated that low-level dosages of the drugs for extended periods of time would provide successful anaplasmosis control to bovine (Pearson et al. 1957). From a developmental standpoint, an efficacy trial was necessary to determine whether therapeutic blood levels could be maintained by a sustained-release bolus formulation. Such bio-assay data would credit this new treatment regimen and provide valuable information to further refine and develop the oxytetracycline bolus. The purpose of the following studies was to determine the efficacy of various drug levels in the prevention of anaplasmosis transmission by infective ticks frequenting cattle and to determine the potential of the oxytetracycline bolus against carrier infections.

Methods and Materials

Experimental animals consisted of Holstein and Jersey dairy cows, 4-7 years of age, which were purchased from an anaplasmosis-free dairy

herd. Prior to exposing the animals to the disease, hematocrits and compliment-fixation (CF) tests were conducted to determine whether the packed cell volumes (PCV) were within the usual range and that there was no serologic evidence of previous exposure to anaplasmosis.

To obtain anaplasmosis infective ticks for disease transmission, Dermacentor andersoni Stiles nymphs were reared and maintained at the Oklahoma State University Entomology Tick Laboratory. To serve as an infective blood source for the laboratory reared nymphal ticks, a splenectomized yearling Holstein calf was inoculated intravenously with a Virginia isolate of A. marginale. These nymphs were placed on the infected calf at a parasitemia of 1 percent. As tick feeding takes 7-14 days, the rapid stage of tick feeding was anticipated to coincide with the increasing parasitemia normally seen 10-12 days post-exposure. After development to the adult stage, the ticks were utilized for disease transmission studies.

Prior to drug therapy, the cattle were divided into several groups with 1 group designated as nonmedicated controls and the others designated as treatments. Animals were weighed 3 times prior to bolus treatment to determine the proper dosage of drug for each animal. All animals were then placed in cattle stanchions and maintained on a cotton-seed hull based ration to minimize gain during the test period. After the adult ticks had completed engorgement, animals were returned to the dry-lot area. Oxytetracycline treatment was administered via 20 percent boluses at the beginning of the test period (day 0). On day 10 all groups were challenged for disease transmission by exposing each animal to A. marginale by feeding infective D. andersoni ticks on these susceptible animals. Disease challenge was initiated on day 10

since therapeutic blood levels were reached after boluses had been in the rumen for 7-10 days. Each animal was infested with 75 pair of the adult D. andersoni ticks. Following exposure to the causal agent (day 10), the status of each animal was evaluated 3 times each week until the first sign of infection was noted. After which, daily blood samples were collected for evaluation by Wright's stained blood slides, PCV, and CF test.

Drug efficacy was ascertained by evaluating the PCV, CF test, and parasitemia values at each sampling to determine the influence of treatment on the ability to prevent disease transmission. Those animals found to be either negative or suspicious for the disease were subjected to further evaluation procedures by sub-inoculation of the treated animals blood into a splenectomized calf (Jones 1968). This calf was then evaluated by utilizing the previously mentioned hematologic and serologic techniques for detection of the disease.

Disease Transmission Studies

In initial testing to determine the effects of various oxytetracycline drug levels in the ability to prevent disease transmission by infective ticks, testing began to establish definite levels of antibiotic dosages for prevention of anaplasmosis transmission. Cattle were divided into 4 groups as shown in Table V. Group I was designated as nonmedicated controls. Oxytetracycline was administered to Groups II, III, and IV at a dosage of 0.23, 0.32, and 0.45 mg/kg of body weight (b.w.) respectively on day 0. After tick challenge on day 10, hematologic and serologic techniques previously described were conducted for drug-efficacy evaluation.

Data recorded during this preliminary trial indicated that the oxytetracycline level was not adequate to prevent anaplasmosis transmission. Therefore, a second study was designed to determine the drug range limits which would prevent disease transmission. In this study, the above mentioned procedures were utilized with the exception that oxytetracycline was administered at 0.35 and 1.0 mg/kg b.w. since Franklin et al. (1967) reported that 0.5-1.0 mg/kg would eliminate anaplasmosis infections.

In summarizing the data obtained during the 2 previous trials it was indicated that although disease transmission was not prevented, the usual prepatent period of the disease was prolonged considerably. Since previously used drug levels failed to prevent disease transmission, another study was designed using higher drug levels. In this trial, 4 groups of animals were utilized (Table IV). Group I were nonmedicated controls while Groups II, III, and IV were administered oxytetracycline at 2.0, 2.5 and 3.0 mg/kg respectively. Following drug administration (day 0) and tick challenge (day 10), all animals were evaluated using the previously described hematologic and serologic techniques.

Carrier Elimination

Tetracyclines have been extensively tested for efficacy in eliminating anaplasmosis carrier infections (Brock et al. 1958). Protection from spreading of the disease is needed during the insect vector season since anaplasmosis transmission can occur from carrier animals and therefore infect the entire herd. It was necessary that the efficacy afforded by the continuous-release of oxytetracycline to

TABLE IV

DRUG THERAPY DESIGN FOR EVALUATING SUSTAINED-RELEASE OXYTETRACYCLINE BOLUS AGAINST ANAPLASMOSIS TRANSMISSION

Group	Oxytetracycline Dosage	Mean Cattle Weight (kg)
I	Non-Medicated Control	749.3
II	2.0 mg/kg	671.0
III	2.5 mg/kg	589.6
IV	3.0 mg/kg	565.0

carier cattle on pasture be determined therefore, early pilot studies were designed to establish the proper dosage of oxytetracycline for removal of carrier infections in cattle.

Seven Holstein, Aeryshire and Jersey dairy cattle which were carriers of anaplasmosis and whose carrier status was determined by a history of disease attack as well as repeated positive reactions to the CF test, were divided into 4 groups with 1 group serving as a control. Treatment groups (I, II, and III) were comprised of 2 animals per group while Group IV was a nonmedicated control group. Oxytetracycline was administered at a rate of 1.0 mg/kg b.w. to Group I. Groups II and III were given oxytetracycline at a dosage of 1.5 and 2.0 mg/kg b.w. respectively. Animals were bolused accordingly with a 20 percent oxytetracycline bolus. Blood was collected weekly from each animal and success or failure of treatments were determined by a CF test and

the subinoculation of treated animals' blood into splenectomized calves when the CF test warranted. After 60 days if no positive results were recorded, animals were rebolused for an additional 60 days.

In subsequent testing, 15 Hereford and Angus cattle determined carriers by CF test were acquired for experimental use. These animals were maintained on 160 acres of native pastureland located in Payne County, Oklahoma. Animals were weighed 3 times prior to drug therapy to determine the proper bolus treatment. Previous studies implied that a minimum drug level of 1.5 mg/kg b.w. was required. Therefore, these animals were divided into 5 groups as shown in Table VIII. With 3 animals in each group, Groups I, II, III and IV received oxytetracycline at a rate of 1.5, 2.0, 2.5 and 3.0 mg/kg b.w. respectively. Due to the fact that the 20 percent oxytetracycline bolus had a longevity of 60 days, additional bolusing was administered at the end of this period in order to provide a 120 day treatment. Group V was designated as nonmedicated controls. After animals were bolused accordingly, blood samples were collected on a weekly basis for evaluation using CF test. Following the 120 day bolus treatment, animals were held for an additional 60 days in order to allow for complete clearance of any oxytetracycline residues which may occur within the animals' tissues. After this period, 2 animals of each group determined negative or suspicious of anaplasmosis infection were prepared for subinoculation procedures. One hundred seventy-five mls of blood from 2 animals in each group were pooled and treated with neoarsphenamine as described by Jones et al. (1968). The blood was treated with this drug since the epirythrizoan complex is usually associated with anaplasmosis infections and thereby making it harder

for identification of the anaplasma bodies. This blood was refrigerated for 24 hours at 5°C before being injected into the splenectomized Hereford calves. PCV, parasitemia values and CF test were conducted on a daily basis to determine results.

Results and Discussion

Disease Transmission

Data recorded during disease transmission studies indicated that the animal serving as a source of infective blood for infection of D. andersoni nymphs reached a peak parasitemia of 48 percent. Since tick feeding occurred during this period, it appeared that a highly virulent infection was obtained for disease transmission via ticks.

Initial testing to establish definite levels of oxytetracycline dosages for prevention of anaplasmosis transmission, indicated that dosages of 0.23, 0.32 and 0.45 mg/kg were not sufficient to prevent disease transmission. The influence of treatment on the developing parasitemia is shown in Table V. The control group (Group I) showed a maximum parasitemia of 13 percent with onset occurring during the normal 20-24 day prepatent period. This parasitemia was greater than that observed among Groups II and IV. Group II had the lowest parasitemia of any treated groups but the dosage of oxytetracycline was not the highest level administered. This was thought to be due to the animals ability to regenerate red blood cells and thereby, warding off the virulence of the infection. It took 58 days for onset of the disease to occur in Group IV which had the highest level of drug. This prolongation in the prepatent period was due to the inhibitory

TABLE V

INFLUENCE OF OXYTETRACYCLINE TREATMENT AGAINST ANAPLASMOSIS TRANSMISSION BY INEFFECTIVE TICKS

	Packed Cell Volume (PCV)		Parasitemia	
Cattle Groups	Mean Preinfection Value	Minimum Percent	Maximum Percent	Prepatent Period
I (Control)	35	14.0	13.0	23
II (.23 mg/kg)	39	28.5	1.8	38
III (.32 mg/kg	34	19.5	16.4	57
IV (.45 mg/kg)	32	21.0	3.8	58

effects that the antibiotic has on the anaplasma organism. All animals converted to a positive CF titer. At no time did any of the treated animals appear critically ill. Constipation was noted in Groups I and III. Oxytetracycline treatment in Groups II and IV were effective in preventing and reducing clinical signs of the disease such as constipation, inappetence, and dehydration.

The use of anaplasmosis infected ticks appeared to produce a highly virulent infection as was noted by a rapid reduction in PCV in Groups I and III once onset occurred. Compared with the control group, response to oxytetracycline therapy by treated Groups II and IV indicated that the severity of the infection was greatly reduced.

Since previous testing illustrated that oxytetracycline levels were not sufficient to prevent disease transmission, a dosage range of

0.30 and 1.0 mg/kg b.w. was used to determine the level of drug that would prevent transmission. Results illustrated that disease transmission occurred at this range. All animals converted to a positive CF titer at an average of 42 days post-exposure. A comparison of drug efficacy is shown in Figure 15 by the PCV values. The severity of the disease was characterized in the control group by a minimum PCV of 18.5 percent. For the 0.3 mg treatment group a maximum parasitemia value of 6.1 percent was recorded with a minimum PCV of 16.5 percent. The 1.0 mg treatment group had a maximum parasitemia of only 0.2 percent while the minimum PCV was 24.5 percent. Even though disease transmission was not prevented, the infectivity level was reduced substantially. More importantly, is the fact that clinical disease was prevented with the 1.0 mg level since the parasitemia never reached 1 percent. Again, oxytetracycline demonstrated its inhibitory effect on the causal agent.

Data for studies reported herein indicated that clinical anaplasmosis could be prevented with a dosage of 1.0 mg/kg. Although previous drug levels proved inadequate in preventing disease transmission, data indicated that a slightly higher level would prevent transmission therefore, the dosage was increased (2.0, 2.5 and 3.0 mg/kg) and response to oxytetracycline therapy is shown in Table VI. In this study the virulence of the infection was characterized by a minimum PCV of 12.5 percent while a maximum parasitemia of 20.6 percent was noted in the control group. The variation of parasitemia in each of the 4 groups was significant. The severity of the infection recorded coincided with the administration of the higher dosage of oxytetracycline. The significance in this study was that disease

Figure 15. Packed Cell Volume of Cattle Treated With 20 Percent Oxytetracycline Boluses

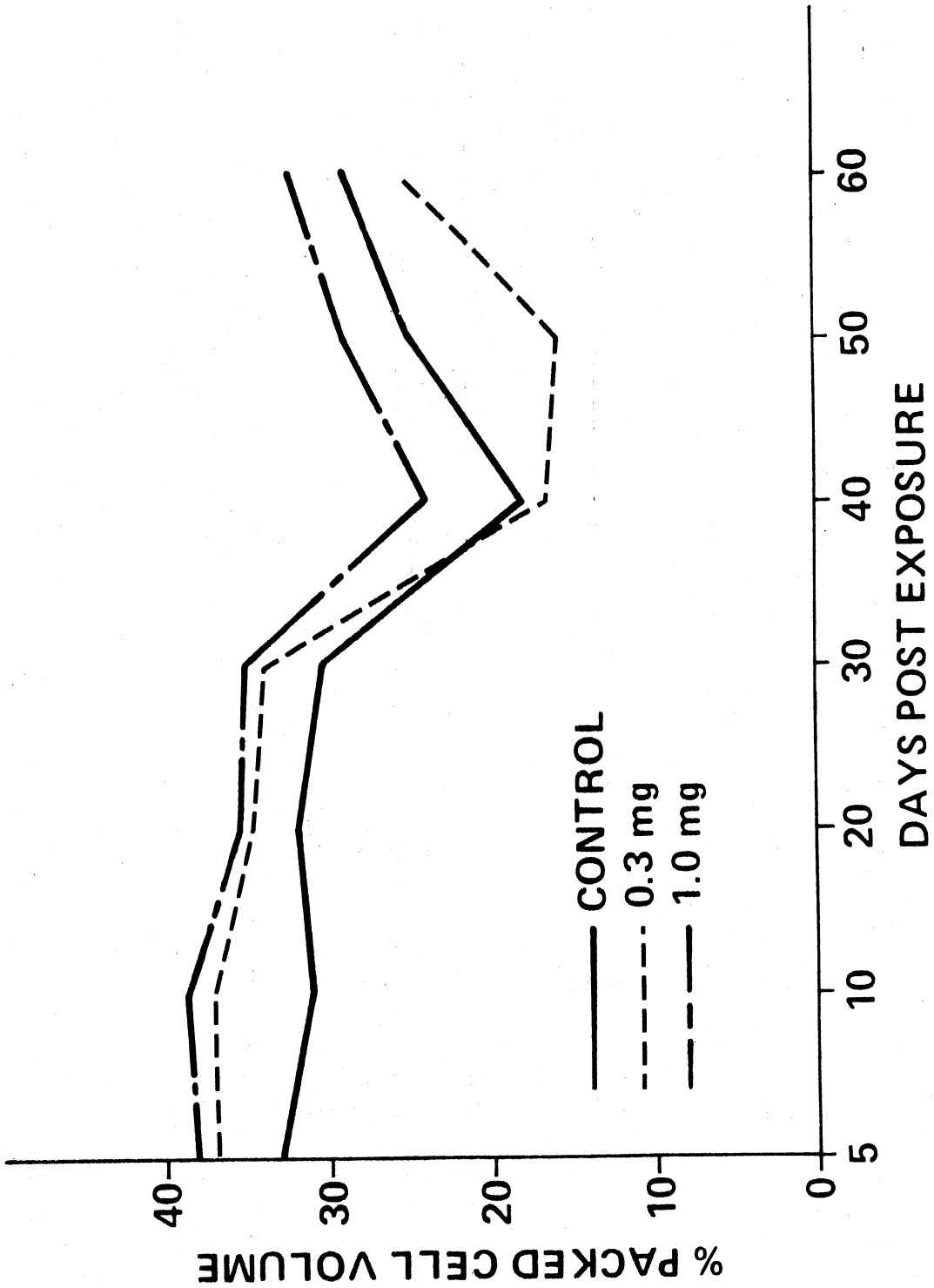

% PACKED CELL VOLUME
40
30
20
10
0
5
10
20
30
40
50
60
DAYS POST EXPOSURE
CONTROL
0.3 mg
1.0 mg

TABLE VI

RESPONSE OF OXYTETRACYCLINE BOLUS THERAPY AGAINST DISEASE TRANSMISSION BY INFECTIVE TICKS

	Packed Cell Volume (PCV)		Parasitemia	
Cattle Groups	Mean Preinfection Value	Minimum Percent	Maximum Percent	Prepatent Period
I (Control)	32	12.5	20.6	30
II (2.0 mg/kg)	37	22.0	5.2	31
III (2.5 mg/kg)	36	30.5	0.4	44
IV (3.0 mg/kg)	34	24.0*	0.0	--

*Low PCV due to severe case of mastitis

transmission was prevented in Group IV at a dosage of 3.0 mg/kg b.w. as was determined by blood slides and CF tests. This animal did have a lowered PCF (24 percent) which was due to development of a severe case of mastitis. This animal was severely affected by this infection but no treatment was administered since the animal was still on study. After 58 days post-exposure to anaplasmosis and prior to subinoculation procedures this animal died due to the mastitis infection. Treatment at all 3 levels demonstrated some effectiveness when compared with the nonmedicated controls. Based on the influence of oxytetracycline therapy on PCV and parasitemia, a pattern of effectiveness from each dosage was observed. The severity of infection was reduced in Groups II and III with only the subclinical disease occurring in Group III. This trial illustrated that the oxytetracycline bolus has the potential

to prevent disease transmission by infective ticks and to prevent clinical anaplasmosis from occurring.

Carrier Elimination

Low levels of tetracyclines have been shown to be effective in eliminating the carrier state of anaplasmosis. It was felt that treatments via a 20 percent oxytetracycline bolus at 1.0-2.0 mg/kg b.w. for 60-120 days would also be effective. In this preliminary trial, Table VII summarizes the results. All groups had high serum titers at the time treatment was given. CF testing occurred on a weekly basis with titers remaining at 4+ (positive reaction) throughout the first 60 day period. Animals were rebolused for another 60 days and Group II cattle showed a 2+ and 3+ titer (suspicious reaction) to the CF test during the last 30 days of treatment. After 120 days from initial bolusing, a splenectomized calf was inoculated with blood from Group II animals. This calf developed anaplasmosis after a prepatent period of 27 days. Results of this experiment indicated that total elimination of the carrier infection was not possible at these dosage levels. However, the treatment was obviously bordering the line between success and failure.

Previous testing implied that animal drug levels were above the 1.5 mg level therefore, efficacy trials for elimination of carrier infections continued. Table VIII shows the drug design and the influence of treatment on the carrier state. It was anticipated that the response to oxytetracycline therapy would be directly related to the drug dosage. However, data illustrated that this did not occur.

TABLE VII

RESPONSE OF CATTLE TO OXYTETRACYCLINE BOLUS TREATMENTS FOR ELIMINATION OF ANAPLASMOSIS CARRIER INFECTIONS

Group	Treatment (mg/kg)	Dosages	Mean CF Titer*	
			At Time of Treatment	120 Days After Treatment
I	1.0	2	160	10R
II	1.5	2	40	< 5S
III	2.0	2	160	10R
IV	control	none	80	45R

*Titers expressed as reciprocal of the highest dilution of sera showing fixation of compliment.

Those animals receiving 1.5 mg oxytetracycline/kg b.w. showed a suspicious (3+) CF titer within the first 30 days of treatment. This group remained either negative or suspicious throughout the 120 day treatment period and remained so for 60 additional days (i.e. 180 days total). Group II animals exhibited positive reactions to the CF test up until the 120 day period, after which 2 animals were observed to be suspicious (2+) for the disease. Those animals in Group II, III, and IV remained positive for anaplasmosis throughout the 180 day test period. It was anticipated that the response to oxytetracycline treatment at the higher dosages was influenced by the fact that the number of boluses required to deliver this rate to a large animal caused release rates to exceed the normal delivery. Studies conducted

TABLE VIII

DESIGN AND INFLUENCE OF OXYTETRACYCLINE TREATMENT ON ANAPLASMOSIS CARRIER INFECTIONS

Group	Weight (kg)	Dosage (mg/kg)	CF Titers		
			At Time of Treatment	120 Days After Treatment	180 Days After Treatment
I	505	1.5	10R	N	N
	460	1.5	20R	S	N
	465	1.5	10R	N	S
II	474	2.0	5R	10R	5R
	453	2.0	10R	S	S
	455	2.0	5R	S	N
III	460	2.5	5R	10R	5R
	483	2.5	40R	40R	20R
	455	2.5	5R	5R	S
IV	383	3.0	5R	10R	10R
	381	3.0	5R	40R	10R
	304	3.0	5R	20R	20R
V	471	control	10R	10R	10R
	433	control	10R	20R	5R
	496	control	10R	20R	10R

concurrently have indicated that a relationship between the size of an animal's rumen and bolus degradation exists (Riner 1980, unpublished data). A 400 kg animal dosed at 3.0 mg/kg b.w. required a treatment of 8 boluses. Previous efforts in developing the oxytetracycline bolus were aimed at a 2-6 bolus treatment. As the increased number of boluses were used, the closeness in proximity of individual boluses caused an increase in release rates. This consequently caused a shortening in bolus longevity thereby decreasing the treatment period. Also, this large a number of boluses rubbing together within the bovine rumen increased the chances for breakage of the bolus. Such occurrences were thought to have happened in Groups IV and V. Another possible explanation was that the interaction of antibiotics with the anaplasma organism caused a false positive CF titer. Other researchers have reported that subinoculation of blood from a CF positive animal into a splenectomized calf resulted in the splenectomized calf showing no signs of the disease.

Since the CF test remained negative in cattle from Group I which later proved to have anaplasma bodies by subinoculation techniques, there were indications that the CF test was not truly accurate for determining the success of animals treated with antibiotics. The splenectomized calf which did not convert to a CF positive titer was thought to have an increased resistance to anaplasmosis. The significance of this phenomenon is not known.

In summarizing the data collected during this experiment, it was noted that the level of the antibiotic maintained in the animal was an important factor in successful eradication of the carrier state. This

trial simulated field situations where cattle of varying ages and varying stages of infection occur. The merits of this treatment program were demonstrated by the fact that the positive CF titers were slowly reduced to the suspicious or negative status.

CHAPTER IV

SUMMARY

The purpose of the experiments described in this thesis was to develop and evaluate a sustained-release oxytetracyline bolus for prophylaxis and control of vector-borne anaplasmosis in bovine. Developmental procedures involved the incorporation of oxytetracycline into an inert matrix consisting of various ingredients, compositions and rates of each. The potential of the sustained-release bolus was determined by comparing each formulation for a predictable and controlled-release of drug for an extended period of time. The efficacy of the oxytetracycline bolus for prevention of anaplasmosis transmission and treatment of carrier animals was evaluated by CF test, Wright's stained blood films and PCV. Further evaluation by subinoculation of host blood into susceptible splenectomized calves was conducted when warranted.

Data indicated that oxytetracycline could be formulated in a sustained-release system to deliver a uniform and consistent release of drug for a 45-60 day period and several parameters examined experimentally support the conclusion. Results indicated that the provision of ca 160 mg/bolus/day from a 20 percent oxytetracycline bolus maintained effective therapeutic levels for 60 days. Furthermore, efficacy trials indicated that dosages of oxytetracycline at 0.5-2.5 mg/kg

administered 10 days prior to exposure to A. marginale inhibited the multiplication of the organism and decreased the level of infectivity. This resulted in the prevention of clinical anaplasmosis. It was also found that a dosage of oxytetracycline at 3.0 mg/kg prevented disease transmission by infective D. andersoni ticks.

The treatment of infected carrier animals with dosages of 1.5-3.0 mg/kg at 60 day intervals decreased and eliminated positive CF titers. This suggested that the carrier state was eliminated but confirmation of these results by subinoculation procedures indicated that the total elimination of the organism was not accomplished. This was thought to be due to some type of interaction of the antibiotic with the causal organism resulting in a false positve CF titer or that the CF test was not truly accurate.

Although current treatment regimens have been shown to prevent and control anaplasmosis, the feasibility and practicality of these programs have not been demonstrated. The phenomena of a controlled-release formulation offers an approach to arthropod and disease control which could potentially solve many of the problems associated with current control methods. By administering an acaricide or therapeutic agent via a sustained-release system sufficient to provide season-long control to the animal, several advantages could be realized. The offering of biologically active compounds only once or twice per season would allow producers to save time and labor thereby, resulting in a greater economic advantage. Compared to repeated and periodic applications as currently used by most treatment regimens, the predictable and consistent release of therapeutic levels via a sustained-release bolus can be maintained for 60 days with only 1 application.

The application of a sustained-release formulation as indicated by these studies are important in preventing the perpetuation and transmission of the disease. The increasing prevalence of anaplasma reactors, the moderate rate of transmission and the possibility of introduction of infected cattle by purchase suggest that a treatment program for anaplasmosis prophylaxis and control should extend over a prolonged period. The use of a sustained-release oxytetracycline bolus affords such a treatment and could provide the basis by which the control and eradication of the disease could be accomplished.

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