SUSTAINED-RELEASE RUMINAL BOLUSES AND FACTORS DETERMINING THEIR RELEASE RATES

By

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CHAPTER I

INTRODUCTION

Chemical control of ticks on cattle can be difficult due to the inaccessibility of the locations where the arthropod feeds. Recommendations call for spraying, dipping, and the use of ear tags and are often based on topical application directly to the site of attachment. These control methods often prove to be laborious and time consuming to the producer due to the repetition required for effective long-term control.

Systemic insecticides have proven effective against insects that feed primarily by drawing blood from their hosts. Effective control is offered as long as the blood concentration of pesticide remains high enough to prove lethal to the target organism without exceeding the toxicity allowances of the host animal.

In past years the only satisfactory means of administering systemic pesticides was by additives via water, mineral blocks, and ration and through the use of injectables and pour-ons. These methods had many draw-backs and often proved insufficient for effective control. Lack of labor and facilities may restrict the use of injectables and pour-ons, especially when the producer must handle his animals several times during a season for adequate control.

Under herd conditions, effective control is difficult when materials are included in water, feed or mineral blocks due to differences in intake. Within a given herd, some animals never consume enough of the agent to achieve therapeutic blood levels while others consume excessive amounts which may cause toxicity. The lack of an efficient means of administering systemic acaracides to supply needed daily doses for extended periods dampened further development of the systemic approach to tick control.

Controlled-release devices which release biologically active compounds uniformly and continuously to the host animal over an extended period of time have been developed recently. Animal health and production has increased in recent years due to the development of several controlled-release systems. Stilbestrol, a hormonal growth stimulant, has been found to offer increased weight gains following one implantation beneath the skin of the ear. Insecticide-impregnated bands and tags have shown promise in controlling certain ectoparasites of livestock. Plastic polymers which are impregnated with various insecticides can be attached to or around the ear and offer season long control of ticks and flies.

Solid oral tablets called boluses which are retained in the rumen of sheep and cattle and release components over an extended period of time can also provide sustained-release of biologically active substances. Supplementary amounts of cobalt for sheep and cattle grazing on cobalt deficient pastures in Australia can be provided through erosion of a ruminal bolus. Utilizing this same principle, other materials such as vitamins, minerals, anti-bloat compounds, and antibiotics can also be supplied to animals for nutritional and therapeutic reasons.

Due to the promise and partial success of implants and ruminal boluses, work began at Oklahoma State University a few years ago in the development of a sustained-release bolus for tick control. The product developed after approximately 3 years of effort was a 35 gram bolus of 50% technical famphur with a specific gravity of 1.62 gm/cm^3.

Development of the 50% famphur bolus raised many questions and problems. Problems of regurgitation, possibly due to the low specific gravity of the bolus, and transverse cracks due to a lack of tensile strength were prominent. Other problems related to variation among animals were encountered but not fully understood.

A new group of compounds called formamidines has been found to possess systemic activity against blood feeding ticks. These compounds when infused into the rumen of sheep caused detachment of adult ticks as well as a reduction in total larvae produced by engorged females. Since there were no systemic compounds unique to bolus formation available, formamidines were tested as possible candidates for future bolus development.

The first of 3 parts of this manuscript was to test formamidines for systemic activity in search of candidate compounds for future bolus development. The second part was to further investigate and make improvements in the famphur bolus by increasing bolus density and tensile strength. The last and perhaps most important part was a series of studies designed to determine the factors involved in the degradation of sustained-release ruminal boluses.

CHAPTER II

EVALUATION OF FORMAMIDINES FOR SYSTEMIC ACTIVITY AGAINST BLOOD-SUCKING ARTHROPODS

Formamidines are a relatively new group of compounds that show insecticidal, miticidal, and ovicidal activities. While observing the effects of chlordimeform, Roulston et al. (1971) noted detachment of the cattle tick *Boophilus microplus* (Canestrini) when topical applications at concentrations as low as .0015% were made. Detached ticks were seen wandering and falling from the host without reattaching.

Knowles and Roulston (1973) found 29 formamidines and related compounds that displayed activity toward the engorged female southern cattle tick, *B. microplus*, by reduction in oviposition and egg viability. Gladney et al. (1974) found that topical applications of very low concentrations (.03%) caused detachment of the lone star tick, *Amblyomma americanum* (L.), the brown dog tick, *Rhipicephalus sanguineus* (Latreille), and the Rocky Mountain wood tick, *Dermacentor andersoni* (Stiles), from guinea pigs.

Teel (1978) tested several formamidine compounds and found that UC55304 infused into the rumen was most effective at a daily dose of 7 mg/kg body weight. The purpose of the following studies was to further investigate UC55304 and related compounds for systemic activity against a number of blood-sucking arthropod species and to compare them to other compounds as candidates for future bolus developments.

Materials and Methods

Study I

Six grade ewe lambs of uniform age and health were fitted with plastic corkscrew-type cannulas (Haver-Lockhart Laboratories, Shawnee Mission, Kansas). These cannulas were applied surgically and provided direct access to the rumen through a 10 millimeter orifice. This orifice was reduced to 7 millimeters by threading latex rubber tubing through the cannula, leaving approximately 4 centimeters externally for constriction with a tubing clamp. Following 10 days post-operative recuperation, lambs were randomly selected and assigned to stanchions in a 6-stall laboratory. Lambs were maintained at $20^{o} \pm 5^{o}$C with a 14 hour photophase and allowed 3 days to acclimate to laboratory conditions. Water and feed was available ad libitum and monitored daily as one criterion of animal health.

The formamidine compound, UC55304, was administered in acetone directly into the rumen at the daily rate of 7 mg/kg body weight to the 3 treatment lambs by procedures of Teel et al. (1977). The 3 control lambs received acetone only. Solutions were infused ruminally at the continuous rate of 1 ml/hour. Following 72 hours of infusion, treatment and control lambs were challenged with _A. maculatum_ (Koch), _A. americanum_, _D. variabilis_ (Say), _D. andersoni_, and the bed bug, _Cimex lectularis_ L.

Twenty adult pairs of each tick species was confined to each lamb in separate stockinette cells which were attached to the animal with contact cement. Approximately 50 nymphs of each species were confined in plexiglas cells attached by the same manner. Adult female mortality was calculated from total ticks attached and includes those that detached and died without successfully reattaching. Adult female repletion, as a percent, was based on those that successfully repleted compared to those that remained attached but did not replete. Engorged females were weighed and placed in individual vials, and held at 92 ± 3% relative humidity, $20^{o} \pm 5^{o}C$, and 14 hour photophase. Replete females were observed for first signs of egg laying and egg mass weights were determined 20 days post-oviposition. Following another 40 days observation (60 days post-oviposition) to allow all eggs to hatch, percent hatch was determined and recorded. In accordance with Drummond et al. (1972) an estimated larval population (EL) was calculated as follows:

EL = g eggs x estimated percent hatch X 20,000

(estimated larvae produced from 1 g of eggs).

Percent mortality of nymphs was determined by comparing the number attached to the number that successfully repleted. Engorged nymphs were held under the aforementioned conditions and molting success was determined and expressed as a percent. Twenty, fourth instar C. lectularius were allowed to feed on each lamb for 30 minutes and held to determine percent mortality at 24 hour intervals.

Study II

Four experimental compounds, UC55304, UC55353, UC55284, and UC58830 were administered via ruminal cannulas at 10 mg/kg body weight/day in acetone at the rate of 10 ml/hour as described by Teel et al. (1977). The 4 treatment and 2 control lambs were challenged with 20 adult pairs and approximately 50 nymphs of the lone star, A. americanum, Gulf

Coast, *A. maculatum* and the American dog tick, *D. variabilis*. Additional methods and measurements were as described in Study I.

Results and Discussion

Study I

Table I summarizes data collected when lambs receiving UC55304 at the rate of 7 mg/kg body weight/day were challenged with 4 tick species. As previously shown by Teel (1978), UC55304 was most effective against the Genera *Dermacentor*. Except for slight control indicated against engorging nymphs, UC55304 was not effective against feeding ticks. Control was evident by high adult and nymphal mortality following repletion and reduced production of viable ova.

UC55304 was most effective against *D. andersoni*, producing the lowest average repletion weight, lowest egg mass weight and lowest percent hatch of the 4 species tested. Adult and nymphal mortality and molt inhibition also were highest with this species. Dying replete females held in the laboratory slowly turned black in color and failed to produce viable ova. Those that successfully produced ova did so at a reduced rate evidenced by the low estimated larval (EL) value.

D. variabilis also was controlled partially by UC55304 at 7 mg/kg body weight/day. No restriction of nymph or female repletion was evident, but drug effects were indicated by reduced egg mass weights, low percent hatch, high nymph mortality, and molt inhibition. Mortality of the *Amblyomma* species was low though some indication of drug effect was evident by increased adult and nymph mortality following repletion, low repletion weights, reduced egg mass weights, and molt inhibition.

TABLE I

MEAN EFFECTS OF UC55304 ADMINISTERED CONTINUOUSLY AT 7 MG/KG/DAY
VIA THE RUMEN ON FOUR TICK SPECIES FEEDING ON SHEEP

	Treatment Control	*Dermacentor variabilis*	*Dermacentor andersoni*	*Amblyomma maculatum*	*Amblyomma americanum*
% Adult female repletion	T	100.0	95.8	100.0	100.0
	C	100.0	100.0	100.0	100.0
% Adult female mortality	T	19.8	42.2	14.1	7.9
	C	3.4	6.1	3.3	0.0
Repletion wt. (g)	T	0.60	1.02	1.21	0.81
	C	0.55	1.16	1.31	0.80
Egg mass wt. (g)	T	0.23	0.11	0.62	0.40
	C	0.33	0.34	0.79	0.44
% Hatch	T	31.7	12.6	66.7	50.6
	C	89.7	26.4	75.6	74.6
Estimated larvae produced (EL)	T	1458.2	277.2	8270.8	4048.0
	C	5920.2	1795.2	11944.8	6564.8
% Nymph repletion	T	79.2	88.2	75.3	81.9
	C	71.5	95.2	100.0	98.0
% Nymph molt	T	25.3	1.9	81.2	46.9
	C	62.2	85.6	98.6	69.2
% Nymph mortality	T	81.6	98.2	11.4	51.6
	C	36.7	15.4	1.5	29.0

What appeared to be fully developed larvae were observed within the egg case of significant portions of egg masses from surviving females of all 4 species fed on treated sheep, however eclosion did not occur. This was not observed among control egg masses reflected by the EL values for UC55304 in Table I. Treatment animals showed no obvious signs of toxicosis throughout the duration of the study and the drug exhibited no activity against the bed bug, C. lectularius.

Overall, UC55304 at the rate of 7 mg/kg body weight/day showed the greatest effectiveness against the Genera Dermacentor and least effectiveness against Amblyomma. Differences in species susceptability as well as latent effects on molting and fecundity reflect the differing properties of this experimental compound.

Study II

Tables II - V summarize data collected when lambs challenged with 3 tick species received 4 experimental compounds at a daily rate of 10 mg/kg body weight via rumen infusion. Treatment values and estimates recorded on all tables were compared to the average response of the 3 tick species on both control lambs. UC55304 expressed the greatest degree of control by only slightly outperforming UC55353. Only moderate indications of control were expressed by UC55248 and UC58830. Anorexia was evident with the lamb receiving UC58830 and was believed due to individual response to laboratory conditions. Data collected from this animal could be biased and may not be valid for comparison.

UC55304 was most effective against D. variabilis by reducing repletion weights, egg mass weights, and percent hatch all reflected by the low EL value (Table II). Control was also evident against

TABLE II

MEAN EFFECTS OF UC55304 ADMINISTERED CONTINUOUSLY AT 10 MG/KG/DAY VIA THE RUMEN ON THREE TICK SPECIES FEEDING ON SHEEP

	Treatment Control	*Dermacentor variabilis*	*Amblyomma americanum*	*Amblyomma maculatum*
% Adult female repletion	T	100.0	85.0	——[a]
	C	100.0	85.9	94.5
% Adult female mortality	T	78.0	41.2	25.0
	C	0.0	11.1	33.3
Repletion wt. (g)	T	0.49	0.45	1.29
	C	0.52	0.58	1.37
Egg mass wt. (g)	T	0.01	0.25	0.63
	C	0.25	0.21	0.67
% Hatch	T	2.67	21.8	12.8
	C	78.4	47.8	41.0
Estimated larvae produced (EL)	T	7.0	1079.2	1624.0
	C	3920.2	2015.0	5523.7
% Nymph repletion	T	70.6	81.8	——
	C	100.0	97.6	86.5
% Nymph mortality	T	94.1	64.7	——
	C	0.0	3.5	18.6
% Nymph molt	T	8.3	22.2	——
	C	100.0	99.0	100.0

[a] Insufficient numbers due to poor attachment

TABLE III

MEAN EFFECTS OF UC55353 ADMINISTERED CONTINUOUSLY AT 10 MG/KG/DAY
VIA THE RUMEN ON THREE TICK SPECIES FEEDING ON SHEEP

	Treatment Control	*Dermacentor variabilis*	*Amblyomma americanum*	*Amblyomma maculatum*
% Adult female repletion	T	100.	52.6	100.
	C	100.0	85.9	94.5
% Adult female mortality	T	89.5	0.0	42.1
	C	0.0	11.1	33.3
Repletion wt. (g)	T	0.48022	0.42601	1.03940
	C	0.51728	0.57748	1.37046
Egg mass wt. (g)	T	0.04700	0.16270	0.10780
	C	0.25001	0.21077	0.67362
% Hatch	T	7.4	25.2	0.4
	C	78.4	47.8	41.0
Estimated larvae produced (EL)	T	69.6	820.0	806.2
	C	3920.2	2015.0	5523.7
% Nymph repletion	T	——	——	100.
	C	100.0	97.6	86.5
% Nymph mortality	T	——	——	100.
	C	0.0	3.5	18.6
% Nymph molt	T	——	——	51.1
	C	100.0	99.0	100.0

TABLE IV

MEAN EFFECTS OF UC55248 ADMINISTERED CONTINUOUSLY AT 10 MG/KG/DAY
VIA THE RUMEN ON THREE TICK SPECIES FEEDING ON SHEEP

	Treatment Control	*Dermacentor variabilis*	*Amblyomma americanum*	*Amblyomma maculatum*
% Adult female repletion	T	100.0	77.8	100
	C	100	85.9	94.5
% Adult female mortality	T	0	7.7	33.3
	C	0.0	11.1	33.3
Repletion wt. (g)	T	0.55773	0.41560	1.32479
	C	0.51728	0.57748	1.37046
Egg mass wt. (g)	T	0.24580	0.18020	0.79800
	C	0.25001	0.21077	0.67362
% Hatch	T	35.9	58.8	60.1
	C	78.4	47.8	41.0
Estimated larvae produced (EL)	T	1764.8	2119.2	9592.0
	C	3920.2	2015.0	5523.7
% Nymph repletion	T	78.6	65.0	——
	C	100.0	97.6	86.5
% Nymph mortality	T	53.6	75.0	——
	C	0.0	3.5	18.6
% Nymph molt	T	59.1	53.9	——
	C	100.0	99.0	100.0

TABLE V

MEAN EFFECTS OF UC58830 ADMINISTERED CONTINUOUSLY AT 10 MG/KG/DAY VIA THE RUMEN ON THREE TICK SPECIES FEEDING ON SHEEP

	Treatment Control	Dermacentor variabilis	Amblyomma americanum	Amblyomma maculatum
% Adult female repletion	T	100.0	78.3	80.0
	C	100.0	85.9	94.5
% Adult female mortality	T	0.0	0.0	29.4
	C	0.0	11.1	33.3
Repletion wt. (g)	T	0.57066	0.53400	1.17000
	C	0.51728	0.57748	1.37046
Egg mass wt. (g)	T	0.23383	0.15808	0.52520
	C	0.25001	0.21007	0.67362
% Hatch	T	56.0	10.2	48.8
	C	78.4	47.8	41.0
Estimated larvae produced (EL)	T	2618.9	322.5	5126.0
	C	3920.2	2015.0	5523.7
% Nymph repletion	T	100.0	85.0	66.7
	C	100.0	97.6	86.5
% Nymph mortality	T	0.0	64.1	33.3
	C	0.0	3.5	18.6
% Nymph molt	T	95.4	82.4	100.0
	C	100.0	99.0	100.0

engorged nymphs by direct mortality and molting inhibition. Mortality of A. americanum and A. maculatum was not evident at this rate.

Table III shows the mean effects of UC55353 at 10 mg/kg body weight/day. Differences may have been greater had the delivery tube not plugged at the site of entry into the rumen reducing the dosage by half for a 20 hour period. As with UC55304, UC55353 demonstrated its greatest effectiveness against D. variabilis. Control was indicated by high mortality of adult females following repletion and reduced egg mass weights. Reduced repletion weights and egg mass weights were also noted for A. americanum and A. maculatum.

Tick mortality was low for UC55248 (Table IV). Some reduced responses were observed but may be attributed to an elevated dosage received by the lamb early in the study. Forty millimeters of UC55248 was delivered in a 20 hour period essentially doubling her dosage. An intraruminal vacuum produced during normal function caused this increased dosage but no signs of toxicity were noted.

Unlike the other compounds tested, UC58830 (Table V) showed its greatest effectiveness against A. americanum. Effectiveness was indicated by reduced repletion weight, reduced egg mass weight, and low percent hatch all reflected by the low EL value. Effectiveness against D. variabilis or A. maculatum was not evident when treated lambs were compared to untreated controls.

Clinical signs of toxicosis were not evident with any of the 4 compounds tested. The lamb receiving UC58830 died late in the study but feed intake records indicated that this lamb had not eaten well since the study began. Anorexia may have been drug related but the author felt it was an individual response to environmental stress.

Although formamidines have shown potential as animal systemic acaricides, required dosages are too high for consideration as candidates for bolus development. The most effective formamidine tested, UC55304, showed effective control of D. variabilis but even at the rate of 10 mg/kg body weight/day some tick survival was evident. Based on these findings the author decided famphur was the best candidate for bolus development and to concentrate efforts toward the improvement of the famphur bolus for systemic control of ticks.

CHAPTER III

IMPROVEMENTS IN THE FAMPHUR BOLUS FOR DELIVERY OF SYSTEMIC COMPOUNDS TO RUMINANTS

Control of ticks on domestic animals can be difficult. Recommendations for spraying or dipping (Hair et al. 1974) are based on topical applications directly to the site of attachment every 2 to 4 weeks from May to August.

The use of systemic insecticides in livestock has proven effective against insects that feed primarily by drawing blood from the host animal. Warbex, for example, is approved as a pour-on for systemic control of lice on cattle and swine. After being absorbed into the blood stream, systemic insecticides are ingested as the parasite takes a blood meal from the host animal. Conventional screening procedures for systemic pesticides were outlined by Drummond (1958) in which guinea pigs, receiving candidate compounds orally and subcutaneously, were challenged with nymphal lone star ticks, *A. americanum*, screwworms, *Cochliomyia hominivorax* (Coquerel), and stable flies, *Stomoxys calcitrans* (L.). The most promising materials were then selected for further testing as oral drenches for sheep and goats.

Later Drummond (1967) found systemic activity of 14 insecticides in screening tests with arthropods parasitizing guinea pigs. Drummond also reported that 8 out of 35 products tested against the cattle grub *Hypoderma* spp. offered greater than 90% systemic control of the grub species. Following the same procedure, Drummond (1970) found control of the common cattle grub was effective with various systemic compounds and that famphur administered as a pour-on offered 93% control of the grub species. Gladney et al. (1972) tested 6 systemic insecticides in feed against the tropical horse tick Anocentor nitens (Neumann) infesting stanchioned cattle and found that famphur at 5 mg/kg/day offered over 98% control as indicated by smaller numbers of engorged females, reduced repletion weight, reduced egg mass weight, and low percent hatch of treated animals.

Famphur, when incorporated into cattle feed for 30 days at 2.5 mg/kg of body weight, offered control of the short-nosed cattle louse, Haematopinus erysternus (Nitsch) (Roberts et al. 1969). At twice this dosage, Drummond et al. (1972) found that treatment provided complete control of estimated larval populations (EL), of the Gulf Coast tick, A. maculatum, over 99.5% control of EL of the lone star tick, A. americanum, but only moderate control of the American dog tick, D. variabilis. Teel et al. (1977) reported similar results when famphur was administered via ruminal infusion to sheep to simulate the sustained-release of systemic acaracides from ruminal boluses. He reported that famphur infused at 7 mg/kg/day provided complete control of A. maculatum, effective control of A. americanum but comparatively little control of D. variabilis.

Systemic insecticides administered as pour-ons, oral drenches, and feed and water additives have generally proven inadequate under commercial conditions. Commercial application of "feed-through" and "water-additive" compounds have limitations, particularly under rangeland situations where intake cannot be adequately monitored. Based on these inadequacies, work has been done in recent years in the development of sustained-release mechanisms for arthropod control.

The principle on which ruminal boluses operate is based on retention in the rumenoreticular sac of the ruminant. Through activities of peristalsis during rumination the active biological agent is released through erosion or leaching and is utilized systemically by the host animal. One of the first developments of this type was by Dewey et al. (1958) in which cobalt was provided to sheep and cattle grazing cobalt deficient pastures in Australia. Pellets of cobaltic oxide and China clay were molded by compression into a cylindrical form. The resulting pellet was capable of releasing cobalt through ruminal activity at the rate of 1 mg/day for over 100 days.

More recently, Teel et al. (1979) demonstrated that a 50% famphur bolus released approximately 300 mg/day and was effective against _A. maculatum_ and _A. americanum_. Hair et al. (1979) reported complete control of two 1-host tick species, _B. microplus_ and _B. annulatus_ (Say), on animals receiving famphur at the rate of 6.82 mg/kg body wt/day via ruminal boluses. Famphur, an organophosphate insecticide, had previously shown effectiveness against _A. maculatum_, _A. americanum_, and _D. variabilis_ by offering 100, 99.5, and 19-83% control of reproduction when administered via ration at the rate of 5 mg/kg body wt/day (Drummond et al. 1972). Teel et al. (1977) infused famphur directly into the rumen of sheep and suggested that at least 7 mg/kg body wt/day was necessary to achieve the same results reported by Drummond et al. (1972).

State-of-the-Art

Teel (1978) suggested that problems of bolus regurgitation and breakage were due primarily to lack of density and tensile strength of the 50% famphur bolus. His formulation contained 30.7% carnauba wax and 69.3% barium sulfate and had a density of 1.62 gm/cm^3. The following studies were directed toward the improvement of the famphur bolus developed by Teel. Studies are reported in chronological order to aid understanding of the developmental procedures.

The first efforts to improve this formulation were directed toward the production of a laminated bolus with a high density inert core. Marston (1962) reported that cobalt pellets may consist of a low density biologically active substance embodied in a low-density, or high-density matrix, the particles of which are molded or compressed around a high density core. If a matrix containing a high percent active ingredient was compressed around a high density inert core, both the needed density and tensile strength might be achieved.

Based on the guidelines of Teel and Hair (1978), matrixes capable of blending and compressing with active ingredients into a conventional ruminal bolus were prepared in the following manner: Carnauba wax was heated in a double boiler to melting point (86^{o}C), at which time the high-density, nontoxic metal derivitive, barium sulfate ($BaSO_4$), was added. Ingredients such as glycols and lubricants were added to promote bolus stability and modify bolus characteristics. The molten mass was poured into aluminum trays to a thickness of approximately 4 mm and allowed to cool to room temperature. After cooling the material was ground to a fine powder with a model 688-3 kitchen blender (Scovill, Hamilton Beach Division) and screened through a 40-mesh U.S. standard sieve to produce a uniform particle size. Aliquots were poured into the open cavity of a steel mold and compacted to the desired pressure, using a hydraulic press (Model CT710X Soil Test, Inc. Evanstan, Ill.).

The mold, described by Teel (1978) was designed from S-7 tool steel and heat treated to a 50 to 55 Rc (Rockwell hardness). The internal parts were machined to a maximum tolerance of 0.0125 mm and all internal surfaces received 0.005 mm industrial hard chrome. The mold was designed to produce a bolus, 75.0 by 21.9 mm with oval ends, (Fig. 1) that could be administered with a conventional balling gun.

Initial efforts toward the production of a laminated bolus began by combining equal aliquots of the matrix described by Teel (Table VI, Matrix 1) with technical famphur. Three laminated boluses were produced by placing 15 grams of this 50% famphur matrix 1 formulation on either side of 15 grams of matrix 2. This inner core of higher density matrix increased bolus density from 1.62 gm/cm^3 to 1.79 gm/cm^3. An additional 3 boluses were produced in a similar manner using 20 grams of matrix 2 for the high density core. These boluses had a slightly higher density of 1.81 gm/cm^3. A 3rd group of 3 boluses was produced by laminating 17.5 grams of the 50% famphur matrix 1 formulation on either side of 10 grams of matrix 2 producing boluses with a specific gravity of 1.62 gm/cm^3.

One bolus of each of these 3 formulations was separately administered via the cannula to 3 fistulated heifers fitted for model #10c ruminal cannulas (Bar Diamond, Inc., Parma, Id.). Boluses were removed via cannula 3 times weekly, blotted dry with paper toweling and weighed on an Ainsworth Model 200 electronic balance. This allowed for calculation of release rates by bolus weight change, assuming there

Figure 1. Sixty % famphur boluses 75.0 mm long by 21.9 mm wide with oval ends weighing approximately 40 grams.

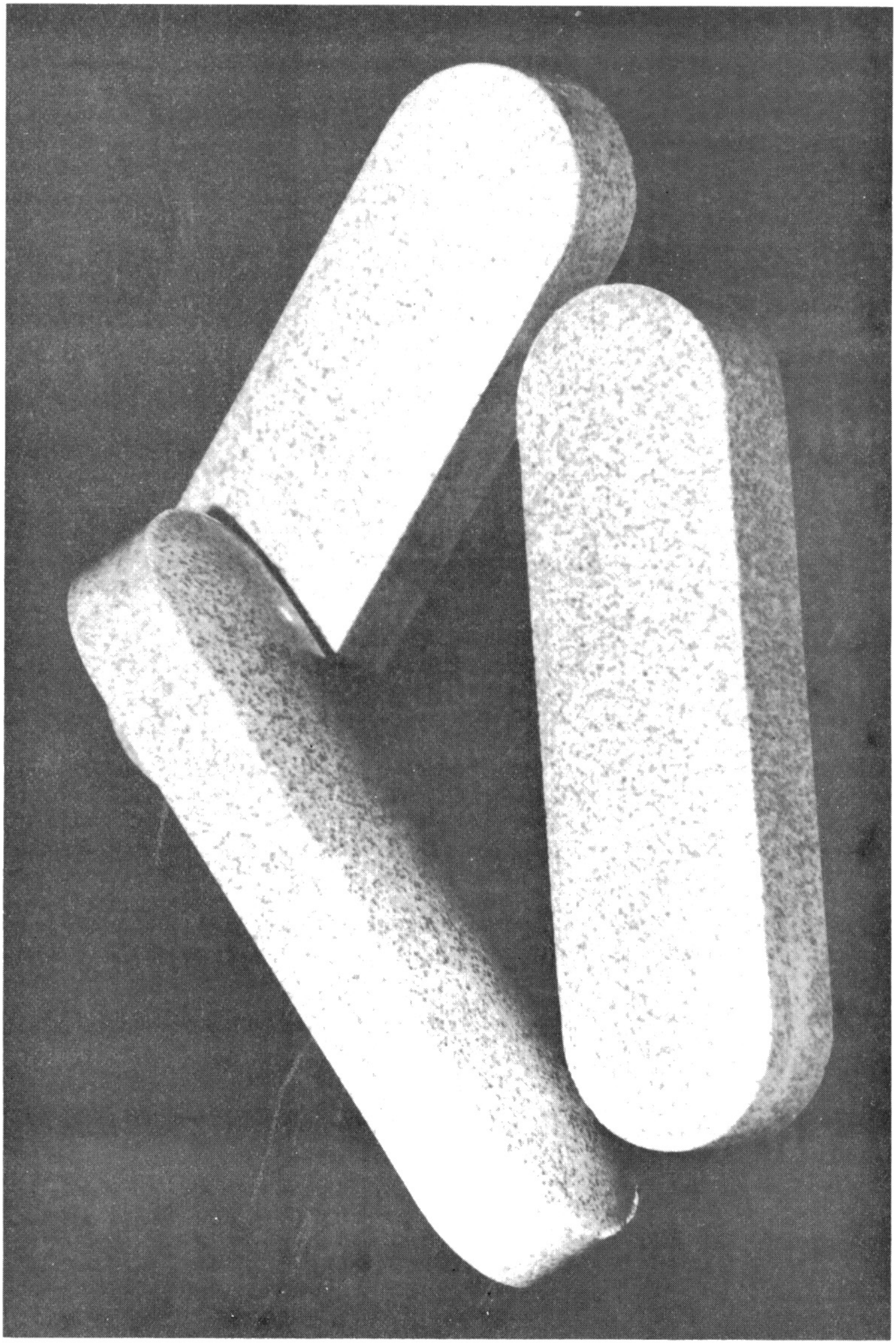


TABLE VI

MATRIXES DEVELOPED AND TESTED WITH FAMPHUR
AS A SUSTAINED-RELEASE BOLUS
FOR TICK CONTROL

Matrix Number	Percent Ingredients in Matrix				
	Carnauba	Beeswax	$BaSO_4$[a]	PEG[b]	Iron Powder[c]
1	30.7	0.0	69.3	0.0	0.0
2	26.7	0.0	73.3	0.0	0.0
3	27.3	0.0	68.2	4.5	0.0
4	27.5	0.0	68.8	3.7	0.0
5	27.5	0.0	69.4	2.8	0.0
6	28.0	0.0	70.1	1.9	0.0
7	28.3	0.0	70.8	0.9	0.0
8	12.6	15.0	71.6	0.8	0.0
9	17.0	11.0	72.0	0.0	0.0
10	21.0	7.0	72.0	0.0	0.0
11	25.0	3.0	72.0	0.0	0.0
12	47.1	0.0	52.9	0.0	0.0
13	35.7	0.0	48.2	0.0	16.1
14	30.8	0.0	51.9	0.0	17.3
15	30.8	0.0	34.6	0.0	34.6
16	7.4	9.3	67.9	0.0	15.4
17	12.1	4.6	67.9	0.0	15.4
18	3.7	13.0	67.9	0.0	15.4
19	0.0	16.7	64.8	0.0	18.5
20	10.2	3.9	57.6	0.0	28.3
21	6.9	2.8	31.4	0.0	58.9

[a] Barium Sulfate

[b] Polyethylene glycol

[c] 40 mesh

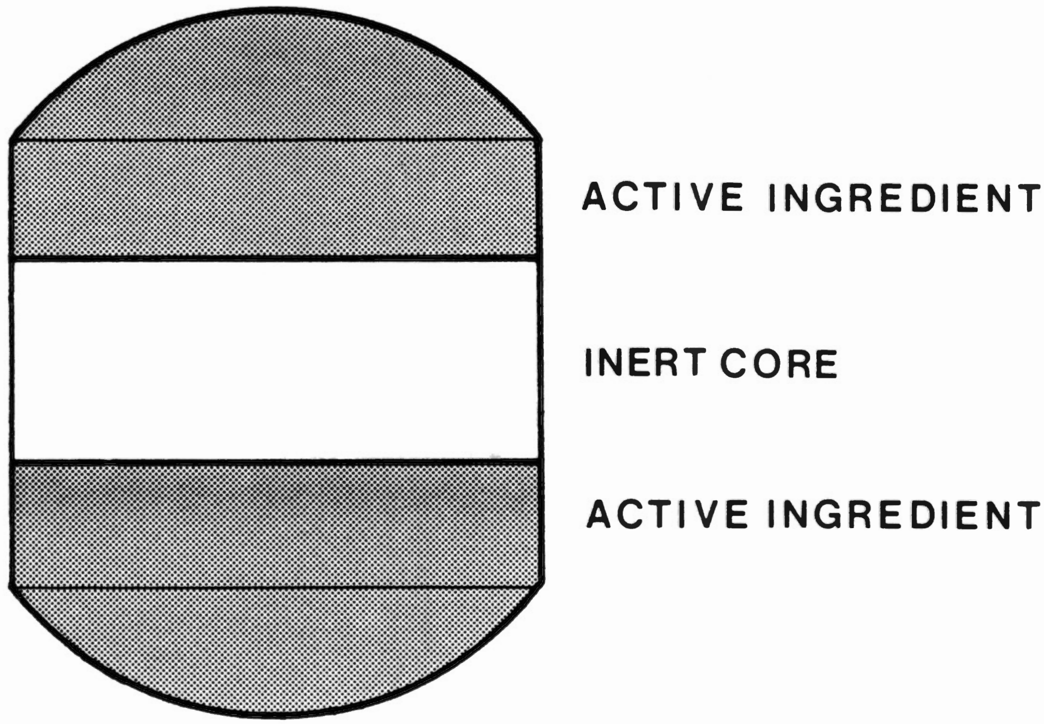
was a uniform distribution of technical material, as well as visual inspection.

Following 18 days observation all 3 of the above formulations showed extensive cracking between the laminated outer layers and the inert core. In some cases, the outer layers had completely separated from the core producing 3 separate wafer-thin boluses. These wafers did not have the expected laminated appearance (Figure 2-top) but rather that of Figure 2-bottom. This may seem of minor significance but is actually very important in understanding lines of stress when powders are compacted.

To keep the laminated layers from separating, the percentage of active ingredient in the outer layers was varied and other materials were added to the inert core. The inert core appeared as if it formed a fluid barrier from which the outer layers would split away. Since no alterations worked successfully, the lamination studies were terminated and development efforts concentrated on the production of new matrixes.

To understand the role of polyethylene glycol (PEG) on bolus erosion, matrixes 4, 5, 6, and 7 (Table VI) were developed and combined with 50% technical famphur and pressed at 572 kg/cm^2. These formulations exhibited problems of a previously described phenomena called "capping" (Train 1956; Train and Lewis 1963; Marshal 1977; Teel 1978). When powders are compacted, especially under high pressures, particles rearrange to conform to the shape of the mold. During this rearrangement, lines of stress are formed which may cause separation of one or both sides of the bolus from the bolus body (Fig. 3). As indicated in Figure 4 the severity of this phenomena increased as the

Figure 2. Laminated boluses showing expected (top) and actual (bottom) layering when compaction was achieved with a single punch press.


ACTIVE INGREDIENT
INERT CORE
ACTIVE INGREDIENT

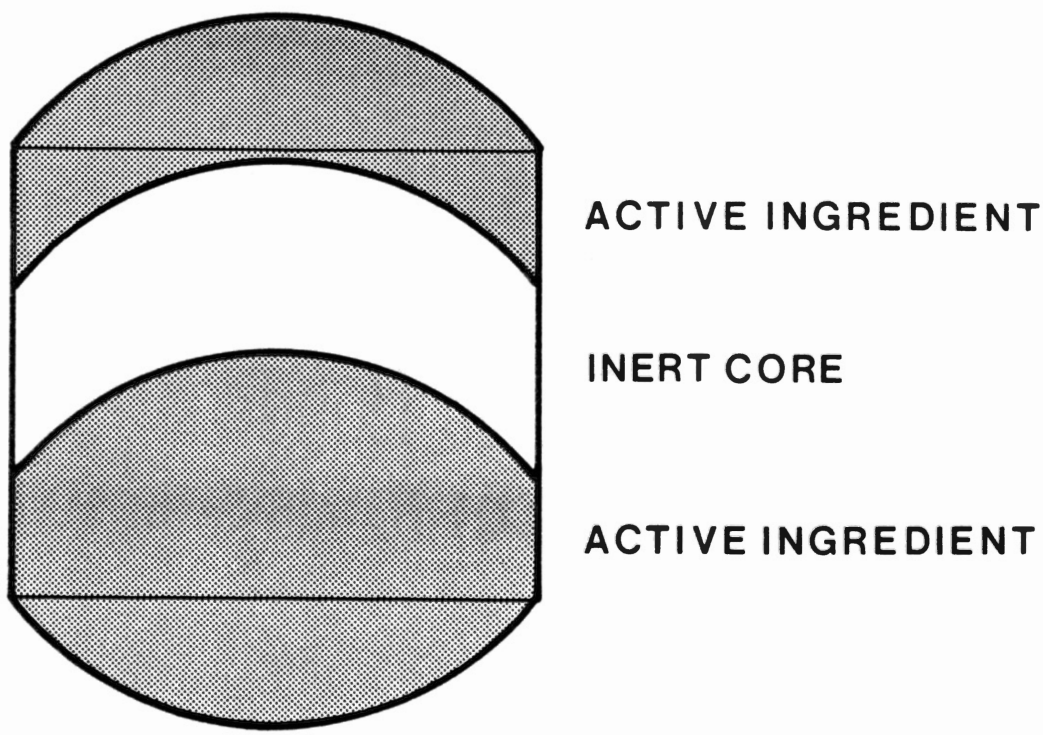
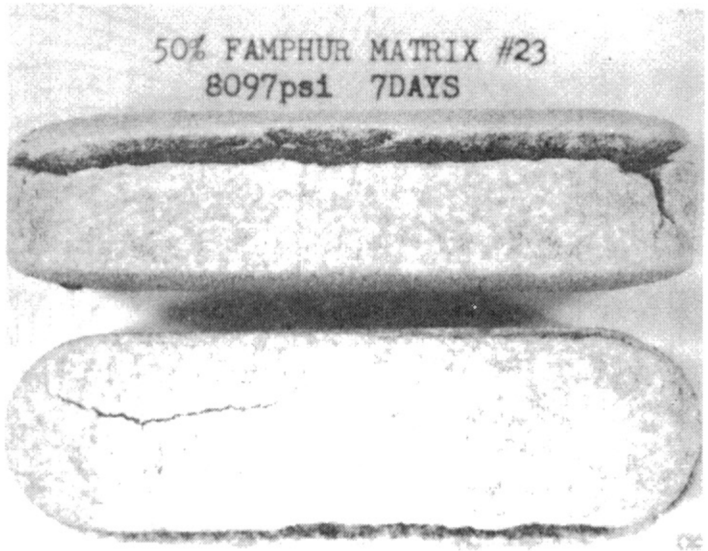

ACTIVE INGREDIENT
INERT CORE
ACTIVE INGREDIENT

Figure 3. Lateral view of boluses showing major cracks along lines of stress created during compaction.


50% FAMPHUR MATRIX #23
8097psi 7DAYS

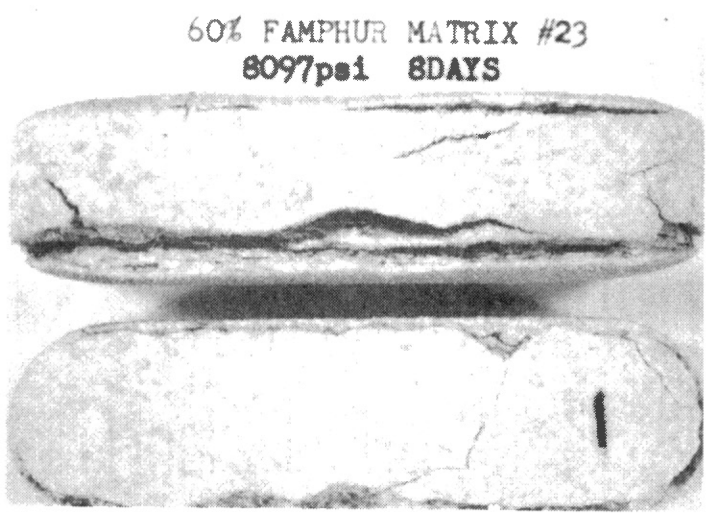
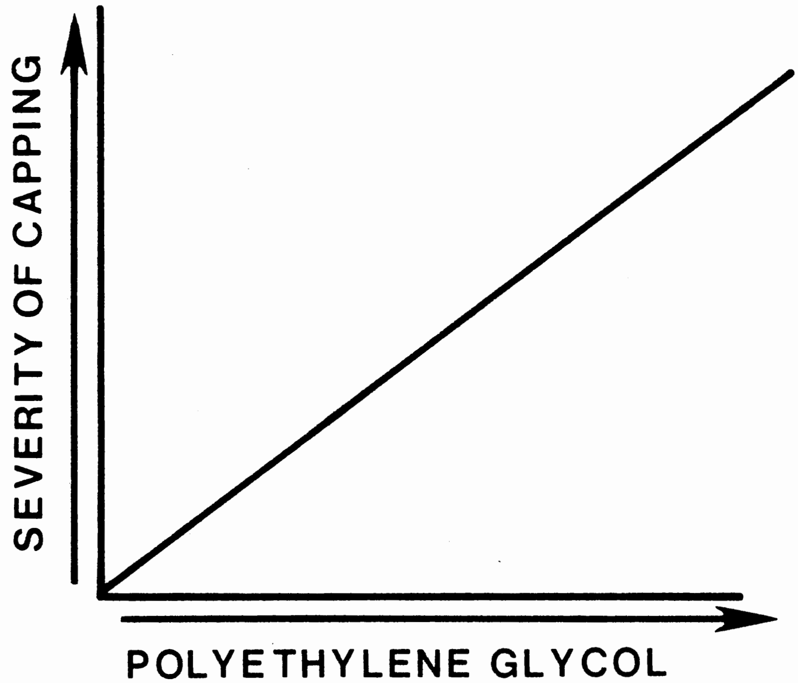
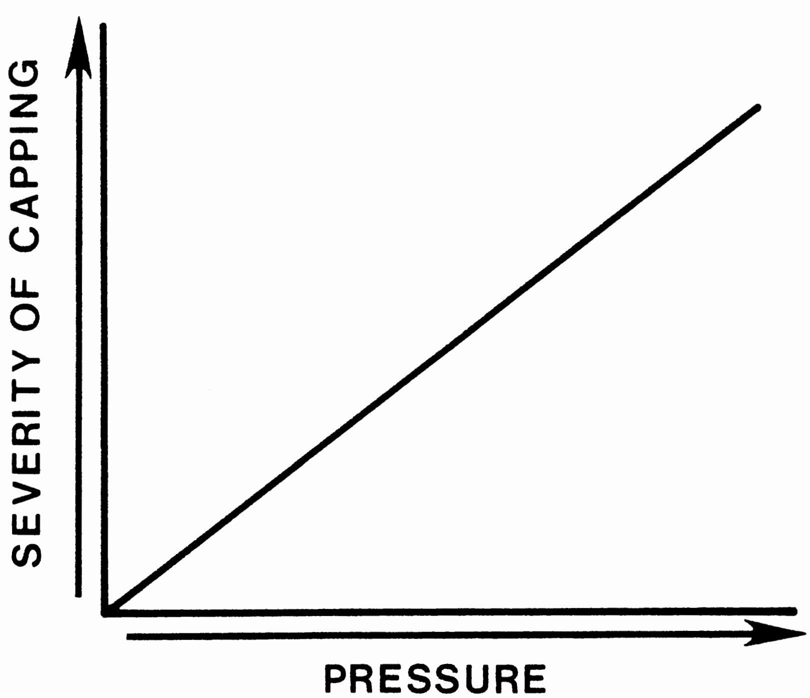

60% FAMPHUR MATRIX #23
8097psi 8DAYS

Figure 4. Qualitative effect of increasing polethylene glycol on capping of boluses.

Figure 5. Qualitative effect of increasing pressure of compaction on capping of boluses.


SEVERITY OF CAPPING
POLYETHYLENE GLYCOL


SEVERITY OF CAPPING
PRESSURE

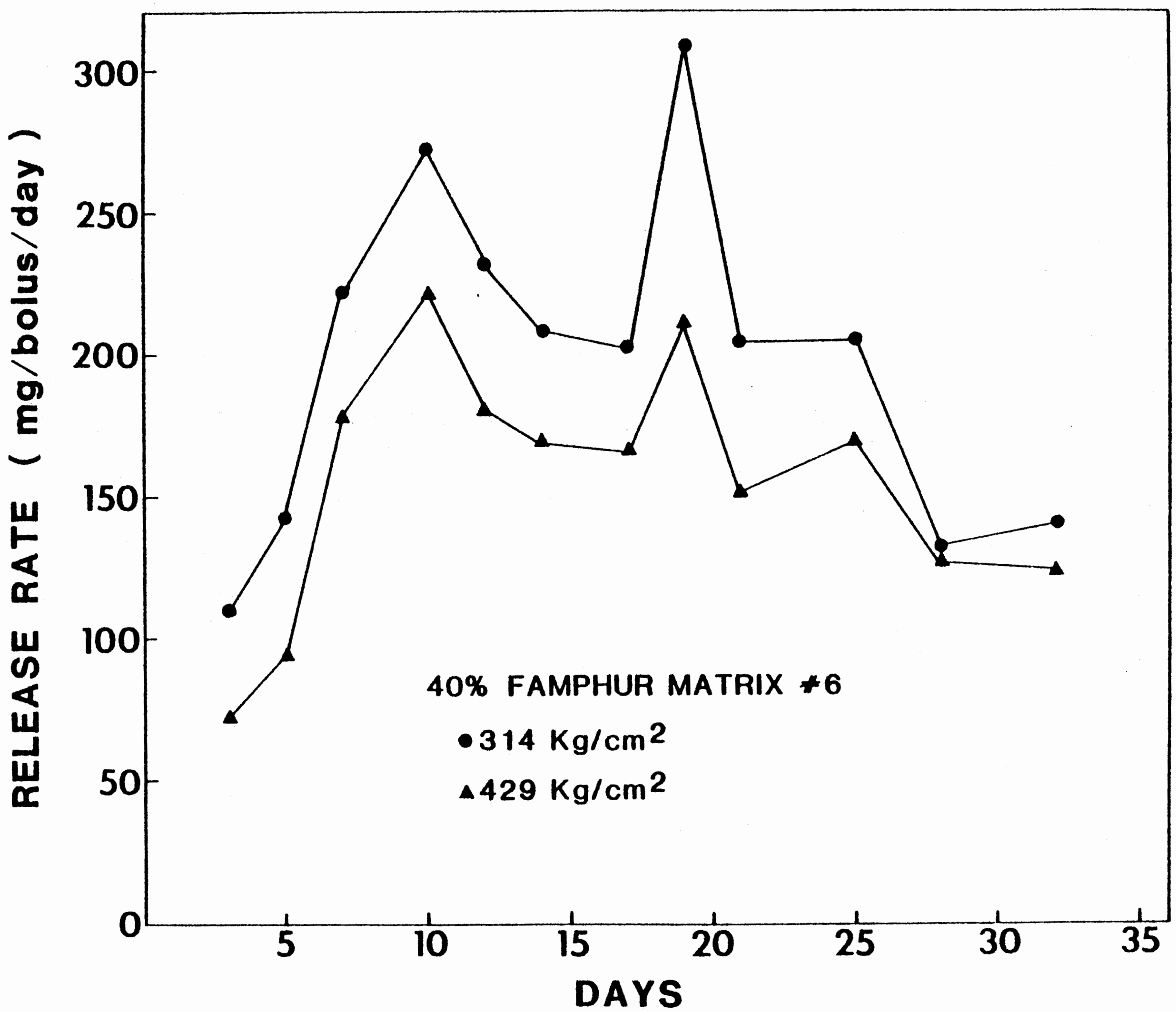
percent of PEG increased. Since PEG is a lubricant, the author felt that its lubricating capabilities caused the sliding of particles instead of cohesion and thus promoted capping.

To determine the effect of pressure on bolus erosion, previously described matrixes 1, 3, 4, 5, 6, and 7 were combined with 50% famphur and pressed at 230, 314, 429, 572, 714, 857 and 1000 kg/cm^2. Several formulations looked promising but capping was evident and directly proportional to pressure. Figure 5 shows the qualitative effects of increasing pressure on capping of boluses. As expected, bolus release rates were inversely proportional to pressure as shown by the 2 formulations in Figure 6.

In search of a softer matrix, compatible with a higher percentage active ingredient, matrixes 8, 9, 10, and 11 were developed. Forty, 50, and 60% technical famphur boluses were formulated with these matrixes and compressed at 230, 314, and 429 kg/cm^2. Fifty % matrix 11 pressed at 314 kg/cm^2 produced the greatest release rates by achieving an excess of 300 mg/day for 23 days at which time the study was terminated due to breakage and regurgitation.

In assessment of results to this point, regurgitation appeared to be the major obstacle to overcome in the successful development of a famphur bolus. Iron powder (100 mesh) was added as a new density ingredient due to its availability and relative low cost as compared to other heavy metals. Initial efforts involved the testing of 2 matrixes previously described by Teel (1978) by combining equal amounts of famphur with matrixes 1 and 3 to produce 50% formulations. Immediately prior to pressing, 2 grams of iron powder was added to each formulation, producing 42 gram boluses. When administered to bovine, both

Figure 6. Release rates of technical famphur (mg/day) from 40% matrix 6 boluses pressed at 314 and 429 kg/cm^2.


RELEASE RATE (mg/bolus/day)
300
250
200
150
100
50
0
5
10
15
20
25
30
35
DAYS
40% FAMPHUR MATRIX #6
●314 Kg/cm2
▲429 Kg/cm2

formulations were broken into as many as 5 pieces by the 5th day after administration but the iron powder showed promise in solving density problems.

To further investigate iron powder as a density material, matrix number 12 was developed and combined with famphur in 43, 46, and 50% formulations. Ten grams of iron powder was added to all 3 formulations prior to pressing at 572 kg/cm^2. All formulations exhibited extensive breakage but a density of almost 1.8 gm/cm^3 was achieved.

It was concluded that the iron powder matrixes had poor compaction properties compared with previously tested wax-based matrixes. To alleviate this problem and retain the necessary density, the iron powder was added to the molten slurry during matrix formulation which caused the particles to become coated with matrix increasing their compressibility. Matrixes 13, 14, and 15 were developed and combined with 44% technical famphur utilizing this new formulation technique. When these formulations were pressed at 572 kg/cm^2 densities in excess of 1.8 gm/cm^3 were expected, but in fact, densities of less than 1.7 gm/cm^3 were obtained. It was discovered that the iron powder had settled out when the molten slurry was poured into the aluminum trays for drying. To alleviate this problem, barium sulfate was added to the matrix until it had a consistency to retain the iron particles in suspension.

In search of a softer, more compressible matrix, number 16 was formulated. This matrix utilized beeswax, as had some earlier formulations, and was combined with 55, 60, and 65% technical famphur and each formulation was pressed at 572 and 857 kg/cm^2. Bolus densities ranged from 1.67 gm/cm^3 for the 65% formulation pressed at 572 kg/cm^2 to 1.75 gm/cm^3 for the 55% formulation pressed at 857 kg/cm^2. Bolus release rates were improved with all formulations remaining functional for 39 days. The best 2 boluses were 55% matrix 16 pressed at 572/kg cm^2 and 60% matrix 16 pressed at 857 kg/cm^2 (Fig. 7). Both of these formulations produced release rates far superior than earlier developments.

In an effort to increase the longevity of the famphur bolus, matrixes 17, 18, and 19 were developed and differed from matrix 16 by different ratios of carnauba to beeswax. These 3 matrixes were combined with 50 and 60% technical famphur and compressed at 572 kg/cm^2. All boluses proved satisfactory in the early stages of erosion but capping became evident in all formulations by the 37th day of erosion except for matrix 17 (Fig. 8) which remained functional at day 55.

Results from the 2 previous studies indicated that boluses in the reticulum eroded more rapidly than boluses in the rumen. In an effort to increase bolus density and therefore retain boluses in the reticulum, matrixes 20 and 21 were developed. These matrixes were combined with 50, 55, 60 and 65% famphur and compressed over a range of pressures from 572 to 1143 kg/cm^2. Though densities of 2.0 gm/cm^3 were achieved, capping was evident with all formulations, especially at the higher pressures.

In view of all the work done on the famphur bolus by Teel (1978) and the author, it was decided that a bolus utilizing famphur may not be feasible. Shortcomings of this drug and considerations of future drugs should include the following: 1) Relative low mammalian toxicity; 2) Effective systemic activity at less than 1 mg/kg body weight/day; 3) High density technical material; and 4) Highly compressible.

Figure 7. Release rates of technical famphur (mg/day) from 55 and 60% matrix 16 boluses pressed at 857 and 572 kg/cm^2, respectively.

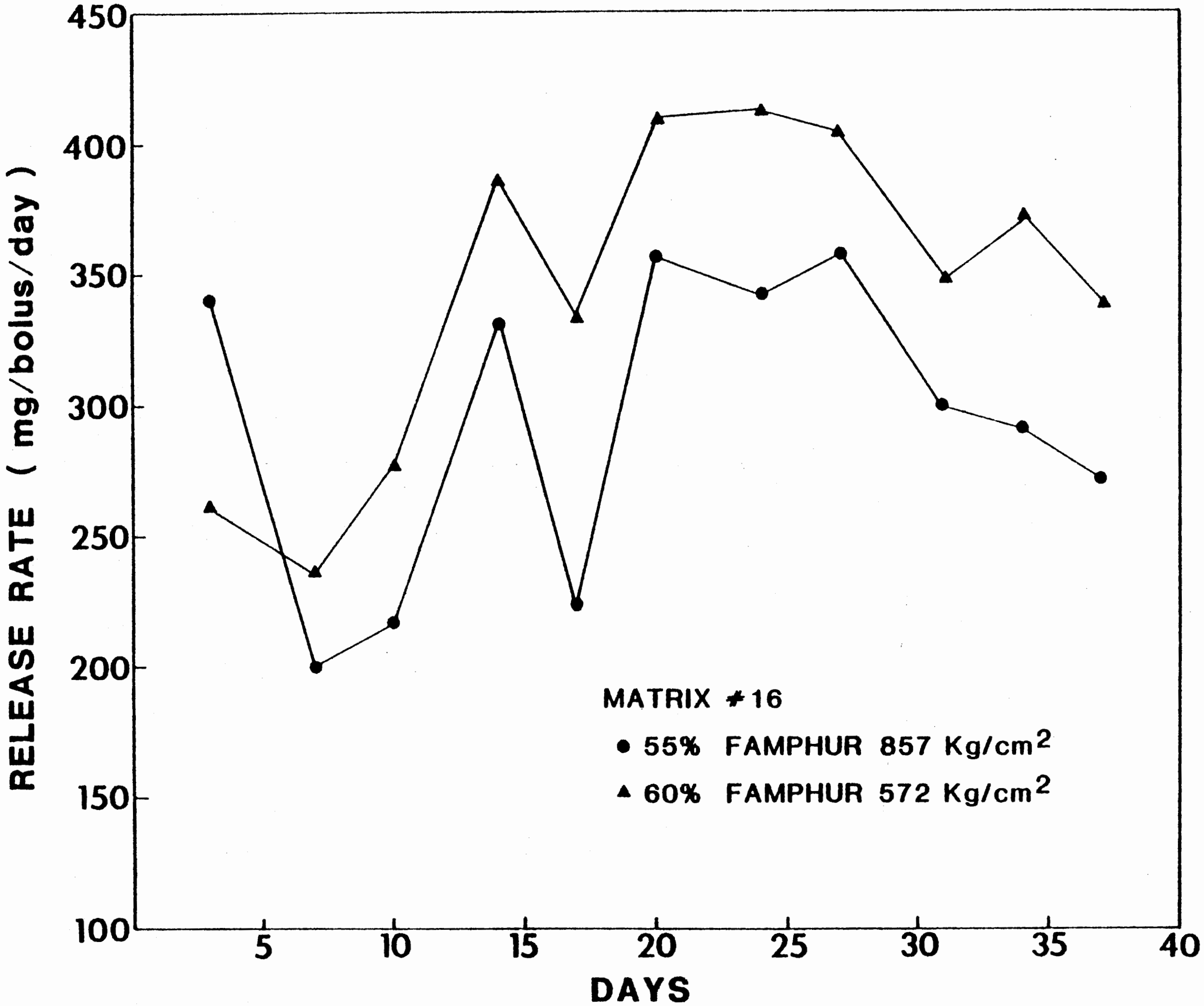
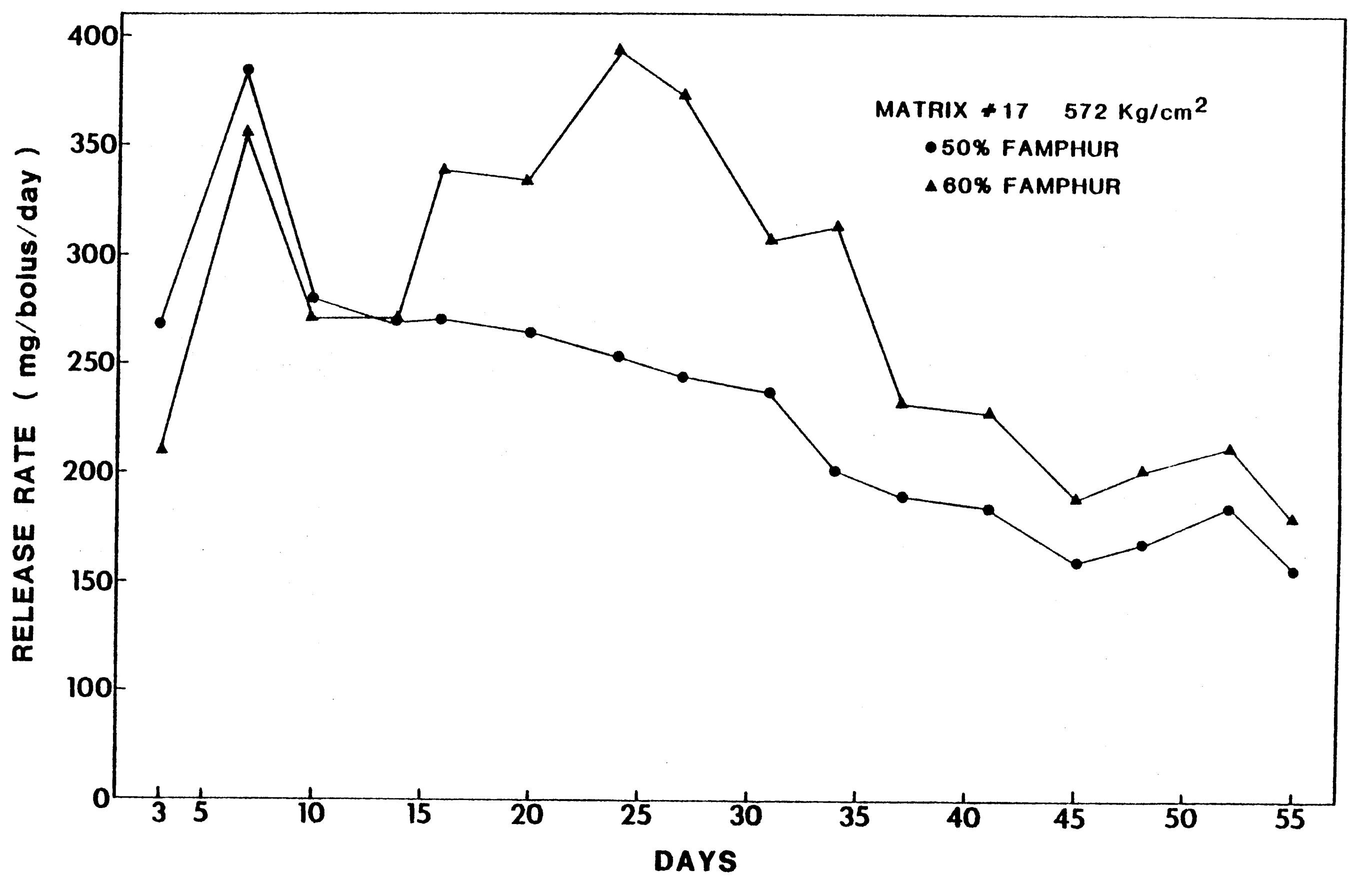

RELEASE RATE (mg/bolus/day)
450
400
350
300
250
200
150
100
5
10
15
20
25
30
35
40
DAYS
MATRIX #16
● 55% FAMPHUR 857 Kg/cm2
▲ 60% FAMPHUR 572 Kg/cm2

Figure 8. Release rates of technical famphur (mg/day) from 50 and 60% matrix 17 boluses pressed at 572 kg/cm^2.


MATRIX # 17 572 Kg/cm^2
● 50% FAMPHUR
▲ 60% FAMPHUR
RELEASE RATE (mg/bolus/day)
DAYS
400
350
300
250
200
150
100
0
3
5
10
15
20
25
30
35
40
45
50
55

CHAPTER IV

THE ROLE OF DENSITY IN DETERMINING STOMACH POSITION OF SUSTAINED-RELEASE RUMINAL BOLUSES

Ruminal boluses as vehicles for chemotherapy of ruminants have recently generated considerable interest among scientists in the animal health industry. The principle on which these devices work is based on retention of the objects in the reticulum or rumen of the animal and the active biological agent is gradually released through erosion or leaching during ruminal peristalsis. These active ingredients may be utilized systemically by the host animal, or if insoluble, may pass through the digestive system in the feces for control of some manure-breeding dipterans. One principal criterion in the successful operation of such devices has been shown to be density.

Dewey et al. (1958) in developing cobalt pellets for sheep discovered that of all the properties required for pellet retention, density was the most important. Marston (1963), in conducting experiments concerning pellet retention in sheep and cattle, indicated that the minimum critical density was approximately 2.5 gm/cm^3 but the preferred density was between 4.0 and 4.5. In testing various nontoxic metal derivities of high density and low water solubility, Siegrist and Katz (1970) suggested that while a density of 1.5 was minimal, 1.9 was preferred. Teel et al. (1979) noted that the direct relationship between bolus density and rumen retention pointed to the shortcoming of his experimental bolus with a density of 1.62. Roebuck and Whitehead (1971) found that once a pellet was introduced into the reticulorumen of sheep and cattle, a density of 2.2 was required for retention.

This author found in studies conducted in Chapter II that when densities were around 1.5 - 1.7 gm/cm^3 the incidence of regurgitation was far greater than when densities of 1.9 or greater were achieved. The objective of this study was to monitor bolus activity as density was fluctuated and determine minimal density requirements for ruminal boluses.

Materials and Methods

Density and Movement

To demonstrate the influence of bolus density on movement between the reticulum and ventral sac of the rumen (Fig. 9) boluses of varying densities were produced from inert materials (Table VII). In anticipation of bolus interaction, especially when multiple boluses are administered to the small confinement of the reticulum, boluses of the 7 densities of Table VII were divided into 3 density groups which were tested in different periods (Table VIII). This treatment regimen allowed boluses representing 2 densities (1.6 and 2.0) to act as common links between periods and as indicators of bolus interaction.

Boluses of period 1 (Table VIII) were lightly etched for identification and one representing each density was placed via a model #1C ruminal cannula into the reticulum of each of 6 mature Hereford heifers. Boluses were placed in the reticulum initially because this is the location in the stomach where heavy objects initially lodge when

Figure 9. Left view of ruminant abdomen showing interior features of the reticulorumen.

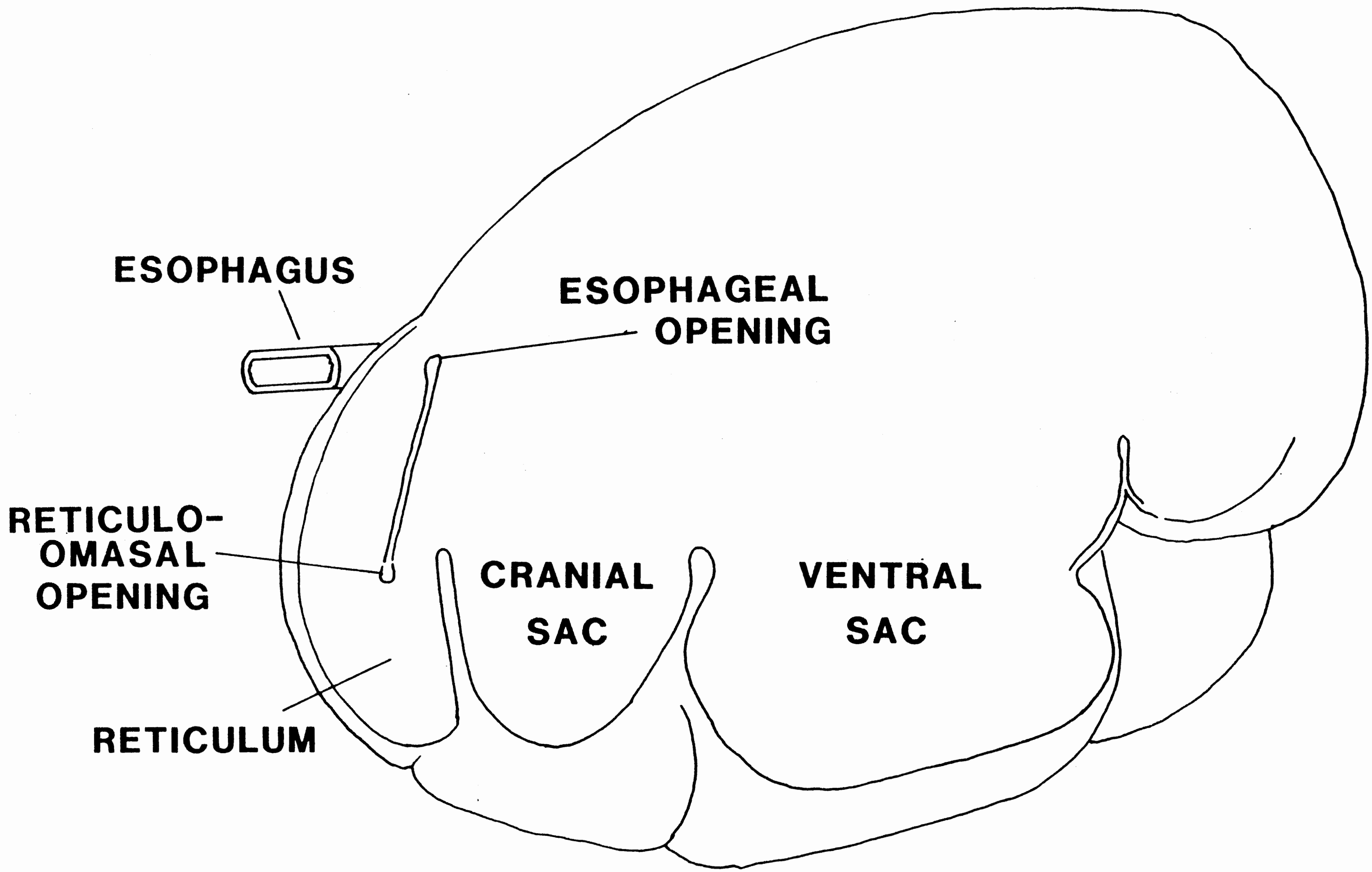

ESOPHAGUS
ESOPHAGEAL OPENING
RETICULO-OMASAL OPENING
CRANIAL SAC
VENTRAL SAC
RETICULUM

TABLE VII

INERT MATERIALS USED IN THE PRODUCTION
OF BOLUSES OF VARYING DENSITIES

Density (gm/cm^3)	Carnauba (g)	Barium sulfate (g)	Polyethylene glycol (g)	Iron Powder (g)	Pressure (Kg/cm^2)
1.2	67.5	22.5	0	0	428.7
1.4	56.3	33.7	0	0	428.7
1.6	18.2	20.5	1.3	0	457.2
1.8	13.8	24.5	1.7	0	442.9
2.0	10.9	27.3	1.9	0	328.7
2.2	9.7	24.4	1.3	4.6	543.0
2.4	8.7	21.8	1.5	8.0	600.2

TABLE VIII

STOMACH POSITION OF INERT BOLUSES OF 7 DENSITIES FOLLOWING ADMINISTRATION TO 6 FISTULATED HEREFORD HEIFERS AND MONITORED FOR 30 CONSECUTIVE DAYS

PERIOD	DENSITY[a] (gm/cm^3)	NUMBER OF OBSERVATIONS[b] Reticulum[c]	Rumen	Missing[d]
	1.2	3	54	123
1	1.4	37	95	48
	1.6	130	31	19
	1.6	85	90	5
2	1.8	133	47	0
	2.0	178	2	
	2.0	153	27	0
3	2.2	180	0	0
	2.4	180	0	0

a Determined by water displacement method.

b Determined by monitoring 6 animals daily for 30 days producing 180 observations per density.

c Includes those found in the anterior dorsal sac of the rumen.

d Observed regurgitated in the holding pens.

swallowed (Dewey et al. 1958, Teel et al. 1979). Boluses were observed daily for 30 days, thus producing 180 observations for each density. Experimental heifers were maintained as a unit under dry lot conditions on wheat straw and a cottonseed-hull based ration containing 30% alfalfa meal pellets, 24.7% cracked corn, 7% cottonseed meal, 7% molasses, 0.3% plain salt, 0.5% dicalcium phosphate, 0.5% calcium carbonate, and vitamin A (3,000,000 IU/907 kg of feed). Heifers also received 0.5 kg of finely ground corn per head per day containing 4.4 g of MGA-100 (Melengesterol) premix per kg of corn for estrus control. Heifers had ad libitum access to water and salt.

Boluses of periods 2 and 3 were administered to the same 6 animals following 30 days observations of periods 1 and 2, respectively. Boluses were monitored and animals were maintained for periods 2 and 3 as described for period 1.

Results and Discussion

Effect of density on bolus movement and stomach position is presented in Table VIII. Boluses found in the cranial sac of the rumen are included in counts with those found in the reticulum since movement between these 2 compartments was frequent but temporary. Boluses not found were recorded as missing and most were found in the holding pens indicating they had been regurgitated.

The incidence of missing boluses, and presumably regurgitation, was higher ($P < .05$) with the lower density boluses. Over one-half of the 180 observations of boluses having densities of 1.2 were recorded as missing and the majority of those retained were found in the rumen. This suggests that the lower density boluses were probably regurgitated when they came into close proximity of the esophageal opening of the reticulum (Fig. 9). If more of the lower density boluses had been retained in the reticulum, the incidence of missing boluses may have been even greater. Loss of boluses decreased as densities increased and 1.8 g/cm^3 was determined the minimal density for retention of boluses in the reticulorumen.

As densities increased from 1.2 to 2.4 g/cm^3, more boluses were retained in the reticulum with all 180 observations of boluses having densities of 2.2 and 2.4 found therein. When the same density was monitored in 2 different periods (i.e. Periods 1 and 2, Density 1.6; Periods 2 and 3, Density 2.0) an interaction between bolus densities was observed. The higher density boluses (1.8, 2.0) of period 2 may have "crowded out" the boluses having a density of 1.6. Therefore, fewer boluses were found in the reticulum than when these boluses were present with boluses of densities 1.2 and 1.4 of period 1. The same trend was indicated with the 2.0 density of periods 2 and 3.

Movement of the lower density boluses appeared to be random but as bolus density increased, so did the number of consecutive observations per stomach location. Twenty-four of the 27 observations in the rumen of the bolus density 2.0 of period 3 were due to presence of a single bolus in the rumen of one animal from day 7 to 30. A similar occurrence involving 3 animals was responsible for 41 of the 47 observations in the rumen for boluses with a density of 1.8.

Results indicate that several factors are important in the development of sustained-release ruminal boluses. A minimal density of 1.8 is required to prevent regurgitation of boluses from the reticulorumen of bovine and a density above 2.0 is needed for retention in the reticulum. Boluses densities below 2.0 moved between stomach compartments and release rates differed with position. Boluses, retained in the reticulum, produced significantly higher release rates than boluses in the rumen. Proper bolus density is needed to maintain stomach position and stabilize the rate of bolus erosion.

CHAPTER V

BOLUS EROSION DIFFERENCES BETWEEN THE RETICULUM AND RUMEN OF CATTLE

Limited observations have been made on the movement of objects within the gastrointestional tract of ruminants but the factors regulating this movement have not been defined. Initial work indicated that when heavy pellets were swallowed by sheep they usually lodge in the cranial sac of the rumen, but soon passed to the reticulum (Dewey et al. 1958). Miller et al. (1977) found that 5 of 6 boluses with densities of approximately 2.1 when orally administered, were found in the reticulum at time of slaughter. Teel et al. (1979) found boluses with a density of 1.62 were initially retained in the reticulum, but by the 7th day, 90% had passed to the ventral sac of the rumen. Riner et al. (1981) in testing a Rabon® bolus for face fly control observed lower release rates when boluses passed from the reticulum to the ventral sac of the rumen. The author also observed movement during the development studies of the famphur bolus (Chapter II). The purpose of this study was to determine the difference in erosion rate of boluses in the reticulum vs. the rumen of cattle.

Materials and Methods

Stomach position may be a factor in erosion of rumen inclusions in bovine since different rates of erosion have been observed

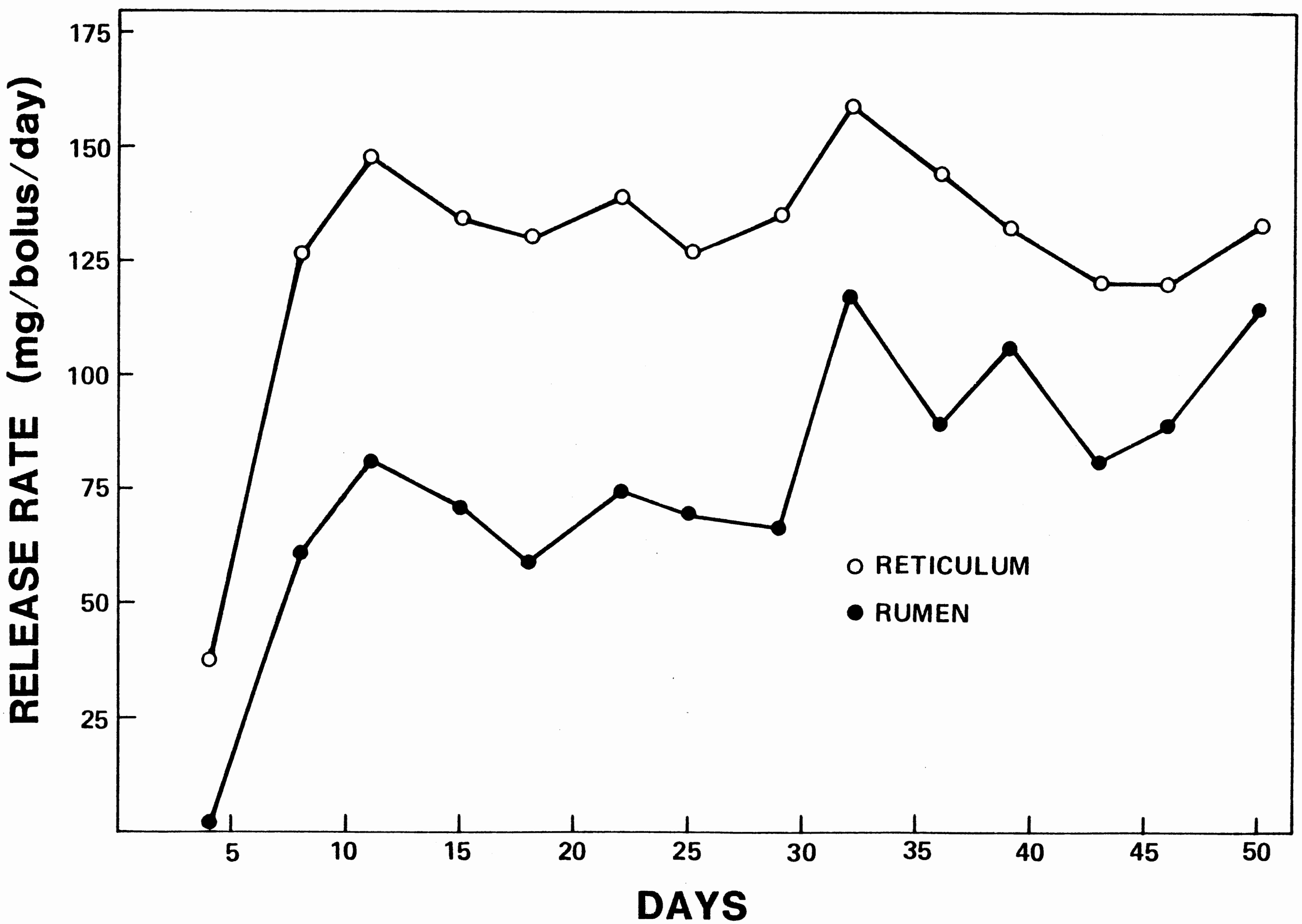
(Riner et al. 1981). To better understand these differences, 20% oxytetracycline boluses were formulated (Byford et al. 1980) and lightly etched for individual identification. Two boluses having densities of 1.8 g/cm^3 were placed in the reticulum and 2 in the rumen of 12 fistulated Hereford heifers (Fig. 9, Chapter IV). Boluses were observed via the cannula daily and those found out of their initial position were returned to the proper location. Boluses were removed twice weekly, blotted dry with paper towling, weighed on an Ainsworth Model 200 (Ainsworth Division, Denver, Colorado) electric balance and returned to their respective locations. Boluses were monitored for 50 days and erosion curves (Fig. 10) were constructed from weight change data amassed from one weigh period to the next. Animals were maintained as a unit under the same regimen as described for the density study (Chapter IV).

Results and Discussion

Release rates of individual boluses eroding in the reticulum are compared to those eroding in the rumen of cattle in Figure 10. Release rates are based on the average daily release of oxytetracycline in milligrams. Boluses in the reticulum and the rumen went through an initial moisture "absorption period" (Days 0-10) followed by uniform release rates skewed to the right as surface area decreased. We have now shown that during the absorption period, mass loss of boluses does occur but due to moisture absorption, weight change is minimal.

Boluses in the reticulum produced release rates significantly higher ($P < .05$) than those occupying the rumen. This was believed to be due to the smaller size of the reticulum which forced a greater degree of contact and abrasion among individual boluses. A significant correlation

Figure 10. Release rates of oxytetracycline (mg/day) from 2 boluses in the reticulum and 2 in the rumen of 12 fistulated Hereford heifers.


RELEASE RATE (mg/bolus/day)
175
150
125
100
75
50
25
5
10
15
20
25
30
35
40
45
50
DAYS
RETICULUM
RUMEN

existed between curves constructed from the 2 stomach locations. Variation in release rates was due to a fluctuation in rumen activity or error in procedures used to construct release curves. The study was terminated after 50 days due to bolus breakage and irregular degradation of some of the boluses in the reticulum.

Rumen inclusions could play an important role in maintaining animal health. As interest grows in the development of such systems, factors which influence their effectiveness and determine their feasibility must be better understood. Bolus density has been shown to play an important role in bolus retention (Chapter IV) and the findings reported herein show density as the major factor in determining stomach location which in turn affects the release rate of a specific object eroding in the reticulorumen.

CHAPTER VI

BOLUS INTERACTIONS AND EROSION

Candidate compounds for bolus formulation differ in the amount of technical material which must be employed to be effective. Therapeutic blood levels of a given compound may be provided by a single bolus, while for other compounds, a multiple treatment of 4 to 6 boluses may be needed.

Previous research indicates that the erosion rate of boluses increases as the number of boluses in the rumen increases. Teel et al. (1977) tested 50% famphur boluses against the Gulf Coast and lone star tick and determined that the abrasion of comparatively large numbers of boluses (4 to 5) increased erosion rates and caused shearing stressed in excess of the tensile strength. While testing the same formulation against _B. microplus_, _B. annulatus_, and _D. albipictus_ (Packard), Hair et al. (1979) found that the removal or addition of a single bolus resulted in a changed erosion pattern of other boluses in the rumen. More recently, Riner et al. (1981) in testing a 60% Rabon® bolus for face fly control reported that erosion of individual boluses increased as bolus number increased and movement of boluses from the reticulum to the ventral sac of the rumen occurred more frequently at the higher treatment levels. The purpose of this study was to monitor bolus activity and determine erosion rate with different numbers of boluses.

Materials and Methods

Twelve mature Hereford heifers weighing approximately 400 kg were fistulated to accommodate model # 1c ruminal cannulas (Bar Diamond, Inc., Parma, Idaho) which would allow direct access to the rumen. The animals were randomly assigned to 3 groups (squares) of 4 animals each and each heifer received either 1, 2, 4 or 6 boluses within a Latin-square design (4 x 4). Twenty % oxytetracycline boluses (Byford et al. 1980) were used in squares 1 and 2 and 60% Rabon® boluses (Riner et al. 1981) were used in square 3. The oxytetracycline boluses had densities of 1.85 gm/cm^3 and were designed to release approximately 200 mg/bolus/day for 45 days when administered at a rate of 2 boluses/animal. The Rabon boluses had densities of 1.95 gm/cm^3 and would release approximately 150 mg/bolus/day for 150 days when administered at a rate of 2 boluses/animal. Boluses of all 3 squares were placed in the reticulum of each animal and monitored for 21 days. At the end of each 21 day period, animals were reassigned random treatments of unused boluses according to the pre-determined design.

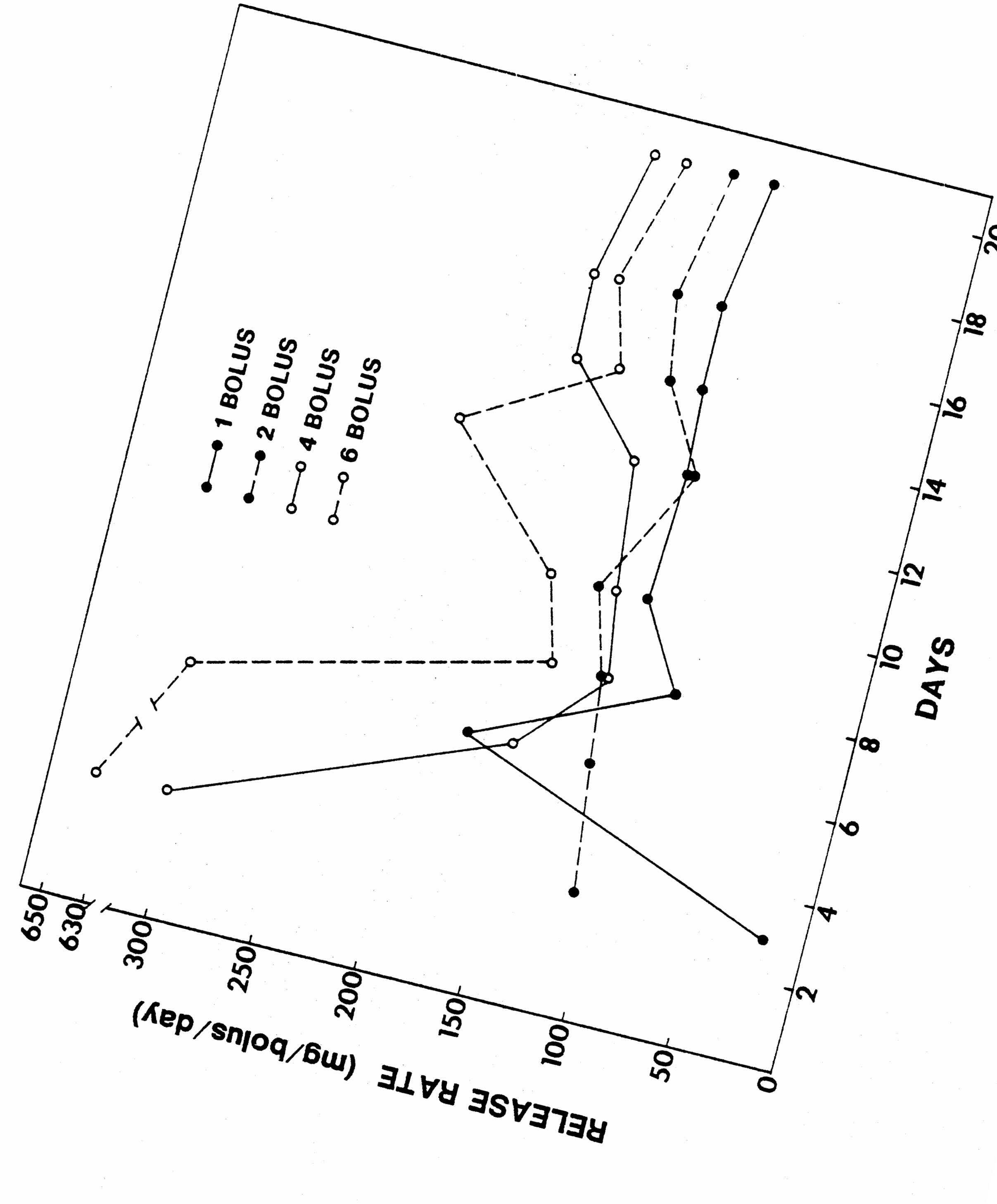
Boluses assigned to each dosage rate were lightly etched according to prearranged code so that each bolus could be individually monitored for the length of the treatment period. Boluses were removed via the fistula triweekly, blotted dry with paper toweling, immediately weighed, and returned to the location within the animal from which they were retrieved. This enabled the determination of dosage by bolus weight change from one period to the next. Experimental animals were maintained under drylot conditions as described in Chapter IV.

Results and Discussions

As discussed in Chapter V, boluses eroding in the reticulum erode at a greater rate over those that pass to the rumen. Due to this difference, it was necessary to discuss boluses eroding in the reticulum separately from those found in the rumen. Figures 11, 13, and 15 show release rates of boluses found in the reticulum at time of weighing of squares 1, 2, and 3, respectively. Figures 12, 14, and 16 show the average release rate of boluses in the reticulum and rumen of the same 3 squares. As expected, boluses eroding in the reticulum show higher release rates than the average of those in the reticulorumen.

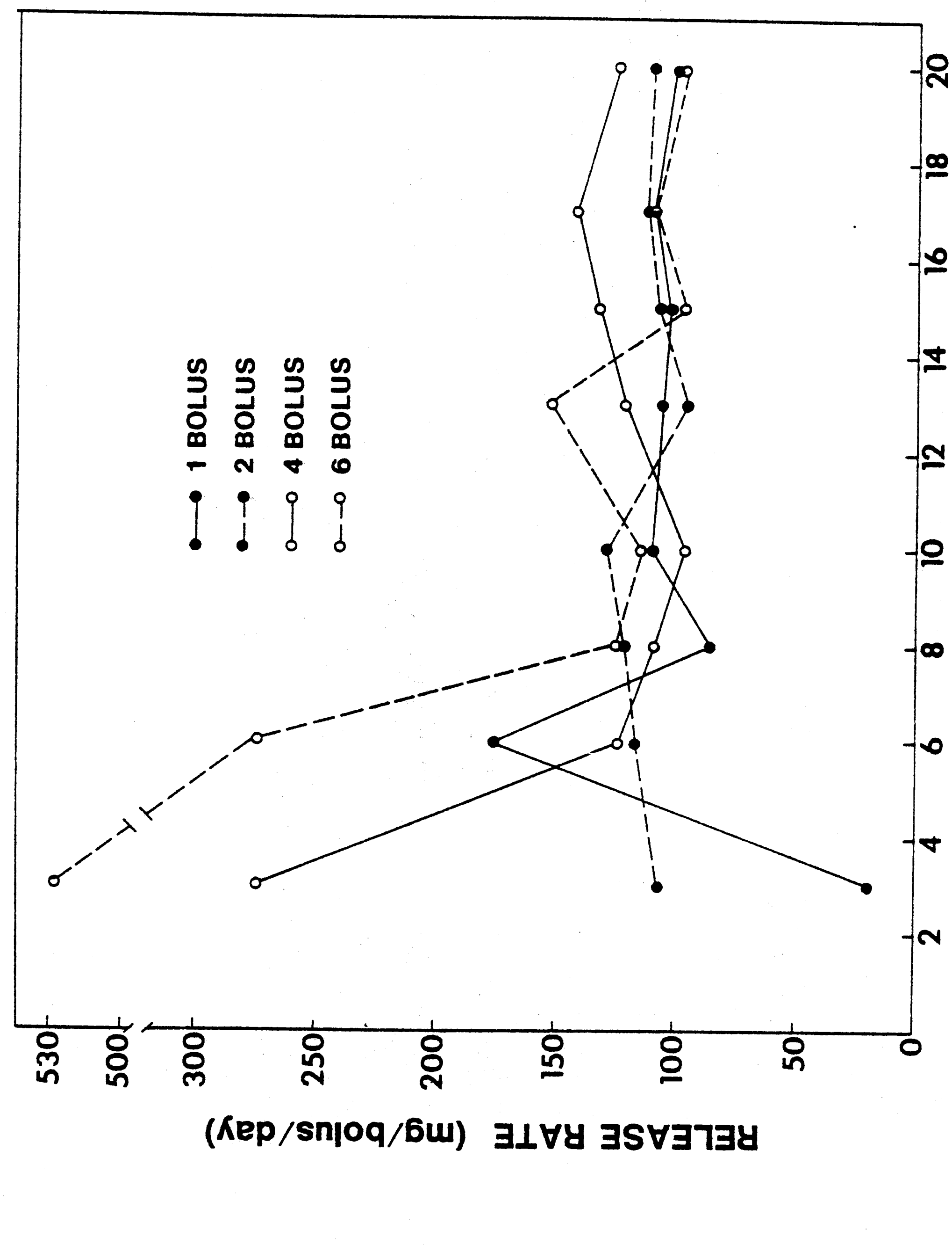
Figures 11 - 14 show the average release of oxytetracycline in milligrams from boluses eroding in the reticulorumen at a dosage of 1, 2, 4, and 6 per animal. The 1 and 2 bolus treatments produced uniform release rates but as bolus number increased, bolus erosion became erratic. Visual inspection of the surface of these boluses showed signs of sloughing and some of the ends appeared to be "chewed off". This irregular degradation may be due to the abrasive posture of the increased dosage. During the first 8 days of erosion, 13% of the boluses of the 4 bolus treatment had broken in half and 22% had passed to the rumen. Seventeen % of the boluses of the 6 bolus treatment had broken in half by the same period, with 21% found in the rumen and 4% recorded as missing. Release rates during the first 10 to 12 days of Figures 11 - 14 are more indicative of the true erosion of the oxytetracycline bolus as later stages of erosion were influenced by the factors mentioned above. When the oxytetracycline treatment of 1, 2, 4, and 6 boluses were analyzed statistically, the error was too large to detect non-linear relationships, but linear curves were found significantly different ($P < .05$) from each other at all

Figure 11. Average release rates of oxytetracycline (mg/day) from 1, 2, 4, and 6 boluses in the reticulum of 4 fistulated Hereford heifers in Square One of a Latin-Square design (4x4).



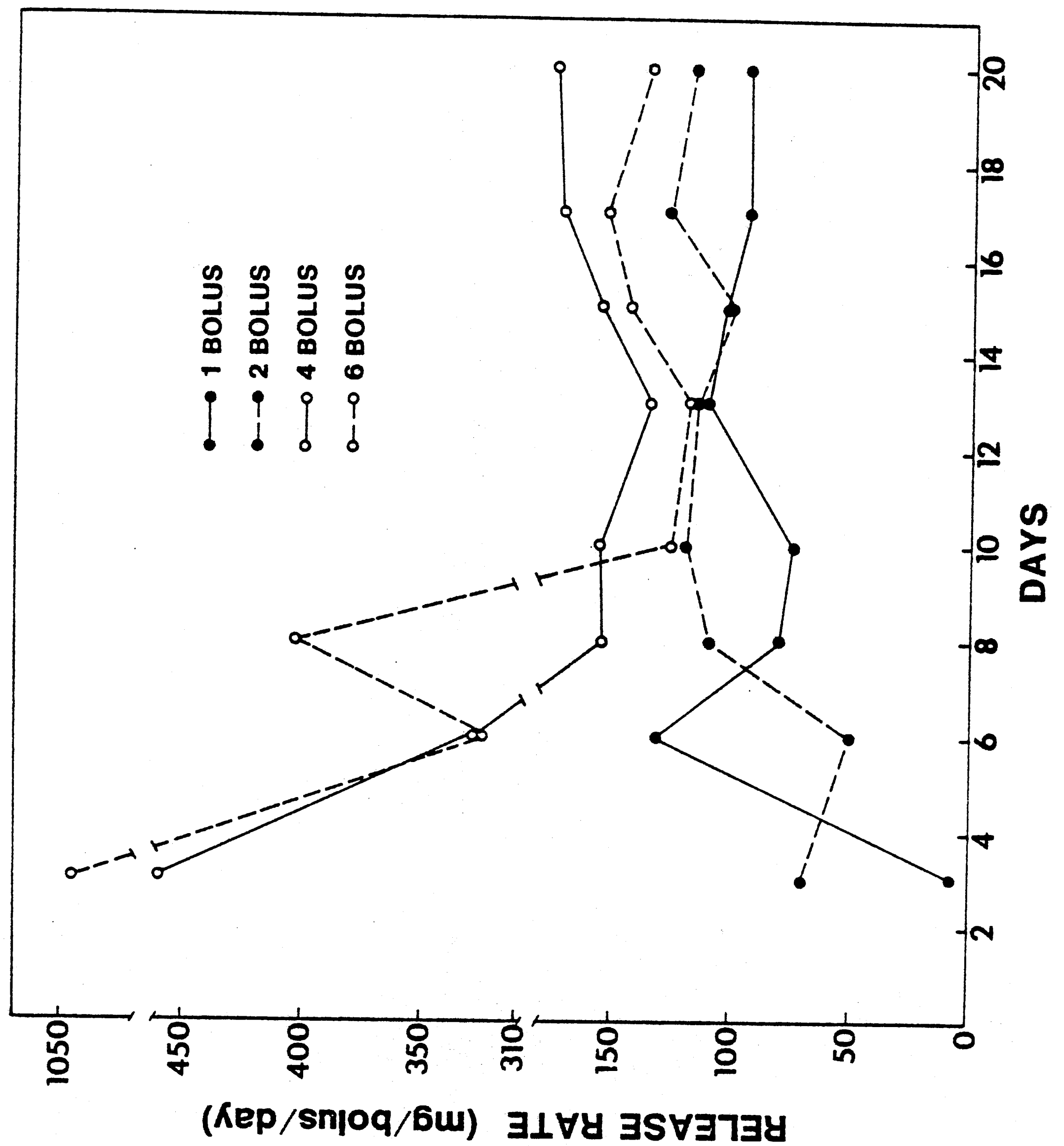
1 BOLUS
2 BOLUS
4 BOLUS
6 BOLUS
RELEASE RATE (mg/bolus/day)
0
50
100
150
200
250
300
630
650
DAYS
2
4
6
8
10
12
14
16
18
20

Figure 12. Average release rates of oxytetracycline (mg/day) from 1, 2, 4, and 6 boluses in the reticulorumen of 4 fistulated Hereford heifers in Square One of a Latin-Square design (4x4).



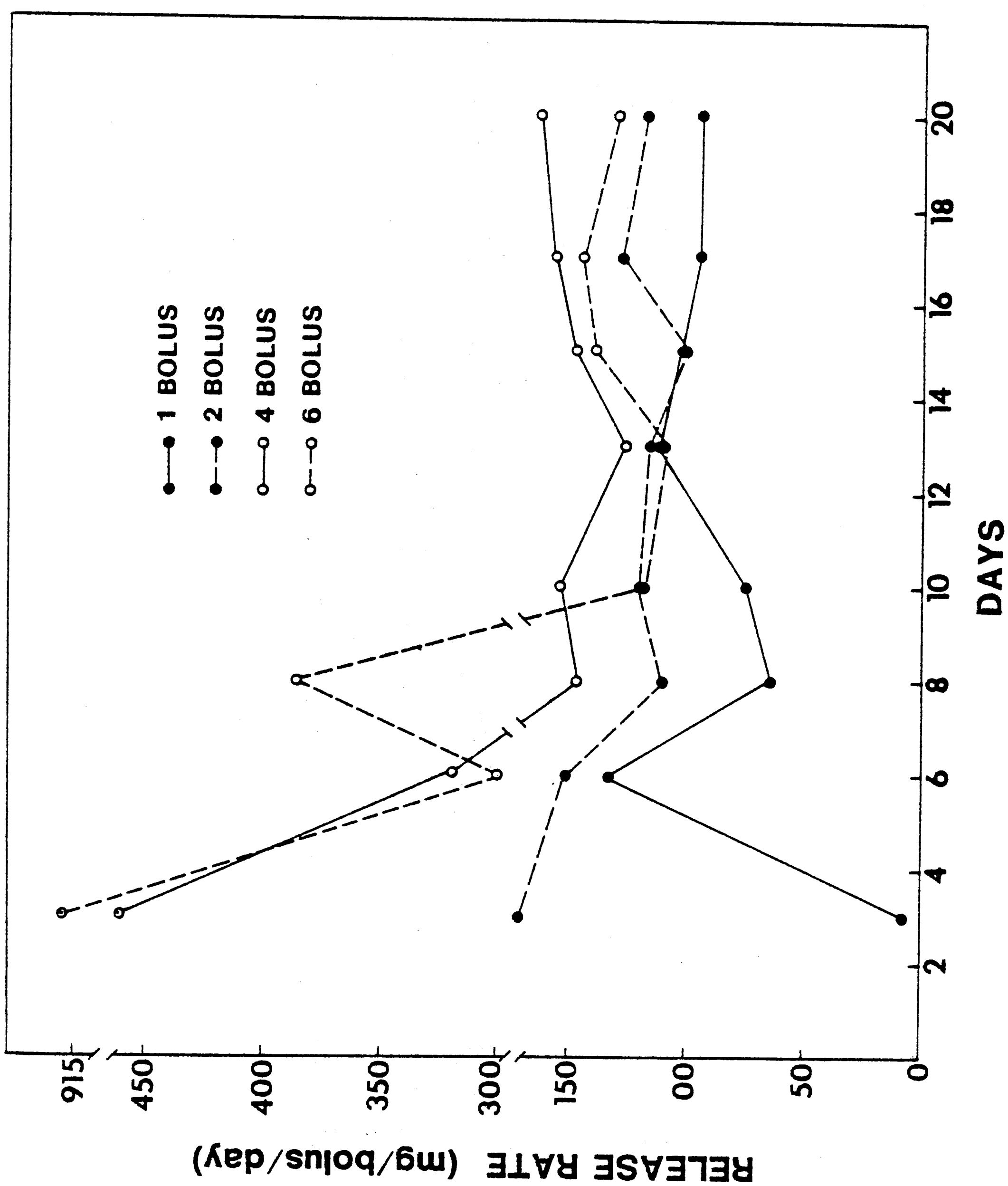
RELEASE RATE (mg/bolus/day)
0
50
100
150
200
250
300
500
530
DAYS
2
4
6
8
10
12
14
16
18
20
1 BOLUS
2 BOLUS
4 BOLUS
6 BOLUS

Figure 13. Average release rates of oxytetracycline (mg/day) from 1, 2, 4, and 6 boluses in the reticulum of 4 fistulated Hereford heifers in Square Two of a Latin-Square design (4x4).



1 BOLUS
2 BOLUS
4 BOLUS
6 BOLUS
RELEASE RATE (mg/bolus/day)
1050
450
400
350
310
150
100
50
0
2
4
6
8
10
12
14
16
18
20
DAYS

Figure 14. Average release rates of oxytetracycline (mg/day) from 1, 2, 4, and 6 boluses in the reticulorumen of 4 fistulated Hereford heifers in Square Two of a Latin-Square design (4x4).



1 BOLUS
2 BOLUS
4 BOLUS
6 BOLUS
DAYS
RELEASE RATE (mg/bolus/day)

Figure 15. Average release rates of Rabon® (mg/day) from 1, 2, 4, and 6 boluses in the reticulum of 4 fistulated Hereford heifers in Square Three of a Latin-Square design (4x4).

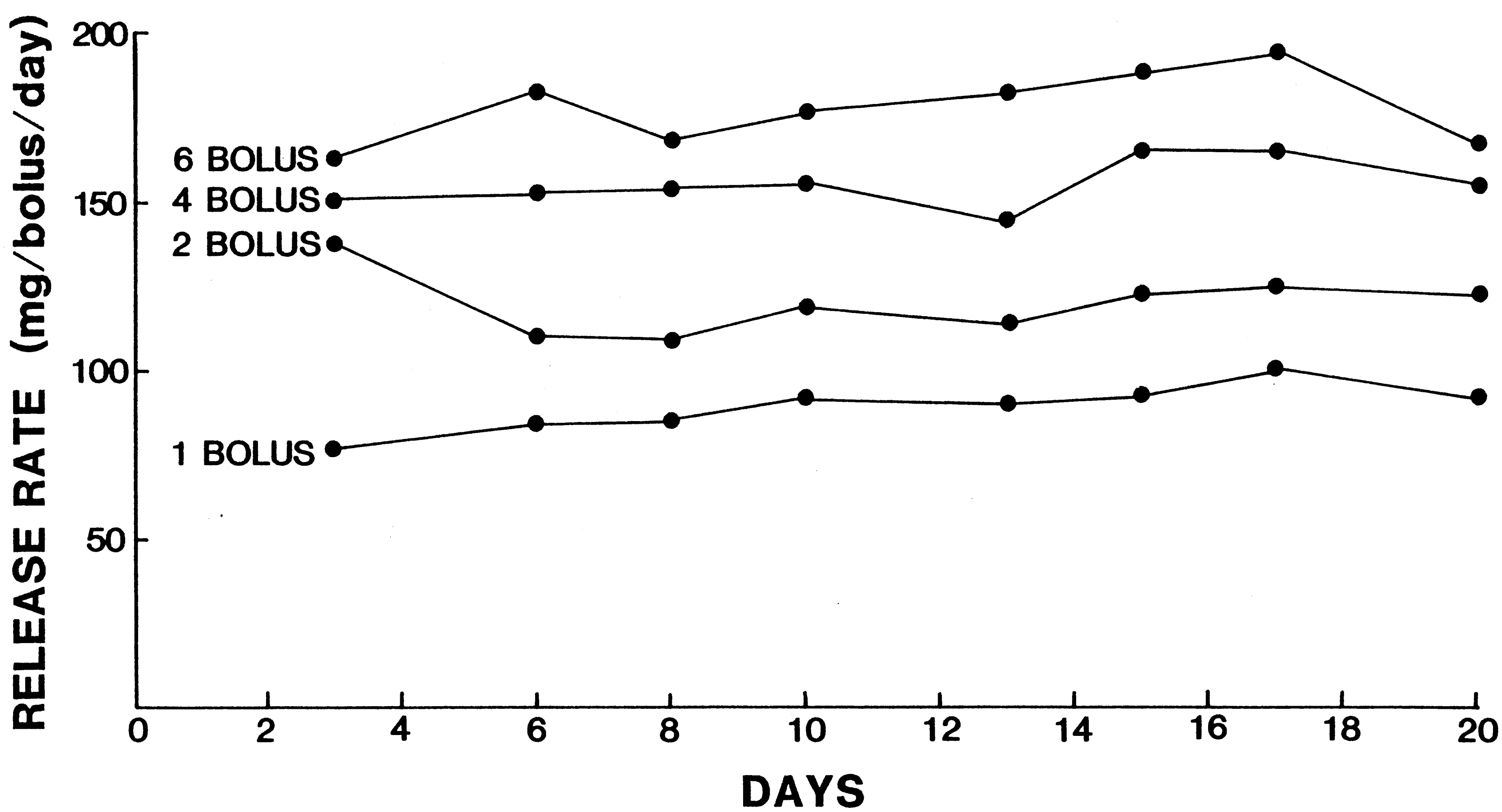
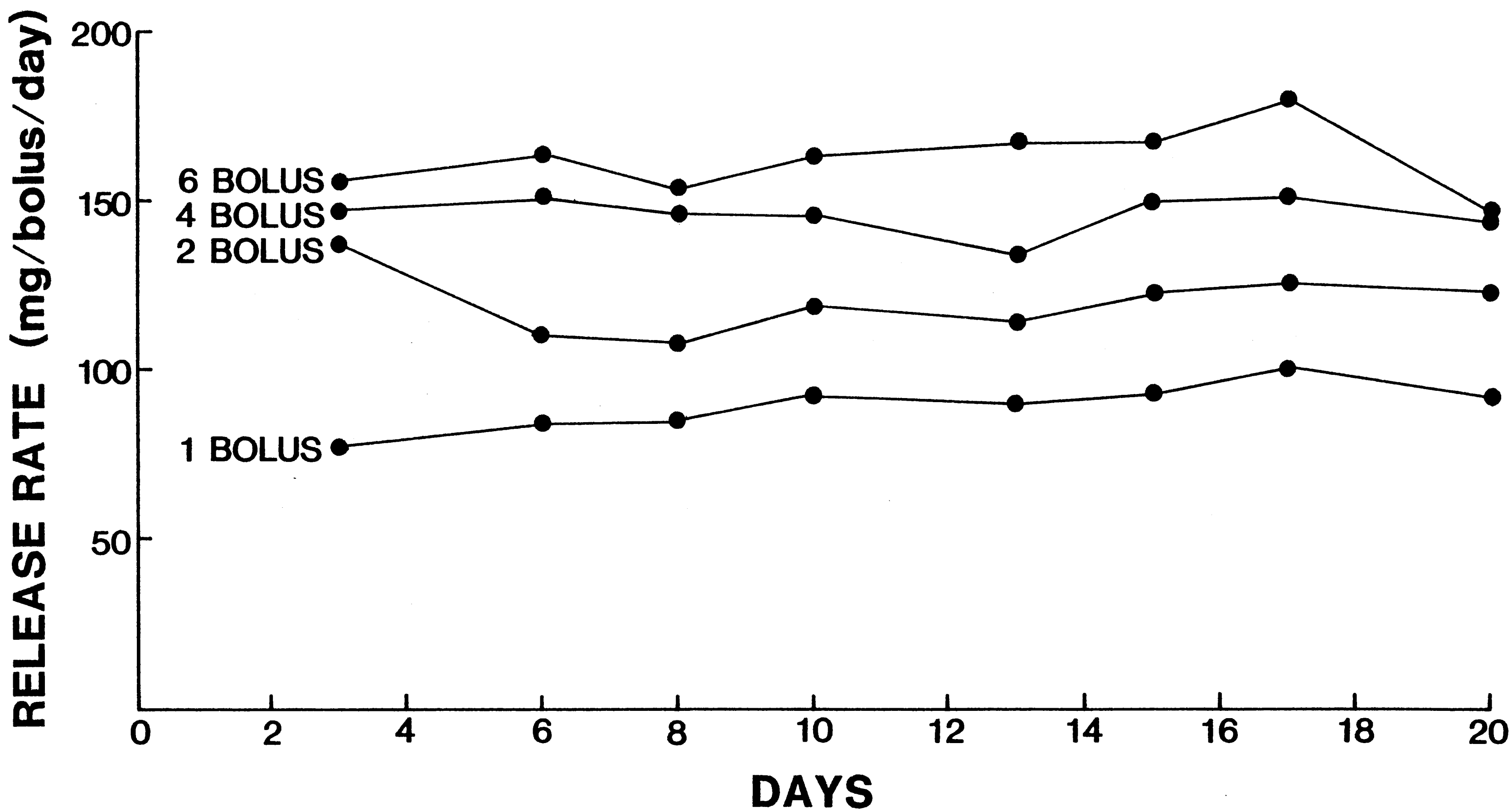

RELEASE RATE (mg/bolus/day)
200
150
100
50
6 BOLUS
4 BOLUS
2 BOLUS
1 BOLUS
0
2
4
6
8
10
12
14
16
18
20
DAYS

Figure 16. Average release rates of Rabon® (mg/day) from 1, 2, 4 and 6 boluses in the reticulorumen of 4 fistulated Hereford heifers in Square Three of a Latin-Square design (4x4).


RELEASE RATE (mg/bolus/day)
200
150
100
50
6 BOLUS
4 BOLUS
2 BOLUS
1 BOLUS
0
2
4
6
8
10
12
14
16
18
20
DAYS

treatment levels. The large error was due primarily to the erratic release rates with the 4 and 6 bolus treatments.

Rabon boluses of figures 15 and 16 (square 3) showed a clearer picture of the difference in treatments of 1, 2, 4, and 6 boluses. Little difference was seen between boluses eroding in the reticulum (Fig. 15) and those in the reticulorumen (Fig. 16) because most of the boluses were retained in the reticulum. Reticulum retention and lack of breakage were contributing factors in the uniform release rates produced by the Rabon bolus. It can be seen from Figure 15 that a Rabon bolus eroding in the reticulum with 5 other boluses will erode at twice the rate than it would alone. Curves were found to be significant ($P < .05$) from each other.

The results above indicate that the number of boluses alter the erosion rate of individual boluses in the reticulorumen. For the formulations tested, erosion rate increased as bolus number increased and the degree of bolus interaction should be determined during the early stages of development of any formulation. Bolus erosion appears to be primarily a physical process during rumination. It's logical that as the number of boluses administered to the small confinement of the reticulum is increased, so will the erosion rate of each individual bolus.

CHAPTER VII

THE INFLUENCE OF ANIMAL SIZE ON BOLUS EROSION

Animal size has been suggested as an important factor in determining release rates of sustained-release boluses. Teel and Hair (1978) indicated the average release rate of a 50% famphur formulation in mature bovine was 200 mg/bolus/day, but when the same formulation was tested in yearlings, Teel et al. (1979) revealed the average release rate was 304 mg/bolus/day. Teel suggested that this increase may be due to a comparatively smaller rumen capacity as boluses tended to lie in closer proximity to each other thereby promoting an accelerated erosion rate. Hair et al. (1979) made some interesting observations while testing the same 50% famphur bolus against Boophilus ticks. He observed that the greater degree of contact between individual boluses in calves or yearlings caused a marked difference in erosion patterns. He suggested that the smaller rumen volume of calves placed boluses in a more abrasive posture, causing a more rapid bolus erosion. The purpose of this study was to determine the difference in erosion rate of boluses in animals differing in size or age.

Materials and Methods

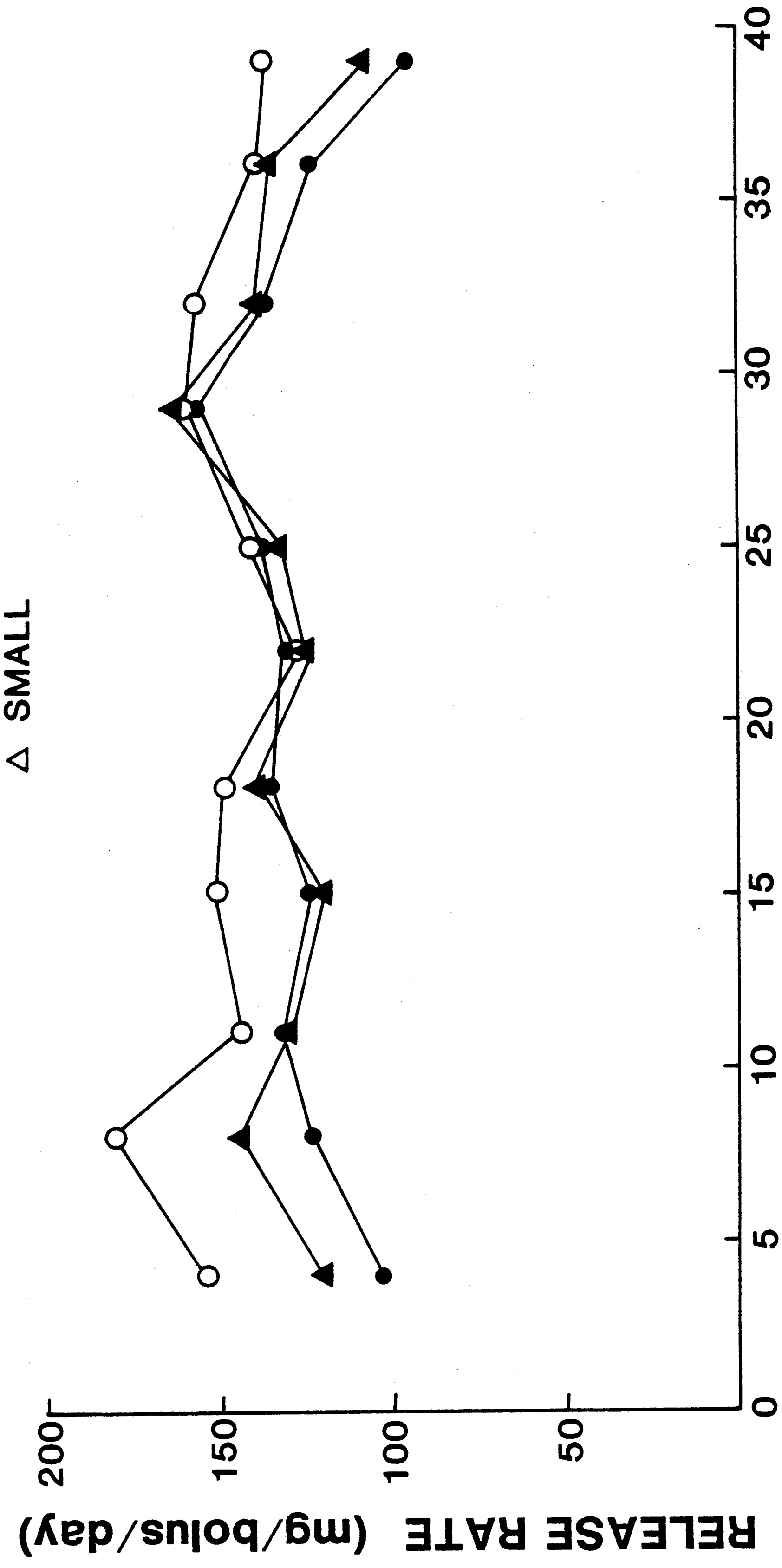
Boluses containing 20% oxytetracycline (Byford et al. 1980) were prepared and administered to 12 Hereford heifers selected by age and size into 3 distinct groups. The small group consisted of 4 yearling calves weighing approximately 220 kg. The medium size group consisted of 4 heifers 2-3 years of age weighing approximately 385 kg. The large group consisted of 4 mature heifers weighing approximately 475 kg. All experimental animals were fitted for model #1c ruminal cannulas to allow direct access to the rumen. Due to the differences in bolus erosion seen between the reticulum and rumen (Chapter V) 2 oxytetracycline boluses were administered, via the cannula, to each compartment of all 12 animals. Boluses were lightly etched for individual identification and observed daily returning any bolus found out of position to its initial location. Boluses were removed twice weekly and weighed over a period of 39 days as described previously. Release rate curves were constructed from weight change data recorded from one weigh period to the next. Animals were maintained under dry lot conditions as previously described.

Results and Discussion

Figures 17 and 18 show release rates (mg/day) of technical oxytetracycline from boluses eroding in the reticulum and rumen, respectively. Tables IX and X correspond to Figures 17 and 18, respectively and show results of statistical analysis of release rates representing the 3 groups of animals. As expected, boluses eroded more rapidly in the reticulum than the rumen. Animal size also had the greatest effect on erosion in the reticulum, being greater for the smaller animals. If the mechanism of erosion is primarily physical, the smaller size of the reticulum of the small group would place the boluses in a more abrasive position thus increasing their erosion rate.

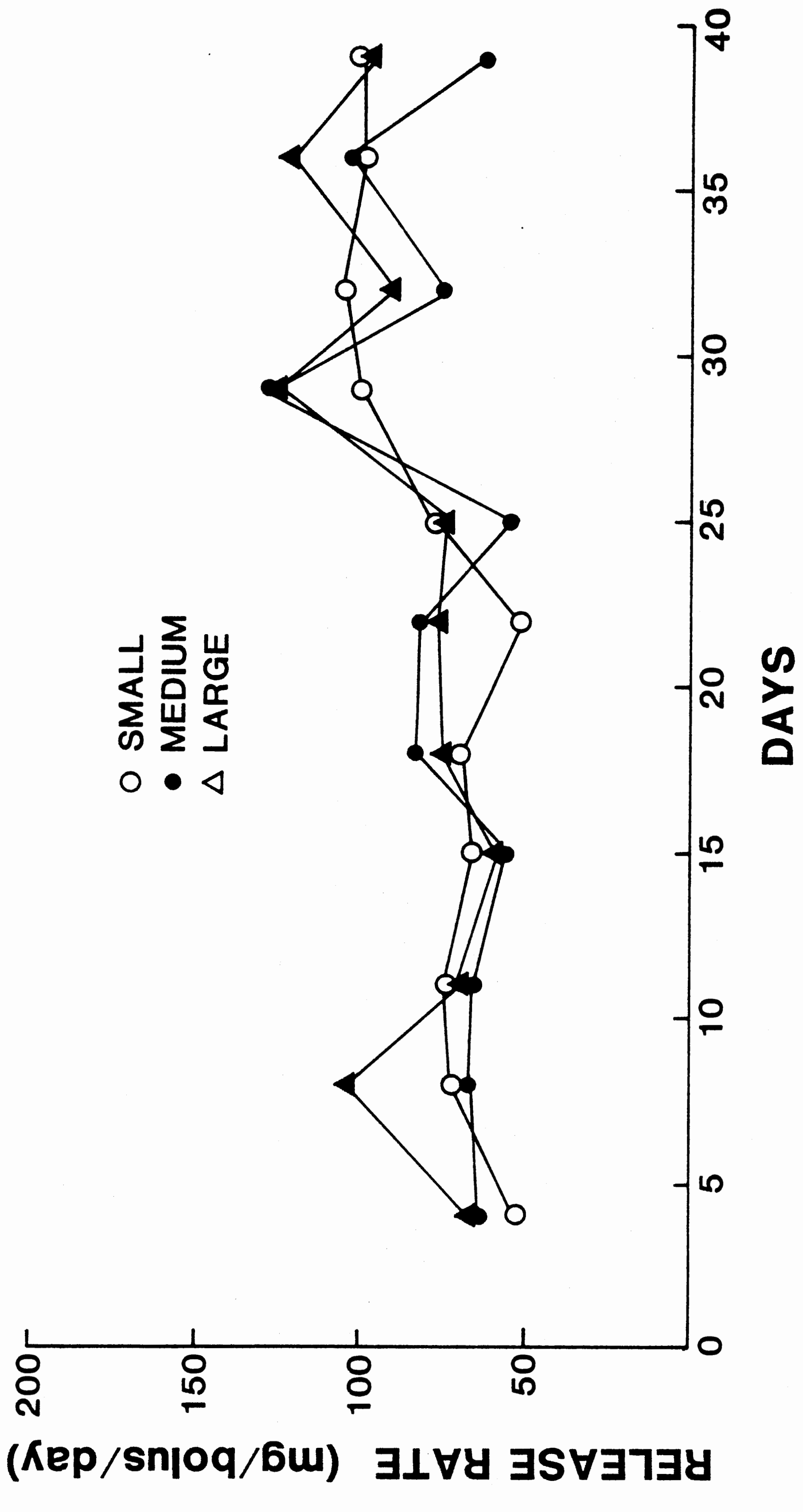
Erosion rates of boluses in the ventral sac of the rumen (Fig. 18,

Figure 17. Release rates of oxytetracycline (mg/day) from boluses in the reticulum of animals of three different sizes.



RELEASE RATE (mg/bolus/day)
200
150
100
50
0
5
10
15
20
25
30
35
40
DAYS
○ LARGE
● MEDIUM
△ SMALL

Figure 18. Release rates of oxytetracycline (mg/day) from boluses in the rumen of animals of three different sizes.



○ SMALL
● MEDIUM
△ LARGE
RELEASE RATE (mg/bolus/day)
200
150
100
50
0
5
10
15
20
25
30
35
40
DAYS

TABLE IX

AVERAGE DAILY RELEASE RATES OF OXYTETRACYCLINE BOLUSES ERODING IN THE RETICULUM OF ANIMALS OF THREE DIFFERENT SIZES

Day Post Administration	Release Rates Per Animal Size (mg/day)		
	Small	Medium	Large
4	159[a*]	104[b]	120[b]
8	180[a]	124[b]	142[b]
11	144[a]	131[a]	131[a]
15	151[a]	124[ab]	122[b]
18	149[a]	136[a]	138[a]
22	129[a]	129[a]	127[a]
25	141[a]	137[a]	134[a]
29	160[a]	155[a]	164[a]
32	157[a]	137[b]	139[b]
36	139[a]	123[a]	135[a]
39	137[a]	96[b]	108[b]

*means in a row with the same letter are not significantly different ($P<.05$)

TABLE X

AVERAGE DAILY RELEASE RATES OF OXYTETRACYCLINE BOLUSES ERODING IN THE RUMEN OF ANIMALS OF THREE DIFFERENT SIZES

Day Post Administration	Release Rates Per Animal Size (mg/day)		
	Small	Medium	Large
4	53[a*]	65[a]	67[a]
8	72[a]	67[a]	104[a]
11	74[a]	66[a]	72[a]
15	66[a]	57[a]	59[a]
18	70[a]	82[a]	75[a]
22	52[a]	81[a]	78[a]
25	77[a]	55[b]	74[ab]
29	100[a]	127[a]	126[a]
32	105[a]	76[b]	91[ab]
36	99[a]	103[a]	121[a]
39	101[a]	62[b]	87[ab]

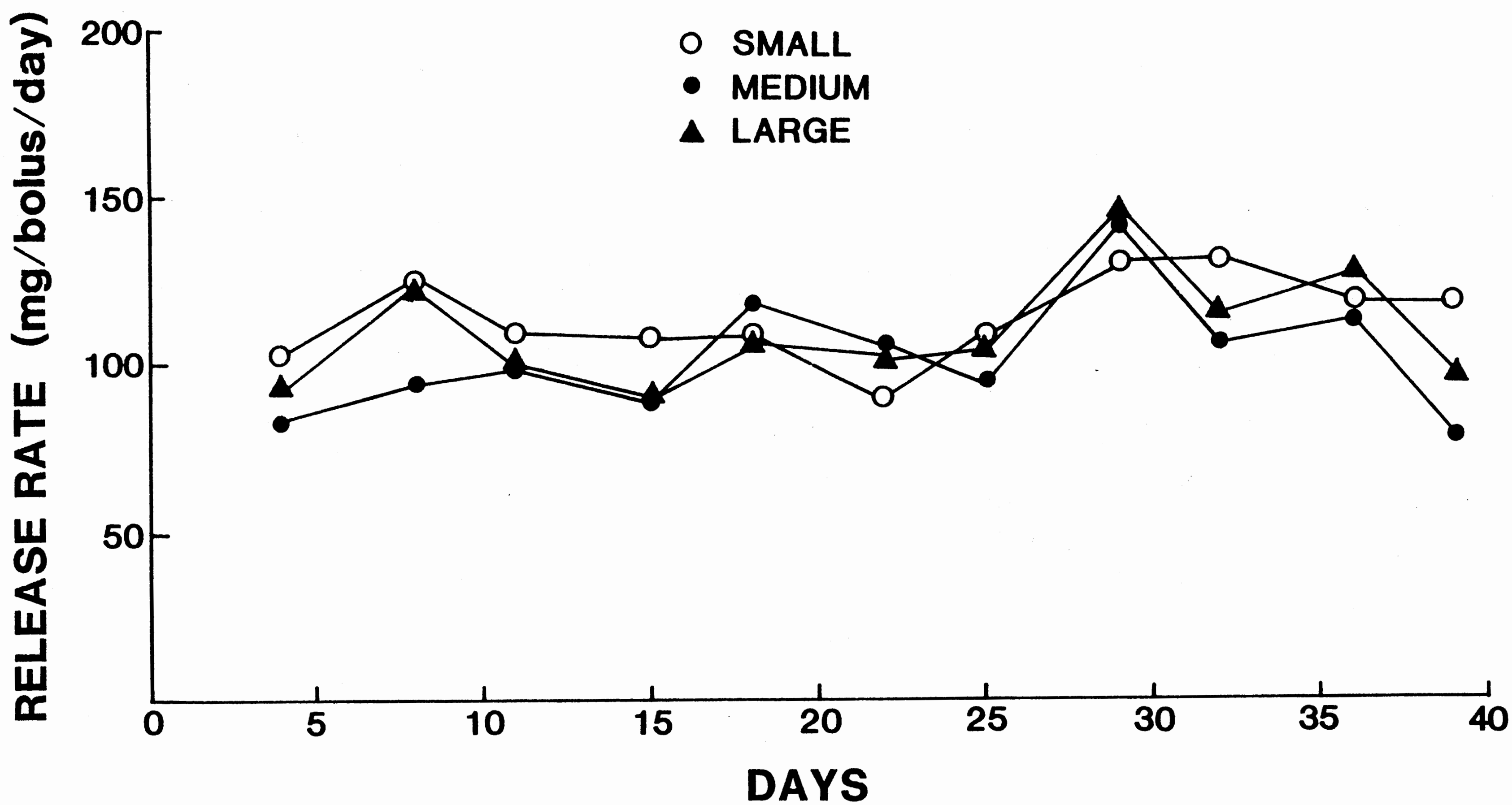
*means in a row with the same letter are not significantly different ($P < .05$)

Table X) were not greatly affected by animal size, presumably due to the comparatively large size of the rumen of all animals. The higher release rates of boluses eroding in the reticulum (Table IX) when compared to those in the rumen (Table X) supports earlier results as discussed in Chapter V.

Figure 19 shows the average release rates of all boluses in the reticulum and rumen. Erosion rates for the smaller heifers was slightly greater than the erosion rates for larger heifers especially the first 15 days.

Differences in erosion rate attributable to animal size was not as pronounced as expected. The author feels that the differences between animal groups reported previously by Teel et al. (1979) and Hair et al. (1979) may be due to an increased dosage and interbolus abrasion rather than animal size. Granted, had a higher dosage rate been used in this study, say 4 or 6 boluses, erosion rate differences between groups may have been more pronounced.

Figure 19. Release rates of oxytetracycline (mg/day) from boluses in the reticulum and rumen of animals of three different sizes.


SMALL
MEDIUM
LARGE
RELEASE RATE (mg/bolus/day)
200
150
100
50
0
5
10
15
20
25
30
35
40
DAYS

CHAPTER VIII

THE EFFECT OF DIET ON BOLUS EROSION

Previous chapters discuss factors involved in the erosion of sustained-release ruminal boluses and most of these factors would apply to any bolus developed for any application. One factor which may be dependent upon the conditions under which the application is made is diet. Bolus erosion is considered to be primarily a physical process affected by particle size, shape, and density of the surrounding foodstuffs in the reticulorumen. Sustained-release bolus may be employed across many areas of animal husbandry with diverse feeding conditions and diet types. One might expect boluses in the rumen of animals on wheat pasture to erode differently than those in the rumen of animals on a feedlot ration. The purpose of this study was to compare erosion patterns of boluses in animals fed distinctly different diets.

Materials and Methods

Fifteen Hereford heifers weighing approximately 400 kg and fitted for model #1c ruminal cannulas were randomly divided into 3 groups of 5 animals each. Groups were randomly assigned to a high concentrate ration, a low concentrate ration or a range situation. The concentrate rations (Table XI) were provided free choice and the range situation was one typical of early-August bermuda grass pasture in central Oklahoma. Two 20% oxytetracycline boluses (Byford et al. 1980) were

TABLE XI

CONCENTRATE RATIONS USED TO MEASURE THE INFLUENCE OF DIET ON BOLUS EROSION

Ingredient	Percent of Material in Ration	
	Low Concentrate	High Concentrate
Crimped Oats	35.2	33.5
Crimped Corn	13.1	30.0
Alfalfa Pellets	10.1	3.0
Cottonseed Hulls	25.1	17.0
Supplement Pellet	11.5	11.5
Cane Molasses	5.0	5.0

administered to the reticulum of all 15 animals and monitored as described previously twice weekly for a period of 40 days. Water and salt was provided free choice.

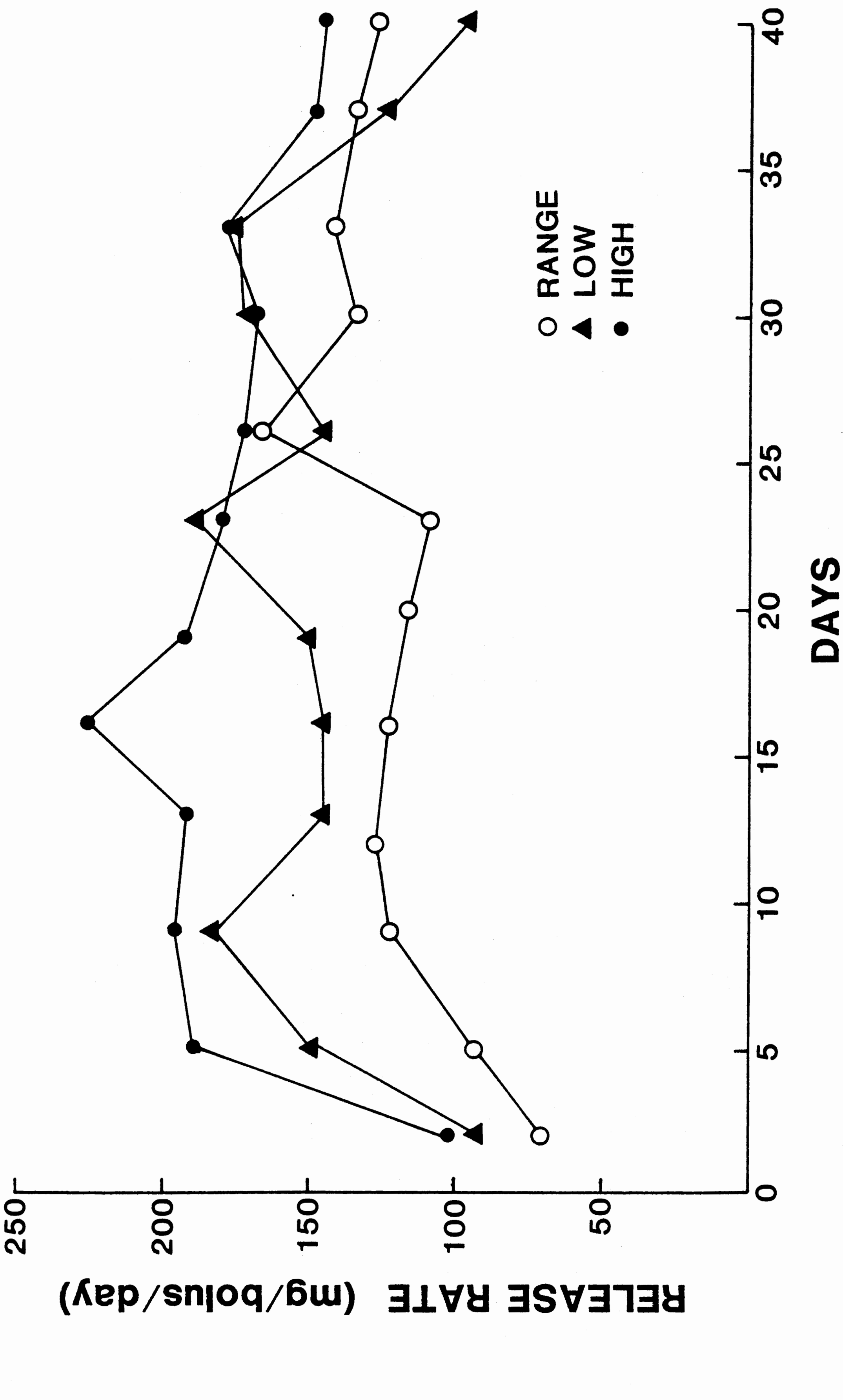
Results and Discussion

Figure 20 shows release rates of boluses eroding in the reticulo-rumen of animals receiving the 3 diets tested. Boluses eroding in the reticulum were analyzed separately from these in the rumen as movement between compartments of this bolus with a density of 1.85 was frequent. When boluses eroding in the reticulum were analyzed statistically, all 3 diets were found significant ($P < .05$) from each other with the high concentrate diet showing the highest release rates, the range situation showing the lowest and the low concentrate diet in between. No significant diet effect was detected for erosion rate of boluses in the rumen and variance was much greater.

The effect of diet on bolus erosion rate may be due to the greater abrasive activity of the high density particles found in the ruminal fluid of animals fed certain diets. High density grain particles move readily between the reticulum and rumen with each peristaltic movement and facilitate bolus erosion. Erosion was also believed to be facilitated by an increased metabolism caused by the higher concentrate ("hotter") ration.

Erosion rates in heifers fed the low concentrate ration were higher than in grazing heifers. The ruminal fluid below the floating pad of animals grazing bermuda pasture has very little particulate material. Lack of particulate material may be the primary reason for the lower release rates of this group. Differences between release rate curves

Figure 20. Release rates of oxytetracycline (mg/day) from boluses in the reticulorumen of animals on three different diets.


RELEASE RATE (mg/bolus/day)
250
200
150
100
50
0
5
10
15
20
25
30
35
40
DAYS
○ RANGE
▲ LOW
● HIGH

gradually decreased over time. This might be expected as the surface area of the more rapidly eroding boluses decreases and reduces the surface area for subsequent erosion.

Results indicate that diet type is an additional factor to consider when determining dosage rates of sustained-release ruminal boluses. The influence of diet was not as dramatic as certain other factors but should be considered in development and testing of sustained-release ruminal boluses.

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