A SUSTAINED-RELEASE SYSTEMIC ACARACIDE BOLUS FOR TICK CONTROL IN BOVINE

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CHAPTER I

INTRODUCTION

Recommendations for chemical control of ticks on cattle in Oklahoma call for spraying or dipping (Hair et al. 1974) and are based on the topical application of an acaracide to the target species at the site of attachment. These control methods have evolved with the development of acaracides and date back to the 19th century (Graham and Hourrigan 1977). These recommendations provide effective tick control only if a thorough program is instituted and well managed; however, they suffer from several drawbacks. The effectiveness of these treatments are short-lived and require repeated applications. During the summer months it has become necessary for ranchers to treat their herds at 10 - 14 day intervals to keep the parasitic tick load under control (Williams et al. 1977). The expense involved can be exorbitant considering the time and labor required, especially where large herds and acreages are involved. With the continual trend toward higher production costs in the cattle industry, tick control practices as are currently invoked, may provide tick relief for livestock but do not provide financial relief from this burden for the producer. From an economic standpoint there is an obvious need for new and improved methods of controlling ticks as well as other livestock ectoparasites.

Estimates of the economic loss to cattlemen from the parasitic effects of tick attack have only recently been attempted. Previously, it

had generally been accepted that heavy tick burdens resulted in production losses brought on by irritation, blood loss and associated unthriftiness. In controlled experiments, weekly infestations of the Gulf Coast tick, Amblyomma maculatum Koch, resulted in a 24 kg difference between infested and tick-free drylot Hereford steers (Williams et al. 1977) and a 12.42 kg difference for infested and tick-free pastured Hereford steers (Williams et al. 1978). Unthrifty cattle and those bearing physical signs of tick parasitism such as "gotch" ear, resulting from heavy infestations of the Gulf Coast tick reduce consumer appeal. Gladney et al. (1977) observed buyer discrimination against gotch-eared calves which brought as much as $4.00 less per hundred weight or resulted in buyer refusal.

The phenomena of controlled release of biological agents offers an approach to tick control that can potentially solve many of the current chemical control problems. By placing a quantity of a systemic acaricide sufficient to provide season-long control within the bovine and release it at a rate to control ticks without harm to the bovine host, several advantages over current control procedures would be realized. By offering season-long control with systemic acaracides administered once or twice per season, cattlemen would achieve a greater economic advantage by saving the time and labor involved in treating 6 to 8 times under the current recommendations. The dependable release of an effective dose from a controlled release system would over time, reduce the total consumption of pesticides applied topically to the host and parasites in order to cope with detoxification and dispersion (Cardarelli 1975).

Lawatsch (1977) suggested that a reliable controlled release system could result in greater reliability of older pesticides with estab-

lished Environmental Protection Agency-approved uses. This authority added that newer compounds whose environmental risk is questionable, due for example to excessive mammalian toxicity at high dosage rates, may ultimately prove more environmentally safe through the more efficient sustained low-level release and yet achieve the compound's therapeutic aim.

The potential of such a system for systemic control of livestock ectoparasites is far reaching. On a world wide basis the loss of livestock to blood-sucking anthropods and the diseases they transmit prevent many third-world countries from becoming efficient producers of animal protein. Without the systematic dipping of cattle in East and South Africa, for example, Theileria parva (Theiler), the causal organism of East Coast fever transmitted by the "brown tick", Rhipicephalus appendiculatus Neumann, can kill in excess of 80% of cattle herds (Soulsby 1968). Similar situations exist throughout the world and serve as reminders of the importance and need for improved animal health care.

It is the scope of this manuscript to describe a series of investigations into the research and development of a controlled-release systemic insecticide bolus for tick control in bovine. The approach was 3-fold, including screening of candidate systemic pesticides for efficacy against 3-host ticks and bed bugs, formulating and testing various matrixes compatible with promising systemic acaracides for release at desirable dosage rates and finally, product testing to determine efficacy against ticks.

CHAPTER II

SCREENING CANDIDATE SYSTEMIC ORGANOPHOSPHATE INSECTICIDES BY RUMEN INFUSION

The use of systemic insecticides in livestock has proved effective against insects that draw their sustenance from the blood of their hosts. Warbex®, for example, is an approved systemic control agent for lice on cattle and swine via pour-on application. After being absorbed into the host's bloodstream systemic insecticides bring about the death of target organisms as they imbibe the material with their blood-meal. Such insecticides can provide effective, continual protection against some blood-sucking arthropods as long as the blood concentration of the material is maintained at a level which proves lethal to the parasite and yet does not endanger the host by exceeding the safety margin for that drug.

Interest in the use of systemic insecticides has generated various screening procedures and provided fruitful results. A screening procedure was outlined by Drummond (1958) in which guinea pigs administered candidate compounds orally or subcutaneously were challenged with nymphal lone star ticks, Amblyomma americanum (L.), screwworms, Callitroga hominivorax (Dqrl.) and stable flies, Stomoxys calcitrans (L.). Promising compounds from this initial screen were administered to sheep and goats as oral drench to further determine their systemic capabilities when challenged with screwworms and lone star ticks. Similar to tests

against cattle grubs (Hypoderma spp.) performed by Kohler and Rogoff (1962), Drummond (1960) began additional screening against cattle grubs where compounds were administered to cattle in capsules, as drench, pour-ons, or intramuscular injection.

Later Drummond (1966) modified these tests to include the administration of candidate insecticides in the feed of cattle for 10 days to determine their efficacy against cattle grubs. This procedure proved effective in controlling the tropical horse tick, Anocentor nitens (Neumann). Trichlorfon in feed at 20 mg/kg/day for 10 days and fenthion in feed at 5 mg/kg/day for 5 days were systemically effective against this tick on horses (Drummond and Graham 1964; Drummond and Ossorio 1966). Gladney et al. (1972) tested 6 systemic insecticides in feed against the tropical horse tick infesting stanchioned cattle and found that famphur at 5 mg/kg/day and fenthion at 1.25 mg/kg/day provided 98.3 - 100% and 97.8% control respectively. Fenthion at 2.5 mg/kg/day proved lethal to the animal. Control was indicated by smaller numbers of engorged females, reduced repletion weights, egg mass weights and low % hatch when compared to those from untreated animals. Famphur has also been observed to control the shortnosed cattle louse, Haematopinus eurysternus (Nitsch) when incorporated in cattle feed at the rate of 2.5 mg/kg body weight and fed for 30 days (Roberts et al. 1969). Drummond et al. (1972) also used this feed additive method to control adults and nymphs of the lone star, Gulf Coast, and American dog ticks (Dermacentor variabilis (Say)) on stanchioned cattle. The most effective treatment in this study was famphur at 5 mg/kg/day. Famphur was shown to have afforded greater than 99.5% control of the estimated larval population of the lone star tick through reduction of egg deposition

and % hatch.

Commercial application of systemic and "feed-through" pesticides have limitations, particularly under rangeland situations where feed and water intake cannot be adequately monitored and animals are not readily accessible. In a geographically isolated location where access to cattle water supplies could be regulated, Miller et al. (1976) demonstrated that the insect growth regulator (IGR) methoprene could be dispensed by tablet erosion to achieve a continuous supply of the medicament for control of the horn fly, Haematobia irritans (L.), in manure.

The principal deficiency of these biological control agents is that no satisfactory means of insuring uniform daily intake exists. Achieving therapeutic levels of biological control agents administered as additives via water, mineral blocks or ration is subject to individual animal intake both in frequency and volume. Within a given herd some animals may never consume enough of the agent to achieve therapeutic blood levels while others may consume excessive amounts resulting in toxicosis. Long term control could be achieved in ruminants with pesticide ladened, sustained-release boluses providing a desired dosage independent of the great variability in animal intake and without the problems of managing animal movements. The problem of producing such an acceptable product is magnified by the fact that despite the research in this area, no materials are currently registered for use as systemic tick control agents.

The first phase of this project evolved around the development of a technique to screen candidate compounds as possible systemic tick control agents by simulating the release of an acaracide from a rumenal bolus. Subsequently, 4 organophosphate compounds were screened for

efficacy.

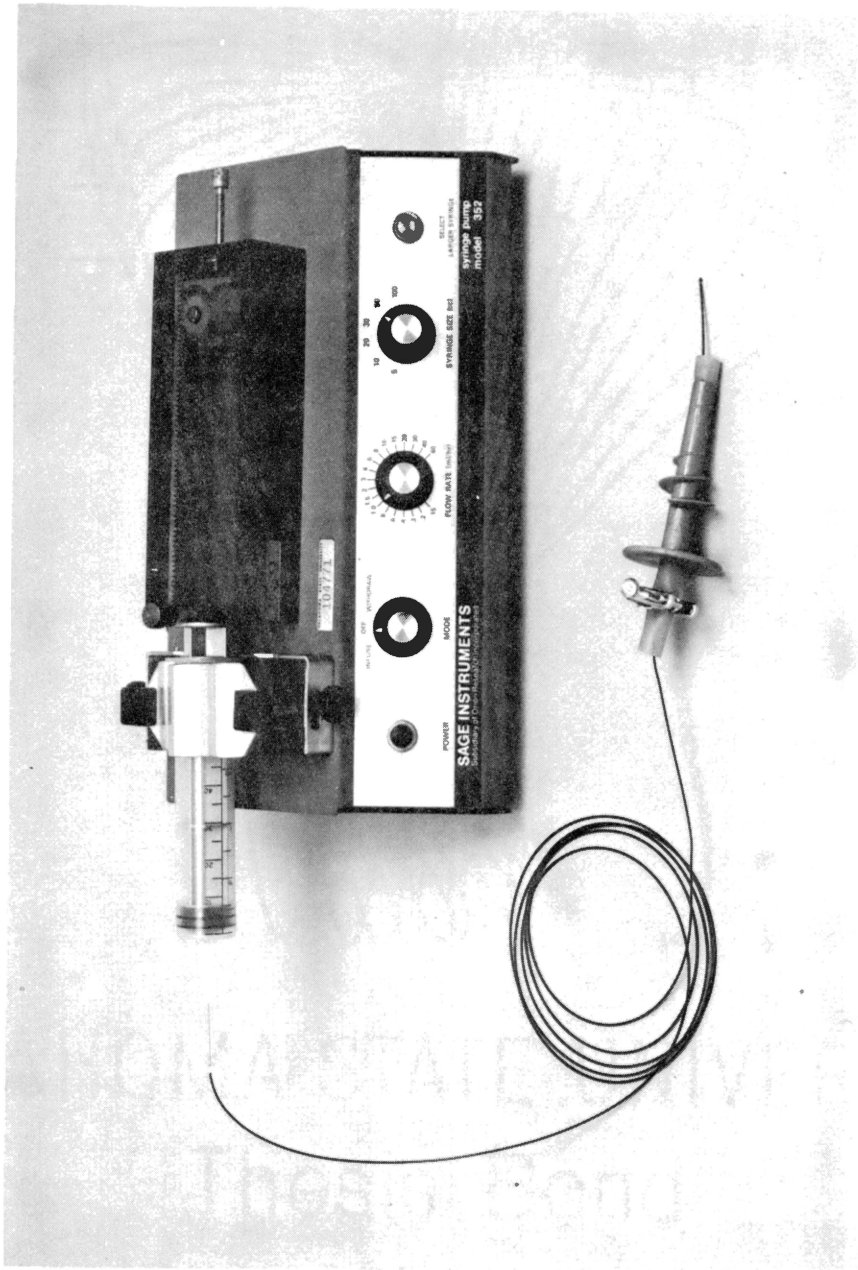
Materials and Methods

Grade ewes of uniform age and health were used to evaluate candidate compounds in these experiments. A preliminary screen was conducted with each compound utilizing 1 ewe at each dosage rate and 1 control to determine the lowest effective dosage rate and any toxicological problems the sheep might experience. Following the preliminary trial, a study was conducted at the rates thought to be most efficacious (Teel et al. 1977). At each dosage rate, 3 treatment and 3 control animals were randomly selected and assigned to stanchions in a 6-stall laboratory maintained at $20^{\circ} \pm 5^{\circ}$C with 14-h photophase.

Plastic corkscrew-type cannulas (Haver-Lockhart Laboratories, Shawnee Mission, Kans.) were inserted through the abdominal and rumen walls to provide direct access to the rumen. The diameter of the fistula was reduced from 10 to 7 mm by threading latex rubber tubing (12 mm OD) through the cannula. The latex tubing extended 4 cm externally to allow a tubing clamp to constrict the outer orifice. Sheep were allowed 10 days postoperative rest following minor surgery for insertion of the cannulas.

Continuous flow of the candidate material was supplied via 60 ml syringes driven by Sage Instruments® model 352 infusion pumps (VWR Scientific, Denver, Col.). Each pump was capable of driving 3 syringes, each delivering 24 ± 2 ml/24 h. Intramedic® polyethlene tubing (1.25 mm OD) (Melton Co., Inc., Oklahoma City, Okla.) carried the material from the syringes through the cannula and into the rumen. The tubing was held in place by constriction of the latex tubing. Figure 1 shows

Figure 1. Assembled rumen infusion system consisting of: infusion pump, syringe, polyethylene tubing, and assembled cannula


SAGE INSTRUMENTS
POWER
MODE
FLOW RATE
SYRINGE SIZE
SYRINGE PUMP
model 352

the assembled unit.

During a 3-day laboratory acclimation period, sheep were bled to determine their preinfusion packed-cell volume, hemoglobin, and red blood cell cholinesterase levels. Cholinesterase determinations were made by the procedure of Radeleff and Woodward (1956) and expressed in ΔpH units. Blood samples were obtained at 3-day intervals following initiation of infusion to monitor animal health. Candidate compounds in ethanol, were delivered to the rumen at the rate of 1 ml/h. Control animals received ethanol only. After 72 h infusion, treatment and control sheep were challenged with A. americanum, A. maculatum, D. variabilis, and the bed bug, Cimex lectularius L.

Twenty adult pairs of each tick species were confined in stockinette cells, and more than 50 nymphs of each species were confined in Plexiglas® cells to each sheep. Percent adult mortality was determined on total numbers attached. Engorged females were weighed, placed in individual vials, and held at 92 ± 3% RH, 20° ± 5°C, and 14-h photophase. Ticks were observed daily for the first date of oviposition. Weights of egg masses were determined 20 days after oviposition began. Another 40 days were allowed to pass to allow sufficient time for all eggs to hatch before % hatch was measured and recorded. In accordance with Drummond et al. (1972) an estimated larval population (EL) was calculated as follows:

EL = g eggs x estimated % hatch x 20,000 (the estimated larvae produced from 1 g of eggs of these 3 species).

Percent mortality of nymphs was determined from those attached. Engorged nymphs were held under the aforementioned conditions to determine % molt. Twenty 4th-instar C. lectularius were fed on each sheep

for 30 min and then held to determine % mortality at 24-h intervals.

At each infusion rate, analysis of variance of the split-plot design was performed, and the treatment and control means compared by LSD.

Results and Discussion

Four technical grade organophosphate insecticides including coumaphos[1] (O, O-diethyl O-(3-chloro-4-methylumbelliferone) phosphorothioate), supona[2] (2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate), famphur[3] (O,O-dimethyl O-(p-(dimethylsulfamoyl) phenyl) phosphorothioate), and fenthion[4] (O,O-dimethyl O-4-methylthio-m-tolyl phosphorothioate) were screened for systemic activity with this model. They were selected on the basis of their potential systemic activity and availability from cooperating companies. Coumaphos previously shown to have systemic activity in guinea pigs (Drummond 1958) but little to no activity in rabbits (Vickery and Arthur 1960) proved lethal to sheep when administered at 3.5 and 5.0 mg/kg/day. A dosage rate of 2.5 mg/kg/day was not lethal to sheep, however, it proved ineffective against all 3 tick species. This ineffectiveness against test organisms and the narrow safety margin of the insecticide administered in this manner precluded further testing.

Supona, a non-sulfur containing organophosphate compound which has

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[1] Chemagro, Kansas City, MO.

[2] Shell Chemical Co., Modesto, Cal.

[3] American Cyanamid Co., Princeton, N.J.

[4] Bayvet Corp., Shawnee Mission, Kans.

been shown to possess excellent acaracidal activity (Drummond et al. 1966, 1976) as a spray or dip was screened at 1.0, 2.0, 3.0, 4.0, 5.0, 7.0 and 10.0 mg/kg/day in this systemic model.

At these 7 rates supona demonstrated no systemic activity against test organisms including the bed bug. Test results from blood samples indicated red blood cell cholinesterase was significantly inhibited only at the 10.0 mg/kg/day rate. The lack of arthropod and cholinesterase response to these treatments suggests that either the parent compound was being metabolized by the host to non-toxic metabolites or was being passed through the alimentary tract without being absorbed.

Preliminary screening of famphur at 5 and 7 mg/kg/day showed promising systemic activity without host toxicosis. Tables 1 and 2 show data collected from secondary screening trials using 3 treatment and 3 control sheep at each rate. Analysis of these data revealed that at 5 mg/kg/day (Table 1), differences in % mortality of female A. maculatum and % hatch of eggs from those surviving females which received famphur were statistically significant ($P < 0.05$) from controls. Significant differences in % mortality of female A. americanum and D. variabilis were not noted. No significant effects on male mortality were noted for any of the 3 tick species. Mortality of A. maculatum nymphs at this rate was significantly higher than that from control sheep ($P < 0.05$). No effect on % molt of engorged nymphs was observed for any species.

Secondary screening of famphur at 7 mg/kg/day (Table 2) provided complete control of A. maculatum adults. Although data from adult A. americanum and D. variabilis were not significant at this rate, an increased % adult female mortality and decreased estimated larval produc-

Table 1. Mean effects of famphur administered continuously at 5.0 mg/kg/day via the rumen on 3 tick species feeding on sheep.

	Treatment			Control		
	A. americanum	A. maculatum	D. variabilis	A. americanum	A. maculatum	D. variabilis
% Adult male mortality	11.00	15.96	36.00	0.00	2.00	26.00
% Adult female mortality	2.00	44.00[a]	5.00	0.00	5.00	4.00
Repletion wt. (g)	0.85	0.98	0.58	0.81	0.97	0.61
Egg mass wt. (g)	0.48	0.47	0.32	0.42	0.49	0.32
% Hatch	83.98	40.83[a]	87.79	82.23	61.77	94.52
Oviposition time (days)	6.8	4.5	4.6	7.1	4.6	4.4
Estimated larvae produced (EL)	7942.0	4142.3	5677.4	6926.6	6662.9	6227.6
% Nymph mortality	1.00	53.00	7.0	15.00	16.00	1.00
% Nymph molt	100.00	100.00	100.00	100.00	100.00	100.00

[a] $P < 0.05$.

Table 2. Mean effects of famphur administered continuously at 7.0 mg/kg/day via the rumen on 3 tick species feeding on sheep.

	Treatment			Control		
	A. americanum	A. maculatum	D. variabilis	A. americanum	A. maculatum	D. variabilis
% Adult male mortality	19.00	77.00[a]	9.0	0.00	5.00	0.00
% Adult female mortality	56.00	98.00[a]	12.00	15.00	7.00	7.00
Repletion wt. (g)	0.69	0.70	0.58	0.69	0.99	0.60
Egg mass wt. (g)	0.35	0.36	0.33	0.38	0.60	0.34
% Hatch	30.60	0.00[b]	84.43	49.73	65.55	89.53
Oviposition time (days)	8.7	5.0	5.4	9.4	4.2	4.7
Estimated larvae produced (EL)	2581.6	0.0	5635.4	4193.2	8203.0	6171.5
% Nymph mortality	76.00[a]	100.00	5.00	0.00	29.00	0.00
% Nymph molt	71.00	------	97.00	100.00	67.00	100.00

[a] $P < 0.05$.

[b] Eggs deposited by surviving A. maculatum females were not viable.

tion (due to reduced egg mass weight and % hatch) were noted for A. americanum. Complete control of A. maculatum nymphs was achieved and % mortality of A. americanum nymphs at 7 mg/kg/day was statistically significant ($P < 0.05$). Famphur showed no effect on % molt of engorged nymphs at this rate. Drummond et al. (1972) noted that 5 mg/kg/day famphur placed in the feed of cattle was highly effective against A. maculatum and less effective against A. americanum and D. variabilis. Although we utilized a different ruminant host, data from our ticks indicate similar results. Famphur may be metabolized more rapidly by sheep, which would necessitate a slightly higher dosage rate to achieve similar control.

Fenthion was administered at 1.0, 1.5, 2.0, 2.5 and 3.0 mg/kg/day in the preliminary screen. There were no signs of clinical toxicosis and the rates of 2.5 and 3.0 mg/kg/day provided positive results prompting secondary screening at these rates. Table 3 shows data collected from the secondary trial at 2.5 mg/kg/day. Mortality of both male and female A. maculatum were significantly different ($P < 0.05$) from the respective control values attributing the difference to the fenthion treatment. Only 1 female A. maculatum successfully engorged on treated sheep, however, she did not produce viable eggs resulting in complete control of this species. Reductions in egg mass weight and % hatch of A. americanum resulted in a significantly ($P < 0.05$) lower estimated larval population (EL) attributed to the fenthion treatment. Increased mortality of both male and female D. variabilis were observed among those ticks from treated sheep, however, these were not significantly different from controls.

The high mortality of adult male A. americanum is attributed to

Table 3. Mean effects of fenthion administered continuously at 2.5 mg/kg/day via the rumen on 3 tick species feeding on sheep.

	Treatment			Control		
	A. americanum	A. maculatum	D. variabilis	A. americanum	A. maculatum	D. variabilis
% Adult male mortality	86.00[a]	100.00[a]	71.00	12.00	16.00	15.00
% Adult female mortality	11.00	82.00[a]	26.00	0.00	0.00	3.00
Repletion wt. (g)	0.55	0.67	0.56	0.75	1.04	0.56
Egg mass wt. (g)	0.28	0.32	0.30	0.38	0.58	0.29
% Hatch	58.27	0.00	81.28	82.49	60.87	78.42
Oviposition time (days)	8.8	5.0	5.4	8.5	3.6	4.9
Estimated larvae produced (EL)	3807.2	0.0	4918.8	6383.9	7628.0	4720.6
% Nymph mortality	41.00	88.00[a]	75.00[a]	4.00	8.00	1.00
% Nymphal molt	68.00	50.00	25.00	96.00	93.00	99.00

[a] $P < 0.05$.

treatment (statistically significant, $P < 0.05$). It should be noted that male mortality was observed to occur at different times for the different species. Periodic mortality determinations indicate that death among male A. americanum and D. variabilis, even though statistically attributed to treatment, occurred after they had successfully mated with females and thereby contributed to completion of female engorgement. In contrast, adult male A. maculatum began dying as early as the 4th day post-attachment and mortality increased thereafter. There appeared to be little to no successful engorgement and mating. These species differences in male mortality may play an important role in assessing the potential success of control programs. Mortality of A. maculatum and D. variabilis nymphs was significantly ($P < 0.05$) higher than control and though not attributed to treatment 41% mortality of A. americanum nymphs was observed. A decreased % molt of replete nymphs was observed with the greatest reduction occurring among D. variabilis.

When fenthion was administered at 3.0 mg/kg/day (Table 4) complete control of A. maculatum was achieved with successful engorgement held in check. The repletion weight and egg mass weight of A. americanum were reduced and the reduction in % hatch was significant at the 1% level. This resulted in a highly significant ($P < 0.001$) reduction (45.4%) in estimated larval production. Again, increased % mortality among male and female D. variabilis was observed but could not be attributed to treatment.

A leak in the intramedic tubing discovered early in the trial eliminated the nymph data from 1 treatment sheep. Consequently nymph data collected from the 3.0 mg/kg/day trial were not subjected to statistical analysis. The nymph data in Table 4 is therefore based on 2

Table 4. Mean effects of fenthion administered continuously at 3.0 mg/kg/day via the rumen on 3 tick species feeding on sheep.

	Treatment			Control		
	A. americanum	A. maculatum	D. variabilis	A. americanum	A. maculatum	D. variabilis
% Adult male mortality	53.00[c]	98.00[c]	61.00	4.00	12.00	17.00
% Adult female mortality	7.00	71.00[a,c]	35.00	0.00	1.00	2.00
Repletion wt. (g)	0.65	-----	0.49	0.712	1.26	0.51
Egg mass wt. (g)	0.27	-----	0.24	0.34	0.756	0.27
% Hatch	40.45[d]	-----	84.81	77.84	62.55	88.86
Oviposition time (days)	8.9	-----	6.5	9.1	4.2	5.7
Estimated larvae produced (EL)	2791.0[e]	-----	4114.7	6145.7	9314.8	4852.3
% Nymph mortality	55.00[b]	85.00[b]	36.00[b]	4.00	4.00	21.00
% Nymphal molt	55.00[b]	48.00[b]	60.00[b]	71.00	96.00	91.00

[a] 28.9% of the A. maculatum were less than replete and remained attached at the end of the study.

[b] Nymph mortality and molt data is based on 2 treatment sheep as a leak was discovered and corrected on the 3rd sheep after a major portion of nymph feeding had transpired.

[c] $P < 0.05$ [d] $P < 0.01$ [e] $P < 0.001$.

treatment sheep. An increased nymph mortality and reductions in % molt of replete nymphs were posted by all 3 tick species.

Table 5 summarizes the data collected from feeding C. lectularius on treatment and control sheep at each rate for these 4 organophosphate insecticides. Coumaphos and supona were ineffective at the dosage rates tested. Famphur provided excellent control at both 5 and 7 mg/kg/day. The % control provided by fenthion illustrates the progressive systemic effectiveness of this insecticide through the rates tested. This dose-response relationship provided complete control of the bed bug at 3.0 mg/kg/day.

The effectiveness of using the bed bug as a treatment bioassay in our screening technique was 2-fold. This insect feeds within minutes and mortality could be obtained within 24-48 h giving a rapid indication of the presence of the insecticide or an active metabolite reaching the periferal blood stream of the host. Being a blood feeding insect, these data may also provide a relative indicator of the effectiveness of these compounds against some biting flies.

Monitoring animal health was a primary concern during these tests. Significant changes in pack-cell volume or hemaglobin levels were not observed for any sheep. A reduction or cessation of feed consumption coupled with cholinesterase depression were the first indications when toxicity occurred. The toxicity of coumaphos to sheep at 3.5 and 5.0 mg/kg/day has been mentioned previously. At death, red blood cell cholinesterase levels for both sheep were 0.06 ΔpH units, depressed from 0.20 ΔpH units for the 6th infusion day for the 5.0 mg/kg/day sheep and 0.22 ΔpH units by the 4th day for the 3.5 mg/kg/day sheep. No clinical signs of toxicity were observed for the sheep receiving coumaphos at 2.5

Table 5. Percent control of the bed bug, Cimex lectularius L. fed on sheep receiving candidate systemic organophosphate insecticides via rumen infusion.

Insecticide	Dosage (mg/kg/day)	Number of test feedings	Percent control
Coumaphos	2.5	2	5.3
	3.5	Lethal to host.	
	5.0	Lethal to host.	
Supona	1.0	2	0.0
	2.0	2	0.0
	3.0	2	0.0
	4.0	2	0.0
	5.0	2	3.8
	7.0	2	0.0
	10.0	2	0.0
Famphur	5.0	8	95.0
	7.0	8	100.0
Fenthion	1.0	2	0.0
	1.5	2	25.0
	2.0	2	73.1
	2.5	8	77.5
	3.0	8	100.0

mg/kg/day whose red blood cell cholinesterase level was depressed from the preinfusion level of 0.18 ΔpH units to 0.04 ΔpH units by the 23rd day of infusion. Cholinesterase remained between 0.04 and 0.06 ΔpH units through the 41st day of infusion without the sheep showing clinical toxicity.

Red blood cell cholinesterase depression was not detected for supona administered at rates from 1.0 - 4.0 mg/kg/day. At 5.0 and 7.0 mg/kg/day supona only mildly suppressed the cholinesterase values from the preinfusion average of 0.20 ΔpH units to 0.15 ΔpH units on the 17th infusion day, however, at 10 mg/kg/day supona lowered the preinfusion average of 0.20 ΔpH units to 0.08 ΔpH units. Clinical toxicosis was not observed for this treatment.

At 5 mg/kg/day, famphur depressed the red blood cell cholinesterase level from an average 0.19 to 0.05 ΔpH units in 19 days infusion. At 7 mg/kg/day, this depression went from an average 0.20 to 0.03 ΔpH units in 19 days infusion. Fenthion depressed the red blood cell cholinesterase from a preinfusion average of 0.16 to 0.03 ΔpH units in 16 days infusion at 2.5 mg/kg/day and from 0.20 to 0.03 ΔpH units in 20 days infusion at 3.0 mg/kg/day. Clinical toxicosis was not observed for either famphur or fenthion at the tested rates.

It should be emphasized that red blood cell cholinesterase depression may not be correlated with tissue level cholinesterase depression therefore care should be exercised in interpreting these data (Radeleff 1970). If the organophosphate insecticide inhibits tissue and blood cholinesterase levels at approximately the same rate, the blood cholinesterase level will provide a more accurate picture than if the enzyme is inhibited at different rates. By monitoring cholinesterase depres-

sion from preinfusion blood levels and from control sheep an indication of rumen delivery and circulatory uptake of the compounds or active metabolites was obtained for each dosage rate.

The overall potential of any candidate acaracide under these test conditions is an accumulation of the estimates of control it offers against each tick stage, i.e. Estimated Cumulative Control (ECC). Using the data collected from infusion trials (Tables 1-4), an ECC for each tick species at each dosage rate is calculated based on an initial population of 100 eggs. The following formulas were derived for determination of ECC values with control parameters indicated by Cnt. and treatment parameters by Trt.:

A) Estimated reduction of larval production:

$$100 \text{ eggs} - \left(100 \text{ eggs} \times \frac{EL_{Cnt.} - EL_{Trt.}}{EL_{Cnt.}}\right) = \#\text{ surviving larvae.}$$

B) Assume 100% survival of larvae to nymphs, as infusion trials did not establish data against this stage. Were larval mortality and molt data available it would be incorporated as data for nymphs described below.

C) Estimated reduction of nymph population due to mortality:

$$\#\text{ surviving nymphs} - \left(\#\text{ Nymphs} \times \frac{\%\text{ engorged}_{Cnt.} - \%\text{ engorged}_{Trt.}}{\%\text{ engorged}_{Cnt.}}\right)$$

$$= \#\text{ replete nymphs.}$$

D) Estimated reduction of nymph population due to unsuccessful molt:

$$\#\text{ replete nymphs} - \left(\#\text{ replete nymphs} \times \frac{\%\text{ molt}_{Cnt.} - \%\text{ molt}_{Trt.}}{\%\text{ molt}_{Cnt.}}\right)$$

$$= \#\text{ replete nymphs molting to adults.}$$

E) Estimated reduction of adult (female) population due to mortality:

$$\#\text{ adult females} - \left(\#\text{ adult females} \times \frac{\%\text{ engorged}_{Cnt.} - \%\text{ engorged}_{Trt.}}{\%\text{ engorged}_{Cnt.}}\right)$$

= # adult females surviving.

Percent female mortality is used here as the best estimate of overall adult mortality from these studies. As females appear to be more resilient to pesticides than males, it is the more conservative estimate.

F) $$ECC = 100 \times \frac{100 \text{ potential adults} - \#\text{ surviving adults}}{100}$$

or ECC = 100 maximum potential adults - # surviving adults. As the base population used in these calculations is 100, the resulting figure may be expressed as percent.

A summary of ECC values is shown in Table 6. Fenthion was indicated as being the most potent acaracide providing more effective control of all 3 species at less than half the dosage rate of famphur. Complete control of A. maculatum was achieved at 2.5 mg/kg/day of fenthion while 7 mg/kg/day of famphur was required to provide equivalent control. Although the 94.6% ECC obtained for A. americanum with famphur at 7 mg/kg/day was not achieved with fenthion, the ECC for fenthion at both 2.5 and 3.0 mg/kg/day was much more effective than famphur even at 5 mg/kg/day.

Fenthion was more efficacious against D. variabilis at both rates tested than either rate tested for famphur. These 3 species are 3-host ticks, therefore, the practicality of these ECC values to a field situ-

Table 6. Estimated cumulative control (ECC) of larval production, nymphs and adults attributed to 3 tick species feeding on sheep receiving famphur or fenthion.

Acaracide	Dosage mg/kg/day	Tick species		
		A. americanum	A. maculatum	D. variabilis
Famphur	5.0	2.0	79.5	15.2
	7.0	94.6	100.0	21.3
Fenthion	2.5	76.9	100.0	95.1
	3.0	84.7	100.0	70.0

ation would have to be used with discretion as the 3 feeding stages are not obligated to feed on bovine. The ECC affords 1 value indicating the total effectiveness of these acaracides under these test conditions. Where 1-host tick species such as Boophilus find all feeding stages on bovine, such ECC values would have more direct relative importance in the field.

Interestingly, a review of Tables 1-4 show that the reproductive capability of female D. variabilis was not greatly affected with either acaricide as was the case for the Amblyomma species. The ECC values for D. variabilis were most greatly accumulated from nymph mortality and molt as well as adult female mortality. Variations in effectiveness, as observed in such a critical analysis of these acaracides may be due to these ticks' genetically inherited ability to metabolize the parent compound or its active metabolite. In studying the genetics of resistance to organophosphate acaricides among several resistant strains of the cattle tick, Boophilus microplus, (Canestrini), Stone et al. (1976a,b) found that decreased cholinesterase activity and decreased cholinesterase sensitivity to inhibitors in adult tick brains as well as increased detoxification in larvae and adult females were genetically controlled. They found the inheritance of fenthion resistance in 1 tick strain to be a single incompletely dominant autosomal genetic factor (Stone et al. 1976b). Hair et al. (1978) found that while famphur administered to Hereford calves at 7 mg/kg/day provided complete control of Boophilus spp., it afforded no control of D. albipictus (Packard). The similarity in our results against the Genera Dermacentor further suggests a genetic ability to handle certain acaracides.

CHAPTER III

EVALUATION OF 5 FORMAMIDINE COMPOUNDS FOR SYSTEMIC ACARACIDAL ACTIVITY

Recent developments have indicated that a relatively new group of compounds hold some promise for controlling ticks. Roulston et al. (1971) made some interesting observations about chlordimeform, a formamidine compound, while searching for new chemicals with different modes of action to control the organophosphorous resistant cattle tick Boophilus microplus in Australia. They noted concentrations as low as 0.0015% caused ticks to detach from their host. They were then seen wandering about on the hair of the animal and eventually were observed falling from the host without reattaching.

This hyperactivity of attached ticks treated with chlordimeform and other formamidines was also described by Atkinson and Knowles (1974). These same researchers associated this tick behavior with compounds which are inhibitors of monoamine oxidase (MAO) of monoaminergic systems within the ticks central nervous system. Atkinson et al. (1974) have shown that high levels of MAO activity exist in the cattle tick, B. microplus, and that this activity was inhibited when formamidines were applied. Holden and Hadfield (1975) agreed that chlordimeform and its metabolite N-desmethylchlordimeform were potent inhibitors of MAO in the cattle tick, however, even with depressed MAO activity, ticks survived indicating that the mode of action of formamidines may involve

other processes.

Topical applications of chlordimeform have been examined by Gladney et al. (1974) as a detaching agent against the lone star tick, the brown dog tick, Rhipicephalus sanguineus (Latreille), and the Rocky Mountain wood tick, Dermacentor andersoni (Stiles) feeding on guinea pigs. Their data indicate that a concentration of chlordimeform as low as 0.03% would cause nymphs and adults to detach within 2 hours. A 0.5% concentration resulted in 85% detachment of adult A. americanum, 49% of which died in 7 days. A 0.125% concentration resulted in 100% detachment of adult R. sanguineus, 20% of which died in 7 days. And a 0.0625% concentration resulted in 100% detachment of D. andersoni, 20% of which died in 7 days. In addition to the detachment phenomenon, Knowles and Roulston (1973) noted that formamidines topically applied to female B. microplus reduced both oviposition and egg viability.

The systemic capabilities of formamidines have not been extensively studied. However, while screening new formamidine compounds against B. microplus on mice, Stone et al. (1974) gave both subcutaneous and intraperitoneal injections of formamidines. Within 2 h over 50% of the ticks had detached and little difference was exhibited between the 2 methods of application.

Although the mode of action is not fully understood, the results of these researchers indicate that the formamidine compounds are definite possibilities as systemic acaricides.

In response to a query, the Upjohn Company (Kalamazoo, Michigan) provided 5 formamidine compounds believed to possess systemic activity; U36059, U2564, U7503, U6506, and U8633. These compounds were screened for acaracidal activity by rumen infusion (Teel et al. 1977) for poten-

tial use as active ingredients in a bolus preparation.

Materials and Methods

Due to the nature of tick detachment associated with these drugs, the screening procedure described in Chapter II was modified by infesting the experimental sheep prior to initiation of rumen infusion.

Twenty pairs of adult A. americanum, A. maculatum and D. variabilis were confined to sheep in separate stockinette cells 72 h before treatment. Samples of 50 - 100 nymphs of these 3 species were confined in separate Plexiglas® cells 48 h before treatment. Attachment was determined just prior to treatment and unattached ticks were removed. Treatment sheep received candidate formamidine compounds diluted in acetone at 1 ml/h via rumen infusion previously described. Control sheep received acetone only. Cells were examined twice each day. Detached and/or replete ticks were removed from cells and held in the laboratory at 92 ± 3% RH, 20° ± 5° C, and 14 h photophase. Efficacy of these compounds was based on the detachment of feeding adult and nymphal ticks prior to complete engorgement, nymph molting success, as well as the oviposition and % hatch of eggs from engorged females.

Results and Discussion

Partially fed (< ½ engorged) adult and nymphal ticks were observed to be detached and wandering about the confines of their cells as early as 6 h after initiation of treatment (U36059 at 40 mg/kg/day) and as latent as 72 h (U7503 at 30 and 40 mg/kg/day).

Four of the 5 formamidine compounds U36059, U2564, U6506 and U7503 demonstrated systemic activity determined by % detachment of feeding

ticks (Table 7). Rapid and complete detachment was observed among all 3 adult tick species feeding on sheep receiving U36059 at 40 mg/kg/day. This compound was less effective in producing detachment of nymphs at the same rate. Ticks feeding on sheep receiving U36059 at 30 mg/kg/day were much less responsive and at 20 and 5 mg/kg/day no response to treatment was observed. There seemed to exist a positive dose-response relationship with U36059 at these rates. The rate of the detachment response to this compound is shown in Figures 2 and 3. Though this compound showed dramatic results against ticks systemically it proved lethal to sheep at 30 and 40 mg/kg/day. U2564 and U7503 were more effective against nymphs than adults. Effectiveness of U2564 against D. variabilis at 15 mg/kg/day was greatly reduced over the higher rate of 30 mg/kg/day, however, its effectiveness against adult A. americanum and A. maculatum was not significantly different at these rates. Sheep administered U2564 at 30 mg/kg/day became lethargic and reduced their feed intake after 6 days infusion. They rapidly returned to normal following termination of the test. U6506 was equally effective against nymphs and adults at the 30 mg/kg/day rate and indicated some selectivity for D. variabilis over the Amblyomma species. At 20 mg/kg/day no detachment was obtained. U8633 showed little to no response against all but A. maculatum adults where 10 and 21 % detachment was recorded at the low and high rates respectively.

There were no differences in mortality of attached adult or nymphal ticks feeding on treated and control sheep. Mortality of detached ticks which were held in the laboratory commenced within 7 days and reached 90% in most tests by 4 weeks. No effect on molting of successfully engorging nymphs could be detected.

Table 7. Percent detachment of three 3-host tick species feeding on sheep receiving experimental formamidine compounds via rumen infusion.

Formamidine and dosage mg/kg/day	Number of sheep	Hours to first detachment	Adult females			Nymphs		
			A. americanum	A. maculatum	D. variabilis	A. americanum	A. maculatum	D. variabilis
U36059								
5.0	1	--	0	0	0	0	0	0
20.0	1	--	0	0	0	0	0	0
30.0	1	24	10	34	25	0	32	14
40.0	1	6	100	100	100	54	95	33
U2564								
15.0	3	24	79	78	11	100	100	100
30.0	3	24	68	65	89	---[a]	---[a]	---[a]
U6506								
20.0	1	--	0	0	0	0	0	0
30.0	1	36	8	56	61	3	20	71
U7503								
30.0	1	72	0	0	0	82	63	0
40.0	1	72	0	0	0	61	---[b]	100
U8633								
20.0	1	36	0	10	0	0	0	0
30.0	1	48	0	21	8	0	0	0
Controls								
Acetone only	6	--	3	3	0	0	1	0

[a] Insufficient nymph attachment (< 10 ticks) precluded adequate test.

[b] Insufficient A. maculatum nymph attachment prevented adequate testing.

Figure 2. Rate of detachment of adult female ticks feeding on sheep receiving the formamidine, U36059, at 40 mg/kg/day by rumen infusion

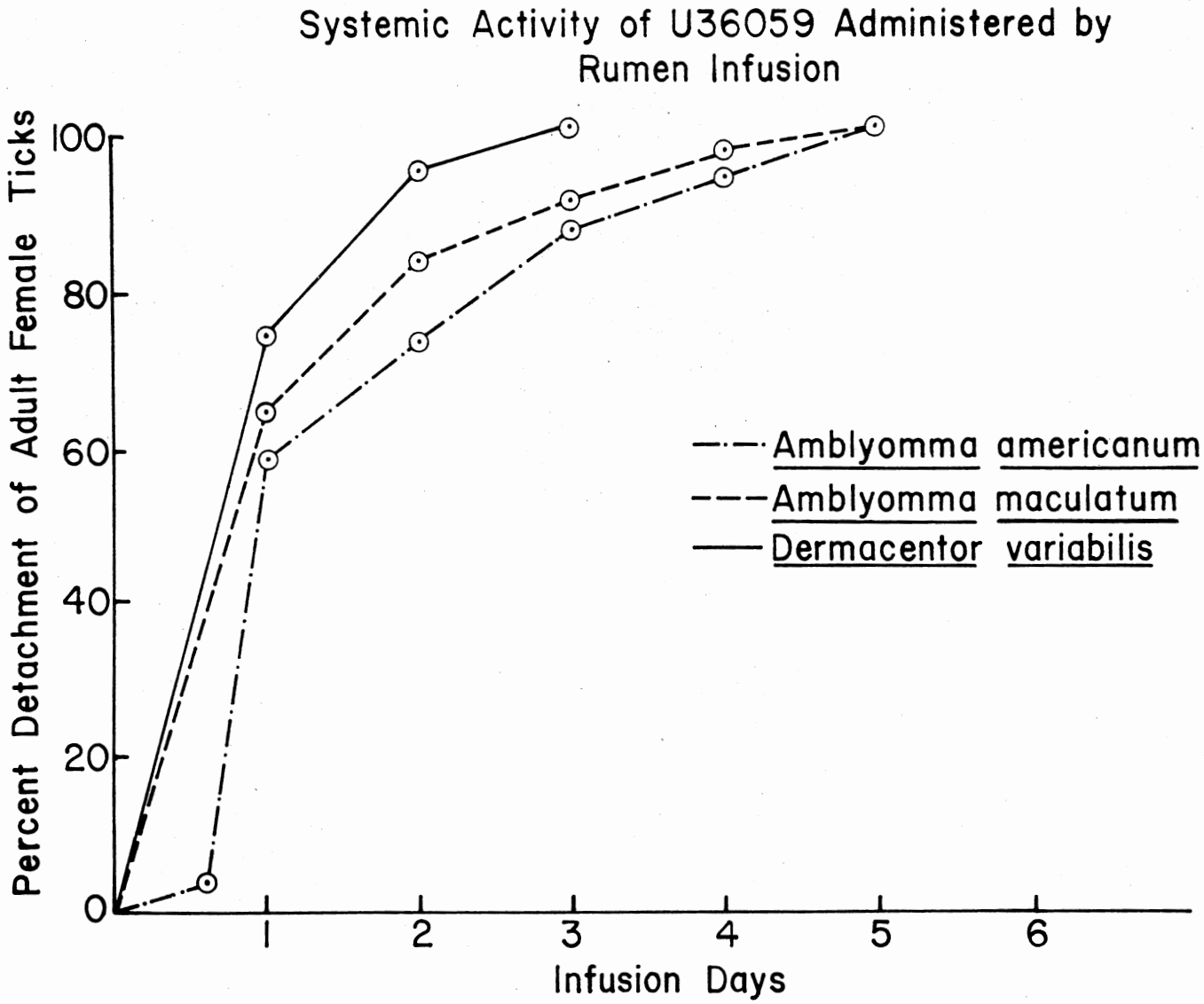
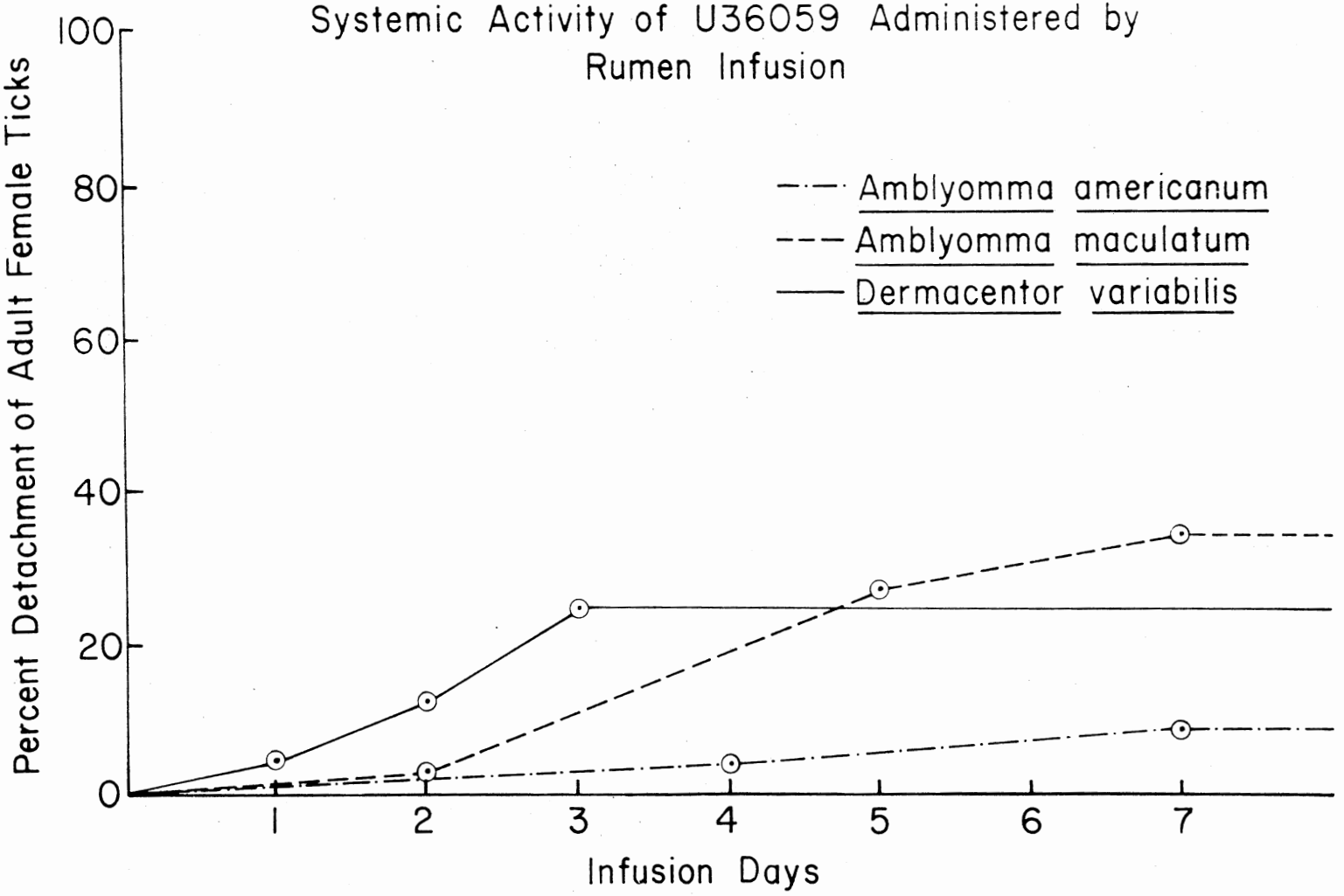

Systemic Activity of U36059 Administered by Rumen Infusion
Percent Detachment of Adult Female Ticks
100
80
60
40
20
0
1
2
3
4
5
6
Infusion Days
Amblyomma americanum
Amblyomma maculatum
Dermacentor variabilis

Figure 3. Rate of detachment of adult female ticks feeding on sheep receiving the formamidine, U36059, at 30 mg/kg/day by rumen infusion


Systemic Activity of U36059 Administered by Rumen Infusion
Percent Detachment of Adult Female Ticks
100
80
60
40
20
0
1
2
3
4
5
6
7
Infusion Days
Amblyomma americanum
Amblyomma maculatum
Dermacentor variabilis

The repletion weight, egg mass weight, and % hatch of females successfully engorging on formamidine treated sheep were examined and found not to be significantly reduced from the same parameters of ticks feeding on control sheep. Knowles and Roulston (1973) have noted that all 29 formamidine and related compounds tested by topical application and injection to B. microplus engorged females resulted in reduced oviposition and egg viability.

The difference in our findings could be attributed to several factors, including metabolism by the host, route of entry and dispersion in the tick, and metabolism by tick species other than B. microplus. Chemical structures of 4 of these formamidine compounds were not provided, so a comparison of activity based on structural differences could not be made. Systemic activity as indicated by detachment indicates that an active form of 4 of these compounds was reaching the parasites.

Unfortunately, the toxicity associated with the 2 most potent detaching agents, U36059 and U2564, as well as the comparatively high dosage rates necessary to achieve a parasite response precludes the use of any of these 5 compounds for bolus development as is currently feasible. However, the demonstration of systemic acaracidal activity among this group of compounds encourages screening other formamidines in search of acaracidal activity at lower rates without host toxicosis.

CHAPTER IV

EVALUATION OF UC55304 FOR SYSTEMIC ACTIVITY AGAINST 3-HOST TICKS

Union Carbide technical data for the experimental miticide UC55304[1] indicated a low order mammalian toxicity and substantial mortality and ovicidal capability against 2-spotted spider mites. This compound, whose mode of action is unknown, was chemically unlike any previously tested. It possesses no halogens or phosphate groups and has no hormonal structures or properties.

Materials and Methods

In a preliminary screen UC55304 was administered continuously via the rumen at 2.0, 5.0, 7.0 and 10.0 mg/kg/day in acetone at 1 ml/h as described by Teel et al. (1977). The 4 treatment and 2 control sheep were challenged with 20 pairs of adults and samples of ca. 100 larvae of the 3 test acarines; lone star, Gulf Coast and American dog ticks. Following the 72 h attachment period for adults and 48 h for nymphs, attachment was determined and non-attached ticks removed. Parameters on tick mortality, repletion, molt and fecundity were determined as explained in Chapter II.

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[1] Union Carbide Corp., Jacksonville, FL.

Results and Discussion

Tables 8 - 10 summarize data collected at the 2.0, 5.0, 7.0 and 10.0 mg/kg/day rates for each tick species. Unlike the organophosphates and formamidines previously tested, UC55304 was most effective against D. variabilis, followed by A. maculatum, and least effective against A. americanum. Both the difference in species susceptability as well as the latent effects on molting and fecundity reflect the differing properties of this experimental compound. Except for A. maculatum, the efficacy of UC55304 was not evident among feeding ticks. Little to no effectiveness was observed against engorging nymphs of all 3 species, or adults of A. americanum and D. variabilis. Adult tick mortality on the host could not be considered significant for any of these species receiving UC55304 at these rates.

UC55304 demonstrated its greatest effectiveness against A. americanum at 7 mg/kg/day, reflected in the lowest average repletion weight, egg mass weight and % hatch at this rate (Table 8). Nymph mortality and molt inhibition were also greatest at this rate. Differences between the 7.0 and 10.0 mg/kg/day rates may be due to the fact that this sheep managed to sever her infusion delivery tube on 3 occasions late in the study when A. americanum typically reach their rapid engorgement phase resulting in low blood levels of UC55304 during that crucial feeding period. This is substantiated by the efficacy data for A. maculatum and D. variabilis which both show a graded response through these infusion rates and both species typically engorge before A. americanum.

Mortality of Gulf Coast ticks (Table 9) was not a significant factor. However, % repletion shows a definite dose-response relationship. At higher delivery rates UC55304 appeared to arrest the engorgement of

Table 8. Mean effects of UC55304 to Amblyomma americanum (L.) feeding on sheep receiving the experimental compound at 4 rates via rumen infusion.

	Dosage rate (mg/kg/day)				
	2.0	5.0	7.0	10.0	Control
% Adult female repletion	100.0	100.0	90.0	100.0	97.4
% Adult female mortality	5.2	0.0	5.0	5.0	7.7
Repletion wt. (g)	0.91	0.91	0.61	0.74	0.90
Egg mass wt. (g)	0.53	0.56	0.27	0.44	0.51
% Hatch	74.7	88.9	11.4	82.4	86.7
Estimated larvae produced (EL)	7924.2	9880.3	613.8	7210.0	8836.5
% Nymph repletion	22.2	100.0	100.0	77.7	100.0
% Nymph mortality	------[a]	0.0	89.3	71.4	2.8
% Nymphal molt	------	100.0	53.8	100.0	97.2
Estimated cumulative control (ECC)	------	0.0	96.2	36.6	------

[a]Inadequate sample size due to poor attachment.

Table 9. Mean effects of UC55304 to Amblyomma maculatum Koch feeding on sheep receiving the experimental compound at 4 rate via rumen infusion.

	Dosage rate (mg/kg/day)				
	2.0	5.0	7.0	10.0	Control
% Adult female repletion	89.4	88.0	33.3	24.0	92.6
% Adult female mortality	5.2	5.8	8.3	4.0	0.0
Repletion wt. (g)	0.93	1.02	0.76	0.88	0.92
Egg mass wt. (g)	0.54	0.50	0.03	0.29	0.50
% Hatch	10.7	46.6	0.4	0.0	72.3
Estimated larvae produced (EL)	1163.1	4694.5	2.8	0.0	7192.4
% Nymph repletion	100.0	100.0	100.0	100.0	100.0
% Nymphal mortality	0.0	0.0	39.0	1.9	0.0
% Nymphal molt	100.0	97.5	92.7	100.0	100.0
Estimated cumulative control (ECC)	84.6	60.0	100.0	100.0	------

Table 10. Mean effects of UC55304 to Dermacentor variabilis (Say) feeding on sheep receiving the experimental compound of 4 rates via rumen infusion.

	Dosage rate (mg/kg/day)				
	2.0	5.0	7.0	10.0	Control
% Adult female repletion	100.0	100.0	75.0	100.0	100.0
% Adult female mortality	0.0	27.3	79.1	94.7	0.0
Repletion wt. (g)	0.50	0.32	0.27	0.39	0.40
Egg mass wt. (g)	0.28	0.07	0.01	0.03	0.24
% Hatch	21.4	0.9	0.0	0.0	93.7
Estimated larvae produced (EL)	1208.7	13.3	0.0	0.0	4463.9
% Nymph repletion	100.0	100.0	100.0	100.0	100.0
% Nymph mortality	34.7	6.6	86.9	85.9	5.9
% Nymph molt	88.4	96.4	21.3	29.7	93.7
Estimated cumulative control (ECC)	74.4	100.0	100.0	100.0	------

a greater number of Gulf Coast ticks. These ticks were of normal color, remained attached but appeared to stop feeding when ca. 1/2 - 3/4 engorged. At termination of the study these incompletely engorged females, totalling 17, were manually removed and held in the laboratory under optimum humidity and temperature for observation. Only 4 of these females produced ova but none were viable. Males appeared to feed normally and mating was observed. With the exception of the 2.0 mg/kg/day rate there was a graded response from 5.0 to 10.0 mg/kg/day for ova production and % hatch. The estimated cumulative control (ECC) of A. maculatum at 7.0 and 10.0 mg/kg/day was complete.

Perhaps the most interesting response to UC55304 was observed for D. variabilis (Table 10) which here-to-fore had been the most resilient tick species to candidate acaracides tested in this model. With the exception of the 7.0 mg/kg/day rate, adult female engorgement was complete and no mortality was observed for males. However, mortality was very evident among replete females fed on treated sheep and held in the laboratory for oviposition, and a prominent dose-response relationship was observed. Dying replete females slowly turned black in color and ova were not produced. This dose-response relationship was also evident in the weight of replete females, ova produced, and % hatch.

What appeared to be fully developed larvae were observed within the egg case of significant portions of egg masses from surviving females fed on treated sheep, however, eclosion did not occur. This was not observed among control egg masses. The EL values for UC55304 reflect these total effects. Nymph mortality and molting ability show the same trend toward increased dosage, although it is not as sharply delineated. Replete nymphs were observed to turn black in color and succumb before

molting, and mortality among newly molted adults increased with dosage rate. The estimated cumulative control based on adult and nymph data was 100% at the 5.0, 7.0, and 10.0 mg/kg/day rates. Clinical signs of toxicity to UC55304 were not observed for sheep at these rates.

Although only a preliminary screen of UC55304 has been conducted to date, results appear very promising. The candidate compound can be obtained in crystalline form for incorporation in slow-release bolus formulations and it was effective against all 3 tick species at 7.0 mg/kg/day, a delivery rate conceivably attainable with a sustained-release bolus. Furthermore, UC55304 is appealing because its mode of action is unlike that of organophosphates. Further testing of UC55304 and related compounds would be therefore desirable.

CHAPTER V

DEVELOPMENT OF AN INSECTICIDE-LADENED SUSTAINED-RELEASE BOLUS

State-of-the-Art

Interest in controlled release devices for animal health and production has increased in recent years, especially with the advent of several successful controlled release systems. Within the cattle industry hormonal growth stimulants such as stilbestrol, is administered once via implantation of a small pellet beneath the skin of the ear. The pellet is made of inert material impregnated with the hormone which the animals receive at a concentration to effectively increase weight gain over a period of 100-120 days.

More recently, insecticide-impregnated bands and tags have been developed as a promising new approach to ectoparasite control for livestock. Various polymers, such as polyvinyl chloride, impregnated with various insecticides and adapted for use as horn bands, ear bands and ear tags were shown to provide season-long protection against the Gulf Coast tick and the screwworm (Gladney 1976; Ahrens et al. 1977). Since the Gulf Coast tick confines its feeding principally to the ears of cattle, this approach to control has proved successful, however, other tick species which feed indiscriminately over the host are not controlled by these devices.

Solid oral veterinary preparations called boluses have been retained

in the rumens of sheep and cattle for varying periods of time to provide the release of different biologically active substances. The search for an alternative method of providing cobalt to sheep in South Australia where pasture treatment with cobalt sulfate was becoming economically infeasible led to the development of a "cobalt bullet" (Dewey et al. 1958). The bolus was retained in the reticulum of the rumen where the cobalt was released through erosion. When the boluses became coated with calcium or silicon salts a "grub screw" was administered with the bolus to successfully prevent the coating by promoting continual erosion (Mr. Pat Brown, "The Grove", Burra, South Australia, personal communication). Sheep require 0.1 mg cobalt per day and blood analysis for vitamin B_{12} indicated boluses maintained therapeutic cobalt levels for 50 weeks (Dewey et al. 1958).

Veterinary boluses have been designed to supply ruminants with biologicals such as vitamins, anti-bloat compounds and antibiotics. Most of these products release their active component within 7 to 30 days as a function of drug need and bolus characteristics; size, composition and release rate.

As a result of successful controlled-release products for animal health and the need for a means of administering an effective acaracide over a substantial time period, the state-of-the-art was investigated in search of possible compositions and combinations to meet this challenge.

Marston (1962) reviewed and described several bolus designs relative to the delivery of biological agents to ruminants. "High density" biological agents (those with a density of at least 2.5 g/cm^3) have been incorporated with various binders and compressed into desirable shapes. These high density agents have also been contained in an open-ended

sheath or in a closed-ended shell enabling the systemically active material to be leached from or to diffuse through the shell. Shell material was required to have a density equal to or less than that of the drug. "Low density" (< 2.5 g/cm^3) biological agents have been embodied in high-density matrixes by compression or by permeating the matrix with the biological agent. Also, low-density biologicals may be contained in a high-density sheath or shell. Additional descriptions for low-density biological agents included compression of the drug alone around a high-density core, or compression around such a core after being incorporated in a matrix of high or low density. Wicks have been inserted in the ends of closed-ended shells containing low or high-density biological agents allowing the gradual release of the active material.

The principle on which these devices operate is based on retention of the boluses in the rumeno-reticular sac of the ruminant. The active biological agent is released through erosion, leaching, solubilization or other means facilitated by the course of peristalsis in rumination. Therefore, a primary factor in the success of these devices is density. Marston (1962) preferred a density between 3.5 and 5.5 as a range providing reliable bolus retention, Rednick and Tucker (1970) preferred 1.5 to 5.0. Siegrist and Katz (1970) suggested the minimum density was 1.5 but preferred a density of 1.9.

The bolus preparation designed by Marston (1962) and used to treat sheep on cobalt deficient pastures (Dewey et al. 1958) was made by compressing cobalt oxide and china clay, 75% and 25% by weight respectively, at 698 kg/cm^2. These pellets were then heated to 1000°C for 10 min to bind the materials into a hard porous mass. Variations in quantities

of ingredients and the use of polyvinyl acetate as binder instead of china clay also provided desirable results. These boluses were designed to provide 0.1 mg cobalt per day for periods up to 1 year.

Firing of the above pellets at high temperatures was essential in producing a product capable of lasting for extended periods as it binded the medication in the bolus. Rednick and Tucker (1970) point to this as a shortcoming for boluses releasing drugs which must be clear of the animal tissues before market and added that the release from prior boluses was unpredictable and uncontrollable. These authors developed a veterinary bolus for the administration of sulfonamides to food producing ruminants several weeks before slaughter. They cite the addition of a "lubricant" as being critical in the formulation of a short term (10 days to 3 weeks) bolus with a predictable, controlled release. Desirable lubricants were fatty acid derived such as stearates (i.e. magnesium stearate) and added to the formulations in small quantities to facilitate the release of the active drug. Their composition comprises a sulfonamide drug which has been mixed with a dense filler (such as iron powder, calcium sulfate, portland cement, or zinc oxyiodide cement) and a binder (such as cellulose esters or polyvinylpyrrolidone). These ingredients were granulated, screened and dried for ca. 12 h at 48.9^{o}C. The desired amount of lubricant (1-3.5%) is then mixed into the screened granules before being compressed with conventional tableting machines. This patent covers the currently available sulfamethazine Spanbolet® boluses (Norden Laboratories, Lincoln, Neb.) used to treat infectious diseases of non-lactating cattle. These boluses were designed to release 22.5 g of active drug in 10 - 20 days from a 36 g bolus.

Siegrist and Katz (1970) extended the use of veterinary boluses to timing estrus in cattle as a herd management tool for efficient utilization of artificial insemination. These boluses required a reliable controlled release rate of a progestational agent with a finite end point to synchronize estrus. Their compositions were designed to disintegrate fully at their end point to allow estrus to occur and permit the administration of another bolus if desired without the concern of leftover active drug or retained inert boluses complicating the release in a repeated administration. Their compositions consisted of 4 components. The first was 1 or more highly water insoluble solid wax, fat, oil, fatty acid amide, fatty acid ester, fatty amide alcohol, or polymer which provided an inert base for the matrix and acted as a binder. Among the examples of the first component mentioned were; carnauba wax, candeilla wax, paraffin, glyceryl monostearate, polyethylene glycol, shellacs, and resins. The second component was a high-density, non-toxic metal derivative added in sufficient quantity to the matrix to bring the final bolus density to at least 1.5, but preferably 1.9 g/cm^3. Barium, bismuth or calcium salts and metal oxides were mentioned as possible ingredients. The final components include the therapeutic agent and if needed, a lubricant or adjunct for the purpose of altering the physical characteristics of another major component or to facilitate drug release.

The procedure used by Siegrist and Katz (1970) for preparing their compositions began by incorporating the therapeutic agent and binder or matrix base together in a melt or through a solvent such as methanol. To this was added the high-density non-toxic metal derivative and any lubricant. The granulation thus formed was screened and the preparation

hydraulically compressed in commercial tableting machines at 69-346 kg/cm^2. An alternative was to pour the molten mass into molds and allow solidification. The bolus products designed by these authors released steroid progestational compounds at ca. 10 mg per day for 2-30 days depending on the composition tested.

These authors noted several relationships important in formulating and producing such compositions that played an important role in attaining the desired release rate. The disintegration rate and therefore the release rate was inversely proportional to the pressure used in compression. They also found that very hydrophobic substances such as carnauba wax produced longer lasting boluses than other materials with lower melting points and less hydrophobic quality. Furthermore, they demonstrated that mixtures of desirable first component materials could provide the matrix base necessary to achieve the release rate whereas either of the 2 used alone would provide too rapid or too slow release rate.

Improvements on prior art boluses provide insight into means of manipulating formulations to attain a more desirable product. In order to attain rapid and high, yet prolonged levels of sulfapyridazines in cattle for antibacterial therapy, Henderson et al. (1973) formulated the commercially available Oblet® (American Cyanamid, Princeton, N.J.) by compressing a mixture of ethylcellulose, bismuth subcarbonate or subnitrate with varying percentages of sustained and fast release drug granulations. Sheth and Stiel (1973) found that antibacterial sulfa boluses containing dibasic calcium phosphate plus a binder and lubricant were unexpectedly free of the deposition of insoluble calcium salts which the authors cited as a major disadvantage to prior sulfa bolus formulations.

Establishing Some Current Criteria for Assessing the Development of a Sustained-Release Bolus

Bearing both commercial production and consumer appeal in mind, Harbeston (1975) outlined some economic considerations which will influence development and have bearing on the ultimate fate of any veterinary preparation. These include:

1. Readily available low-cost materials
2. Simple and low cost manufacturing process
3. Utility of matrix with other drugs
4. Patent protection for matrix
5. Frequency of application
6. Ultimate consumer cost
7. Potential consumer demand

Based on the nature of the problem and the prior art, bolus development was pursued with consideration given to a number of additional factors which would play a role in its success. These include:

1. Bolus density
2. Practicality of bolus size
3. Non-toxic nature of matrix ingredients
4. Practicality of production from the manufacturer's view
5. Attaining a desirable release rate
6. Achieving sufficient longevity of therapeutic delivery
7. Determination of finite end point

Developmental Formulating and Testing of Potential Matrix Ingredients for a Sustained-Release Acaracidal Bolus

Initial tests were conducted to observe the characteristics of various potential binders, high-density metal derivatives and lubricants or adjuncts when placed in the rumen of a fistulated cow. These inert ingredients were mixed together while the binder was in liquid form, either in a melt or by the addition of water. The mass was then poured into molds made of polyethylene tubing (8 cm long x 2.5 cm diameter) and allowed to solidify at room temperature. The ends of these boluses were dome shaped by carving or filing the sharp edges on the ends of the cylinders. Boluses were placed in the reticulo-rumen via the fistula and retrieved periodically. Notes were kept on the degradation of these combinations, general appearance and rumen location at each inspection in order to determine relative durability and potential as a matrix for an organophosphate bolus.

During an initial search for ingredients, boluses were prepared of dental and artist's plaster of Paris, clays, and paraffins embodying steel shot or ball bearings to attain sufficient bolus weight. When placed in the rumen these boluses were found to be short-lived (i.e., less than 14 days) and therefore considered inferior as potential binders for a lasting bolus.

In testing conducted by Siegrist and Katz (1970) it was noted that carnauba wax produced a durable composition capable of lasting in excess of 100 days. Carnauba wax, a product of the Brazilian palm, Copernicia cerifera, was supplied by S. C. Johnson & Son, Inc. (Racine, Wisc.). The least expensive grade, Type 4, Filtered, was used throughout these

experiments.

When carnauba boluses were prepared from a melt as previously described using either magnesium oxide (MgO) or barium sulfate ($BaSO_4$) to increase bolus density, little to no weight loss could be detected even after 4 weeks in the rumen. The surface of these boluses remained very polished and smooth showing no indication of degradation. The durability of the carnauba boluses appeared promising. Consequently, tests were run to determine densities attainable and types of adjuncts or lubricants that could be added to initiate and stabilize the degradation of these boluses.

A 33% MgO, 66% carnauba bolus provided a density of 1.4 g/cm^3 and a 66% $BaSO_4$, 33% carnauba bolus provided a density of 1.9 g/cm^3. Barium sulfate was less expensive and if successful with an active component added, would reduce the cost of a final product. Boluses of this nature possessed a density of 2.2 g/cm^3 when a composition of 75% $BaSO_4$, 25% carnauba wax was formed. Furthermore, the increased quantity of $BaSO_4$ did not facilitate a weight change when boluses were tested in the rumen.

To the 75% $BaSO_4$, 25% carnauba bolus melt were added various additives to study compatibility while attempting to facilitate the erosion of this bolus material. Agar added at 7.6% proved incompatible by promoting presolidification during the melt and resulting in complete bolus degradation in 2 days. The lubricant glyceryl monostearate added at rates of 6.9, 13.8 and 31.0% resulted in all boluses gaining ca. 3 mg weight per day during the 18 day test period. A composition with 10% sucrose eroded at ca. 2 mg per day while 20% sucrose boluses of this type eroded at ca. 5 mg per day. Gelatin was added to the melt at rates of 1.0, 2.5, 5.0, 10.0 and 20.0% and the results show the relationship

between a lubricant and its concentration in the bolus. Gelatin at 1.0% provided an average daily degradation of 14.9 mg; at 2.5%, 3.77 g/day; at 5%, 5.54 g/day; and at 10 and 20%, the 34 g boluses lasted only 3 days in the rumen before being completely disintegrated. The surface of these boluses eroded by sluffing minute chips suggesting incompatability and contributing to the day to day variability observed during the test.

Polyethylene glycol (PEG) (Mol. wt. 4000) was incorporated into the melt at 6 rates. At 1.0, 2.5, 5.0 and 10.0% no degradation could be detected. At 50% PEG the boluses completely degraded in the rumen in 24 hr. The 25% PEG composition provided a relatively stable release averaging 195 mg per day. In addition, the bolus surface appeared to be eroding uniformly without the pitting observed with gelatin boluses. As a result of these tests PEG was considered to be the most advantageous additive to facilitate degradation of the materials under consideration.

Formulation, Production and Testing of Experimental Organophosphate Boluses

Problems arise in incorporating biologically active agents into a matrix heated to the melting point of carnauba wax, 86^{o}C. The activity of most pesticides would be reduced if not completely destroyed when heated to this temperature. Combining the matrix and pesticide would therefore have to be accomplished at near room temperatures. Because of this consideration and the tableting characteristics indicated for the ingredients tested, formation of boluses by direct compression was selected. This alternative offered the most commercially adaptable process and therefore guided other decisions during development.

A review of efficacy data from candidate compounds screened by

rumen infusion suggested that the organophosphate insecticides, famphur and fenthion, were both favorable acaracides for bolus development. Although fenthion was the more potent acaracide providing equivalent control at less than half the rate of famphur, fenthion was only available in a liquid state. Famphur was readily available as a white crystalline solid and possessed good tableting characteristics.

In order to span the tick season in Oklahoma, boluses would need to provide the acaracide for systemic action for periods up to 120 days. With this in mind, development and testing began with 3 objectives:

1. To develop a method for incorporating the acaracide uniformly throughout the matrix,

2. To develop a mold for direct compression of the matrix into a desirable shape, and

3. To test various bolus compositions to determine their suitability for achieving a sustained release of from 5-7 mg/kg/day for famphur in bovine.

At the outset of our experiments fistulated cows were in short supply, therefore an artificial rumen was developed as an *in vitro* alternative in initial tests. It also provided a means whereby initial organophosphate boluses could be tested without the concern of toxicity to live animals if the boluses dissolved or eroded too quickly. As confidence was developed in the boluses and fistulated animals became available emphasis in testing was shifted to *in vivo* analysis.

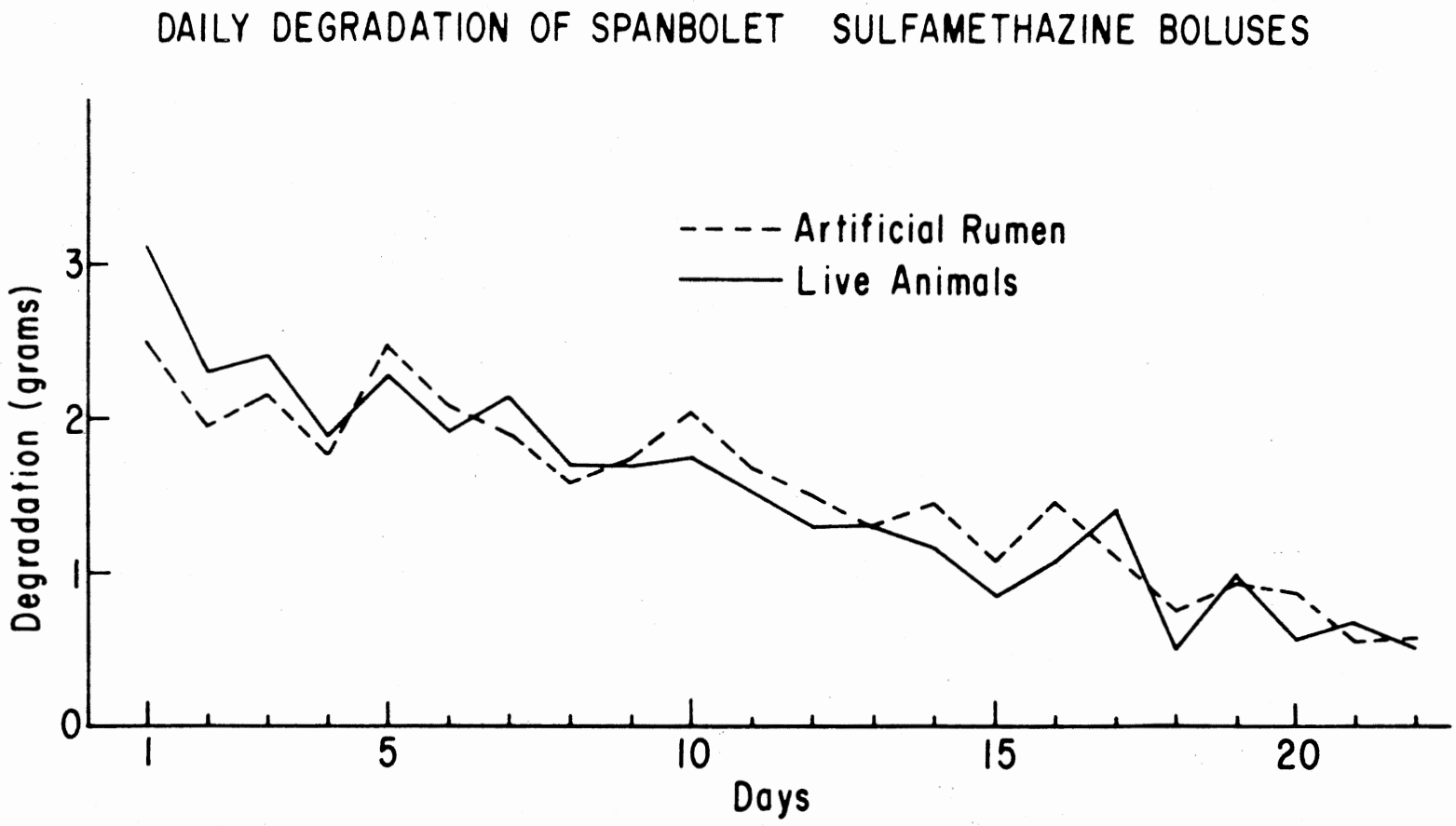
Polyethylene carboy bottles (7.6 litres) with 3 equally spaced internal wooden baffles, 3.5 cm in height, were half filled with rumen contents from fistulated cows. Each bottle was fitted with 2 traction bands 5 cm wide cut from a rubber inner tube. A fermentation gas escape

hole (8 mm in diameter) was drilled in the polyproplyene lid. Bottles were cradled on their sides between steel shafts rolling on ball bearings. One steel roller was chain driven by a low rpm electric vending machine motor. The speed of this drive shaft could be regulated by changing motors and sprockets and was adjusted to calibration until the bottle degradation of a commercially available bolus used as a standard (Sulfamethazine Spanbolets®, Norden Laboratories, Lincoln, Neb.) was the same as degradation observed in 2 available fistulated cows. The bottles and rotating apparatus were housed in a military CONEX box having ca. 6 cm insulation. The box was equipped with a vent fan and heater on dual thermostat to provide the internal rumen temperature of 39° ± 5°C (Barnett and Reid 1961).

Figure 4 illustrates the comparative degradation obtained when 2 Spanbolets® were placed in the rumeno-reticular sac via the fistula of each of 2 cows and 1 Spanbolet® in each of 2 bottles when the drive shaft speed was adjusted to 0.5 rpm.

Matrixes of various compositions suitable for cool dry blending of active ingredients and compression were prepared as follows: a melt was made of the hydrophobic binder, carnauba, in a double boiler heated above 86°C for the purpose of incorporating the high-density, non-toxic metal derivative ($BaSO_4$). When needed, additional ingredients such as lubricants or adjuncts for modifying bolus character were added to the melt. The molten mass was thoroughly mixed, poured into aluminum trays to a thickness of ca. 4 mm and allowed to cool to room temperature before being ground to a powder using a standard kitchen model blender. The ground matrix powder was screened through a U.S. Standard 40 mesh sieve to provide a more uniform matrix and reduce the conceivable varia-

Figure 4. Comparative rates of degradation for Spanbolet® sulfamethazine boluses in mature fistulated cows and artificial rumens


DAILY DEGRADATION OF SPANBOLET SULFAMETHAZINE BOLUSES
Artificial Rumen
Live Animals
Degradation (grams)
0
1
2
3
1
5
10
15
20
Days

bility in bolus performance as a result of variation in matrix particle size. The active ingredient, famphur, was then blended into the powdered matrix at desired rates again using a standard kitchen model blender. A convenient aliquot of the final formulation (i.e., 35 g) was hydraulically pressed in a steel mold using a model UTM-3B hydraulic press (Vega Interprises, Decatur, Ill.) to form an oblong conventional veterinary bolus suitable for administration with a balling gun.

Through the developmental period 2 molds were constructed to meet the needs of hydraulically compressing bolus materials for veterinary preparations. The author designed the single press punch molds to produce veterinary boluses similar to those commercially available. It was felt that data collected from a product produced close to manufacturing specifications might more nearly approximate its true potential, possibly create commercial interest and provide quality laboratory analysis as well.

After reviewing the description of commercial tableting machines with particular interest paid to punches and dies (Kibbe 1966; Browning 1967) and evaluating the needs and circumstances under which these experimental acaracide boluses would be produced, a bolus mold design was drawn.

Figure 5 provides a 3-dimensional concept and Figure 6 the dimensions used in the construction of the first mold. Construction material was S-7 tool steel. Because of the nature of the single punch press and the difficulty anticipated in force pressing the bolus from the mold after compression, a split die was designed held together by four 9.4 mm hex-head cap screws. The horizontal surface area of the die bore was 13.8 cm^2. A 0.075 to 0.100 mm tolerance was used in the con-

Figure 5. Three-dimensional view of the single punch press bolus mold with split die

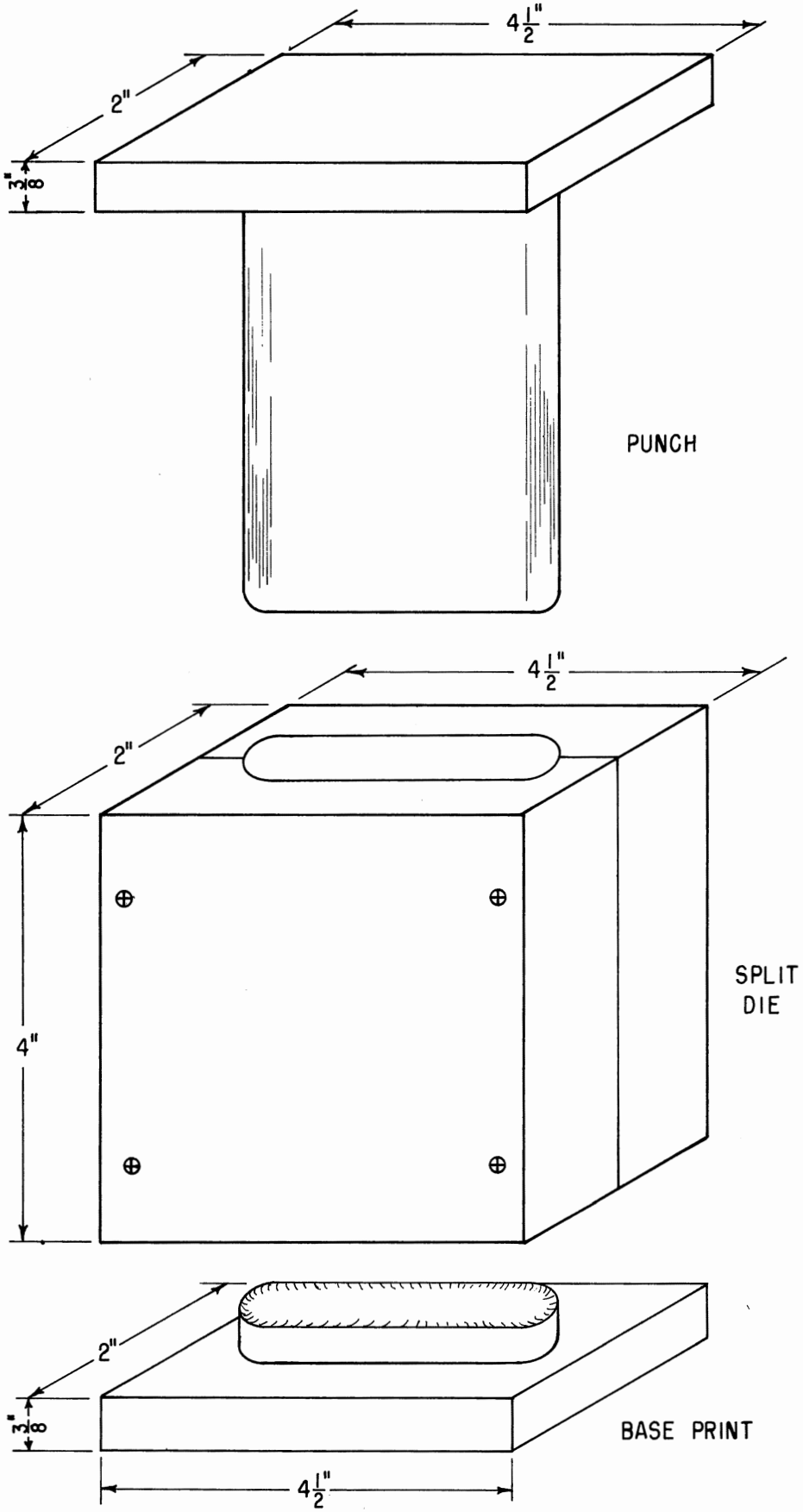
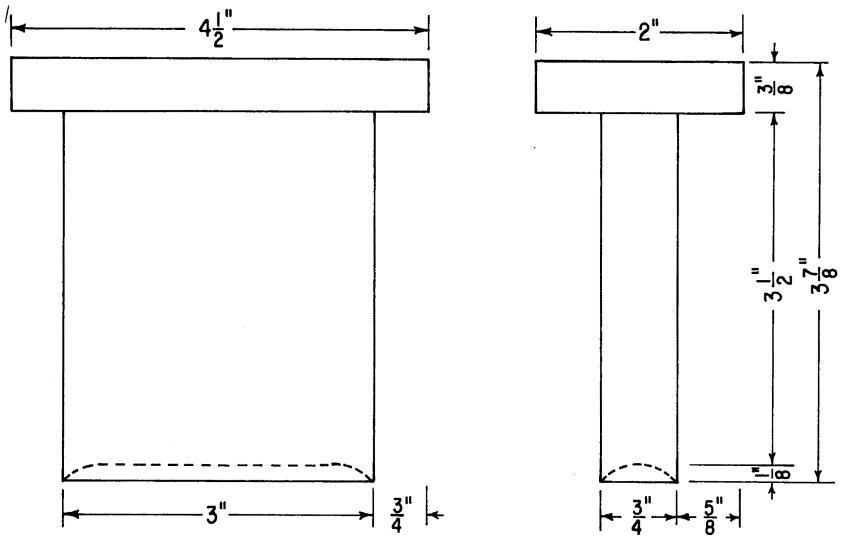
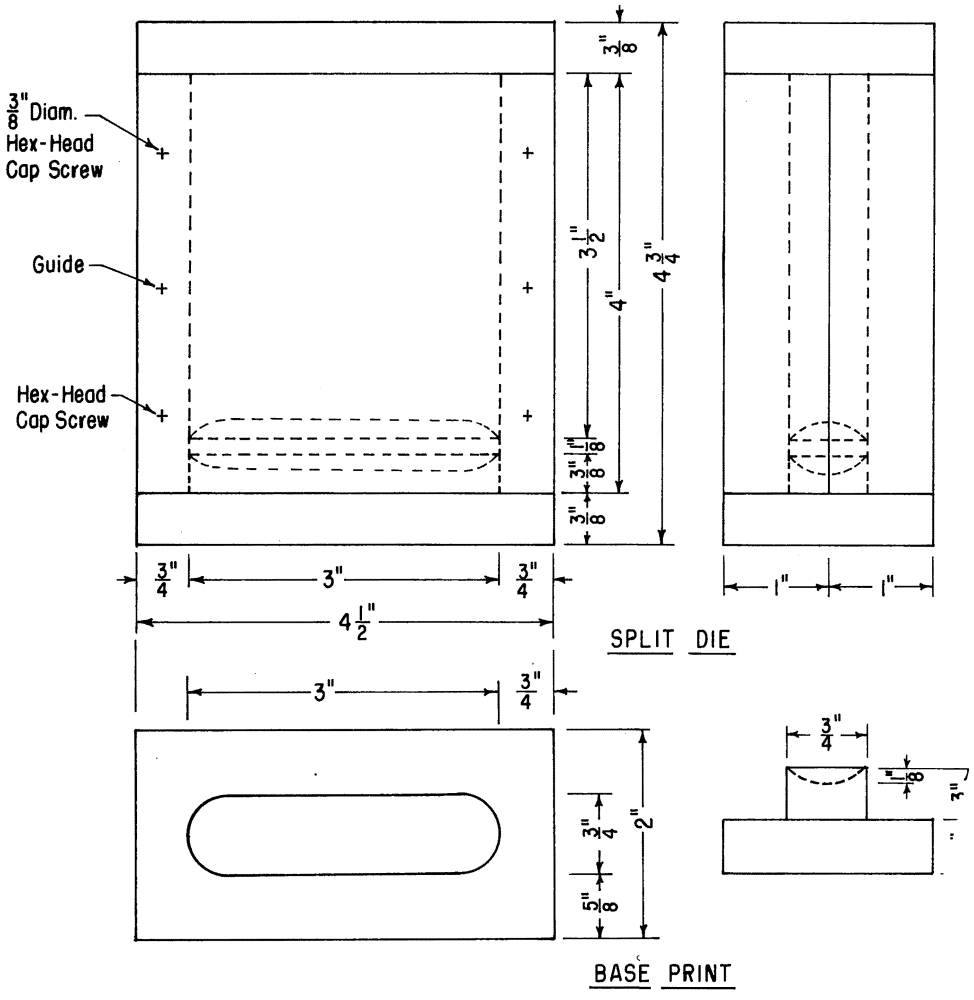

4 1/2"
2"
3/8"
PUNCH
4 1/2"
2"
4"
SPLIT
DIE
2"
3/8"
BASE PRINT
4 1/2"

Figure 6. Design and dimensions for construction of the first single punch press bolus mold



PUNCH



SPLIT DIE

BASE PRINT

struction of internal parts. Even though the internal surfaces were highly polished, the first compressions revealed that the steel was too porous. The compressed bolus material adhered so tightly to the die and punch that the bolus was torn apart trying to remove it from the mold. To solve this problem the internal surface of the die and concave surfaces of the base plate and punch were coated with 0.005 mm industrial hard chrome. The porosity of these surfaces was thereby reduced and coefficient of release increased permitting the compressed boluses to be removed easily without breakage.

Production of boluses by direct compression was accomplished by loading aliquots of bolus ingredients into the die with the base plate in place. The ingredients were leveled and the punch inserted into the die. The unit was then placed between the compression plates of the hydraulic press and pressure applied to the base plate and punch in a vertical plane. The press was capable of producing 11,364 kg total pressure with an accuracy of ± 91 kg. Famphur boluses were made using the procedure just described with various matrixes. Following dry weight and density determinations these boluses were placed in the artificial rumen bottles. Boluses were retrieved at least twice weekly to examine the physical appearance and determine the average daily release of famphur by recording bolus weight lost through degradation. At each weighing fresh rumen contents were pumped from fistulated cows to recharge the bottles of the artificial rumen. Each bottle was "fed" a portion of the ration fed to the fistulated cows to promote normal rumen microflora growth and function.

The amount of famphur released was calculated by multiplying the bolus weight lost by the % famphur blended into the matrix during formu-

lation. This assumes that the famphur is evenly distributed throughout the bolus and that the acaracide is released in direct proportion with the matrix through degradation. This procedure cannot account for the possibility of the acaracide leaching from the matrix before it can be physically released through erosion.

In an initial test for varying matrix composition, 35 g boluses were prepared of 5 g famphur blended in 30 g matrix (i.e., 14.3% famphur bolus). Four boluses were made of each of the matrix formulations I-III in Table 11. The boluses were compressed at 651 g/cm^2 resulting in average bolus densities of 1.8 for matrix I, 1.9 for matrix II and 1.9 for matrix III.

These boluses were placed in the artificial rumen and monitored as described. On the 9th day of inspection all matrix III boluses were discovered broken in as many as 4 pieces. On the 14th day, 2 matrix I boluses were broken and the others cracked. Through this period matrix II boluses were calculated to be releasing 12 ± 3 mg of famphur per day between the 7th and 14th days. Confidence about this composition prompted placement of 3 of these same matrix II boluses from the bottles in the reticulums of separate available fistulated cows to monitor the degradation progress for 35 days. By the second weighing of boluses from these cows famphur was being released at 22 ± 8 mg per day. Average famphur release of these matrix II boluses throughout this <u>in vivo</u> analysis was ca. 27 mg per day. One bolus was lost through passage or regurgitation after 30 days into the trial. On the 35th day the remaining 2 boluses were calculated to be releasing 26.5 and 24.9 mg famphur per day.

The results of this trial were very encouraging. Matrix II demon-

Table 11. Carnauba wax based matrix compositions tested for development of sustained-release famphur boluses for tick control.

Matrix number	Percent matrix ingredients		
	Carnauba	$BaSO_4$	Polyethylene glycol
I	27.3	63.6	9.1
II	27.3	68.2	4.5
III	20.0	60.0	20.0
IV	21.8	72.7	5.5
V	30.7	69.3	----

strated excellent capabilities in releasing famphur at a sustained rate. After 3 to 5 days in the rumen contents the surface of these boluses took on the appearance and texture of sandstone and all surfaces appeared to wear evenly. The practicality of the 14% famphur matrix II bolus for delivering famphur at 5-7 mg/kg/day to a 180 kg cow for example, was beyond consideration. Too many boluses would be required.

A second experiment was begun to determine the effect on the release rate of famphur from matrix II when increased amounts of acaracide were blended into the matrix. Three 35 g boluses formulated with matrix II were produced at each of 5 increasing concentrations of famphur (14, 23, 27, 30 and 33% famphur). Boluses were again pressed at 651 kg/cm^2. Densities ranged from 1.9 g/cm^3 for 14% famphur boluses to 1.8 for 33% famphur boluses. These were placed in the artificial rumen and monitored for 2 weeks. Average famphur release rates for the second week were 9 mg/day from 14%, 11 mg/day from 23%, 33 mg/day from 27%, 38 mg/day from 30%, and 70 mg/day from 33% boluses. Although the release of famphur was again steady, the rate of release was still not high enough to make treatment with such boluses practical.

In order to determine the maximum amount of famphur that could be loaded into matrix II a similar experiment was designed. Thirty-five gram boluses formulated with matrix II and varying amounts of famphur were produced as described above. In this test, increasing increments of famphur ranged from 33% to 50% and 4 boluses were produced at each formulation. Densities ranged from an average 1.8 for 33% famphur boluses to 1.7 for 50% famphur boluses. Table 12 shows the effect of increasing the amount of acaracide on the release rate from matrix II boluses. As increasing amounts of famphur were loaded into the matrix,

Table 12. Effects of increasing the proportion of famphur in a 35 gram bolus prepared by compressing matrix II and famphur at 651 kg/cm^2 tested over 135 days in artificial rumens.

Percent famphur in bolus composition	Total famphur released (mg)	Average daily release (mg) over 135 day period	Highest average daily release achieved (mg)
33.0	2797.2	20.7	105.1
36.8	3085.2	22.9	112.7
40.0	3293.1	24.4	125.5
42.9	3652.5	27.1	135.1
45.5	3935.2	29.2	136.9
47.8	4088.1	30.3	154.7
50.0	4334.9	32.1	153.4

a progressively larger amount of total famphur was released. This progression was also evident in the average daily famphur released and the highest recorded daily release over the entire test period. This average daily release includes the pre-plateau rise and post-plateau decline. Figures 7-9 provide more insight into the behavior of these boluses showing the pre- and post-plateau rise and decline. Figure 9 also indicates that the 50% famphur bolus maintained an average release rate above 100 mg per day from day 10 to day 80. The release-degradation curves of the other 6 formulations, Figures 7 and 8, follow the same pattern and produced plateaus indicative of the relative quantities of famphur incorporated into the matrix.

As the amount of famphur was increased, less binder (carnauba) was available in the 35 g bolus to hold the systemic acaracide resulting in slightly higher average degradation rates, therefore higher release rates. Physically these boluses appeared much the same as the initial test. The sandstone surface developed in ca. 5 days and boluses wore evenly on all surfaces throughout the test. At the termination of the study 2 boluses of each composition were dried at 21^{o}C for 7 days and then weighed. From their previous "wet" weights it was determined that on the average, 7.6% of that weight was due to moisture absorption. The average bolus weight at termination of the study was 8.3 g. Figures 10 and 11 show the progressive degradation of these boluses. The crevice on the top edge of these boluses persisted with nearly all boluses produced as earlier described. Following compression of each bolus it was noticed there was enough space between the die wall and punch that some of the compressed material "flowed" out of the compression area and up this void. It was along this edge of the bolus that the crevice was

Figure 7. Release of famphur through the *in vitro* degradation of 17.5, 22.5, and 27.5% famphur boluses

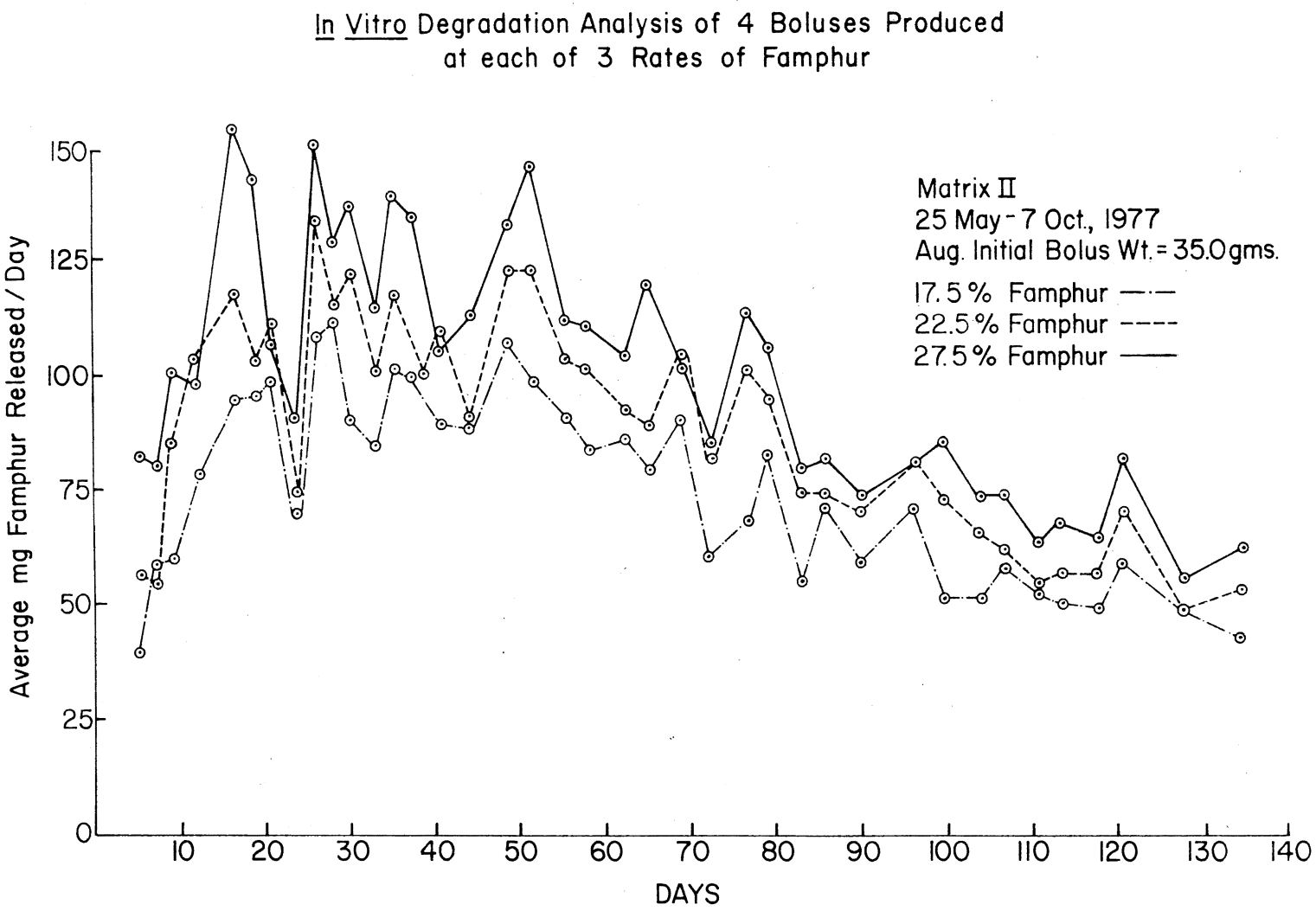

In Vitro Degradation Analysis of 4 Boluses Produced at each of 3 Rates of Famphur
Average mg Famphur Released / Day
150
125
100
75
50
25
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
DAYS
Matrix II
25 May - 7 Oct., 1977
Aug. Initial Bolus Wt. = 35.0gms.
17.5% Famphur
22.5% Famphur
27.5% Famphur

Figure 8. Release of famphur through the *in vitro* degradation of 33.0, 40.0, and 45.5% famphur boluses

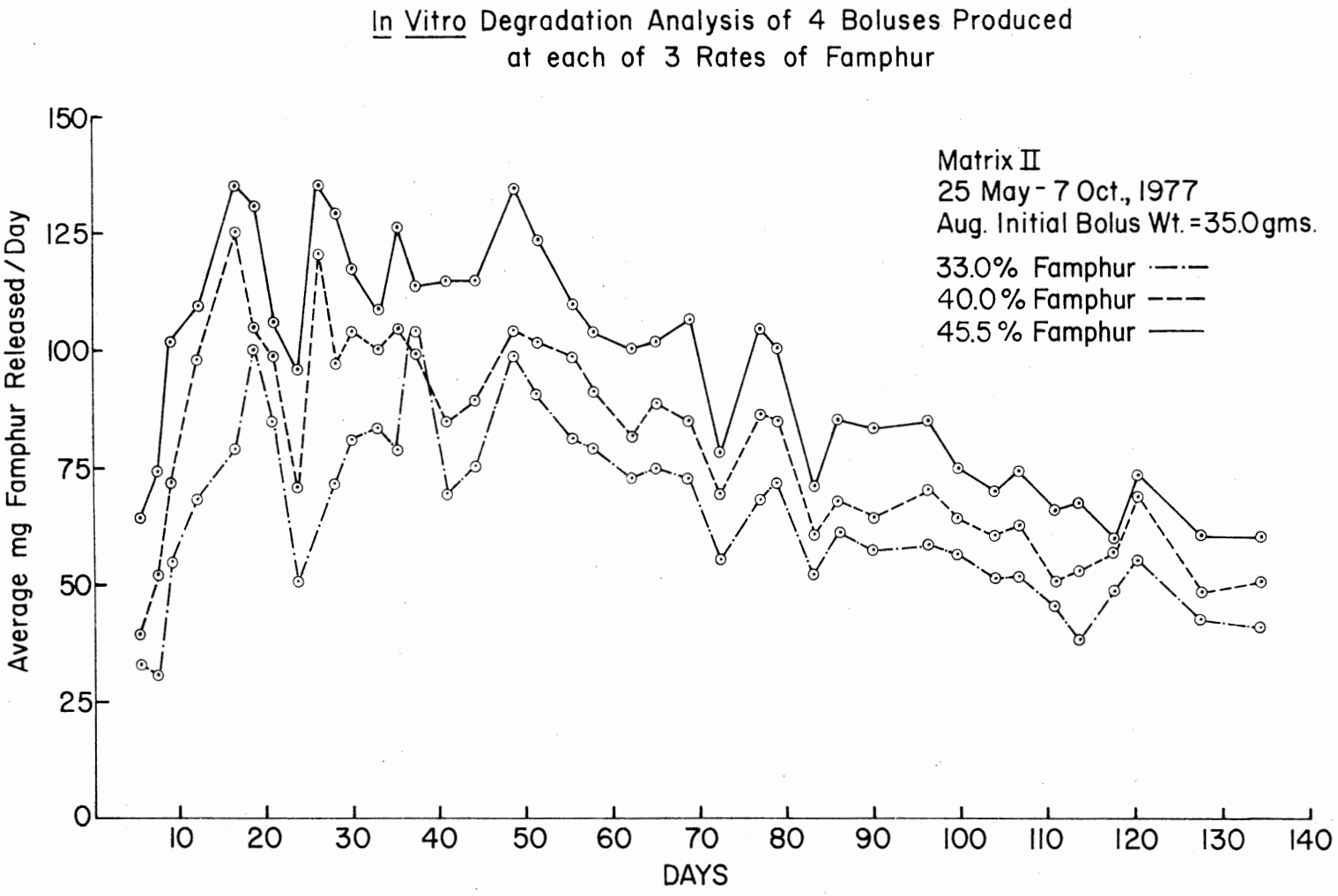

In Vitro Degradation Analysis of 4 Boluses Produced at each of 3 Rates of Famphur
Matrix II
25 May - 7 Oct., 1977
Aug. Initial Bolus Wt. = 35.0 gms.
33.0% Famphur
40.0% Famphur
45.5% Famphur
Average mg Famphur Released / Day
150
125
100
75
50
25
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
DAYS

Figure 9. Release of famphur through the *in vitro* degradation of 50% famphur boluses

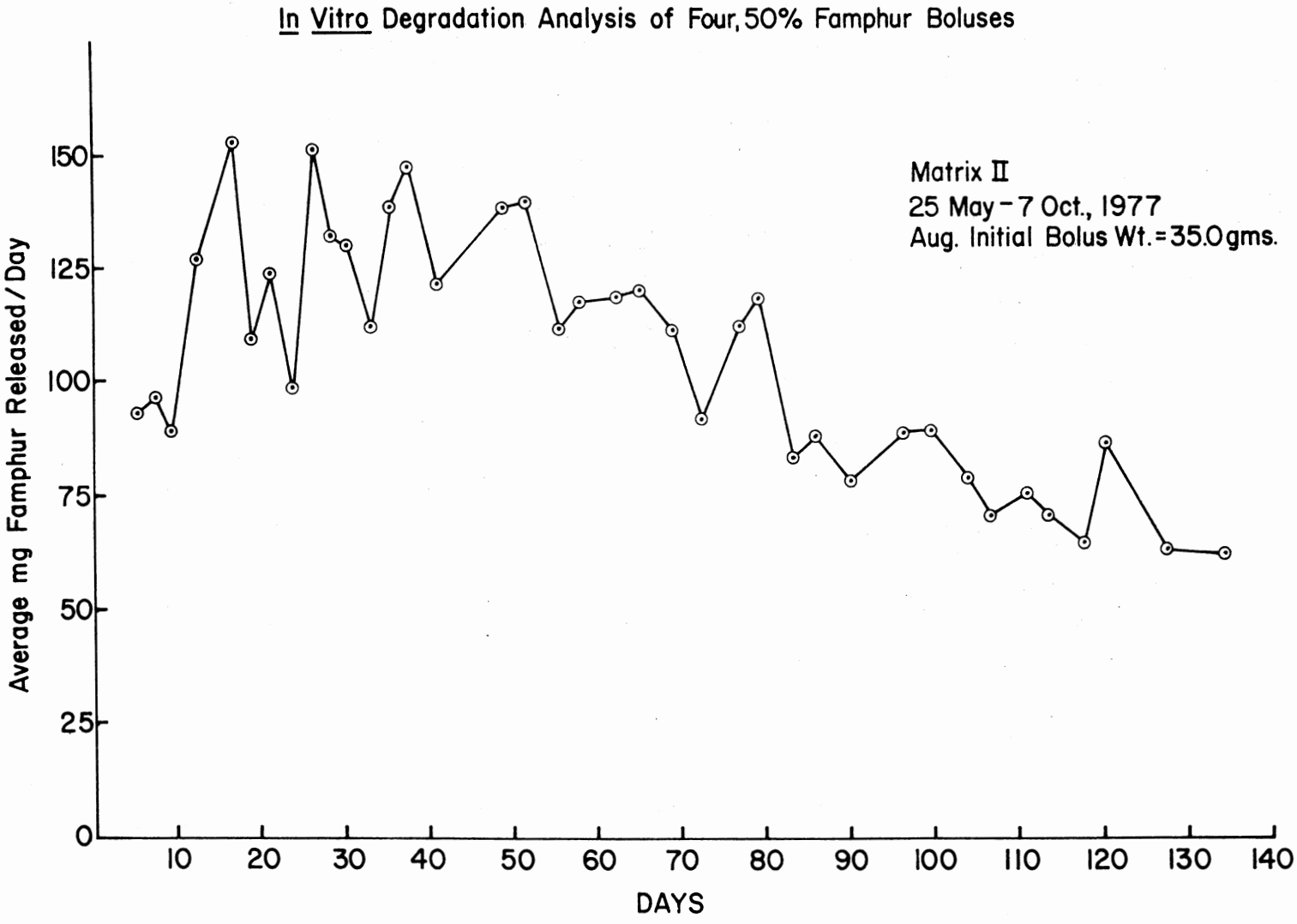
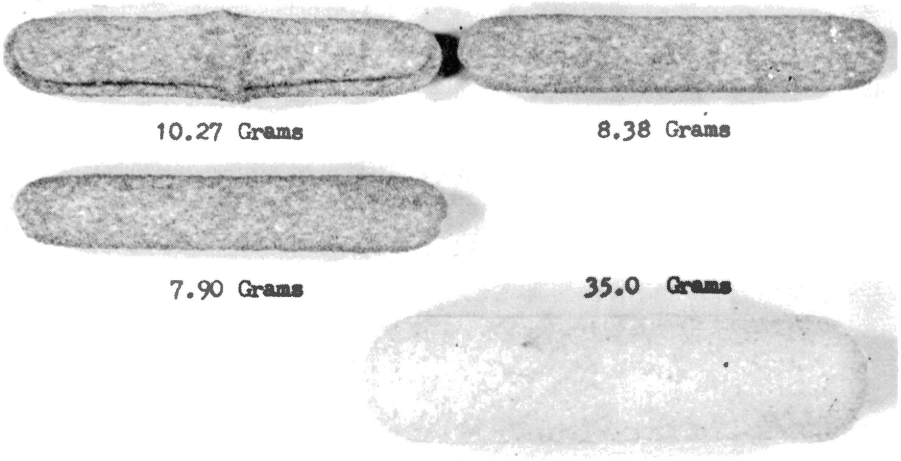

In Vitro Degradation Analysis of Four, 50% Famphur Boluses
Matrix II
25 May - 7 Oct., 1977
Aug. Initial Bolus Wt. = 35.0 gms.
Average mg Famphur Released / Day
0
25
50
75
100
125
150
10
20
30
40
50
60
70
80
90
100
110
120
130
140
DAYS

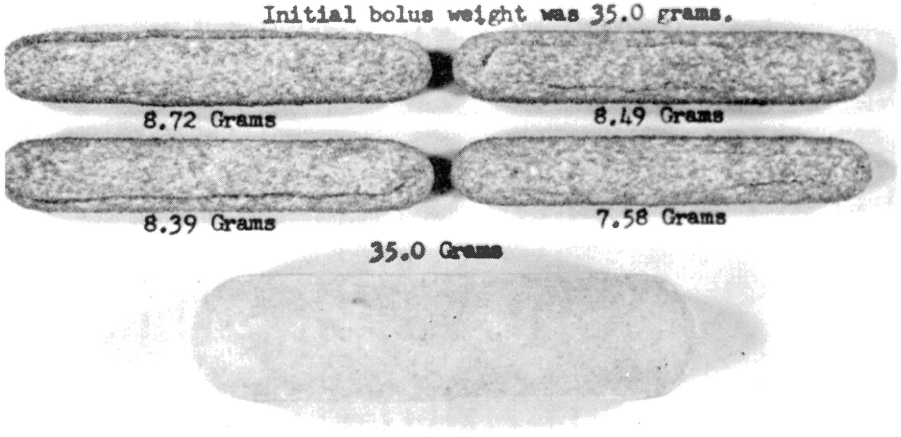
Figure 10. Comparison of 33% famphur bolus size and appearance following 134 days degradation in artificial rumens

Figure 11. Comparison of 50% famphur bolus size and appearance following 134 days degradation in artificial rumens

33% Famphur, Matrix #2
134 Days in Artificial Rumen.
Pressed with 9400 psi.
Initial bolus weight 35.0 grams.



50% Famphur, Matrix #2
134 days in artificial rumen.
Pressed with 9400 psi.
Initial bolus weight was 35.0 grams.



forming. This became a concern due to its yet unknown effect on degradation and perhaps weakening of bolus structure.

The successful ability of matrix II to handle 50% famphur prompted the production of 60% famphur matrix II boluses. When tested in the artificial rumen these boluses fell into ca. 12 pieces on the 4th day. An alternative for increasing the release rate of the 50% famphur boluses was the addition of more lubricant (PEG). Matrix IV (Table 11) was formulated with a 1% increase in PEG and 4.5% increase in $BaSO_4$. Fifty percent boluses were prepared with this matrix compressed at 651 kg/cm^2 and placed in the artificial rumen. On the 4th day these boluses split in half longitudinally. This longitudinal split separated the bolus through the previously observed and persistent crevice which in this case appeared to penetrate through the bolus and contribute to its failure.

The suitability of the promising 50% famphur matrix II boluses received further testing in vivo with fistulated cows. Using the same composition and production procedure eight 50% famphur matrix II boluses weighing ca. 35 g and having a density of 1.7 were placed in the reticulums of 4 fistulated cows, 2 per animal. By the 9th day 2 of the boluses were broken in half and the others severely cracked. The cracks appeared to be extensions of the previously described crevice and were still believed to be associated with the mold itself. The more rigid physical test of the live rumen proved to be too strenuous either for the formulation or for the physical abberation associated with the crevice.

During the test described above four 50% famphur boluses of matrix V (Table 11) containing no PEG were placed one each in the reticulums of the 4 fistulated cows. These boluses had been compressed at 651 kg/cm^2 yielding an average density of 1.7 g/cm^3. These boluses proved to be

more durable than the matrix II boluses. Figure 12 shows the average mg famphur released per day for the 48 day test period. Between the 5th and 35th day the average daily release remained between 150 and 250 mg/bolus. Between the 5th and 48th day the average daily release did not dip below 100 mg/bolus. On the 48th day these boluses weighed ca. 15 g leaving enough remaining material for more than 10 additional days of bolus life.

Despite these results 2 problems persisted. First the bolus crevice was still detectable though not as severe when matrix V was used and second, the release rate remained too low to make the famphur bolus commercially attractive. Live animal evaluation indicated shorter bolus longevity than for boluses observed during the artificial rumen evaluation. Although the artificial rumen provided the safety needed in analysis of initial bolus formulations, it presented a more static rumen environment than is actually experienced in the live animal. Variation in rumen content due to passage rates, feed and water intake, and overall rumen peristalsis which could not be duplicated in the artificial rumen probably contributed to this difference.

An evaluation of the data and problems experienced indicated that several improvements and modifications in the mold design might resolve these problems. The design for the second mold is shown in Figure 13. This design includes increased die wall thickness, heat treating the S-7 tool steel to 50-55 Rc (Rockwell hardness), and the use of four 22 mm hex-head cap screws for the die halves, all to support greater compression if needed. Internal surfaces again received 0.005 mm industrial hard chrome. In an attempt to correct the problem of bolus material back-flow in the die during compression and the crevice phenomenon be-

Figure 12. Degradation analysis of four 50% famphur, matrix V boluses placed in the rumens of fistulated cows

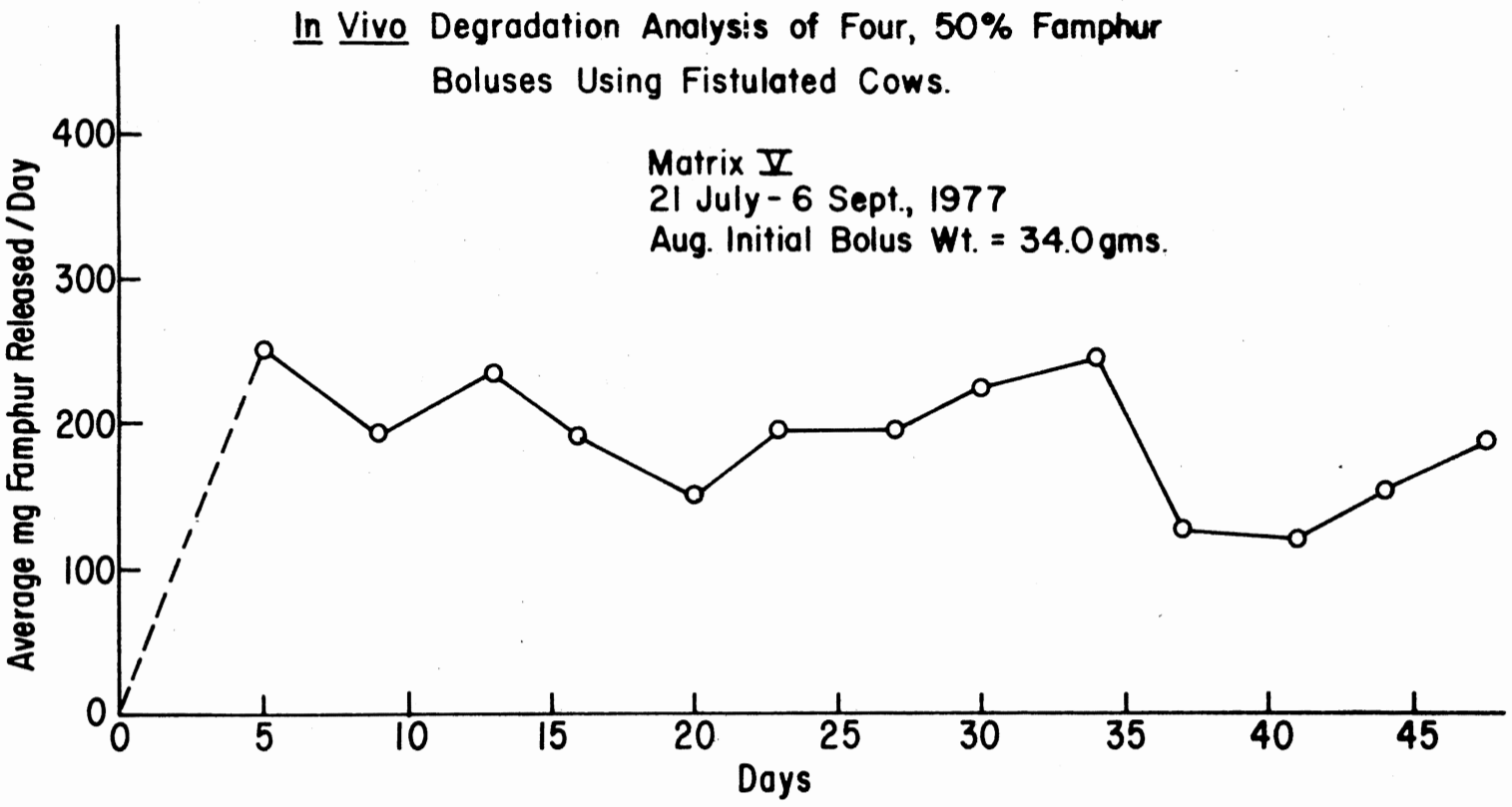
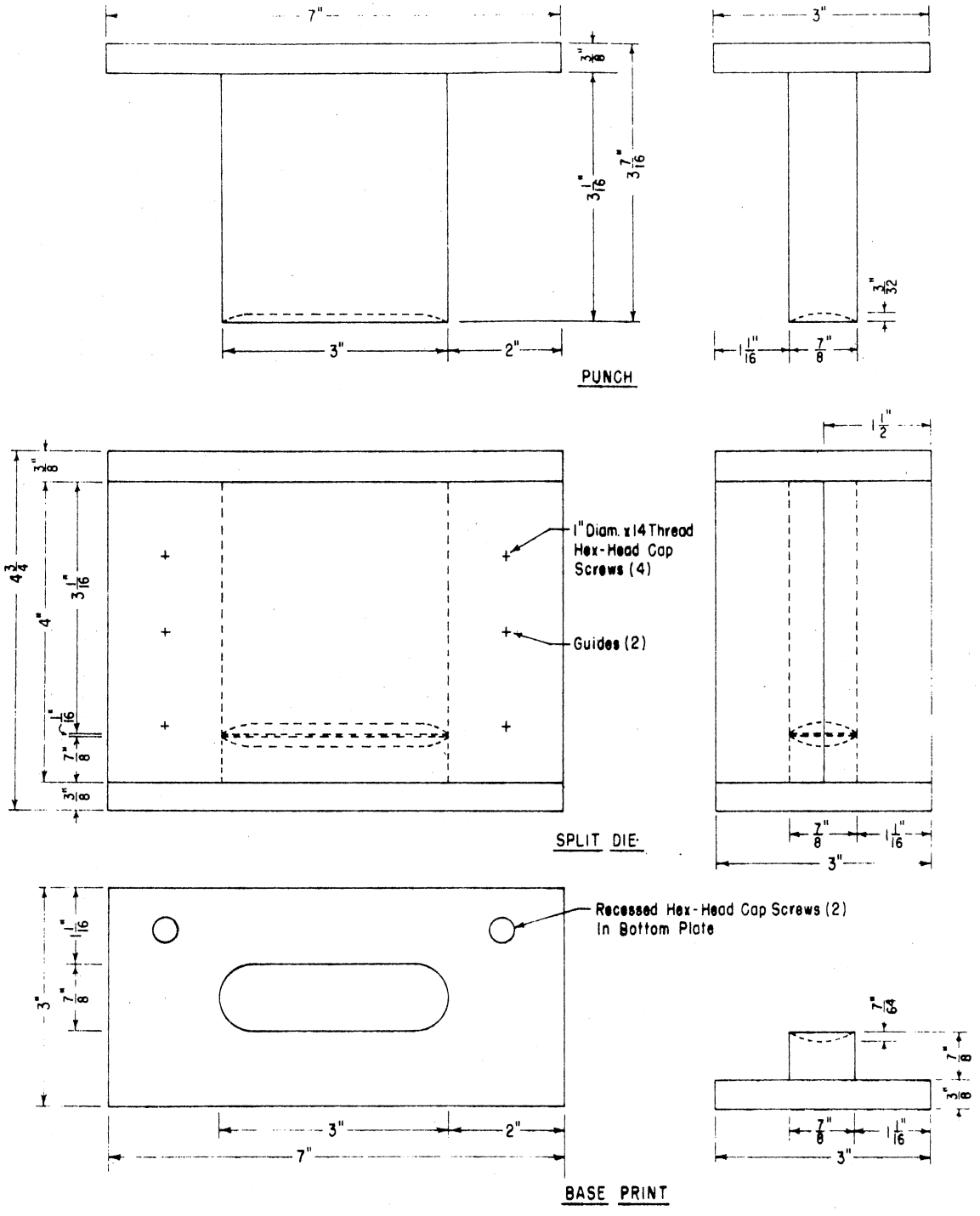

In Vivo Degradation Analysis of Four, 50% Famphur Boluses Using Fistulated Cows.
Matrix V
21 July - 6 Sept., 1977
Aug. Initial Bolus Wt. = 34.0 gms.
Average mg Famphur Released / Day
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
Days

Figure 13. Design and dimensions used in construction of the second single punch press bolus mold


7"
3"
2"
PUNCH
1" Diam. x 14 Thread Hex-Head Cap Screws (4)
Guides (2)
SPLIT DIE
Recessed Hex-Head Cap Screws (2) In Bottom Plate
BASE PRINT

lieved associated with it, the internal mold parts were machined to a maximum tolerance of 0.0125 mm. The size of the die bore, punch and base plate faces were increased in order to produce a bolus 75.0 x 21.9 mm with oval ends. The horizontal area of the die bore was 16.0 cm^2. The increase in size was made to increase bolus surface area and total bolus size. Increased surface area was incorporated for the purpose of increasing the erosion and therefore the release rate. The opportunity for increased bolus size was considered as a means of increasing bolus longevity to offset increased release rates, and of increasing bolus strength against breakage. Recessed 6.3 mm hex-head cap screws were added to hold the base plate firmly to one-half of the die body. The die body was slightly tapered at the top to facilitate guiding the punch into the die.

Upon completion of this mold, six 50% famphur, matrix II boluses were compressed at 563 kg/cm^2 yielding boluses with an average density of 1.66 gms/cm^3. There was no flow of material back up the die nor any visual evidence of cracks around the top bolus edge. These were placed in the reticulum of 6 fistulated cows. These boluses were retrieved via the fistula after 3 days to find that every bolus had developed severe cracks around the top bolus edge formed by the punch and die walls. Lesser cracks were evident around the opposite edge formed by the base plate and die walls. In some cases these cracks were severe enough to result in the top of the entire bolus becoming separated from the rest of the bolus. In 2 cases, the ends of boluses had been broken away revealing that the cracks penetrated to the center of the bolus forming 4 wedge-shaped longitudinal pieces. From this observation it appeared that internal forces stored in the bolus during compression were manifesting

themselves when placed in the warm liquid environment of the rumen.

In reviewing the characterization of powders Marshal (1977) outlined 3 stages in a force-volume relationship undergone during the compression process. The first stage refers to the repacking of particles after the compressing force is first applied. Particles essentially move in a random process to fill small voids between larger particles. As this closer packing occurs larger particles may be reduced in size while leading to size enlargement. When the particles have repacked themselves under initial pressure a stage of elastic deformation is entered in which there is very little decrease in the porosity of the particle mass. This stage is characterized by the elastic recovery of the particle mass if the pressure applied is released. When the limit of elasticity is passed, a stage of plastic deformation begins. Under this pressure particles continue size enlargement through cohesion. Furthermore, Marshal (1977) adds that there is a relatively narrow optimum porosity range within the plastic deformation stage at which adequate mechanical strength is attained while allowing a measure of water uptake to promote good disintegration characteristics. If one continues to apply pressure beyond this range, weaknesses occur.

Train and Lewis (1962) noted that a complex balance of applied and induced stresses exist following agglomeration by compaction giving rise to areas of high and low density within the compaction. Density variation in cylindrical compacts were extensively studied earlier by Train (1956). In lubricated compacts he observed regions of greater density in the top corners and about two-thirds of the way down in the center of the compaction body. Regions of lower density were found in the lower corners and near the top center of the compaction. He further observed

that the regions of high density were connected by planes of lower density. When the maximum applied pressure is released some elastic relaxation takes place producing stress within the compaction where the material is less dense. This author adds that this is the suggested cause of the phenomenon called "capping", a condition where the top of a compaction detaches itself from the body of the tablet. Three causes were cited as a possible basis for capping; excessive pressure, entrapped air, and insufficient binder.

With respect to the famphur bolus capping, excessive pressure seemed to be the most probable cause. Examination of previous boluses did not reveal evidence of entrapped air and boluses of the matrix II composition pressed with the first mold appeared to have sufficient cohesion even though cracks appeared on the bolus.

To test excessive pressure as the probable cause of bolus capping, a set of three 35 g, 50% famphur boluses prepared of matrix II were compressed at each of 4 different pressures; 197, 394, 563 and 704 kg/cm^2. The average densities of these compactions; 1.55, 1.60, 1.66 and 1.66 g/cm^3 respectively, indicate the extent to which compaction was complete. Four days after these boluses were placed in the reticulums of fistulated cows all but those boluses compressed at 197 kg/cm^2 showed definite characteristics of capping. Figure 14 shows an end view of one of the capping boluses revealing the separation probably associated with the lines of stress described by Train (1956). Figures 15 and 16 show the final result of boluses exhibiting the capping phenomena and Figure 17 the intact boluses without capping that were pressed at 197 kg/cm^2.

The release of famphur through analysis by degradation of the intact 50% famphur, matrix II boluses compressed at 197 kg/cm^2 continued

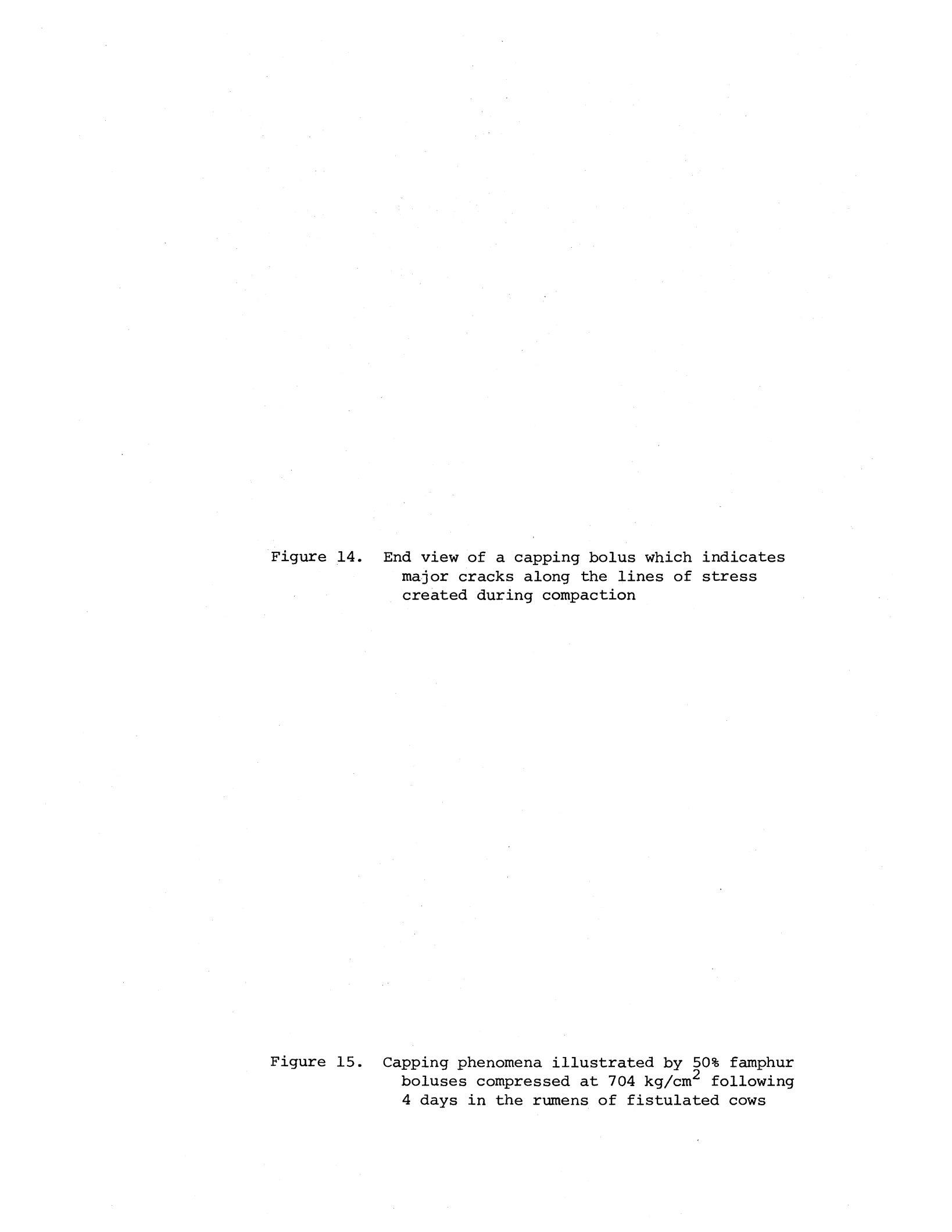
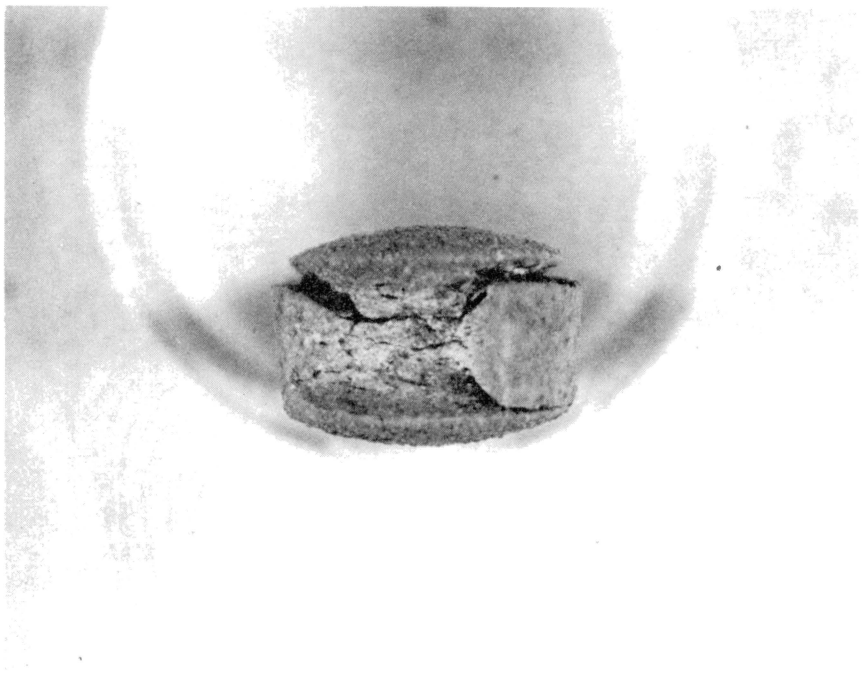


Figure 14. End view of a capping bolus which indicates major cracks along the lines of stress created during compaction

Figure 15. Capping phenomena illustrated by 50% famphur boluses compressed at 704 kg/cm^2 following 4 days in the rumens of fistulated cows



50% FAMPHUR
MATRIX #2
25,000 TOTAL lbs. PRESSURE
4 DAYS IN Vivo

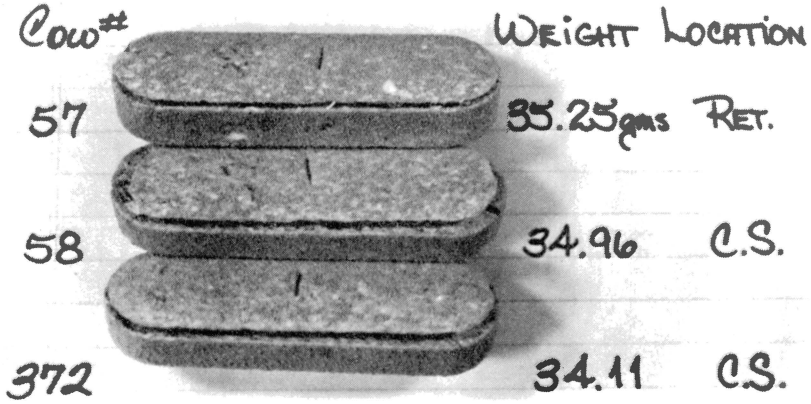
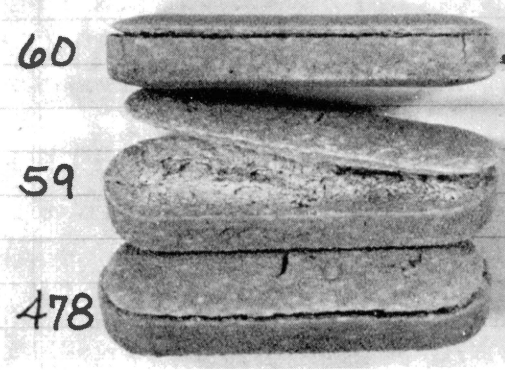


Figure 16. Capping phenomena illustrated by 50% famphur boluses compressed at 563 kg/cm^2 following 4 days in the rumens of fistulated cows

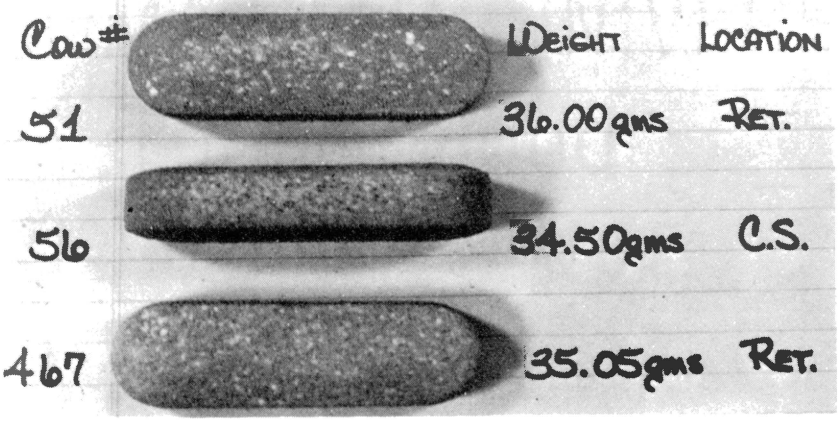
Figure 17. Intact 50% famphur boluses compressed at 197 kg/cm^2 showing no signs of capping after 4 days in the rumens of fistulated cows

50% Famphur
Matrix #2
20,000 Total lbs. Pressure For 30 Sec.
4 Days In Vivo



Cow #	Weight	Location
60	30.68	C.S.
59	34.95	C.S.
478	35.38	C.S.

50% Famphur
Matrix #2
7000 Total lbs. pressure
4 Days In Vivo



Cow #	Weight	Location
51	36.00 gms	Ret.
56	34.50 gms	C.S.
467	35.05 gms	Ret.

for 45 days (Figure 18). These boluses released substantially more famphur per bolus over a shorter time period than previous boluses of this type produced with the first mold. In addition, this preparation did not establish a plateau of sustained release, but increased its release rate to a peak and steadily declined thereafter. The increased surface area of boluses produced in the newer mold and the lower compaction pressure probably contributed to this bolus release behavior. Siegrist and Katz (1970) point out that the maximum pressure of compaction, within limits, is inversely proportional to disintegration rates. Consequently, lower pressure of compaction can result in higher release rates.

A comparison study was begun with 50% famphur boluses prepared from matrix V containing no PEG. Six 35 g boluses were formed under compression at 563 kg/cm^2 in the redesigned mold. The average density of these boluses was 1.65 gms/cm^3. These boluses were placed in the reticulums of 6 fistulated cows and the release of famphur monitored for 66 days. Figure 19 shows the famphur release pattern observed for these boluses. With the exception of the period from day 24 to 28 these boluses maintained an average release rate in excess of 200 mg famphur/day for 31 days, and above 125 mg/day for 64 days.

Table 13 summarizes the data collected in this study. Day to day observations indicated that variability among bolus release rates could largely be attributed to animal-to-animal variation in rumen content. Some of the fistulated cows retained a more fluid filled rumen while others maintained the stratified layers described by Smith et al. (1955) with varying degrees of dry matter feed. Tracking the location of individual boluses indicated that boluses retained in the reticulum had

Figure 18. Degradation analysis of 50% famphur, matrix II boluses compressed at 197 kg/cm^2 and placed in the rumens of fistulated cows

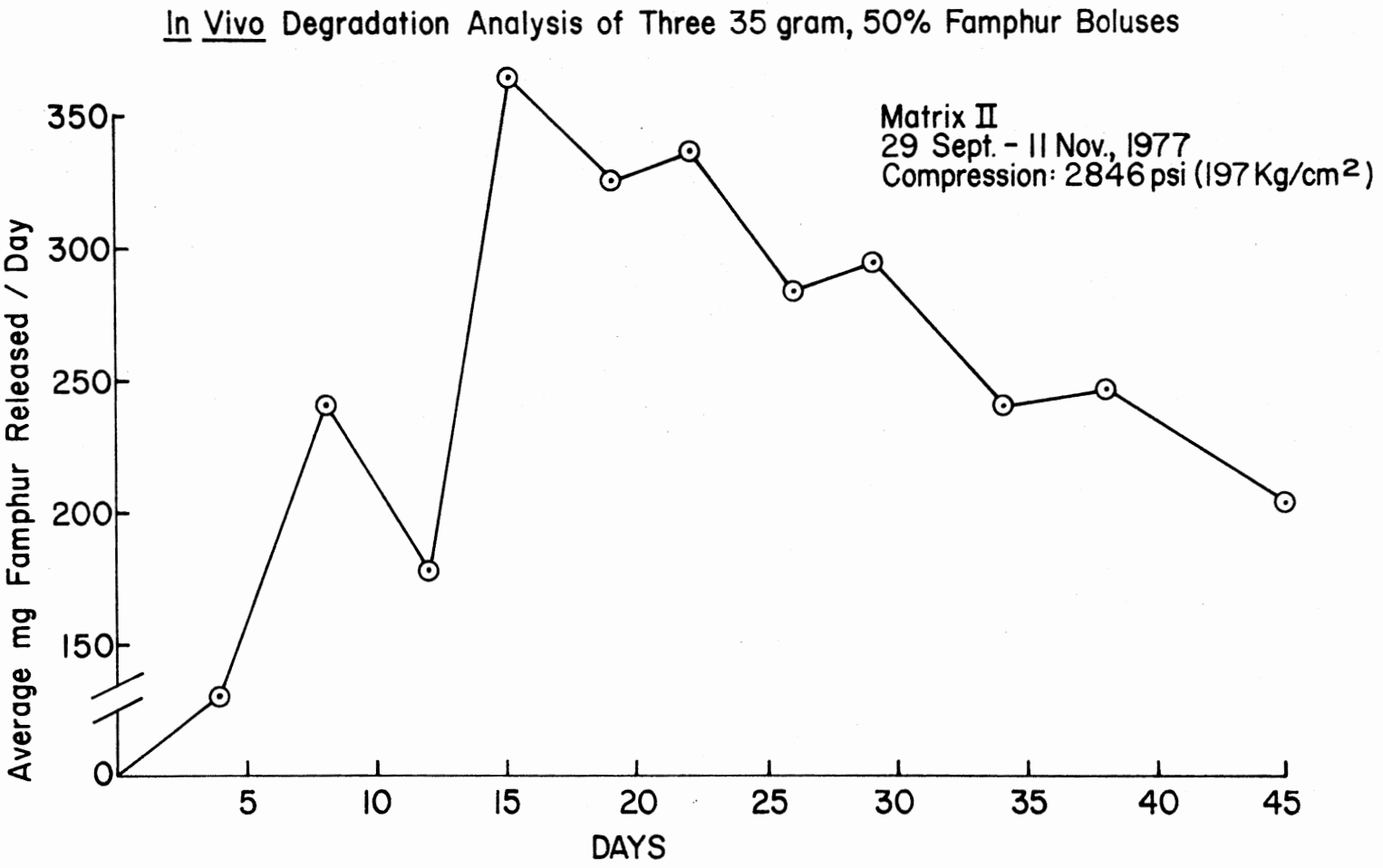

In Vivo Degradation Analysis of Three 35 gram, 50% Famphur Boluses
Matrix II
29 Sept. - 11 Nov., 1977
Compression: 2846 psi (197 Kg/cm²)
Average mg Famphur Released / Day
350
300
250
200
150
0
5
10
15
20
25
30
35
40
45
DAYS

Figure 19. Degradation analysis of 50% famphur, matrix V boluses compressed at 563 kg/cm^2 and placed in the rumens of fistulated cows

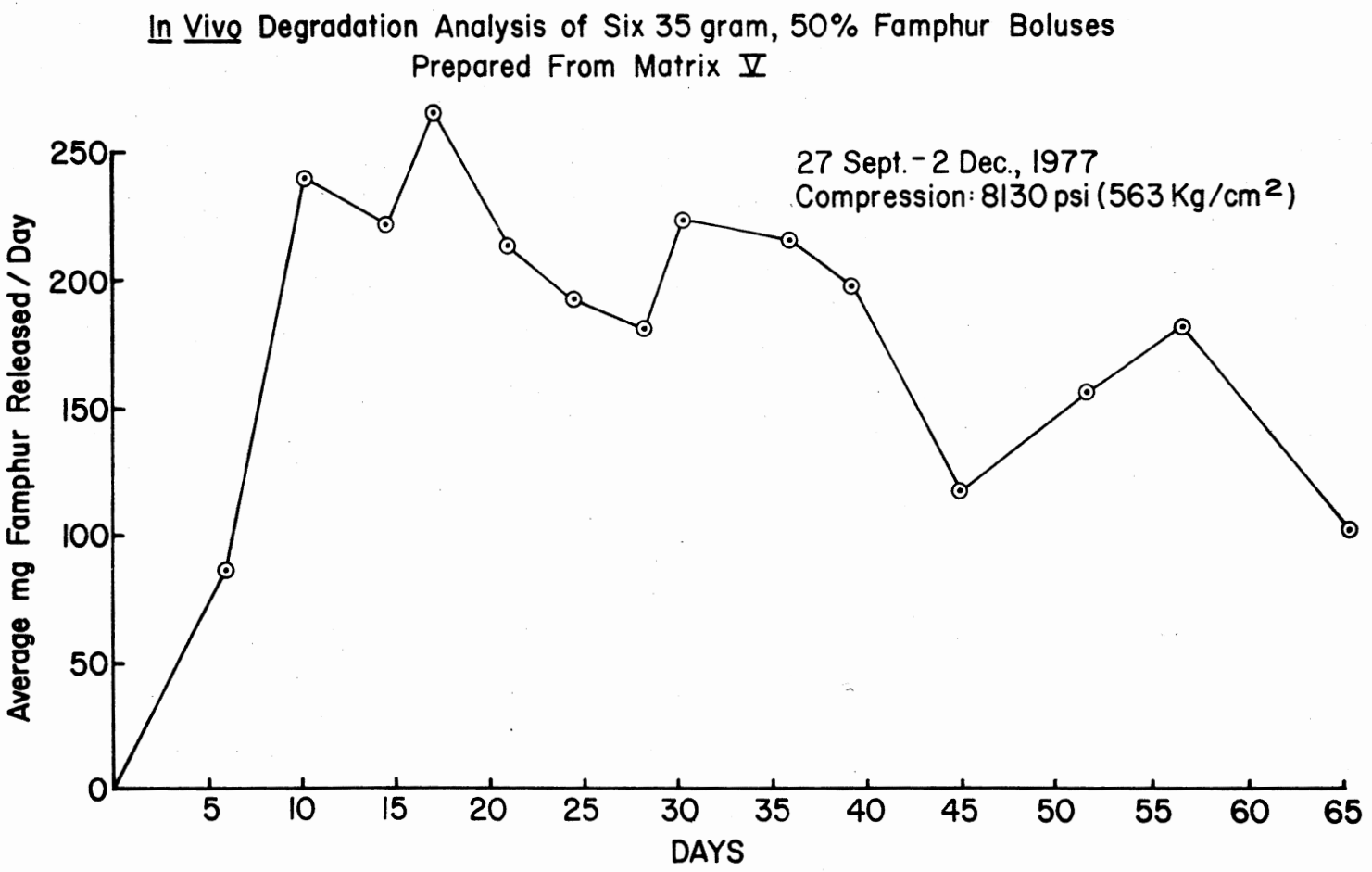

In Vivo Degradation Analysis of Six 35 gram, 50% Famphur Boluses
Prepared From Matrix V
27 Sept. - 2 Dec., 1977
Compression: 8130 psi (563 Kg/cm2)
Average mg Famphur Released / Day
0
50
100
150
200
250
5
10
15
20
25
30
35
40
45
50
55
60
65
DAYS

Table 13. Degradation and retension data for 50% famphur boluses prepared of matrix V, compressed at 563 kg/cm^2 and placed in the reticulums of 6 mature fistulated cows.

Day	Average (X) daily release of famphur	± S.D.	Total number of boluses	Rumen location		Total number of boluses broken in half
				Reticulum	Ventral sac	
6	88.5	49.8	6	2	4	0
10	241.0	50.2	6	1	5	0
14	222.2	86.0	6	4	2	0
17	266.0	100.1	6	2	4	0
21	213.0	120.4	6	2	4	0
24	193.0	68.2	6	2	4	0
28	184.0	69.0	6	4	2	0
31	246.0	92.9	6	4	2	0
36	218.0	54.0	6	1	5	2
39	199.8	35.9	6	3	3	2
45	119.0	34.0	5[a]	2	3	2
52	156.0	36.7	4[b]	1	3	2
57	182.5	21.9	3[a]	1	2	3
66	103.5	26.2	2[b]	0	2	3

[a]Change in total bolus number reflects boluses lost within the animal through, passage, regurgitation or disintegration.

[b]Change in total bolus number reflects manual removal of boluses broken in half.

somewhat higher release rates than those found passed to the ventral sac and therefore probably contribute another factor to the variability of release rate.

Even though boluses were replaced in the reticulum after each inspection to determine the rate of passage to the ventral sac, no boluses were lost until after the 39th day. At that time the boluses that were lost were those that had broken in half. Initially these halves weighed ca. 10 g and remained in the rumen until they approached ca. 5 g. At about this weight they seem to be lifted from the rumen floor by the ingesta of the rumen and probably passed.

The frequency with which these boluses were passed from the reticulum to the ventral sac indicate that eventually most all of these slow-release boluses would be retained in the ventral sac.

The capping phenomena observed among the matrix II boluses did not surface in this study even though boluses were compressed with 563 kg/cm^2 pressure. Matrixes II and V differ in that matrix V contains no PEG. It seems quite possible that the PEG, whose melting point is lower than that of carnauba facilitates the capping phenomena by "lubricating" the powders along the lines of stress described by Train (1956). Therefore, any matrix incorporating PEG or other similar additives will probably be more apt to exhibit the capping phenomena.

The results obtained from the last study were very attractive. Data contained in Table 13 and illustrated in Figure 19 indicated that if a dosage rate of 5-7 mg/kg/day of famphur could be maintained with the 50% famphur matrix V boluses, some measure of control against ticks might be possible for a period of ca. 60 days.

CHAPTER VI

EFFICACY OF A 50% FAMPHUR RUMENAL BOLUS AGAINST LONE STAR AND GULF COAST TICKS ON HEREFORD HEIFERS

Previous data collected from 50% famphur boluses prepared from a matrix containing 30.7% carnauba wax and 69.3% barium sulfate (Chapter V) as monitored in mature cows weighing ca. 364 kg indicated the provision of at least 200 mg famphur/bolus/day for ca. 30 days and 125 mg/bolus/day for more than 30 additional days. Efficacy data on famphur administered continuously by rumen infusion (Chapter II) indicated a dosage rate of 7 mg/kg/day was necessary to provide complete control of Gulf Coast and 95% control of lone star ticks. Based on providing a therapeutic dosage of 7 mg/kg/day from a sustained-release bolus delivering 200 mg/bolus/day, a treatment rate of 28.6 kg of animal weight per bolus was derived. This treatment rate was considerably higher than cattlemen are normally accustomed using currently available, more rapid-release veterinary boluses. With this dosage requirement the likelihood of commercial acceptance of this famphur bolus is doubtful. However, from a developmental standpoint, an efficacy trial was needed to determine if this dosage rate could be delivered and maintained by sustained-release from a bolus. Such bio-assay data, if successful, would add credence to this new approach to tick control and add valuable information needed to further refine and develop the bolus. A study was

subsequently designed to test the efficacy of these boluses against Gulf Coast and lone star ticks over the first 60 days of bolus longevity in bovine.

Materials and Methods

Nine of 18 grade Hereford heifers weighing ca. 180 kg were fistulated for model NU4 rumenal cannulas (Bar Diamond, Inc., Parma, Id.) made of Plastisol® which provided entry to the rumen through a permanent orifice ca. 12.5 cm in diameter. Surgery was followed by 10 days post-operative antibiotic therapy. The cannulated and non-cannulated heifers were maintained together under drylot conditions on oat hay and a 30% cottonseed hull based ration including 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 30,000 IU/g Vitamin A provided at 200 g/2 ton mix.

Fifty percent famphur boluses were prepared using matrix V as previously described in Chapter V and by Teel and Hair (1978). The matrix was prepared in 200 g quantities which, after being pulverized and screened through a U.S. Standard 40 mesh sieve, were mixed together by screening through a 10 mesh sieve to obtain uniform particle distribution throughout the bulk of the matrix. Famphur from the same production lot number was blended into the matrix in equal parts by weight. Thirty-five gram aliquots of the blended preparation were hydraulically pressed at 507 kg/cm^2 to produce a bolus with an average density of 1.62 ± 0.03 g/cm^3. Pressure applied to these boluses was reduced 10% from that used in the production of earlier boluses of the same composition to reduce internal shearing stresses, yet retain the same release

rate.

Cannulated heifers were administered these boluses orally with a conventional, multiple balling gun, capable of delivering up to 5 boluses per insertion, at the dosage rate of 1 bolus per 28.6 kg animal weight. Bolus dose was determined by treatment weight derived from each heifers weight at time of treatment plus 20% estimated gain over the 60 day testing period. On the average, this regime required 7 boluses per calf. Boluses were periodically removed via the cannula for physical examination and determination of famphur released by bolus weight lost through degradation. The boluses in each dose were lightly etched according to a prearranged code in order that each bolus' degradation behavior could be monitored. Animal weight records were kept as an indicator of bolus effect on rate of gain. Pretreatment red blood cell cholinesterase determinations were made followed by bi-weekly determinations thereafter, using the procedure of Radleff and Woodward (1956).

Treated heifers were returned to drylot for 7 days to allow the boluses sufficient time to develop the previously observed sandstone-like appearance and bring the blood level of famphur to therapeutic dose.

Three cannulated treatment and 3 non-cannulated control heifers representing Group I, were randomly selected on the 7th day post-treatment and randomly assigned to 1 of 6 metabolism stalls in a laboratory maintained at $20^{\circ} \pm 5^{\circ}C$ and 14 h photophase. Water and the cottonseed-hull based ration were available ad libitum. Oat hay was fed daily in order that dietary maintenance of laboratory animals remained similar to those remaining on drylot.

Two samples of 20 pairs of laboratory reared A. americanum and A.

maculatum were confined over the withers of these 6 heifers in stockinette cells. Duplicate samples of ca. 100 larvae and nymphs of these tick species were confined to each heifer's topline in Plexiglas® cells. Attachment was determined in 72 h and 48 h for adults and nymphs respectively. Unattached ticks were removed in order that mortality be determined on feeding ticks. Mortality was determined at 4 day intervals and replete larvae and nymphs were removed to temperature-humidity chambers maintained at 92 ± 3% RH, 20° ± 5°C and 14 h photophase for determination of % molt. Engorged adult females were weighed, placed in individual oviposition vials and held under the same environmental conditions. Egg mass weight, % hatch, estimated larval production (EL) and estimated cumulative control (ECC) values were determined as described in Chapter II. Data from this split-plot design were subjected to analysis of variance to determine any effects attributable to the famphur bolus treatment. Percent mortality of ca. 30 C. lectularius was ascertained after feeding the bugs on each heifer after entry and just prior to their release from the laboratory.

Group I heifers were maintained in the laboratory for 15 days to allow sufficient time to collect tick data. At the end of this period Group I was released to drylot and 3 treatment and 3 control heifers randomly selected and assigned to stalls to compose Group II. Ticks feeding on Group II heifers challenged the famphur bolus during its 22nd to 37th day of longevity. Sequentially, the remaining 3 treatment and 3 control heifers comprising Group III challenged the bolus past the 37th day. By sequentially challenging the longevity of the bolus with cattle not previously exposed to ticks, the interference of resistance developing through repeated challenges on the same animals reported by

Williams et al. (1977, 1978) could be held to a minimum and allow the full potential of the famphur to be analyzed.

Results and Discussion

Immediately following administration of the boluses, each rumen was examined via the fistula to determine bolus distribution. All were retained in the rumeno-reticular sac except 1 or 2 boluses in each heifer which were passed to the cranial or ventral sacs. By the next examination (day 7) more than 90% of the boluses had been passed posteriorly to the ventral sac of the rumen. Upon each examination throughout the course of the study boluses were replaced in the anatomical location where they were found. Tables 14-16 summarize the famphur dosage data calculated from bolus weight changes brought about through degradation. Alteration of bolus numbers in Table 14 indicate the mechanical removal to achieve the desired dosage rate or the result of animal regurgitation. During the challenge period for group I heifers, the famphur delivery rate remained above 7 mg/kg/day for 2 of the 3 treatment heifers (Table 14). Heifer number 72 dipped below the desired delivery rate when the number of boluses was decreased to 5 following the regurgitation of a bolus.

Maintenance of the bolus dosage rate became a problem among the heifers remaining on drylot. The regurgitation rate among these heifers not confined to the laboratory stanchions was somewhat higher and regurgitated boluses could not be identified to owner. In the course of regurgitation, boluses were occasionally masticated rendering them non-replaceable. In addition, after 21 days an increasing number of boluses began breaking in half transversely, in some cases followed by yet a

Table 14. Calculated famphur dosages delivered to group I treatment calves by 50% famphur boluses.

Days in treatment	Ave. dosage mg/kg/day (no. boluses)		
	Calf 72	Calf 91	Calf 96
1 - 7	8.2(6)	10.3(6)	12.4(8)
7 - 10	9.0(5)	9.7(7)	16.2(8)
10 - 14	6.3(5)	8.1(6)	11.6(7)
14 - 17	6.7(5)	8.5(6.5)	10.2(7)
17 - 21	6.2(5)	11.4(6.5)	7.7(7)
21 - 31	6.2(5)	8.7(5)	7.4(7)

Table 15. Calculated famphur dosages delivered to group II treatment calves by 50% famphur boluses.

Days in treatment	Ave. dosage mg/kg/day		
	Calf 89	Calf 92	Calf 99
21 - 27	6.3	5.7	9.2
27 - 31	---	5.0	8.6
31 - 34	---	6.0	----
34 - 35	7.7	4.6	----
35 - 39	---	---	----
39 - 42	---	---	10.0
42 - 45	---	6.0	9.3

Table 16. Calculated famphur dosages delivered to group III treatment calves by 50% famphur boluses.

Days in treatment	Ave. dosage mg/kg/day		
	Calf 97	Calf 73	Calf 71
46 - 47	---	7.1	7.9
47 - 48	5.6	5.2	4.8
48 - 50	7.9	10.5	8.5
50 - 52	7.1	7.4	5.7
52 - 54	---	----	7.5
54 - 58	6.2	----	5.3
58 - 59	7.6	11.3	5.3
59 - 61	7.4	10.7	4.3
61 - 64	7.6	----	6.1

second transverse break at a later date rendering the original bolus in 4 pieces. Bolus pieces this size were more subject to being moved from the rumen floor as they approached ca. 5 g in weight and either regurgitated or eliminated through the digestive tract.

As a result of these previously unobserved problems it was at times impossible to determine the delivery rate of famphur. Table 15 shows the famphur delivery determinations from group II heifers and indicates the difficulties encountered in determining this parameter. It became evident among this group that, on the average, those animals with the smaller rumen sizes experienced more regurgitation and bolus breakage than those with larger rumens. Prior to this experiment, only mature cows (ca. 364 kg) had been used in bolus development and no more than 2 famphur boluses were ever tested in a rumen at one time. Placing the needed bolus dosage of 6-8 boluses in these smaller animals promoted a greater erosion rate as the boluses tended to lay in closer proximity to each other and enhance bolus-to-bolus abrasion. The average erosion rate of intact boluses was 304 mg/day as opposed to 200 mg/day observed for this bolus formulation in previous testing (Chapter V, Table 13). The expected bolus longevity at this degradation rate remained in excess of 60 days.

The problem of regurgitation could probably be alleviated by increased bolus density. Siegrist and Katz (1970) suggested that while a density of 1.5 g/cm^3 was minimal, they preferred a density of 1.9. The direct relationship between bolus density and rumen retention points to the shortcoming of this experimental bolus with a density of 1.62.

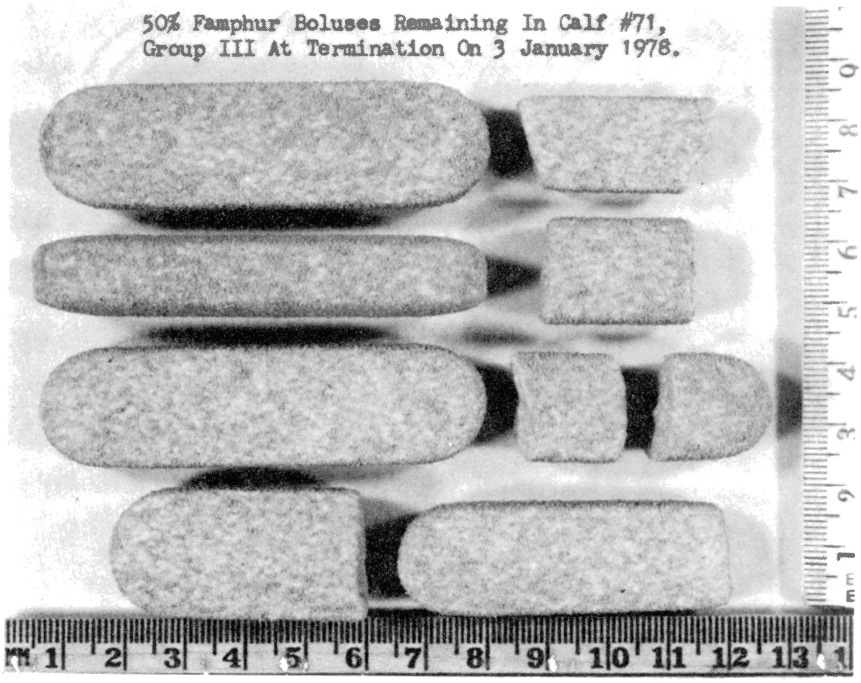
Marshal (1977) noted that compacted powders characteristically possess low tensile strength. When these experimental boluses were re-

tained in the liquid rumen environment for extended periods, the tensile strength of the original compaction was reduced as judged by how easily such boluses could be manually broken. As the mass of boluses on the rumen floor were rolled together by peristalsis it seemed quite conceivable that the abrasion exceeded the tensile strength of the boluses and thereby facilitated the transverse cracks and breakage. The absorption of liquid by these boluses in the rumen decreases the tensile strength of the original compaction. After being in the rumen for extended periods, boluses weighing ca. 15 g were dried to constant weight and found to have contained 7.6% moisture.

The famphur dosage rate for group III heifers was maintained with whole boluses collected from previously challenged animals. Table 16 shows the calculated famphur dosage delivered to these heifers. The desired 7 mg/kg/day rate was maintained for 2 of the heifers while heifer number 71 maintained a dosage rate above 5 mg/kg/day. Figure 20 shows famphur boluses and bolus pieces removed at termination of the study to illustrate the relative sizes of fragments following breakage.

The delivery rate for famphur of 7 mg/kg/day was a limiting factor in achieving a successful bolus. Although this rate could be achieved with this matrix, such a large quantity of the active ingredient must be incorporated into the preparation to achieve a sufficiently high release rate yet provide substantial longevity to make the approach feasible, that density was diminished and less binder was available per unit volume to offer sufficiently strong cohesion of the mass. These problems must be overcome through continued development. A more effective systemic acaracide that would be compatible with this matrix would enhance consumer acceptance by reducing the number of boluses required for

Figure 20. Fifty percent famphur boluses removed from fistulated Hereford heifers illustrating the bolus breakage believed associated with low tensile strength


50% Famphur Boluses Remaining In Calf #71,
Group III At Termination On 3 January 1978.

treatment. Less acaracide would be required per bolus making a higher density more easily attainable.

Even though the technical problems of regurgitation and breakage appeared during this study, the organophosphate acaracide remained sufficiently bound in the matrix of the broken parts to provide a continuous release to the bovine. Tables 17-19 show the efficacy data collected from the 2 challenging acarines. Famphur released from boluses of this preparation was more effective against A. maculatum than A. americanum as previously observed by Drummond et al. (1972) and Teel et al. (1977).

Significant differences attributed to bolus treatment were observed for several tick parameters. Complete control of A. maculatum males was attained in heifer groups I and II and 99% male mortality in group III. Male mortality occurred soon after attachment as previously observed and described in Chapter II. Adult female mortality of A. maculatum was significant for groups I and II. Reductions in repletion weights, egg mass weights, % hatch and EL values were obtained from females feeding on heifers in these groups. Complete control of A. maculatum larvae was attained from group I and marked reductions in larval populations from heifer groups II and III were observed. Also, reductions in nymph populations were posted for all 3 groups when compared to those tick populations feeding on control heifers. The overall efficacy is reflected in the ECC values of 100, 99, and 99% for the 3 experimental groups respectively.

Although the differences in individual tick parameters between treatment and control A. americanum were not as great as observed for A. maculatum, the cumulative effects indicated by the ECC values show

Table 17. Mean effects of 50% famphur, matrix V sustained-release boluses on two 3-host tick species feeding on Hereford heifers (group I) from 7 to 25 days after bolus administration.

	Treatment		Control	
	A. americanum	A. maculatum	A. americanum	A. maculatum
% Adult male mortality	69.00[a]	100.00[a]	0.00	7.00
% Adult female mortality	13.00	61.00[a]	1.00	4.00
Repletion wt. (g)	0.74	0.56[a]	0.82	0.91
Egg mass wt. (g)	0.39	0.21[a]	0.43	0.48
% Hatch	74.28	8.18	80.92	51.10
Oviposition time (days)	12.4	15.3[b]	12.0	7.5
Estimated larvae produced (EL)	6157.3	851.1	7013.8	4950.8
% Larval mortality	39.00	100.00[b]	9.00	0.00
% Larval molt	76.00	------	91.00	100.00
% Nymph mortality	27.00	98.00[a]	1.00	3.00
% Nymphal molt	98.00	100.00	100.00	100.00
Estimated cumulative control (ECC)	70.90	100.00	-------	-------

[a] $P < 0.05$.

[b] $P < 0.01$.

Table 18. Mean effects of 50% famphur, matrix V sustained-release boluses on two 3-host tick species feeding on Hereford heifers (group II) from 26-45 days after bolus administration.

	Treatment		Control	
	A. americanum	A. maculatum	A. americanum	A. maculatum
% Adult male mortality	87.00[b]	100.00[b]	1.00	7.00
% Adult female mortality	24.00	69.00[b]	2.00	3.00
Repletion wt. (g)	0.65	0.99	0.81	1.03
Egg mass wt. (g)	0.33	0.60	0.42	0.61
% Hatch	72.87	37.46	83.32	57.44
Oviposition time (days)	7.9	7.0	8.7	6.4
Estimated larvae produced (EL)	5352.2	4079.1	7177.8	7231.0
% Larval mortality	62.00	70.00[b]	14.00	1.00
% Larval molt	96.00	91.00	89.00	99.00
% Nymph mortality	56.00	68.00	0.00	1.00
% Nymphal molt	92.00	98.00	100.00	100.00
Estimated cumulative control (ECC)	86.20	99.00	-------	-------

[a] $P < 0.1$.

[b] $P < 0.05$.

Table 19. Mean effects of 50% famphur, matrix V sustained-release boluses on two 3-host tick species feeding on Hereford heifers (group III) from 45-60 days after administration.

	Treatment		Control	
	A. americanum	A. maculatum	A. americanum	A. maculatum
% Adult male mortality	48.00	99.00[a]	7.00	0.00
% Adult female mortality	33.00	40.00	6.00	5.00
Repletion wt. (g)	0.83	1.12	0.85	1.08
Egg mass wt. (g)	0.42	0.65	0.44	0.63
% Hatch	76.28	60.08	73.28	64.56
Oviposition time (days)	10.2	6.7	9.5	6.6
Estimated larvae produced (EL)	6776.1	8433.9	6616.3	8552.8
% Larval mortality	54.00	94.00[a]	30.00	8.00
% Larval molt	64.00	73.00	70.00	94.00
% Nymph mortality	20.00	73.00	11.00	13.00
% Nymphal molt	97.00	100.00	96.00	99.00
Estimated cumulative control (ECC)	60.20	99.00	-------	-------

[a] $P < 0.05$.

71, 86, and 60% control of these tick populations when treated with famphur boluses of this formulation. Treated animals provided complete control of the bed bug at each of the 2 feedings per experimental group through the entire study.

Clinical signs of toxicity were not observed among any of the treated animals during the study, even though the dosage rate of 7 mg/kg/day was exceeded. The herd pretreatment red blood cell cholinesterase average of 0.34 ΔpH units was depressed to an average 0.06 ΔpH units by the 25th day where it remained throughout the remainder of the study. Control heifers lost an average 3.4 kg while cannulated treatment heifers lost an average 6.5 kg during the laboratory phase of the study. Both control and cannulated heifers gained their average expected 20% increase in body weight over the course of the study.

Although some refinements are necessary to produce a commercially attractive famphur bolus of this nature, the demonstration that a systemic acaracide can be released continuously at a controlled rate from an inert matrix within bovine for tick control indicates that such a commercially successful achievement is within our grasp. In addition, Miller et al. (1977) recently reported on an experimental demonstration of a plaster of Paris--iron fillings--stretcher matrix composition for a slow-release insect growth regulator bolus effective for 10-12 weeks against horn flies developing in manure further illustrating the interest and potential of this approach to pesticide delivery. The potential of the phenomena of controlled release via bolus preparations for ectoparasite control lends impetus to the search for safer, more effective systemic acaracides and compatible matrixes for their reliable, sustained release.

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VITA 2

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