INVESTIGATIONS INTO THE USE OF CONTINUOUS LOW-LEVEL MEDICATION FOR THE CONTROL OF HELMINTHS IN THE RUMINANT

A thesis submitted for the degree of Doctor of Philosophy

by

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ABSTRACT

The work presented in this thesis is concerned with the chemotherapy of parasite infestations in the ruminant with the anthelmintic thiophanate /diethyl 4,4'-o-phenylene bis (3-thio-allophanate)/. A new concept of administration was investigated, namely continuous low-level anthelmintic medication.

Two aspects were studied which divide conveniently into two sections.

In Section 1, following preliminary trials undertaken to assess the low-level anthelmintic activity of thiophanate, the effects are assessed when this drug is administered continually directly into the rumen of parasitised sheep. Thiophanate was infused at dosages between 3.0 and 5.0 mg per kg bodyweight over various periods of medication against various developmental stages of gastro-intestinal parasites. The daily drug release rate required to inhibit egg hatch and eliminate the worm burden is established. A minimum daily release rate of 3.0 mg per kg bodyweight was shown to be required to completely inhibit egg hatch and 4.5 mg per kg for effective vermicidal activity.

In similar experiments, the anthelmintic activities of levamisole (s-(-)-2,3,5,6-tetrahydro-6-phenylimidazo (2,1-b) thiazole), febantel (N-(2-(2,3-bis-(methoxycarbonyl)-guanidino)-5-(phenyl-thio)-phenyl)-2-methoxy-acetamide) and briefly oxfendazole (5-(phenylsulfinyl) -IH-benzimidazol-2yl) carbamate) were also examined.

In Section 2, the development of an intra-ruminal bolus incorporating thiophanate and designed to release drug at a pre-determined rate over an extended period of time is described. Experiments were carried out in sheep to assess the bolus density required for retention within the reticulo-rumen (monitored by direct bolus recovery), to compare various density factors (iron powder, iron bar core, iron shot and sand) and to assess the average drug release rate from different matrix formulations (based on fatty acids, palmitic acid and paraffin wax) when the boluses were dosed singly or in pairs. The development of a stable "carrier" on which to load a suitable matrix is also described, the majority of the experiments undertaken utilising this "carrier". The effect on the drug release rate of incorporating various "leaching aids" (digestible materials, wetting and soluble agents) into the matrix is examined and preliminary anthelmintic trials undertaken in experimentally infected lambs.

The required drug release is achieved when the boluses are administered in pairs. The anthelmintic activity is confirmed. Possible matrices suitable for use as a single bolus administration were produced.

The advantages of this form of anthelmintic medication over the single therapeutic dose are discussed along with some indications for further studies that emerged.

CONTENTS

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1. GENERAL INTRODUCTION

Ruminants, in particular cattle, are an extremely important link in the world food chain by converting grass and other fibrous plants into animal protein. Their ability to do so efficiently is inhibited by internal parasites, an inevitable factor of the grazing environment. Each infected animal contaminates its food and the food of its flock-mate by passing eggs or larvae in the dung, sheep and cattle being at risk from the time they start to graze. The most important group of internal parasites are the roundworms which comprise many species and the seasonal differences in their peak activity levels makes infection an almost all year round problem.

Recent trends in agricultural practices have increased the hazard of helminthiasis in young animals. Disease occurs when an infection is acquired at an excessive rate, that is, when an animal grazes a pasture that is too heavily infested. The majority of sheep and cattle are infected and in certain circumstances, these infections may kill the host but the effects of roundworms, particularly in the gastro-intestinal tract are often subclinical and go unnoticed as no signs of disease are obvious. However, even a small worm burden reduces the host's ability to convert herbage and prevents the animal from reaching its productivity potential (Sykes & Coop, 1977; 1979; Sykes, 1978; Coop & Angus, 1981). Subclinical, rather than acute disease, is the cause of most economic loss.

The conventional management response to parasitic disease has been to concentrate drug therapy during the period of greatest exposure. Sometimes several repeat doses are given at monthly intervals but more often one or more doses are administered only when disease actually occurs. Therefore by the time therapy is implemented the animals are infected and the parasites have already had an adverse effect. Since conventional anthelmintic treatment often has no preventive or residual effect,

reinfection occurs immediately grazing resumes.

Eradication of most helminth infections is not practical and generally not required in order to control the economically important helminth diseases of livestock. The aim is to ensure that parasite populations do not exceed levels compatible with economic production. Effective control measures must be based on the application of knowledge of the life cycles, larval ecology and epidemiology to husbandry practices designed to prevent or limit contact between parasite and host.

Advances in the knowledge pertaining to the epidemiology of gastro-intestinal parasites have been considerable in the past decade, and efficient prophylactic control programmes incorporating a combination of drug therapy with anthelmintics and management procedures have evolved.

Since the introduction in the early 1960's of the first broad spectrum anthelmintic thiabendazole, followed shortly afterwards by tetramisole, there has been a procession of new, highly effective anthelmintics and their uses are well documented, with several general reviews being published (Gibson, 1975; Armour & Urquhart, 1974; Prichard, 1978; Corwin, Talent & McDowell, 1980; Armour & Bogan, 1982; Cawthorne, 1984).

Despite the introduction of these highly active nematocidal agents, parasitic disease is still prevalent, causing approximately £30 million of damage a year and a possible further £120 million in reduced milk yield and weight gain while more than £20 million a year is being spent on drugs to contain these parasites (Anon, 1984).

Anthelmintics are available as different preparations and administration is therefore possible by various routes. An effective therapeutic dose is best guaranteed when the drug is administered as a drench, paste or by injection. With intensely kept stock, medication

via the feed or drinking water is labour saving, but there are several disadvantages. Unless animals feed or drink individually there is no guarantee that each animal will receive a therapeutic dose and sub-therapeutic doses may promote the development of drug tolerance. Also, since depression of appetite is a common consequence of internal parasitism, the quantity of anthelmintic consumed may be sub-optimal. The labour involved in assembling and handling large numbers of stock is a major consideration which has given rise to the search for drug delivery systems that are easier to administer or need to be given less frequently. An attractive approach would be to administer a drug in a form that would lodge within the rumen and steadily release the active ingredient over the critical period of parasite infestation during a grazing season.

The work presented in this thesis was carried out in order to investigate this concept of drug application by formulating a bolus which would be implanted in the reticulo-rumen of sheep and cattle and used to deliver a predetermined dosage of drug over a known period of time.

The anthelmintic thiophanate* was selected as the active ingredient for incorporation into such a bolus.

*'Nemafax' May & Baker Ltd.

2. PARASITES AND PARASITIC GASTRO-ENTERITIS

Parasitic gastro-enteritis (PGE) occurs in ruminants throughout the world and is caused by species of trichostrongylid nematodes found in the gastro-intestinal tract. Mixed infections are usually present and clinical proportions are reached when tens of thousands of worms are present in the tract.

The main members of this family which may cause PGE in sheep and cattle are Haemonchus, Ostertagia, Nematodirus, Trichostrongylus and Cooperia.

An example of the classification of these nematodes is set out below:

PHYLUM	:	Nemathelminthes
CLASS	:	Nematoda
ORDER	:	Rhabditida
SUBORDER	:	Strongylata
SUPER FAMILY	:	Trichostrongyloidea
FAMILY	:	Trichostrongylidae
GENUS	:	Haemonchus
SPECIES	:	contortus

Trichostrongylidae are bursate nematodes with a direct life cycle generally causing infection by ingestion of third stage larvae.

Fig. 1 is a representation of a typical life cycle in cattle and sheep.

Grazing animals become infected by ingesting infective larvae with the herbage. The larvae develop within the alimentary tract through intermediate stages to egg laying adults. The eggs pass via the host's faeces on to the pasture and develop into the third stage infective larvae (L_3). Nematode control systems are based on the knowledge that in many geographical zones, the number of L_3 fluctuate seasonally. For example, in temperate countries, the L_3 overwinter on

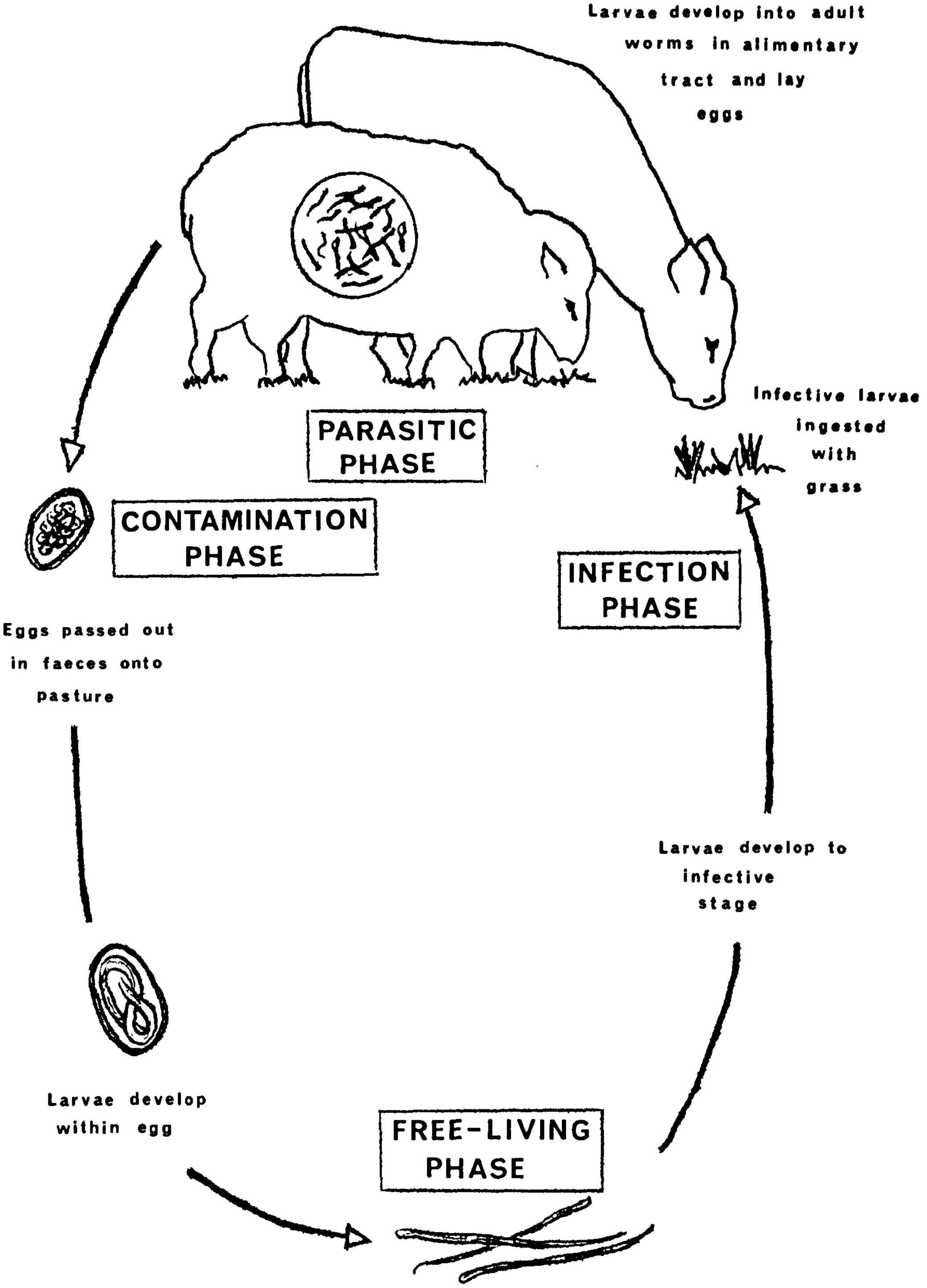


FIG. 1 NEMATODE LIFE CYCLE IN SHEEP AND CATTLE

the herbage and infect grazing animals in early spring; by late spring the overwintered L_3 succumb and from mid-July onwards are replaced by fresh L_3 which develop from eggs deposited in the spring. Many L_3 ingested during the autumn can become inhibited during their development within the host, remaining as fourth stage larvae until development is restimulated.

The epidemiology and control of nematode infections of grazing animals is well documented, several reviews being published (Michel, 1969; 1976; Gordon, 1973; Brunsdon, 1980; Armour, 1980).

With cattle, the major source of infection during the first seasons grazing arises from overwintered pasture larvae which, when ingested, propagate within the animal to produce high levels of contamination during the summer grazing.

With sheep, infection is slightly more complicated. World-wide epidemiological studies have shown that much of the roundworm infestation is in an arrested stage of development within the host during the winter months. At lambing time, these inhibited larvae are stimulated to develop en masse into egg laying adult worms which contaminate the pasture with large numbers of viable eggs. This phenomenon, known as the post-parturient rise, is nature's way of ensuring a supply of infective larvae for the susceptible lamb crop to recycle and thus provide for the parasites' continued survival. Because lambs have little or no immunity to worms, they are very susceptible to their effects. Initially, with the exception of the genus Nematodirus, much of their challenge is from the worm eggs passed by the ewes. This fact has been well researched under various grazing management conditions (Heath & Michel, 1969; Gibson & Everett, 1973; Herd, Streitel, McClure & Parker, 1983) including in-housed sheep (Spedding & Brown, 1956; Field, Brambell & Campbell, 1960; Connan, 1967).

When the larval pick-up has had time to mature within the lambs, the latter start to act as sources of reinfection for themselves. An alternative source of infection may also be provided by overwintered larvae (Gibson & Everett, 1967; Boag & Thomas, 1970) and their effect on lambs has been investigated (Gibson & Everett, 1967; 1975).

The genus Nematodirus is highly seasonal and can be regarded as a lamb to lamb parasite capable of causing severe and sudden losses amongst young stock. Eggs passed by one seasons lambs remain unhatched on the pasture throughout the autumn and winter months and hatch en masse the following spring usually coinciding with the time when the new lambs start to graze (Boag & Thomas, 1975).

The seasonal incidence of PGE in sheep, the species involved and factors affecting acquisition, and the development of differing levels of infection have been well researched (Parnell, 1954; Crofton, 1963; Reid & Armour, 1975). Explanations for the observed seasonal succession of species based on pasture larval populations and ability of some species to overwinter is evident from the work of Rose (1965), Gibson & Everett (1967; 1971), Boag & Thomas (1970; 1971; 1973) and Thomas & Boag (1972). These observations have also been extended into late autumn-early winter (Thomas & Waller, 1979).

The life cycles and pathogenicity of individual nematodes are well documented so only short descriptions are included here. Excellent reviews on their general biology are also readily available (Soulsby, 1965; Dunn, 1978).

a) Haemonchus

Site - Abomasum

Lambs are particularly affected by haemonchiasis. The voracious blood sucking habits of the adults, if present in sufficient numbers, rapidly produce severe anaemia and death. The importance of this species is associated with two characteristic features:-

1 The parasite has a high biotic potential and faecal egg counts are typically many times higher than in most other species.

2. The development of eggs on pasture to the infective stages given a temperature around 22°C and adequate moisture is extremely rapid - 2 to 3 days.

The most significant member of this genus is H.contortus.

b) Ostertagia

Site - Abomasum

Ostertagiasis is an important disease, mainly in cattle, occurring in two forms - Type I during the summer and autumn in grazing stock and Type II in the spring in housed or out-wintered stock.

The emergence of the developing worms from the gastric glands (18days post infection) causes changes in the secretary epithelium which results in an elevation of the pH of the abomasal contents due to a loss of function by the HCl-producing cells and an increased permeability of the mucosa to macromolecules, for example, serum proteins. These combined effects produce diarrhoea and loss of weight.

The important species are O.ostertagi in cattle and O.circumcincta in sheep.

c) Nematodirus

Site - Small Intestine

Nematodiriasis, mainly caused by the species N.battus, is of special importance as a disease of young lambs. The two most important features of this infection are:-

1. The capacity of the infective stage for survival on pasture.
2. The critical hatching requirements which ensure the appearance of a large number of larvae on the pasture simultaneously around May and June.

The entire pathogenic effect is attributable to the larval stages which, after ingestion, cause mass invasion and destruction of the intestinal mucosa. The most acute phase is seen 10 to 12 days after infection when young adults are emerging from the mucosa. There is diarrhoea and dehydration and mortality may be high.

The species represented in the trials outlined in this thesis is N.spathiger which does not require the critical hatching requirements.

d) Trichostrongylus

Site - Abomasum and Small Intestine

This genus is rarely a primary pathogen in the ruminant but is usually a component of general trichostrongylidosis which may typically occur in either spring or autumn. The former outbreaks are associated with the high capacity of Trichostrongylus to survive the winter, while autumn outbreaks are associated with the ingestion of third stage larvae which had been deposited as eggs by the ewes in early summer.

The abomasal dwelling species has a similar effect on the host as Ostertagia spp (i.e. pH change, permeable mucosa), with intestinal trichostrongylosis the effect is obscure.

The main species are T.axei in the abomasum and T.colubriformis and T.vitrinus in the small intestine.

e) Cooperia

Site - Small Intestine

As with Trichostrongylus, Cooperia is never a primary pathogen and is considered to be of very little importance.

The main species present are C.curticei in sheep and C.oncophora and C.punctata in cattle.

3. EXPERIMENTAL STUDIES - MATERIALS AND METHODS

3.1. Experimental animals

Because of their size for ease of handling, housing and feeding, sheep were selected as the ruminant of choice in all the studies undertaken in this thesis.

Suffolk or Finnish crossbred or Dorset Horn purebred lambs, offspring from housed ewes, were bought in parasite free, having never been on pasture, at 8 weeks of age.

The animals used in the experimental studies outlined in this thesis were aged from 17 weeks to yearlings, with liveweights ranging from 25.0 to 80.0 kg. At the laboratories they were maintained parasite free prior to and during each study (unless otherwise stated), being housed on concrete floored pens. A ruminant cereal diet mix* was fed twice daily with hay and drinking water ad lib.

Where used, cannulated sheep were individually housed in metabolism crates, access to feed being as for the floor penned sheep.

To safeguard against cross infection, all metabolism crates and pens were cleaned out regularly and steam cleaned between each new group of sheep.

3.2. Experimental helminth infections

For all the anthelmintic activity studies, 'laboratory strains' of helminths were used. These strains were sensitive to commercial anthelmintics at the distributors recommended dosage rates under the experimental conditions used in this laboratory.

The species used included H. contortus, O. circumcincta, T. colubriformis and N. spathiger as single or mixed infections.

*Milled feed consisting of 73 per cent barley, 20 per cent wheat, 5 per cent molasses and 2 per cent mineral supplement.

3.3. Maintenance of helminth strains

Fresh supplies of infective third stage larvae were maintained by passage through susceptible donor lambs.

Worm-free lambs were infected with third stage larvae of a single species of either 5,000 H.contortus or 15,000 O.circumcincta, T.colubriformis or N.spathiger. At patency (approximately 3 weeks) the lambs were individually housed in metabolism crates and their total faecal output collected daily.

For all species except N.spathiger, the faeces were soaked, mixed with coarse vermiculite for moisture retention and aeration and placed in trays covered with a glass sheet. After incubation at 22°C for 7 days, during which time the cultures were kept moist and mixed to break up any fungal growth, the larvae were recovered by Baermann's apparatus, cleaned, by drying on filter paper and re-Baermannised. The clean larvae were stored in water at 4°C until required.

For N.spathiger infections, the faeces were soaked overnight, broken down and passed through a 100 mesh sieve, the sediment being discarded. After passing the filtrate through a 300 mesh sieve the nematode eggs collected on the sieve were washed off into a large vessel and allowed to sediment. After syphoning off the supernatent the sediment was resuspended in a saturated salt solution. The upper layers containing the suspended nematode eggs were pipetted off and washed in a 300 mesh sieve. After incubation at 25°C for 10 - 14 days in a small quantity of water in a petri dish, the hatched larvae were stored as for the other helminth species.

3.4. Preparation and administration of helminth infections

The infection doses were counted by a dilution technique. Three samples of 0.05 ml aliquots taken from a known volume were microscopically counted from each bottle of larvae used.

Worm-free lambs were orally infected via a plastic syringe with a known number of larvae of one or more species, suspended in water, the dose size being dependent on the helminth species as previously mentioned in Section 3.3.

3.5. Faecal examinations

3.5.1. Egg counts and hatching rates

Individual rectal samples were collected at varying intervals throughout each study where infected animals were used. Helminth egg counts were conducted by the modified McMaster technique (Ministry of Agriculture, 1971) but 2 gms of faeces in 28 mls of water were used instead of the stated 3 gms in 42 mls. These counts were expressed as eggs per gram of faeces (e.p.g.).

Helminth eggs, recovered from each sample by centrifugation in saturated saline on to a microscope cover slip, were incubated in distilled water for 3 days at 22°C for assessment of the hatch rate by microscopic examination of embryonation.

3.5.2. Worm counts

Total worm counts were conducted on the abomasum and small intestine of the infected animals that were slaughtered at the end of a treatment period. The worms were counted by seperately diluting the contents of each abomasum and small intestine to give one litre of suspension from which a one-tenth aliquot was removed. The worms were stained with iodine and counted; if no worms were found in the aliquot, the total suspension was checked. When more than one species was present in a sample, these were counted seperately. All helminths were at the adult stage when the animals were killed.

A proportion of the helminths recovered post mortem were microscopically examined for any reproductive abnormalities.

3.5.3. Drug assay

The detection of excreted drug in the faeces was carried out using a chloroform extraction by the method developed by the author (Appendix 1.). The many variables occurring in this test, for example the speed of growth of Penicillium and the dilution of drug in faeces from the differing weights of faecal matter excreted by individual sheep, did not allow for accurate drug levels to be recorded but served only as a very good indication of drug being passed in the faeces and the regularity of such.

Faecal samples were collected at varying intervals from treated sheep and controls in each study.

Individual samples were obtained from floor penned sheep by manually induced defaecation. If necessary, samples were collected more than once during the same day for bulking together to obtain larger quantities.

Samples from lambs in metabolism crates were collected off the faecal trays which were cleaned daily.

3.6. Administration of drugs

The dose rates and formulations used are indicated in the relevant sections.

Suspensions and solutions were prepared immediately before use, while boluses were formulated in advance.

3.6.1. Infusions

Liquids were administered continually at a predetermined rate, via a peristaltic pump, through polythene tubing connected to a ruminal cannula.

Non-soluble drugs were suspended in tragacanth mucilage, soluble drugs being dissolved in distilled water.

The drug reservoirs were changed daily to monitor any fluctuations in fluid intake so maintaining a known steady dose rate.

3.6.2. Boluses

Boluses were orally administered using a rigid polythene sheep balling gun.

Treated sheep were observed for a short period of time after dosing to ascertain correct swallowing and retention of the bolus.

Bolus densities were determined prior to dosing using water displacement for volume measurement.

Examples of photographs taken of the boluses prior to dosing and at recovery have been included, where appropriate, throughout this thesis as an extra visual, descriptive aid.

3.7. Surgical techniques

3.7.1. Rumen cannula insertion

The sheep were prepared by clipping the wool over an area on the left flank measuring approximately 10 x 10 cms immediately posterior to the last rib and ventral to the lumbar transverse process. The skin was cleansed and approximately 10 mls of local anaesthetic* was injected into the area. A metal trochar and cannula (8 cms long x 0.5 cms diameter) was thrust into the centre of the area at right angles to the skin and to the full depth of the cannula. After withdrawal of the trochar, the position of the cannula in the rumen was confirmed by the back-flow of ruminal fluids. A rigid polythene tube was inserted through the cannula to a depth of approximately 20 cms. Back-flow of rumen fluid again confirmed that the tube end was correctly positioned. The cannula was removed and the polythene tube was secured by sutures to the skin, the open end being connected to the tubing from a peristaltic pump.

*"Lignavet Plus" 2 per cent lignocaine hydrochloride B.Vet.C. C-Vet Ltd.

3.7.2. Rumenotomy procedure

Rumenotomies were carried out on treated sheep at certain time intervals during the medication period to recover the boluses for assessment of retention and drug release. Prior to surgery the sheep were deprived of food overnight and anaesthetised using xylazine* injected intramuscularly at the rate of 0.2 mls per 20 kg bodyweight. With the sheep in a lateral recumbency, the wool was clipped as for the rumen cannula insertion, the exposed area being closely shaved and sterilised by the application of methylated spirit to the skin. Approximately 20 mls of local anaesthetic was infiltrated into the incision site. An incision was made through which the rumen was exposed and exteriorized. The bolus was manually removed through an incision made in the rumen and returned via the same route if a further assessment period was required. The incisions were closed by seperate suture of the rumen, peritoneum, muscle and skin layers. Post-operatively, the sheep received 3 - 5 mls of penicillin**. The skin sutures were removed 10 days later.

3.8. Thiophanate

The anthelmintic thiophanate† (classed as a pro-benzimidazole compound) was the drug used in the experimental work reported in this thesis and is briefly described here.

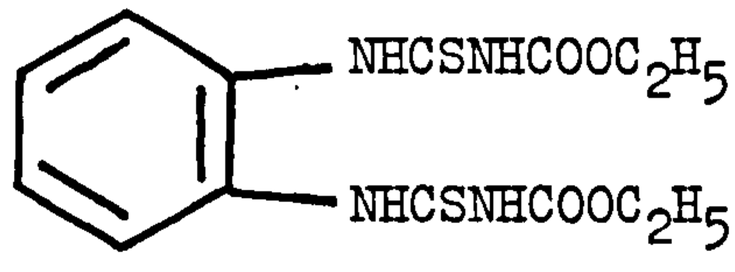
Thiophanate [diethyl 4,4'-o-phenylene bis (3-thioallophanate)] is a stable, pale yellowish-brown crystalline solid, melting with decomposition at 195°C. It is slightly soluble in water, more so in methanol, ethyl acetate and acetone and readily soluble in chloroform.

* "Rompun" 2 per cent xylazine. Bayer Ltd.

** 'Lentrax' Procaine and benzathine penicillin. May & Baker Ltd.

† 'Nemafax' May & Baker Ltd.

It has the structural formula:



Administered orally at dosages of 50 to 100 mg per kg bodyweight thiophanate is a highly efficient anthelmintic against adult and larval stages of the gastro-intestinal nematodes of ruminants (Eichler, 1973; Baines & Colegrave, 1977) and is well tolerated (Eichler, 1974; Baines & Colegrave, 1977). It has also been shown to be ovicidal (Rosa, Niec, Lekovich & Rovere, 1975).

In pigs, a single dose of 50 - 100 mg per kg administered via the feed is highly effective against adult and larval stages of Oesophagostomum spp, Hyostrongylus rubidus and Trichuris suis (Baines, Dalton & Eichler, 1976) and is also ovicidal. It is well tolerated in all types of feed. A dosage of approximately 6 mg thiophanate per kg administered for 14 days via the feed is additionally effective against adult and larval stages of Ascaris suum in the gut (Baines, Evans, Lake & Frape, 1979).

In all the studies reported in this thesis, the pure veterinary grade of thiophanate was used.

SECTION A

ANTHELMINTIC ACTIVITY OF THIOPHANATE WHEN GIVEN AT A LOW-LEVEL

1. INTRODUCTION

Low-level feeding of anthelmintics has been widely used for many years in an attempt to control parasitic gastro-enteritis. From about 1940, low-level dosages of phenothiazine were administered on a self-help basis via a salt-mix (Peterson, Kammlade & Webb, 1944; Harbour, Morgan, Sloan & Rayski, 1946; Page, 1949) and considerable work was undertaken in South Africa with phenothiazine-lick blocks and crumbles (Thomas, 1959; Dent, 1972). Variable results were obtained, however, due to the limited anthelmintic efficacy and potency of the drug itself. Gibson (1950) found that small daily doses were of no value in reducing worm burdens in infected sheep but the inhibition of larval development from the eggs passed in the faeces prevented pasture contamination. The importance of this fact had been previously demonstrated by Harbour, Morgan, Sloan & Rayski (1946) using phenothiazine-salt licks. The use of such a lick was successful in controlling the nematode species Haemonchus but other species were controlled to a lesser extent (Thomas, 1959; Snijders, Stapelberg & Muller, 1964).

Kuttler, Marble & Matthews (1962) and Snijders, Stapelberg & Muller (1964) compared the effectiveness of continuous low-level feeding of phenothiazine with thiabendazole (an anthelmintic with a far broader spectrum) and subsequently the latter has been studied for continuous small dose anthelmintic therapy with greater success (Ross, 1965; 1968; Ciordia, 1969; Elliot, 1970; Snijders, Stapelberg & Muller, 1971). Gibson (1975) reviewed the literature on this subject.

Further trials have indicated that parasitic infections can be controlled with the anthelmintics pyrantel (Cornwell & Jones, 1969),

morantel (Downey, O'Shea & Spillane, 1974; Jones, Pott & Cornwell, 1978) and levamisole (Downey & O'Shea, 1977) when administered at low dosages over various periods of medication. Of the organophosphates, low-level coumaphos has been shown to be effective in cattle when administered for 3, 6 or 49 days (Cox, Mullee & Allen, 1969; Ciordia, 1972) and dichlorvos has also been found to be active when dosed over 29 days (Poeschel & Todd, 1972) or 47 days (Bris, Dyer, Howes, Schooley & Todd, 1968).

The introduction of a series of benzimidazole-carbamate anthelmintics has occasioned additional multiple small dose testing in ruminant animals, the compound fenbendazole being the most widely used (McBeath, Best, Preston & Thompson, 1977; Crowley, Foreyt, Bliss & Todd, 1977; Gaenssler, Wilkins & O'Donnovan, 1978; Thomas, 1978; McBeath, Preston & Thompson, 1979) and is now obtainable incorporated into a feed block* for short-term medication of sheep and cattle.

As an alternative to infeed administration, continuous anthelmintic medication via the drinking water has been shown to suppress the faecal output of trichostrongylid eggs by calves so limiting the parasitic contamination of herbage early in the grazing season (Downey, O'Shea & Spillane, 1974; Downey & O'Shea, 1977; 1981).

Prolonged administration of anthelmintic drugs at low-levels may extend the time during which drug therapeutic plasma levels are maintained and/or drug/parasite contact occurs, thus increasing the anthelmintic efficacy. Activity against the inhibited larval stages is influenced by the length of exposure of the nematode to effective levels of anthelmintic (Prichard, Donald, Dash & Hennessy, 1978). If a drug bypasses the rumen and goes directly into the omasum or abomasum the period of exposure is reduced (McEwan & Oakley, 1978).

*Rumevite Wormablok. Rumenco Ltd.

In contrast, when the drug reaches the abomasum via the rumen, the period of exposure of the nematode to the critical level of drug is prolonged. Prichard, Hennessy & Steel (1978) demonstrated a 92 per cent removal of inhibited O. ostertagi larvae from cattle by prolonged low-level administration of thiabendazole. Similar studies with oxfendazole (Anderson & Laby, 1977) showed that levels between 0.1 and 0.2 mg per kg per day for 8 days resulted in anthelmintic efficacy equivalent to a single dose of 2.5 mg per kg against adult and inhibited O. ostertagi.

This same enhanced effect against inhibited O. ostertagi in cattle has been demonstrated with the anthelmintic thiophanate when administered at a daily dose rate of 20 mg per kg for 5 days (Duncan, Armour, Bairden & Baines, 1979).

Since thiophanate is an ovicidal compound and appears to be effective when adminstered as a split daily dose over an extended medication period it was considered to be an ideal candidate for low dosage administration.

The first section of this thesis briefly describes the preliminary experiments undertaken to assess the low dosage concept using thiophanate (published and unpublished work by the author). Expanding from this, studies were then undertaken to determine the continuous low daily dosage of thiophanate required to prevent the hatching of nematode eggs and to eliminate a worm infestation. Results from preliminary continuous low-level medication trials undertaken with two other selected anthelmintics are also discussed.

2. LOW-LEVEL MEDICATION OF LAMBS

2.1. Establishment of an effective daily dose

These experiments are only briefly summarised as an introduction to the low-level medication with thiophanate since this work has been published by the author (Dalton*, 1978) and is included at the end of this thesis.

2.1.1. In experimentally-infected, housed lambs

The effects of daily doses of thiophanate (1,3 or 5 mg per kg bodyweight) on faecal egg output and egg viability were tested in lambs infected with either T.colubriformis or with H.contortus and N.spathiger.

The results are summarised in Table 1.

Six daily doses of thiophanate at 1 mg per kg bodyweight had little effect on T.colubriformis faecal egg count and no significant effect on the hatching rate, but 3 mg per kg showed activity with a transient although incomplete action on egg viability.

Six daily doses at 5 mg per kg suppressed faecal egg output by H.contortus and N.spathiger both during and after cessation of the course of treatment.

*Now Dobbins

TABLE 1 : The effects of small daily doses of thiophanate on the faecal worm egg output and egg viability of lambs experimentally infected with trichostrongyle nematodes. Experiment 2.1.1.

Group	Nematode species	Number of doses x dose rate (mg/kg)	Number of lambs	Mean percentage* reduction in faecal egg count (egg hatching rate)		
				After 4 treatments	Day of final treatment	28 days after first treatment
1	T.c.	6 x 1.0	2	53 (84)	44 (96)	23 (95)
2	T.c.	6 x 3.0	2	91 (32)	83 (43)	89 (100)
3	T.c. H.c.	6 x 5.0	2	>99 (x) >99 (x)	>99 (x) >99 (x)	>99 (x) >99 (x)

T.c. = Trichostrongylus colubriformis

H.c. = Haemonchus contortus

x = Insufficient number of eggs available

* Compared with pre-treatment on day 0 (Hatching rate = percentage of eggs hatching compared with day 0 (= 100 per cent))

2.1.2. In lambs exposed to a natural infection

The effects of daily doses of thiophanate (50 or 200 mg per head) or phenothiazine (500 mg per head) on the faecal egg output, egg viability, parasite burden, bodyweight and incidence of clinical helminthiasis were tested in groups of 5 lambs (including a control water-only group) grazing together for 14 weeks on pasture contaminated with infective nematode larvae. The effects on the above parameters were also measured from lambs treated with thiophanate at 200 mg per head on alternate days. Phenothiazine was used as the reference standard since much information already exists on its low-level anthelmintic properties.

The results are summarised in Tables 2 and 3.

The mean daily dose of thiophanate required to prevent death from helminthiasis and the excretion of nematode eggs in first year lambs was between 50 and 200 mg per head and greater than 5 mg per kg bodyweight.

A daily dose of 200 mg per head improved productivity when compared with the control group. The phenothiazine and lower thiophanate groups were not noticeably different from the controls.

Daily doses of thiophanate at 50 mg per head or phenothiazine at 500 mg per head did not inhibit faecal egg output to a marked degree. Thiophanate at 200 mg per head per day either inhibited egg output or worm development, or removed the worms before they reached maturity.

Both the thiophanate treatments, but not phenothiazine, reduced or retarded the rate of increase of the final parasitic worm burdens.

Thiophanate at a dose of 200 mg per head (corresponding to a mean intake of approximately 10 mg per kg bodyweight) administered every second day was sufficient to suppress the faecal egg output during

TABLE 2 : The effects of daily, oral low dose treatment with either thiophanate or phenothiazine on first year naturally infected* lambs. Experiment 2.1.2

Treatment	During 14 weeks treatment (4 or 5 lambs/group)**						During 5 weeks after treatment (3 lambs/group)				
	Mean dose rate (mg/kg) (range)		Deaths from helminthiasis	Mean weight gain of survivors at week 14 (per cent) (range)	Mean faecal egg output (e.p.g.) (range)	Mean percentage egg hatch (range)	Mean faecal egg output (e.p.g.) (range)		Mean worm burden (range)	Mean efficiency (per cent) (range)	
	Week 0	Week 14					Week 14	Week 19			
Control	-	-	1	+6 (-28 - +39)	3300 (2650 - 3786)	93 (89 - 97)	4200 (2850 - 4850)	7317 (5500 - 8350)	16453 (9820 - 21110)	-	
Thiophanate (mg/day) 50	4.9 (2.9 - 7.7)	3.4 (2.9 - 4.5)	1	+43 (+14 - +78)	580 (<50 - 1104)	46 (38 - 53)	250 (50 - 350)	950 (550 - 2050)	4700 (2040 - 7340)	72 (55 - 88)	
Thiophanate (mg/day) 200	15.3 (11.1 - 23.5)	8.5 (6.2 - 12.9)	0	+79 (+52 - +90)	<50 (<50 - 50)	x	50 (<50 - 50)	1008 (400 - 1600)	2790 (1520 - 4460)	83 (73 - 91)	
Phenothiazine 500 mg/day	30.8 (27.0 - 100)	23.5 (16.7 - 52.5)	1	+31 (+30 - +71)	1200 (956 - 1342)	19 (6 - 29)	2231 (2000 - 3500)	2250 (1100 - 3050)	17160 (15550 - 19030)	0 (0 - 5.4)	

* Number of larvae per kg herbage varied from 10 in late May to 119 in mid-September

** Dead animals excluded throughout

x= Insufficient number of eggs available

TABLE 3 : <u>The effects of low dose treatment with thiophanate every second day on first year, naturally infected lambs</u>. Experiment 2.1.2.

Group	Number of lambs	Mean faecal egg counts (e.p.g.) (range)			Mean percentage egg hatch (range)		
		Weeks 2 - 9	Week* 9	Week 14	Weeks 2 - 9	Week* 9	Week 14
Thiophanate 200 mg/head alternate days	3	<50 (<50 - 53)	<50 (<50 - 175)	2850 (1650 - 3850)	81 (76 - 88)	85 (79 - 92)	91 (90 - 93)
Control	2	3485 (1650 - 5320)	3700 (2130 - 5270)	4800 (3480 - 6120)	95 (91 - 99)	93 (91 - 95)	95 (92 - 98)

* Removal from pasture

treatment but had no effect on the viability of the few eggs recovered from the faecal samples (Table 3).

2.2. Preliminary self-medication experiment using a mineral-lick "carrier"

Using 3 groups of 17 lambs each, grazing separate (but parasitologically comparable) pastures, the effects on faecal egg output, egg viability, parasite burden, bodyweight, clinical condition and pasture contamination of the following treatments were compared:-

Group A.- Access to unmedicated mineral-lick and monthly treatment with thiophanate (mean dose rate of 75 mg per kg bodyweight).

Group B - Access to mineral-lick containing 3.3 per cent w/w thiophanate.

Group C - Access to unmedicated mineral-lick only.

Pasture larval counts were conducted every 2 weeks and the larvae were differentiated by genera. Lambs were weighed and faecal samples collected at weekly intervals throughout the 20 weeks on pasture. Mineral-licks were also weighed every 2 weeks.

The results are summarised in Table 4.

Daily low-level medication with thiophanate in a mineral-lick "carrier" or routine monthly treatment at a standard dose rate of approximately 75 mg per kg bodyweight prevented deaths due to helminthiasis. Pasture contamination with viable eggs was not prevented to any degree. Both thiophanate treatments improved productivity when compared with the control group. The rate of increase of the final worm burdens was reduced or retarded to a similar degree. This was due mainly to the susceptibility of T.colubriformis but routine monthly treatment was more efficient than low-level treatment against Nematodiru species.

TABLE 4 : The effects of self-medication with thiophanate via a mineral-lick compared with monthly treatment on first year naturally infected lambs. Experiment 2.2.

Group	Deaths due to helminthiasis	Mean percentage weight change of survivors at week 17 (range)	Mean faecal egg count (e.p.g.) (range)	Mean percentage egg hatch (range)	Mean worm burden at week 17 (range) [No. of lambs]	Mean percentage efficiency (range)	Mean worm burdens of predominating parasites (range) [No. of lambs]					
							Week 17			Week 20		
							O.c.	T.c.	N.spp.	O.c.	T.c.	N.spp.
A Monthly treatment (75 mg/kg)	0	+53 (+24 - +100)	213 (<50 - 474)	96 (92 - >99)	13467 (3200 - 29240) [3]	71 (37 - 93)	9063 (2140 - 17248)	18 (0 - 50) [3]	773 (90 - 1170)	-	-	-
B Self-medication with thiophanate*	0	+67 (+52 - +125)	608 (32 - 1124)	77 (26 - 97)	19554 (12460 - 26920) [5]	58 (39 - 91)	10115 (501 - 15783)	232 (0 - 1131) [6]	6462 (3280 - 11029)	10665 (6620 - 12351)	185 (39 - 304) [5]	8347 (641 - 14018)
C Control	4	+14 (-25 - +44)	1137 (121 - 2322)	96 (93.8 - 99.2)	46716 (31480 - 58680) [4]	-	18896 (11732 - 23218)	20122 (12584 - 30905) [4]	7148 (1073 - 16328)	-	-	-

* Mean intake of thiophanate = 1.73 - 6.38 mg per kg bodyweight per day

O.c. = Ostertagia circumcincta

T.c. = Trichostrongylus colubriformis

N.spp. = Nematodirus species

Cessation of treatment did not result in noticeable increases in the worm burdens indicating that treatment did not cause selective removal of adult worms.

The mineral intakes and pasture larval counts are summarised in Table 5.

The mineral-licks were consumed more rapidly in the presence of good grazing. The thiophanate-medicated licks were as palatable as the plain licks. The routine monthly anthelmintic treatment prevented the challenge from reaching the levels seen on the paddocks of Groups B (self-medication) and C (controls).

2.3. Discussion

Treatment with thiophanate at a daily dose of 200 mg per head and ad lib via a mineral-lick "carrier" conferred protection against clinical helminthiasis and promoted weight gains in lambs when compared with control animals. These results were obtained under conditions of set-stocking when the pasture challenge increased considerably to levels which are probably rarely met with in practice (Experiment 2.2.). In this experiment the mean thiophanate intake level was generally low (less than 4 mg per kg per day) when the challenge was high (Table 5). However, in Experiment 2.1.2., the group treated with 50 mg thiophanate per head per day (giving a mean daily intake of a similar order of about 4 mg per kg) failed to make a comparable response during the first 2 months when the challenge was relatively light. It appears, therefore, that a higher treatment level (about 6 mg per kg per day as in Experiment 2.2.) is probably required for susceptible lambs; this can be reduced as resistance to nematodes is acquired.

Prevention of pasture contamination by daily medication of the lambs was only achieved at a daily dose rate of 200 mg thiophanate per head (Experiment 2.1.2.) where detectable faecal egg output was

TABLE 5 : Mineral and thiophanate intakes by lambs, pasture contamination levels and grass availability. Experiment 2.2.

Period	Mean thiophanate intake - Group B (mg/kg/day)	Mean mineral intake (mg/kg/day)			Number larvae*/kg herbage			Grazing
		Group A	Group B	Group C	Group A	Group B	Group C	
2 - 16 May°	5.97	139.3	185.0	168.6	20	20	30	+++
17 - 30 May					30	20	40	+++
31 May - 13 June°	6.38	175.7	197.8	152.1	30	40	60	++±
14 - 28 June	4.98	139.3	154.3	167.1	30	50	60	+++
29 June - 11 July°	3.89	121.4	120.7	140.7	480	1410	1930	++
12 - 25 July	2.21	139.3	68.6	112.1	140	470	570	+
26 July - 8 Aug°	1.73	90.0	53.6	190.0	380	1240	1370	+
9 - 22 Aug	4.52	145.7	140.0	203.6	590	1710	1770	++
23 Aug - 5 Sept	2.23	17.1	69.3	200.0	980	2900	3610	++
Means	3.99	121.0	123.7	166.8				

A = Monthly treatment

B = Self-medication

C = Control

* Ostertagia and Trichostrongylus predominated on all paddocks throughout the experiment. From July onwards, Ostertagia predominated over Trichostrongylus on Group B paddock. Nematodirus was present on all grass samples, but Haemonchus was not found after July. Oesophagostomum and Chabertia occurred occasionally in small numbers.

+++ = Sufficient good quality grass

++ = Grass deficient in quantity and/or quality (many seed heads)

+ = Poor grazing

° = Group A lambs treated on these dates.

completely suppressed. A proportion of eggs failed to hatch in the 50 mg per head per day group as also with the group exposed to medicated mineral-licks. This was, however, probably of little significance in practice in the light of the rapid increase of contamination following faecal egg output in summer. Phenothiazine suppressed pasture contamination with moderate efficiency but did not prevent clinical helminthiasis. This observation agrees with that reported by Gibson (1950).

In Experiment 2.1.2. the faecal egg count increased after treatment with 200 mg thiophanate per head per day had ceased when the lambs were removed from pasture and relatively large worm burdens were recovered. This suggests that except for T.colubriformis, treatment was only partially nematocidal and faecal egg count suppression was due to the reduction in egg laying and/or the selective removal of adult worms. Similar findings in relation to the susceptibility of T.colubriformis were made when lambs in Experiment 2.2. were autopsied. Also, both the paddocks of the medicated mineral-lick and the monthly treatment groups had smaller proportions of Trichostrongylus larvae on the herbage than the control group paddock towards the end of summer.

The mechanism of protection by daily low-level medication with thiophanate is unclear. Heavy burdens were obtained and in Experiment 2.2., the faecal egg count data in the medicated mineral-lick group suggested an early acquisition of burden comparable to that of the controls. The faecal egg count did not, however, rise as high as the controls towards the end of the experiment and the smaller total worm burdens correlated with this pattern. The composition of the burdens may be significant, in that T.colubriformis, not present in large numbers in any thiophanate treated group, did occur in large numbers in the respective control groups.

3. LOW-LEVEL SELF-MEDICATION IN LAMBS

Low-level self-medication of lambs with thiophanate via a mineral-lick "carrier" produced improvements in weight gains and prevention of clinical helminthiasis under conditions of heavy parasitic challenge (Experiment 2.2.). Of the nematode species present, T.colubriformis appeared to be particularly susceptible to low-level treatment under normal challenge conditions. The following study was undertaken to monitor the effects in lambs of mineral-licks incorporating thiophanate at 2 concentrations (5.0 and 3.3 per cent) and monthly treatments with thiophanate drench on pastures where T.colubriformis did, or did not predominate.

3.1. Experimental data

Two permanent pastures (A and B) that had each held grazing ewes and lambs during the previous year were each divided by wire netting fencing into 12 equal sized plots. The plots were "seeded" weekly for 5 weeks with equal amounts of sheep faeces containing H.contortus, O.circumcincta and N.spathiger eggs to supplement the existing challenge. In addition, 6 plots on each pasture were contaminated with T.colubriformis eggs.

Grazing throughout the experiment was adequate for the lambs on Site A; supplementary feeding of hay was provided during the latter months to lambs on Site B.

Groups of 5 weaned, worm free lambs were placed on each plot for a period of 20 weeks (mid-May to mid-October).

The allocation of the treatments on each pasture was as follows:-

Seed infection on plot	Treatments Mineral-lick percentage thiophanate 5.0	3.3	Monthly treatment	Control
H.contortus O.circumcincta N.spathiger	2*	2		2
H.contortus O.circumcincta N.spathiger T.colubriformis		2	2	2

*2 = Number of plots

At fortnightly intervals, each lamb was weighed and individual faecal samples collected for assessment of egg output and viability.

The mineral-licks were weighed every 2 weeks to determine the mineral and thiophanate intakes.

At the end of the medication period and after 3 weeks off pasture and medication, 3 lambs from each treatment group (excluding the monthly treated group) on each site were killed and differential worm counts made of the lumen dwelling nematodes.

3.2. Results

The results obtained are summarised in Tables 6 and 7.

Daily low-level medication with thiophanate in a mineral-lick "carrier" or routine monthly treatment at a standard dose rate of approximately 75 mg per kg bodyweight prevented deaths due to helminthiasis. Pasture contamination with viable eggs was not prevented.

Both percentage thiophanate licks reduced the final worm burdens (Table 7), the 5.0 per cent lick being slightly more effective. The predominant species in the controls (O.circumcincta and T.colubriformis) were greatly reduced, even by the 3.3 per cent lick, on plots where T.colubriformis predominated.

TABLE 6 : The effects of self-medication of thiophanate via mineral-licks on first year naturally infected lambs. Experiment 3.

Measurements	No additional Trichostrongylus on plots			Plus Trichostrongylus on plots		
	3.3 per cent thiophanate lick	5.0 per cent thiophanate lick	Control	3.3 per cent thiophanate lick	Monthly treatment	Control
Deaths due to helminthiasis	0	0	3	0	0	2
Mean percentage weight change of survivors at week 20 (range)	23.7 (-10.0 - +72.2)	31.1 (-3.5 - +126.7)	23.6 (-20 - +60.0)	37.6 (0 - +133.3)	36.1 (-11.4 - +100.0)	22.4 (-16.1 - +106.0)
Mean faecal egg count (e.p.g.) (range)	409.6 (<50 - 3250)	266.6 (<50 - 2550)	1135.1 (<50 - 21300)	335.6 (<50 - 3200)	166.2 (<50 - 1600)	739.2 (<50 - 11350)
Mean percentage egg hatch (range)	83.2 (3.6 - 100.0)	86.3 (0 - 100.0)	95.5 (70.4 - 100.0)	85.9 (0 - 100.0)	93.6 (72.7 - 100.0)	95.9 (80.0 - 100.0)
Mean pasture contamination (larvae/kg herbage)	340.7	230.0	1084.0	288.3	155.6	708.9
Mean residual worm burden (range) [Number of lambs]	1876.0 (300 - 4976) [6]	1191.6 (210 - 3590) [6]	27365.4 (11490 - 43410) [7]	7778.3 (3180 - 19250) [6]		33645.8 (16160 - 60090) [7]
Mean percentage efficiency (range)	93.4 (84.9 - 98.2)	95.6 (89.1 - 99.4)		76.9 (43.4 - 90.6)		
Mean mineral intake (mg/kg/day)	92.9	81.3	120.8	95.5	89.0	118.0
Mean intake of thiophanate (mg/kg/day)	3.07	4.07		3.15		

TABLE 7 : The effects of self-medication of thiophanate via mineral-licks on the worm burdens of first year naturally infected lambs. Experiment 3.

Mean residual worm burden of individual species (number of lambs)	No additional Trichostrongylus on plots			Plus Trichostrongylus on plots	
	3.3 per cent thiophanate lick	5.0 per cent thiophanate lick	Control	3.3 per cent thiophanate lick	Control
Haemonchus contortus		25 (1)	79 (1)	134.5 (2)	113.7 (3)
Ostertagia circumcincta	589.2 (6)	338.4 (5)	7799.3 (7)	195.3 (6)	2227.1 (7)
Trichostrongylus axei	218.2 (4)	166.5 (2)	1081.0 (6)	364.2 (5)	3016.8 (7)
Trichostrongylus colubriformis	682.6 (5)	646.2 (5)	15672.0 (7)	2565.0 (6)	26013.3 (7)
Nematodirus species	515.7 (4)	248.2 (6)	3553.7 (7)	2635.0 (6)	5262.3 (3)
Chabertia ovina	95.0 (2)		48.0 (5)	60.0 (3)	105.0 (2)
Oesophagostomum venulosum	212.5 (4)	67.5 (4)	176.0 (5)	272.0 (5)	110.0 (4)
Trichuris ovis	13.3 (6)	27.5 (4)	38.3 (6)	30.0 (4)	48.3 (6)

Faecal egg counts were kept at a low level by all treatments.

The thiophanate-medicated mineral-licks at both levels were as palatable as the plain mineral-licks.

3.3. Discussion

Treatment with thiophanate available ad lib in a mineral-lick "carrier", confirmed protection against clinical helminthiasis on both sites and promoted weight gains in lambs when compared with control animals using a 5.0 per cent w/w thiophanate lick.

The mean thiophanate intake levels from the 3.3 and 5.0 per cent licks gave comparable results but against T.colubriformis and O.circumcincta (the predominant species at post mortem) the 3.3 per cent lick was less effective.

Some reduction of pasture contamination was achieved by both thiophanate levels. The significance of this should be tested in a follow-up experiment designed to monitor this effect using the same plots and treatments.

It must be remembered in assessing the egg viability that the time of faecal sampling is important. A sample collected later than 72 hours post-treatment will show no effect on egg hatch (personal observation). This may account for the wide ranges in the percentage egg hatch recorded.

A heavy pasture challenge was present and the faecal egg count data suggested that over the first 8 weeks the acquisition of worm burdens by the medicated mineral-lick group was similar to that of the controls. After 8 weeks, the egg count in the controls indicated that the burden rose when compared with that of the medicated lambs. The smaller total worm burdens correlated with this pattern.

The pasture on Site B was of poor quality when the lambs were placed on the paddocks. Mid-summer proved to be very dry compared to

previous years resulting in the "poaching" of the pasture by the lambs, so much so that they were put on supplementary feeding of hay during the last 2 months of the experiment. This fact accounts for the low weight changes achieved by the groups on this site compared with those on Site A.

It was concluded that the results obtained with thiophanate-medicated mineral-licks indicated that this form of low-level medication is effective if an adequate intake can be guaranteed. Since the consumption of a mineral-lick by grazing stock can be influenced by many factors, for example, quality of the grazing, the anthelmintic intake will be variable, some animals not taking the medicated lick, and these will perpetuate the infection on the pastures to the detriment of all animals in the flock/herd.

In addition to the work reported in Experiments 2 and 3, low-level thiophanate also reduces the nematode faecal egg output, egg hatching rate and parasitic worm burdens in treated lambs and ewes. Repeated daily doses of 3.5 to 10 mg per kg for periods varying between 7 and 28 days reduced the output of viable nematode eggs with no marked resurgence after completion of treatment (Baines & Dalton, 1978). Where the worm burden was not eliminated, microscopic examination of the surviving nematodes showed changes in the reproductive system, suggesting that the medication was acting, directly or indirectly, on the nematode reproductive tract and that this was more susceptible to the anthelmintic than other vital functions. A more complete and consistent worm kill was achieved when a dosage of 2.5 mg per kg was administered twice daily. A noticeable reduction in pasture contamination due to the drugs ovicidal effect and a marked reduction in the post-parturient rise has been observed in ewes receiving thiophanate via feed blocks (Baines & Dalton, 1978; Fowler & Fitt, 1978). In cattle, thiophanate incorporated in a liquid feed supplied on a short-term self-help basis has also been shown to be effective in the field (Lovelidge, 1979).

4. CONTINUOUS LOW-LEVEL MEDICATION WITH THE ANTHELMINTIC THIOPHANATE

On the basis of the previous results it was considered feasible that a continual drug input would possibly enhance the effect achieved with low-level daily medication.

To ascertain the daily release rate of thiophanate that would be required to prevent the output of viable eggs and eliminate the worm burden, studies were undertaken in cannulated, parasitised sheep. Drug suspensions of known dose rates were infused directly into the rumen on a continual basis via a peristaltic pump.

A daily dosage of 3 mg thiophanate per kg bodyweight was selected as the guide line in the initial experiments. Thiophanate was suspended in tragacanth mucilage (1.25 gms tragacanth + 2.5 mls chloroform per 100 mls diluted 1 in 3).

4.1. Effect of a daily dose rate of 3 mg per kg on various ages of helminth infections over limited periods of drug exposure.

Lambs were each infected with either H.contortus larvae alone or with a mixed larval suspension containing O.circumcincta, T.colubriformis and N.spathiger.

Thiophanate was infused over periods of between 6 and 31 days. To assess the activity against various developmental stages during the nematode life cycle, the drug was administered over 7 day periods running consecutively throughout the helminth's life cycle, in a group of sheep simultaneously infected at the beginning of the experiment.

As a control for the diluent, one lamb was infused with plain tragacanth mucilage over a period of 23 days starting 4 days after a mixed larval infection had been administered. An infected, untreated control lamb was run concurrently with each infusion study.

The daily flow rate from the peristaltic pump was calibrated on diluted tragacanth mucilage prior to each drug infusion.

The daily liquid volume intake was monitored and corrections made as necessary to maintain a steady drug level. Occasionally the peristaltic pump tubing had to be replaced while running. At such time the fluid intake varied slightly until corrected, causing a wider range in the mean daily dose rates recorded for certain lambs.

Faecal samples were collected from each lamb at regular intervals before, during and after the medication period to assess the nematode egg count and egg viability and to monitor the excreted drug level.

The lambs were killed when the helminth infections had reached maturity and/or after a period off medication. The reduction in worm burden was assessed compared to that of the control. A random sample of any remaining worms found in each lamb was collected for microscopic examination.

A summary of the results obtained from the treated sheep is shown in Table 8.

The daily dose rate of 3 mg thiophanate per kg bodyweight was consistently effective against H.contortus even when medication was administered for only 7 days (an approximate total dosage of 21 mg per kg). Where a faecal egg count was recorded positive prior to medication, a drop in the count to <50 e.p.g. occurred during the first 5 days. A similar reduction in the percentage of eggs hatching was also recorded.

When infused continually prior to infection and through the helminth's life cycle to patency, this dose rate was completely effective against all nematode species on test except O.circumcincta (mean per cent reduction of 83.5). The eggs still being passed in the faeces at patency were non-viable and remained so while the lambs were

TABLE 8 : Effect of 3 mg/kg/day on various ages of helminth infections over limited periods of exposure. Experiment 4.1.

Sheep No.	Mean daily dose rate (mg/kg) infused (range)	Period of drug exposure (days)	Infection	Age of infection at start of infusion* (days)	Faecal egg count	Percentage egg hatch	Anthelmintic efficacy**			
							H.c.	O.c.	T.c.	N.s.
1622	3.4 (3.3 - 3.45)	7	H.c.	Adult	<50 by +4 days	0 after 3 days	100.0			
3650	3.02 (2.82 - 3.59)	11	H.c. O.c.	Adult -5	<50 by +5 days +ve 19 days PI	0 after 2 days Normal		Not killed		
3597	3.15 (2.04 - 5.4)	31	M	-4	+ve 16 days PI -ve N.s. eggs	0 - 7.6 while on infusion	100.0	86.7	100.0	100.0
3606	3.0 (2.81 - 5.25)	31	M	-4	+ve 17 days PI -ve N.s. eggs	0 while on infusion	100.0	80.4	100.0	100.0
1665	2.68 (2.03 - 2.88)	7	H.c. M	Adult -1	<50 by +4 days +ve 20 days PI	0 after 3 days Normal	100.0	81.8	92.1	65.4
3637	2.9 (2.78 - 2.95)	7	H.c. M	Adult -1	<50 by +4 days +ve 22 days PI	0 after 3 days Normal	100.0	85.6	99.4	89.8
3641	2.8 (2.7 - 2.82)	7	H.c. M	Adult +7	<50 by +3 days +ve 20 days PI	34.5 after 2 days Normal	100.0	8.6	57.6	0
3643	3.0 (2.97 - 3.04)	7	H.c. M	Adult +7	<50 by +3 days +ve 24 days PI	0 after 2 days Normal	100.0	9.5	71.2	0
1664	2.9 (2.86 - 2.99)	7	H.c. M	Adult +14	<50 by +3 days +ve 22 days PI	0 after 2 days Normal	100.0	33.7	98.3	86.9
3651	2.7 (2.65 - 2.86)	7	H.c. M	Adult +14	+ve throughout +ve N.s. 17 days PI	0 - 4.0 while on infusion. N.s. eggs non-embryonated	100.0	41.8	92.6	82.7
3622	2.8 (2.5 - 2.98)	9	M	Adult	+ve throughout	0 - 23.1 after 3 days	100.0	75.8	100.0	96.2
3635	3.0 (3.0 - 3.06)	9	M	Adult	+ve throughout	0 - 26.8 after 3 days	100.0	25.6	100.0	99.6

* Where negative figures given indicates infection administered after the start of medication.

** $\frac{\text{Control worm burden - treated worm burden}}{\text{Control worm burden}} \times 100$ per cent

M = Mixed infection

PI = Post infection

H.c. = Haemonchus contortus

O.c. = Ostertagia circumcincta

T.c. = Trichostrongylus colubriformis

N.s. = Nematodirus spathiger

on medication, normal hatching returning by 4 days after the end of the medication period. A daily dosage of 99 mg had been administered over the 31 days which was equivalent to a total dosage of 93 mg per kg bodyweight.

When infused over a period of 7 days from infection (third stage larvae moulting through to fourth stage) the anthelmintic effect was slightly reduced against T.colubriformis (95.7 per cent) and more so against N.spathiger (77.6 per cent) but the same level of efficacy was achieved against O.circumcincta (83.7 per cent). A daily dosage of 85.5 mg was administered over the 7 days but this was only equivalent to a total dosage of 21 mg per kg bodyweight compared to the 93 mg previously. Against the following 7 day developmental stage (fourth stage larvae moulting through to fifth stage) the efficacy was substantially reduced, no effect being observed against O.circumcincta or N.spathiger and only a 64.4 per cent reduction against T.colubriformis. During the final 7 day period before patency (fifth stage larvae moulting through to adults) effective anthelmintic activity was again recorded against T.colubriformis and N.spathiger but not against O.circumcincta (37.7 per cent reduction). A daily dosage of 99 mg administered for 9 days against the adult stages (equivalent to a total dosage of 27 mg per kg bodyweight) was effective against all species but O.circumcincta (50.7 per cent reduction).

When microscopically examined, all the samples of worms collected at random from the post mortem counts appeared normal, no damage to the reproductive tract being apparent. The non-viable eggs were regularly detectable in the sexual organs of the female worms. Blastomers of varying size were conspicuous and the gastrula was atypical.

A nematode faecal egg count from the diluent control lamb was recorded 18 days after infection, the eggs being viable. The level of eggs passed and their hatching rates remained comparable to that of

the control lamb. No effect on the worm burden was recorded at post mortem.

The diameters of the zones of drug activity measured from the plate assay during the first seven days on medication are shown in Table 9, a mean figure of 4 duplicate samples being recorded. Drug was monitored in the faeces of all treated sheep after one to two days on medication. Activity zones were measurable on the plate assays from all samples throughout the treatment period, the presence of drug disappearing by two to three days after the cessation of medication. Faecal samples assayed from the diluent and non-medicated controls were recorded negative throughout.

TABLE 9 : Diameters (cms) of the zones of drug activity measured from the plate assay of faecal samples collected from sheep infused continually with a daily dose rate of 3 mg per kg. Experiment 4.1.

Sheep No.	Day on medication						
	1	2	3	4	5	6	7
1622	1.6	2.5	2.8	2.6	2.5	2.6	3.3
3650		2.2	2.3	2.5	2.4	2.4	
3597		1.9	2.3	2.1	2.4	2.7	2.6
3606		1.8	2.1	3.1	2.4	3.2	3.2
1665	2.9	2.2	2.8	2.8	2.7	2.7	2.3
3637	2.1	1.7	2.8	2.8	2.7	2.2	3.0
3641		1.8	1.5	1.8	1.5	1.8	1.7
3643			2.0	2.0	2.7	2.9	1.8
1664	1.9	1.8	1.6	1.9	2.2	2.1	1.7
3651	1.5	2.7	3.4	2.3	2.9	3.3	2.8
3622	1.6	2.6	2.8	2.1	2.7	3.4	2.7
3635	1.5	1.7	2.9	2.6	2.1	2.1	1.9
Mean	1.87	2.08	2.44	2.38	2.43	2.62	2.45

4.2. Effect of varying the daily dose rate on a mixed immature helminth infection

From the previous experiment (4.1.) it was apparent that a continual daily dose rate of 3 mg thiophanate per kg bodyweight was insufficient to completely control a mixed helminth infection. The species most difficult to eradicate appeared to be O.circumcincta so all but one of the lambs in this experiment were infected with this species only.

Following the same procedure as outlined in 4.1., the lambs were treated with thiophanate at a daily dose rate of either 4.0, 4.5 or 5.0 mg per kg bodyweight. The same parameters were monitored as previously.

The results obtained from each treated lamb are summarised in Table 10.

Variable anthelmintic activity was recorded when a daily dose rate of 4.0 mg per kg was administered one to two days prior to infection and continually through to nematode maturity. (A range of 32.0 to 100.0 per cent worm reduction). A daily dosage of 144 to 204 mg thiophanate had been administered for 24 to 29 days which was equivalent to a total dosage per kg bodyweight of 116 to 96 mg.

Both daily dose rates of 4.5 and 5.0 mg per kg infused continually over a period of 23 and 25 days were completely effective against an infection administered after one or two days on medication. A decline in efficacy was recorded when the drug was administered 8 days after infection (fourth stage larvae present) at the dose rate of 4.5 mg per kg (89 per cent worm reduction). A daily dosage of 189 mg thiophanate had been administered for 13 days equivalent to a total dosage of 58.5 mg per kg bodyweight. This compared with the results obtained in the previous experiment using a daily dosage of 3 mg per kg.

TABLE 10 : Effect of varying the daily dose rate of thiophanate on a mixed helminth infection. Experiment 4.2.

Sheep No.	Daily dose rate (mg/kg)	Mean daily dose rate (mg/kg) infused (range)	Period of drug exposure (days)	Age of infection at start of infusion* (days)	Faecal egg count	Percentage egg hatch	Anthelmintic efficacy*** O.c.
3617	4.0	3.9 (3.4 - 4.5)	24	-2	-ve throughout		99.5
3650		3.99 (3.82 - 4.57)	24	-2	-ve throughout		100.0
1681		3.97 (3.88 - 4.03)	24	-1	+ve 16 days post infection	0 while on infusion	Not killed
16		3.89 (3.43 - 4.3)	29	-1	+ve 19 days post infection	0 while on infusion	32.0
1696	4.5	4.4 (3.93 - 4.97)	29	-1	-ve throughout		99.0
25		4.51 (4.16 - 5.63)	23	0			99.6
7		4.45 (4.31 - 4.69)	23	0**			99.3 (100.0 for H.c., T.c. and N.s.)
1678		4.45 (4.38 - 4.59)	13	8			89.0
3660	5.0	4.93 (4.37 - 5.5)	25	-2	-ve throughout		99.3
3640		4.87 (4.33 - 6.0)	25	-2			96.4
6		4.97 (4.81 - 5.11)	24	-2			100.0

* Where negative figures given indicates infection administered after the start of medication

** Mixed infection

*** $\frac{\text{Control worm burden - treated worm burden}}{\text{Control worm burden}} \times 100$ per cent

O.c. = Ostertagia circumcincta

H.c. = Haemonchus contortus

T.c. = Trichostrongylus colubriformis

N.s. = Nematodirus spathiger

Any nematode eggs passed in the faeces were non-viable while the lambs were receiving medication. All the samples of worms collected at random appeared normal in structure when microscopically examined but the majority were devoid of eggs or contained damaged eggs, confirming the previous observation.

The diameters of the zones of drug activity measured from the plate assays of samples taken during the first 26 days on medication are shown in Table 11, a mean figure of 4 duplicate samples being recorded. Drug was monitored in the faeces of all sheep on medication as previously recorded.

Fig. 2 illustrates the mean diameter of the zones of drug activity measured for each group of sheep at each dose rate. The zone size remained fairly steady throughout the medication period after the initial three to four days, an accumulative drug effect not being noticeable.

TABLE 11 : Diameters (cms) of the zones of drug activity measured from the plate assay of faecal samples collected from sheep infused continually with a daily dose rate of either 4.0, 4.5 or 5.0 mg/kg. Experiment 4.2.

Daily dose rate (mg/kg)	Sheep No.	Day on medication																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
4.0	3617		2.9	3.3	3.6	3.7	3.8	3.9	3.8	3.0	3.4	4.0	3.7	3.3	3.2	3.4	3.3	3.4	4.1	3.4	4.2	4.2	3.9	3.4	3.4		
	3650		2.6	3.0	3.2	3.7	3.5	4.1	3.5	3.7	3.7	3.7	3.9	4.2	3.9	3.7	3.9	3.7	3.3	3.7	4.2	4.5	3.8	3.9	3.7		
	1681		1.7	1.9	2.3	2.5	2.4	3.2	3.2	3.0	2.9	2.8	2.5	3.9	4.0	3.4	3.6	3.3	3.1	2.1	2.6	3.1	2.6	2.9	2.5		
	16			2.6	2.6	2.9	3.0	3.2	4.3	3.8	3.6	3.9	3.6	3.1	3.0	2.0	3.1	3.0	3.9	4.1	2.9	3.2	3.3	3.3	3.3	3.0	3.1
	Mean		2.4	2.7	2.92	3.2	3.17	3.6	3.7	3.37	3.4	3.6	3.42	3.62	3.52	3.12	3.47	3.35	3.6	3.32	3.47	3.75	3.4	3.37	3.27	3.0	3.1
4.5	1696			2.9	3.3	3.7	3.4	3.8	3.2	3.5	3.0	3.6	3.6	3.6	3.8	2.9	3.2	2.9	3.0	3.2	3.0	3.6	2.7	3.0	3.9	3.1	3.6
	25	2.8	3.3	3.1	3.1	3.3	3.7	3.8	2.6	3.0	3.0	3.3	4.0	4.1	3.9	3.4	3.5	3.1	3.2	3.3	3.9	3.6	3.8	3.2			
	7	1.9	3.2	3.2	3.5	3.1	3.5	4.3	3.5	3.9	3.9	3.3	3.9	4.0	3.7	3.5	3.4	3.5	3.9	3.4	3.8	4.0	3.5	3.9			
	Mean	2.35	3.25	3.07	3.3	3.37	3.53	3.97	3.1	3.47	3.3	3.4	3.83	3.9	3.8	3.27	3.37	3.17	3.37	3.3	3.57	3.73	3.33	3.37	3.9	3.1	3.6
5.0	3660		2.6	2.3	2.7	3.4	3.3	3.8	3.3	3.3	3.3	4.3	4.3	4.2	4.3	5.1	4.3	5.5	4.5	4.8	5.3	5.3	4.3	4.6	4.4	4.7	
	3640		4.1	4.2	4.5	4.1	4.1	4.1	4.3	5.2	5.4	4.2	4.7	5.0	5.3	5.4	5.2	4.5	4.2	4.5	4.4	4.3	5.3	5.2	5.1	5.0	
	6	1.5	3.4	3.0	2.9	4.2	4.3	4.1	4.9	4.5	4.9	4.9	4.9	5.2	5.2	5.3	4.8	4.6	4.5	4.3	4.5	4.7	4.3	4.1	3.7		
	Mean	1.5	3.37	3.17	3.37	3.9	3.9	4.0	4.17	4.33	4.53	4.47	4.63	4.8	4.93	5.27	4.77	4.87	4.4	4.53	4.73	4.77	4.63	4.63	4.4	4.85	

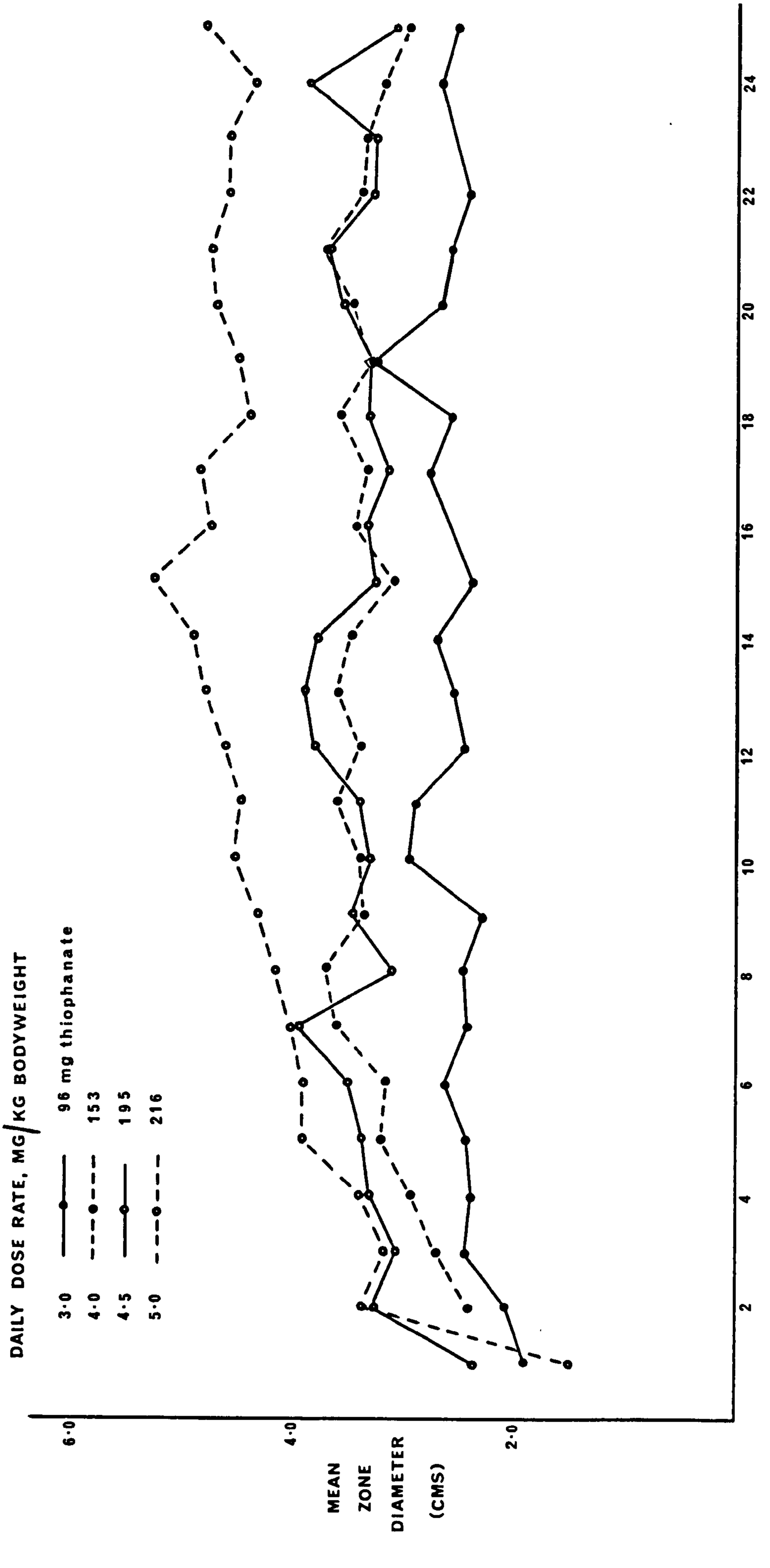


FIG. 2 EXCRETED DRUG ACTIVITY ZONES MEASURED FROM SHEEP ON CONTINUOUS MEDICATION

5. PRELIMINARY INVESTIGATIONS ON THE LOW-LEVEL ACTIVITY OF ALTERNATIVE ANTHELMINTICS

When administered continually via a rumen cannula, thiophanate was effective against a mixed helminth infection at a low daily dosage equivalent to 10 per cent of the single therapeutic dose rate.

As a comparison of efficacy, 2 alternative anthelmintics, one from a different family of compounds, were selected - levamisole, a non-benzimidazole, and febantel, a pro-benzimidazole. Both these drugs were infused continually for varying periods in parasitised sheep at different dose rates and time intervals after infection.

The peristaltic pump flow rate was calibrated on the appropriate diluent prior to each drug infusion.

The lambs were each infected with a mixed suspension of third stage larvae of H.contortus, O.circumcincta and N.spathiger.

Faecal samples were collected from individual lambs on test at intervals before, during and after the infusion periods for assessment of nematode egg counts and egg viability.

All lambs were killed when the infections were mature and/or after a few days off medication. The reduction in worm burden was assessed by comparison with an infected, untreated control lamb run alongside each drug infusion. A random sample of any remaining worms was collected for microscopic examination.

5.1. Levamisole*

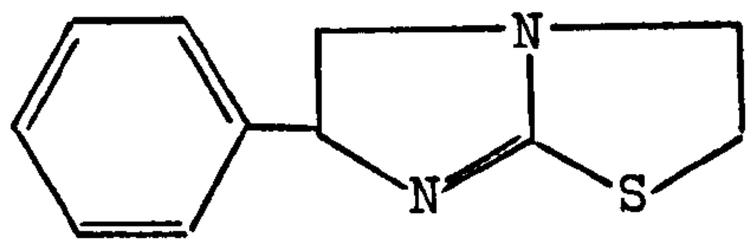
Levamisole (classified as an imidazothiazole compound) is a highly effective soluble anthelmintic, active against immature and adult stages of all the major gastro-intestinal nematodes (including benzimidazole resistant strains) and lungworms at a single dose rate of 7.5 mg per kg bodyweight administered either sub-cutaneously or orally.

*"Nemicide" 7.5% w/v levamisole hydrochloride. I.C.I. Ltd.

It has the chemical formula:

s-(-)-2,3,5,6-tetrahydro-6-phenylimidazo (2,1-b) thiazole

with the structure:



In this study, levamisole was continually infused daily in solution at dose rates of 0.25 to 1.0 mg per kg bodyweight.

The peristaltic pump flow was calibrated on water prior to each drug infusion.

By acting on the adult parasites' nervous system levamisole exhibits no ovicidal activity (Prichard, 1978), so its effect on egg viability was not assessed.

The results obtained for each treated lamb are summarised in Table 12.

A daily dose rate of 1.0 mg per kg bodyweight (13.3 per cent of the single therapeutic dose rate) was required to achieve effective anthelmintic control, the drug being infused against an adult infection over a period of 13 days (a total dosage of approximately 13 mg per kg). The efficacy was lowered when the drug was administered 15 days after infection (fifth stage larvae present). As observed with thiophanate, O.circumcincta appeared to be the most resistant species against drug treatment.

The worms recovered at post mortem appeared as normal when microscopically examined, no damage to the reproductive tracts was apparent. The eggs in situ also appeared normal.

TABLE 12 : Summary of the results obtained from a continual infusion of levamisole in parasitised sheep. Experiment 5.1.

Sheep No.	Daily dose rate (mg/kg)	Mean daily dose rate (mg/kg) infused (range)	Period of drug exposure (days)	Age of infection at start of infusion (days)	Faecal egg count	Anthelmintic efficacy* (per cent)		
						H.c.	O.c.	N.s.
Y22	0.25	0.25 (0.24 - 0.27)	21	0	+ve 16 days post infection	71.9	21.4	10.3
Y46	0.5	0.51 (0.48 - 0.59)	21	0	+ve 16 days post infection	56.2	30.5	17.8
Y20		0.49 (0.37 - 0.52)	10	14	+ve 16 days post infection	100.0	68.1	87.5
Y30	0.75	0.74 (0.67 - 0.78)	10	14	+ve 21 days (15 days for N.s. eggs) post infection	100.0	4.0	97.0
68		0.75 (0.73 - 0.76)	13	15	+ve 16 days post infection	97.5	44.5	98.1
67		0.77 (0.74 - 0.79)	13	19	+ve throughout	87.5	32.2	98.5
1672	1.0	1.01 (0.99 - 1.04)	13	8	-ve		100.0	
58		1.01 (0.93 - 1.11)	13	15	+ve 20 days post infection	87.5	82.5	99.7
64		1.01 (0.98 - 1.06)	13	19	+ve throughout	97.9	95.9	100.0

* $\frac{\text{Control worm burden - treated worm burden}}{\text{Control worm burden}} \times 100$

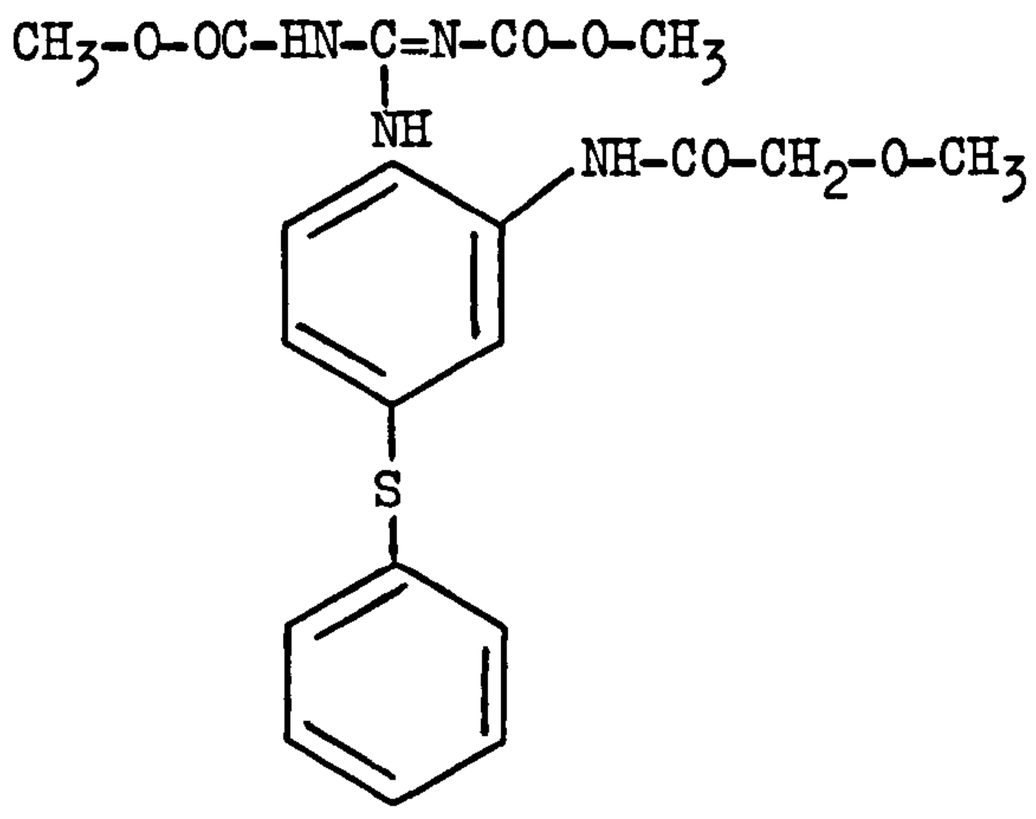
H.c. = Haemonchus contortus

O.c. = Ostertagia circumcincta

N.s. = Nematodirus spathiger

5.2. Febantel*

Febantel (classified as a pro-benzimidazole) is a guanidine derivative with the chemical formula: N-[2-(2,3-bis-(methoxy-carbonyl)-guanidino)-5-(phenyl-thio)-phenyl]-2-methoxy-acetamide and structure:



It has a wide spectrum of efficiency against gastro-intestinal nematodes of ruminants and other domestic animals at a single dose rate of 5 - 10 mg per kg bodyweight administered orally.

In this study, febantel was continually infused in suspension at dose rates of 0.25 to 0.5 mg per kg bodyweight per day.

The peristaltic pump flow was calibrated on diluted tragacanth mucilage prior to each drug infusion.

The results obtained for each lamb are summarised in Table 13.

A daily dose rate of 0.3 mg per kg bodyweight (6 per cent of the single therapeutic dose rate) was required to achieve complete anthelmintic control, the drug being infused for 13 days, beginning 7 days after infection (a total dosage of approximately 3.9 mg per kg). At a dose rate of 0.25 mg per kg, febantel was completely effective against an adult infection but lower activity was achieved against a fourth stage larval infection. N.spathiger appeared to be the most resistant species against febantel treatment.

* "Bayverm" 2.5% suspension febantel. Bayer U.K.Ltd.

TABLE 13 : Summary of the results obtained from continual infusion of febantel in parasitised sheep. Experiment 5.2.

Sheep No.	Daily dose rate (mg/kg)	Mean daily dose rate (mg/kg) infused (range)	Period of drug exposure (days)	Age of infection at start of infusion (days)	Faecal egg count	Anthelmintic efficacy* (per cent)		
						H.c.	O.c.	N.s.
Y40	0.5	0.498 (0.46 - 0.52)	17	8	-ve	100.0	100.0	87.5
Y38		0.51 (0.5 - 0.52)	8	23	<50 after 2 days (5 days for N.s. eggs)	100.0	100.0	100.0
Y35	0.4	0.4 (0.39 - 0.41)	19	0	-ve	100.0	100.0	99.8
Y31		0.41 (0.39 - 0.44)	13	7	-ve	100.0	100.0	100.0
Y45	0.3	0.31 (0.28 - 0.34)	13	7	-ve	100.0	100.0	100.0
Y24	0.25	0.251 (0.246 - 0.274)	19	0	-ve	100.0	100.0	84.2
Y28		0.254 (0.247 - 0.262)	17	8	+ve 21 days post infection (18 days for N.s. eggs)	100.0	91.5	51.3
Y25		0.252 (0.248 - 0.261)	8	23	<50 after 6 days	100.0	100.0	100.0

* $\frac{\text{Control worm burden - treated worm burden}}{\text{Control worm burden}} \times 100$

H.c. = Haemonchus contortus

O.c. = Ostertagia circumcincta

N.s. = Nematodirus spathiger

When examined microscopically, the worms recovered at *post mortem* appeared as normal. A few worms were devoid of eggs and a small percentage of the eggs *in situ* were damaged.

6. DISCUSSION ON INFUSION STUDIES

The two lambs not slaughtered in Experiment 4 were treated with a single therapeutic dose of thiophanate (50 mg per kg bodyweight) to check the susceptability of the infection after continual drug exposure. This single treatment was completely effective in both animals.

The results obtained from continual low-level medication with thiophanate suggest that of the nematode species involved, H.contortus was most sensitive to this form of medication and O.circumcincta the least sensitive. The same susceptability to low-level medication by these nematodes was shown by Ross (1968) using thiabendazole. Also, the adult worms appeared to be the most susceptible to extended drug exposure while the fourth stage larvae were the least. At this stage, the worms are found in the gastric glands. The epithelium lining these glands may act as an effective barrier protecting the larvae from the drug which would not readily penetrate from the gut lumen. The presence of drug already in the body system at the time a helminth infection was acquired appeared to make no difference to the development of the nematodes.

The anthelmintic effect of thiophanate was enhanced by this method of administration when compared to the equivalent results obtained from a single daily dose (Dalton, 1978). Damage to the reproductive tract in the surviving worms recovered at post mortem was not apparent in these experiments on a superficial microscopic examination, but since the nematode species H.contortus was continually eliminated, the effect could not be compared with that achieved by the intermittent dosing (Baines & Dalton, 1978).

Within each dose rate group, although the same daily dosages of thiophanate were administered, the diameters of the zones of activity measured from excreted drug in the faeces varied between animals. There

was no noticeable difference in the zone sizes obtained from sheep receiving a daily dose rate of either 4.0 or 4.5 mg per kg (Table 11). These results emphasised the fact that amounts and consistency of faecal matter excreted alters considerably between sheep and the plate assay method could only accurately be used as confirmation of a steady drug throughput.

From the results obtained using continuous medication with levamisole, a daily dose rate of greater than 1.0 mg per kg bodyweight would be required for effective helminth control (13.3 per cent of a single therapeutic dose rate). These results were comparable to those reported by Hass, Holloway & Brown (1982) who showed that, in sheep, levamisole did not effectively control established clinical parasitic infections at daily oral dose levels equal to or less than 1.0 mg per kg of bodyweight for 30 days and/or a feeding level equal to or less than 10 mg per kg of feed. Levamisole is considered to be a ganglion stimulant in helminths resulting in paralysis (Coles, 1977). Bogan, Marriner & Galbraith (1982) suggested that as such it would be expected that the maximum concentration achieved rather than the duration of concentration would be more relevant to activity. This fact may account for the higher dose rate required when administered on a low daily dosage basis.

From the results obtained in Experiment 5.2., a daily dose rate of 0.3 mg febantel per kg bodyweight was required for effective vermicidal activity (6 per cent of the single therapeutic dose rate). Although febantel is a compound which exhibits ovicidal activity when administered as a single therapeutic dose, in this experiment, at the very low dose levels tested, the ovicidal activity was not apparent. There are no published low-level activity reports available on febantel with which to compare the results reported here.

In addition to the experiments undertaken with levamisole and febantel, two lambs were infused with the benzimidazole compound oxfendazole* at a daily dose rate of 0.25 mg or 0.15 mg per kg bodyweight (5 and 3 per cent of the single therapeutic dose rate). Medication began 7 days after a mixed infection was administered and continued for 13 to 17 days. Both drug levels were completely vermicidal under the conditions used in these studies. These preliminary results were comparable to those reported by Hass, Holloway & Brown (1982) who showed that 0.25 mg per kg was effective in the control of clinical parasitism when administered in the feed for 30 days. Prolonged administration of oxfendazole by intra-ruminal controlled release capsules has been reported to be effective against both susceptible and benzimidazole-resistant strains of parasite at dose rates ranging from 2.9 down to 0.41 mg per kg against the resistant strains and 1.6 down to 0.09 mg per kg against susceptible strains (Le Jambre, Prichard, Hennessy & Laby, 1981). Anderson, Laby, Prichard & Hennessy (1980) have also shown oxfendazole to be highly effective against non-resistant nematodes when administered continually at daily dose rates approximately one-tenth of the single oral dose.

Jones, Pott & Cornwell (1978) achieved a good control of parasitic infections with the anthelmintic morantel when administered at a daily dose level approximately one-seventh of the normal therapeutic dose for gastro-intestinal nematode infections. A similar ratio was demonstrated by Ross (1965) working with thiabendazole.

Of the three main anthelmintics studied in this thesis, febantel appeared to be the most effective when administered as a continual low daily dosage but from the preliminary experimental work undertaken with oxfendazole, it was apparent that this drug could be the most potent at low-level dosages and as previously reported has the

* "Systamex" 2.26% suspension oxfendazole. The Wellcome Foundation Ltd.

added advantage of being ovicidal at these low concentrations (Anderson, Laby, Prichard & Hennessy, 1980).

From the results obtained with thiophanate in this section it was concluded that a minimum daily release rate of 3 mg per kg bodyweight would be required to completely inhibit egg hatch and a daily dose rate of 4.5 mg per kg for effective vermicidal activity. That is assuming an average sheep weight of 40 kg, a daily drug input of 120 to 180 mg of thiophanate.

SECTION B

CONTINUOUS LOW-LEVEL ANTHELMINTIC MEDICATION

BY A RUMINAL BOLUS

1. INTRODUCTION

When lambs and calves consume a diet containing roughage they develop, by about 6 weeks of age, a functional reticulo-rumen with a typical pattern of motility and an extensive microbial population.

The rumen, the largest of the four stomach compartments, serves as an important location for the metabolic breakdown of ingested foodstuffs through the action of the micro-organisms present therein. Numerous internal folds within its walls construct various chambers through which the ingesta are circulated by means of muscular contractions. Solid matter is retained until it is reduced to a size that will pass readily through the reticulo-omasal orifice and omasum to the lower gut. Breakdown of solids is achieved by a combination of chewing during eating and rumination, microbial attack and attrition while being churned by the rumen contractions. The more digestible fibrous particles break down quickly and are retained for only a few hours; the more refractory ones may be retained for days. Any dense particles ingested with the food gravitate to the rumen floor and are gradually propelled forward into the reticulum where they will lodge if dense enough. It follows, therefore, that ruminants receiving anthelmintic treatment to control a naturally acquired parasite infestation will have an established ruminant digestive system consisting of a compartmented stomach suitable for the retention of heavy objects.

One of the first applications based on this theory was the development of a cobalt bolus weighing only 5 gms but with a density of 4.1 (Dewey, Lee & Marston, 1958). These dense pellets were retained for many months or even years by virtue of their high specific gravity.

Heavy pellets made from finely divided iron and elemental selenium were also developed with densities ranging from 4.4 to 5.7 (Kuchel & Buckley, 1969).

A magnesium alloy bullet* is commercially produced and marketed for sheep and cattle and user trials have been well publicised. Both types of bullet have a density of approximately 3.0 and weigh 30 gms and 85 gms for the sheep and cattle bullet respectively. Their effective life, however, is estimated at only about 4 weeks in cattle and 3 weeks in sheep. Retention is variable, a loss of twenty per cent has been recorded from sheep (Wilson, Maguire & Poole, 1962) with sitings of bullets on pasture and in dung pats (Kemp & Todd, 1970).

Successful retention has been achieved in cattle with famphur (an organophosphate systemic insecticide) boluses with a density of only 1.62 but weighing around 25 gms (Teel, Hair & Stratton, 1979; Hair, Gladney, Davey, Drummond & Teel, 1979).

More recently, boluses with a density of only 1.9 but weighing 40 - 45 gms have been experimented with in cattle (Byford, Riner & Hair, 1980; Riner, Byford & Hair, 1981) but as erosion or breakage occurred, lowering the weight to less than 10 gms, the boluses were lost.

Bolus location within the reticulo-rumen in relation to density and the rate of erosion has been studied by Riner, Byford, Stratton & Hair (1982) who showed that, in cattle, a minimum density of 1.8 was required to prevent regurgitation of particles from the rumino-reticulum and a density of 2.0 or greater was necessary for retention within the reticulum. Higher release rates were also obtained from boluses retained in the reticulum than from those in the rumen.

As well as incorporating a dense material, experiments have also been undertaken with ruminal boluses utilizing a shape considered

*"Rumbul" Magnesium Bullets. Agrimin Ltd.

to be impossible for a ruminant to regurgitate. Anderson & Laby (1979) have reported trials using three syringe barrels held together by water disruptable paper tapes, which, after administration, burst, allowing the capsule to revert to an open tripod configuration so making it too large for regurgitation.

Stuedemann, Wilkinson & Lowrey (1984) have recently reported trials with a new form of magnesium bolus, 17.3 cms long and 3.7 cm in diameter, designed to unfold lengthwise after entry into the rumen. The 2 parts are held together by a rubber hinge.

Much interest has been generated in the field of slow release medication but the majority of the trials have been conducted in cattle. The work reported in the second section of this thesis was undertaken to formulate a ruminal bolus, primarily for sheep, that would be dense enough to lodge in the reticulo-rumen and incorporating the anthelmintic thiophanate. The objective was to achieve a release of drug over an extended period of time at a level calculated from the previous experiments in Section A to prevent pasture contamination by the faecal output of non-viable nematode eggs, to prevent the establishment of an infection and to eliminate an existing worm burden. Since thiophanate is an insoluble drug, its release from a matrix would need to be achieved by bolus erosion through either continuous rubbing by ingested hay, straw etc., breakdown by endogenous celluloses or the use of water soluble leaching agents.

2. RUMEN BYPASS METHOD - PETER MÖLLER

In preparing nutrients and medicaments intended for administration to ruminants it is important to protect the active ingredients against the environmental conditions of the rumen, i.e. microbial degradation and the effects of a pH of approximately 5 - 7.

A Norwegian company, Peter Möller A/S, of which ruminant nutrition is one of their major fields, hold a patent on a matrix designed to protect active ingredients and allow an unaffected passage through the rumen rendering the ingredients fully available for digestion in the lower parts of the alimentary tract.

The company's basic idea was to use protective materials which were in themselves end products of the ruminal fermentation process so were natural to the animals and their environment and consequently readily acceptable to health authorities as well as those involved in husbandry.

The components for the Peter Möller matrix are selected blends of long-chain fatty acids, a breakdown ingredient from the ruminant diet, which are not altered in composition to any great extent. The protective coating, therefore, consists of easily digestable nutrients of particularly high energy content.

A slow release bolus must be designed to retain an active ingredient within the rumen for an extended period of time and protection against the enzymatic activity of the rumen flora is a necessary requirement. The Peter Möller matrix appears to afford this protection and in collaboration with the company, initial experiments were designed around this matrix to formulate such a bolus.

The boluses tested in the trials described in Experiments 3, 5 and 6 were made by Mr. B. Campbell-Kelly of Peter Möller A/S, while all others were produced by the author in the laboratories at Ongar Research Station, May & Baker Ltd.

3. EXPERIMENTS TO INVESTIGATE TECHNIQUES FOR BOLUS RETENTION WITHIN THE RETICULO-RUMEN USING THE PETER MÖLLER MATRIX

From the work conducted with trace element bullets, as mentioned in the introduction to this section, it is apparent that only dense objects will be retained within the rumen for any length of time.

The Peter Möller matrix incorporating thiophanate in the ratio 50 : 50, only has a density of approximately 1.0.

A series of experiments were therefore designed to test various techniques aimed at retention, by increasing the density and maintaining the structure of a bolus within the reticulo-rumen.

All boluses contained 50 per cent thiophanate unless stated otherwise.

3.1. Preliminary helminth activity and bolus retention

14 lambs were each infected with H. contortus larvae and 23 days later dosed with one of the following formulations designed using weight or shape as the retention factor.

Two lambs were treated per group:-

Group 1 - 10 gm bolus containing steel ball bearings

Group 2 - 10 gm bolus (25 per cent thiophanate) containing 25 per cent barium sulphate (dense, X-ray opaque medium)

Group 3 - Two 5 gm boluses containing steel ball bearings joined together by a 15 cm length of nylon thread

Group 4 - 10 gm bolus containing steel ball bearings with 2 nylon anchor threads attached

Group 5 - Plain 10 gm bolus as control

Group 6 - 10 gm bolus containing an added softener

(5 per cent oleic acid)

Group 7 - Untreated, infected controls

Examples of these formulations are illustrated in Plate 1.

Bolus retention of groups 1 - 4 (those containing an X-ray opaque medium) was assessed by X-rays* taken 4 hours following treatment, then twice weekly until the boluses were lost or had disintegrated.

Faecal samples were collected at intervals following treatment for assessment of anthelmintic activity by faecal egg count and egg viability and drug release by the faecal plate assay. Three days faecal samples were pooled together for each sample assayed.

The X-ray results are summarised in Table 14 below:-

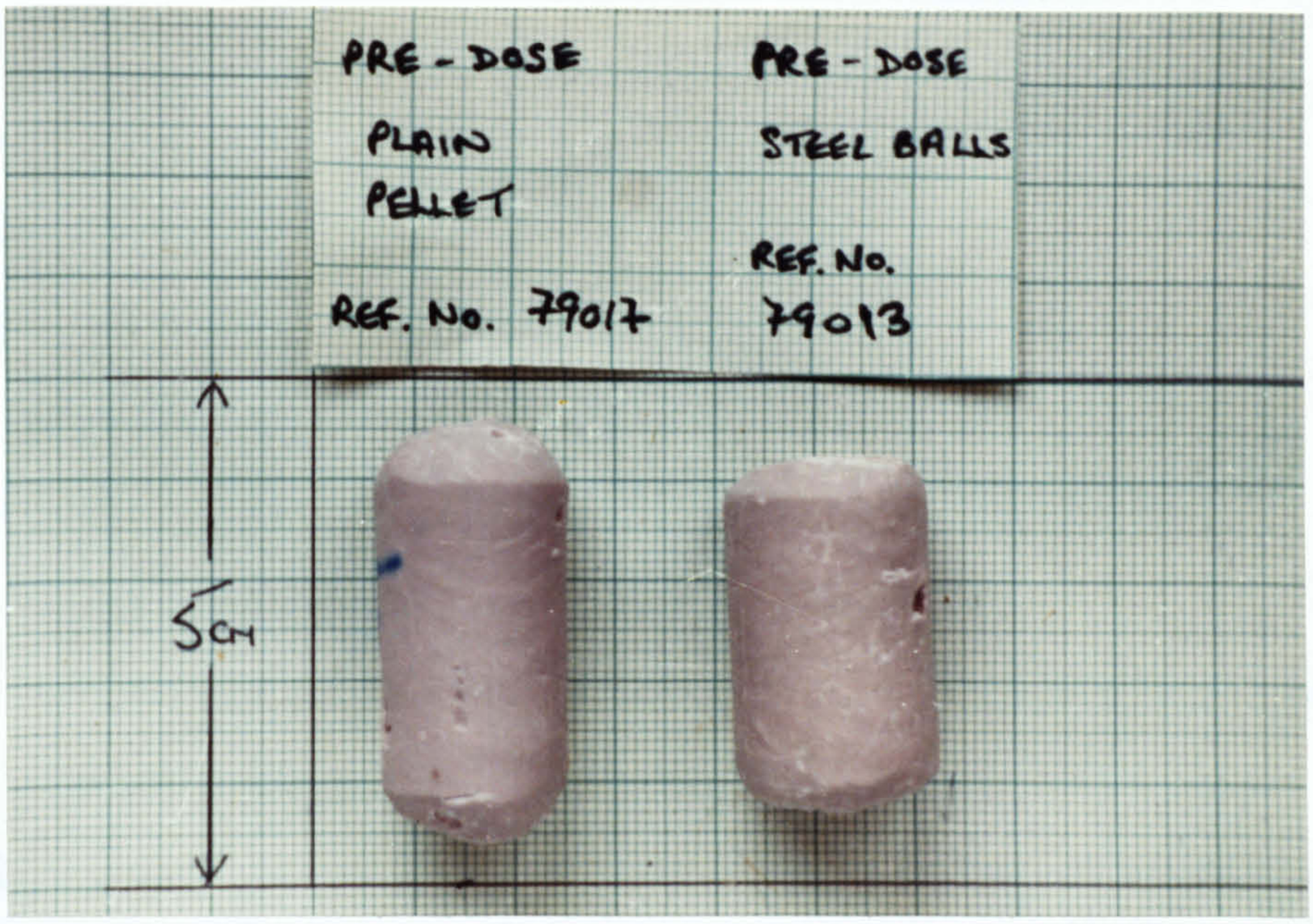
TABLE 14 : Summary of X-ray results. Experiment 3.1.

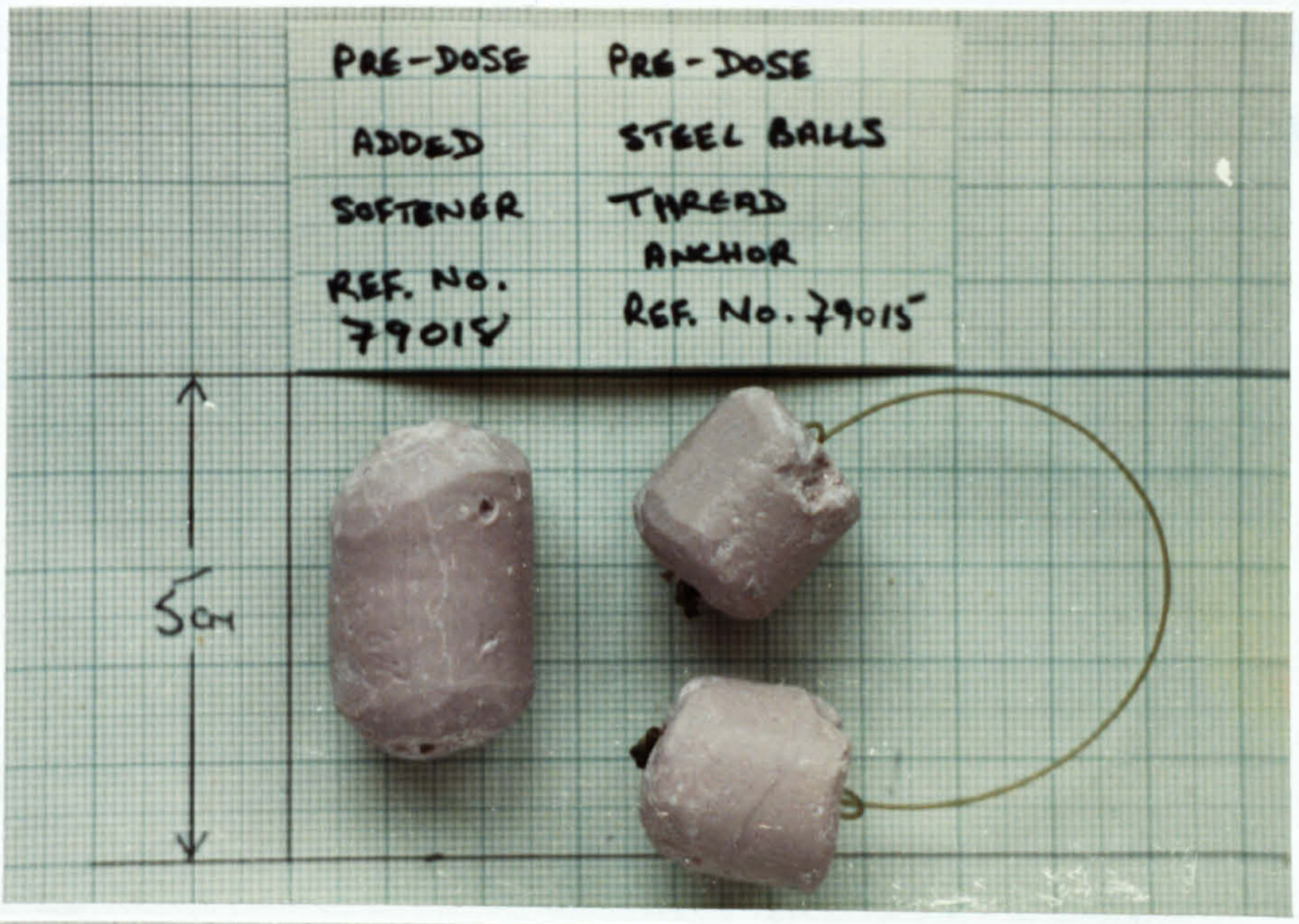
Group	Lamb No.	Conclusions drawn from X-rays
1	1545	Bolus present to day 26. A small portion detectable to day 34
	1569	Bolus broke after 5 days. Halves still detectable to day 34
2	1571	Bolus present to day 5
	1576	Bolus present to day 9
3	1535	Bolus present to day 5. Thread broke. Halves persisted to day 14
	1538	Bolus present to day 5. One half remained to day 16
4	1532	Bolus present to day 5
	1577	Bolus present to day 9. Split. Half remained to day 16

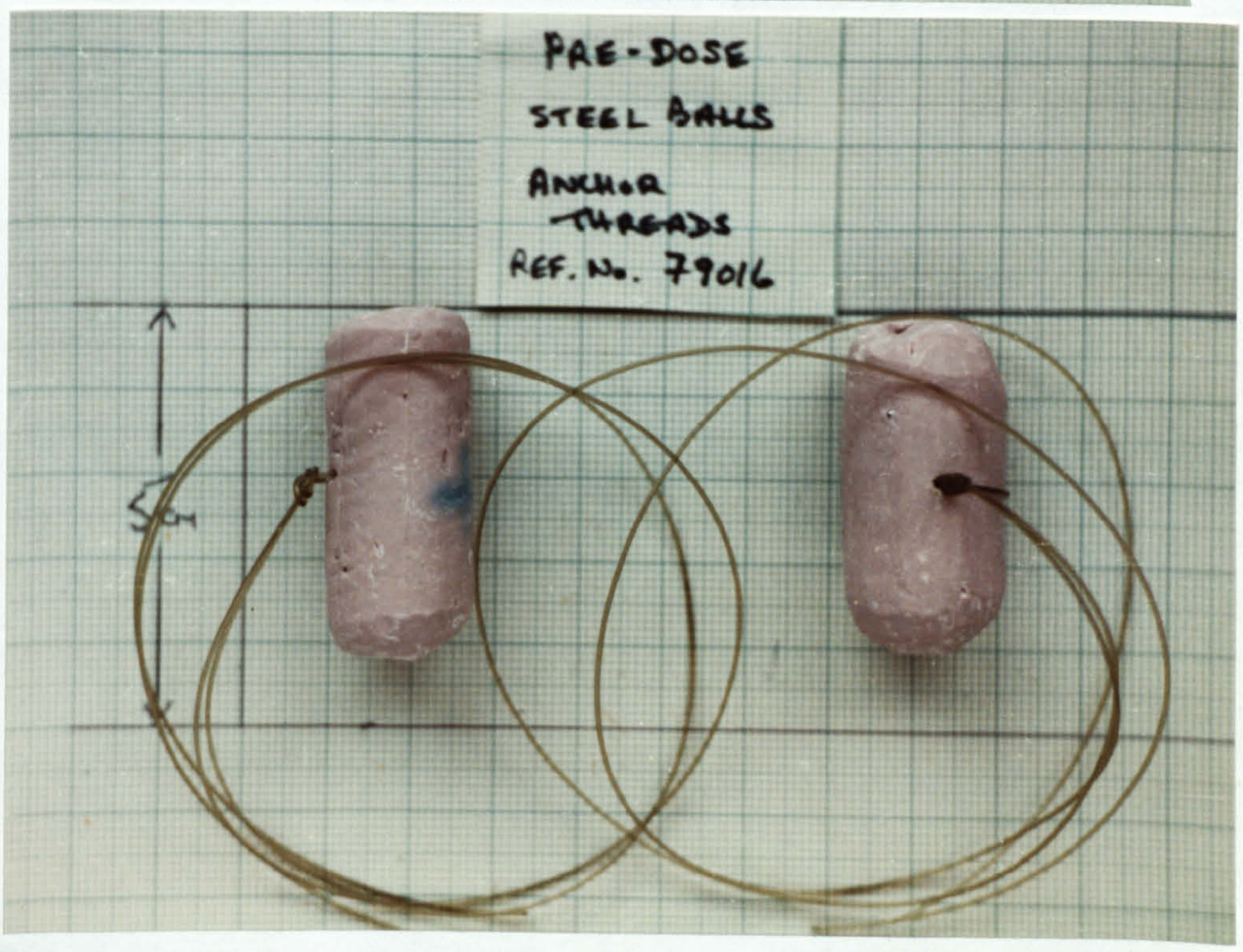
*X-ray facilities were kindly provided by Cambridge Veterinary Hospital, Madingley Road, Cambridge.

PLATE 1 : BOLUSES DESIGNED FOR RUMEN RETENTION. Experiment 3.1.

Pre-dose







The majority of boluses had been lost 14 days after administration. This was confirmed by the faecal plate assay which recorded no excreted drug present in the faeces after 16 days.

Table 15 summarises the anthelmintic activity results. In all but 2 of the lambs, the nematode faecal egg count had dropped to <50 e.p.g. by days 3,5 or 7 following treatment and remained so throughout the remainder of the experiment.

In the two remaining lambs the nematode faecal egg count was considerably lowered compared to the controls and remained low throughout the experimental period (Fig.3.). Both bolus formulations had released sufficient drug to prevent a proportion of the eggs from hatching within the first week (Fig.4.) but the variable effect on the hatching rate indicated an erratic resease of drug.

TABLE 15 : Summary of the anthelmintic activity results. Experiment 3.1.

Group	Lamb No.	Faecal egg count (e.p.g.)		Percentage egg hatch	
		Pre-treatment	Post-treatment[1.]	Pre-treatment	Days post-treatment[2.]
1	1545	10900	See Fig.3.	97.9	See Fig.4.
	1569	10400	<50 by day 3	98.1	+1 = 23.5
2	1571	700	<50 by day 3	100.0	+1 = 6.3
	1576	3900	<50 by day 5	89.7	+1 = 0
3	1535	400	<50 by day 3	96.5	+1 = 0
	1538	4050	<50 by day 5	96.5	+1 = 20.4 +2 = 0 +3 = 0
4	1532	2700	<50 by day 5	93.5	+1 = 0 +2 = 0
	1577	16750	<50 by day 7	92.0	+1 = 25.0 +2 = 42.9 +3 = 0
5	1562	2900	<50 by day 3	97.6	+1 = 0 +2 = 0
	1580	850	<50 by day 3	100.0	+1 = 8.0
6	1556	6700	See Fig. 3.	95.7	See Fig. 4.
	1573	950	<50 by day 7	94.0	+1 = 2.7 +2 = 51.7 +3 = 0
7	1553	7850	See Fig. 3.	93.8	See Fig. 4.
	1563	150		100.0	

1. Counts remained at <50 throughout the experiment unless otherwise stated.

2. After the initial drop, too few larvae were recovered to give a valid result.

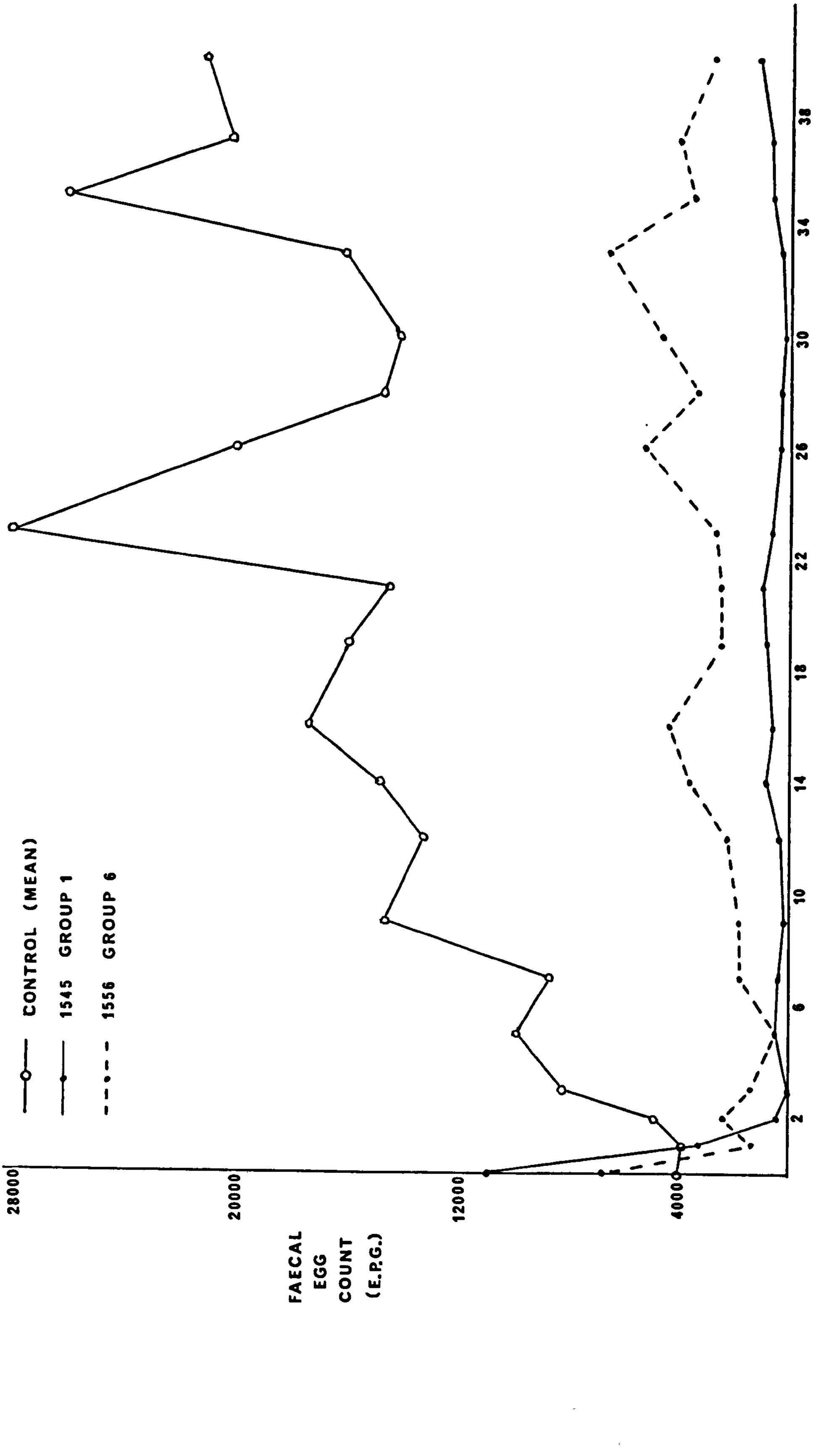


FIG.3 NEMATODE EGG COUNTS EXPERIMENT 3.1.

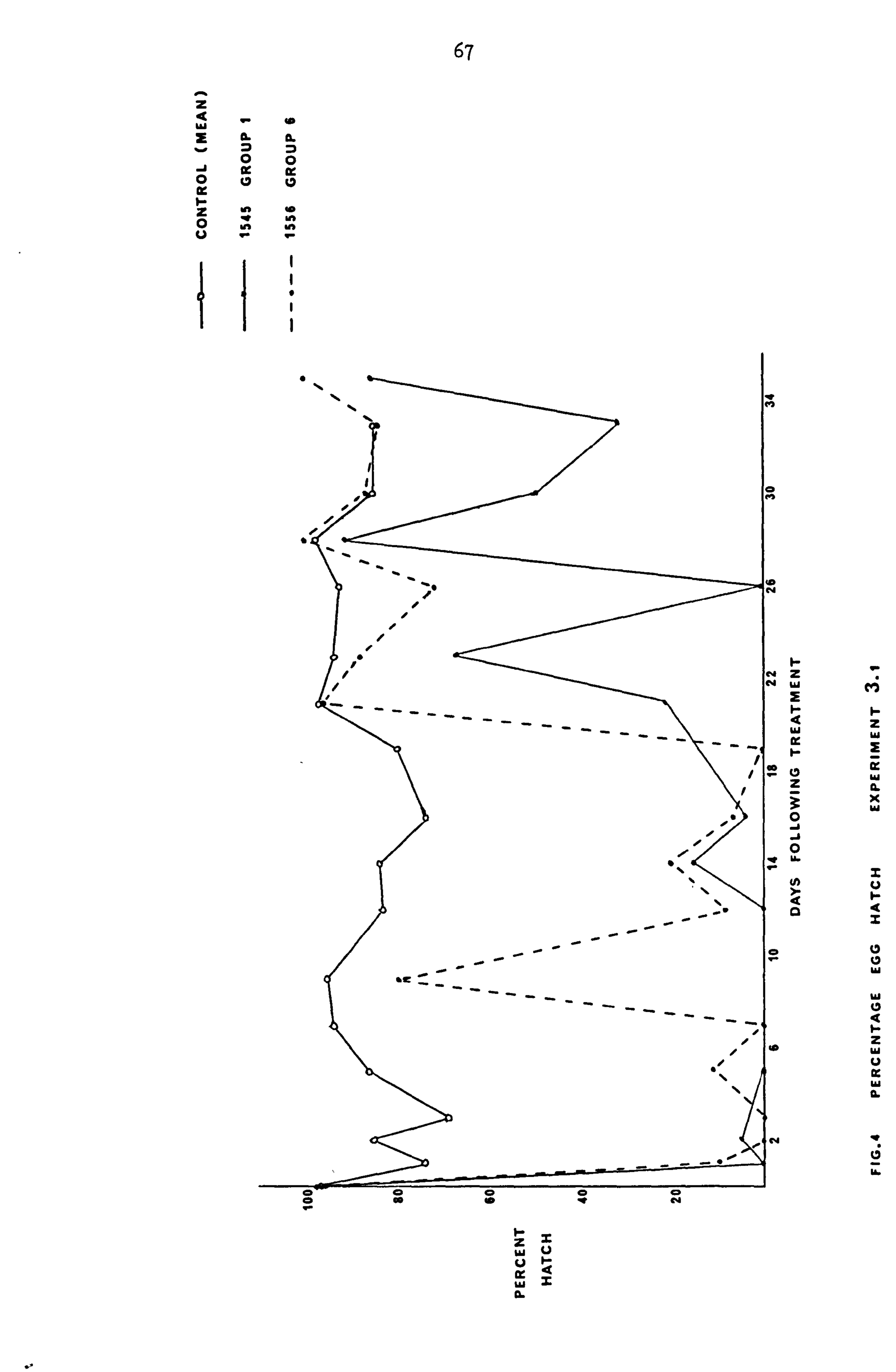


FIG.4 PERCENTAGE EGG HATCH EXPERIMENT 3.1

3.2. Effect of bolus density on ruminal retention

Lambs were dosed with boluses (Plate 2.) incorporating varying sizes of ball bearings to give varying weights and densities.

PLATE 2 : PRE-DOSE EXAMPLES OF DENSITY PELLETS. Experiment 3.2.

Retention was confirmed by X-rays taken weekly with final recovery by rumenotomy.

The results obtained are summarised in Table 16.

These indicate that boluses with densities greater than 1.5 and a weight exceeding 20 gms will be retained in the reticulo-rumen of sheep for periods up to or longer than 69 days.

Examples of the boluses after recovery are illustrated in Plates 3 and 4.

TABLE 16 : Summary of the recovery results for the density boluses.
Experiment 3.2.

Ball bearing diameter (mm) and quantity per 100 gms matrix (gms)	Density	Lamb No.	Bolus weight (gms)	Bolus recovery
Contained a few 1.5 mm balls	1.1	1553	13.5	Regurgitated + 14 days
		1566	12.7	Regurgitated + 6 days
3.175 mm 43.2 gms	1.48	1563	16.8	Regurgitated + 20 days
		1559	25.8	Recovered + 54 days
		1587	22.5	Recovered + 36 days
		1535	20.5	Recovered + 36 days
3.97 mm 110 gms	2.0	1566	26.1	Recovered + 47 days
		1553	26.4	Recovered + 46 days
		1561	21.4	Recovered + 54 days
		1532	22.3	Recovered + 69 days
5.56 mm 184.7 gms	2.49	1563	29.6	Recovered + 49 days
		1560	40.4	Recovered + 54 days
		1573	32.4	Recovered + 69 days

PLATE 3 : <u>EXAMPLES OF THE DENSITY BOLUSES AT RECOVERY</u>. Experiment 3.2.

PLATE 4 : EXAMPLES OF THE DENSITY BOLUSES AT RECOVERY. Experiment 3.2.

3.3. Comparison of two density agents

Lambs were dosed with boluses incorporating either steel shot or an iron bar core as alternatives to ball bearings for achieving the required density of 2.0. The matrix also contained 20, 30 or 40 per cent of a softener to aid the drug release.

Faecal samples were collected at intervals to monitor the drug throughput.

The recovery results after a 30 day retention period are summarised in Table 17.

Nothing was recovered from Group 1.

Only one bolus was recovered intact from Group 2. This was replaced into the rumen and recovered again after a further 33 days. The daily drug release rate was calculated to be an average of 38.5, 44.5 and 50.0 mg thiophanate for the 0 - 30, 0 - 63 and 30 - 63 day periods respectively. A green wax-like substance had been deposited around the middle section of the bolus apparently keeping it intact.

Drug was measured in the faeces at intervals during the first 14 days from both groups. The drug output level was more erratic from lambs in Group 2 but was monitored at intervals throughout the experimental period of 30 days.

TABLE 17 : Summary of the recovery results of boluses incorporating either steel shot or an iron bar core. Experiment 3.3.

Group	Bolus type	Per cent softener	Bolus weight (gms)	Lamb No.	Recovery + 30 days
1	Steel shot	20	19.29	1566	All disintegrated
			20.3	1559	
		30	19.17	649	
			19.69	1573	
		40	19.62	647	
			19.09	772	
2	Iron bar core	20	22.13	771	Iron bar only
			21.66	774	Iron bar only
		30	21.05	770	Intact*. Replaced. Recovered + 63 days
			21.95	1563	Iron bar only
		40	21.95	646	Iron bar only
			21.39	773	Iron bar only

* + 30 days wt. - 18.74 gms

Loss - 2.31 gms 77.0 mg/day 38.5 mg thiophanate/day

+ 63 days wt. - 15.44 gms

Loss - 5.61 gms 89.0 mg/day 44.5 mg thiophanate/day

+ 30 - + 63 days

Loss - 3.3 gms 100.0 mg/day 50.0 mg thiophanate/day

3.4. Maintenance of bolus structure within the rumen

Boluses were formulated to incorporate various aids to prevent rapid disintegration of the matrix but still allow slow erosion to occur. External, centrally placed metal, plastic or rubber rings were used for holding the bolus together along its central axis where cracking predominantly appeared. Since little erosion seemed to occur around the middle of a bolus as indicated from previous samples, a ring covering the centre would not reduce the erosion rate substantially, covering only about 15 - 20 per cent of the matrix. Short hair fibres were also incorporated within the matrix to provide additional strengthening.

Lambs were dosed with boluses incorporating the following aids:-

Group 1 - Iron bar and rubber ring

Group 2 - Iron bar and fibre reinforcing

Group 3 - Iron bar and plastic ring

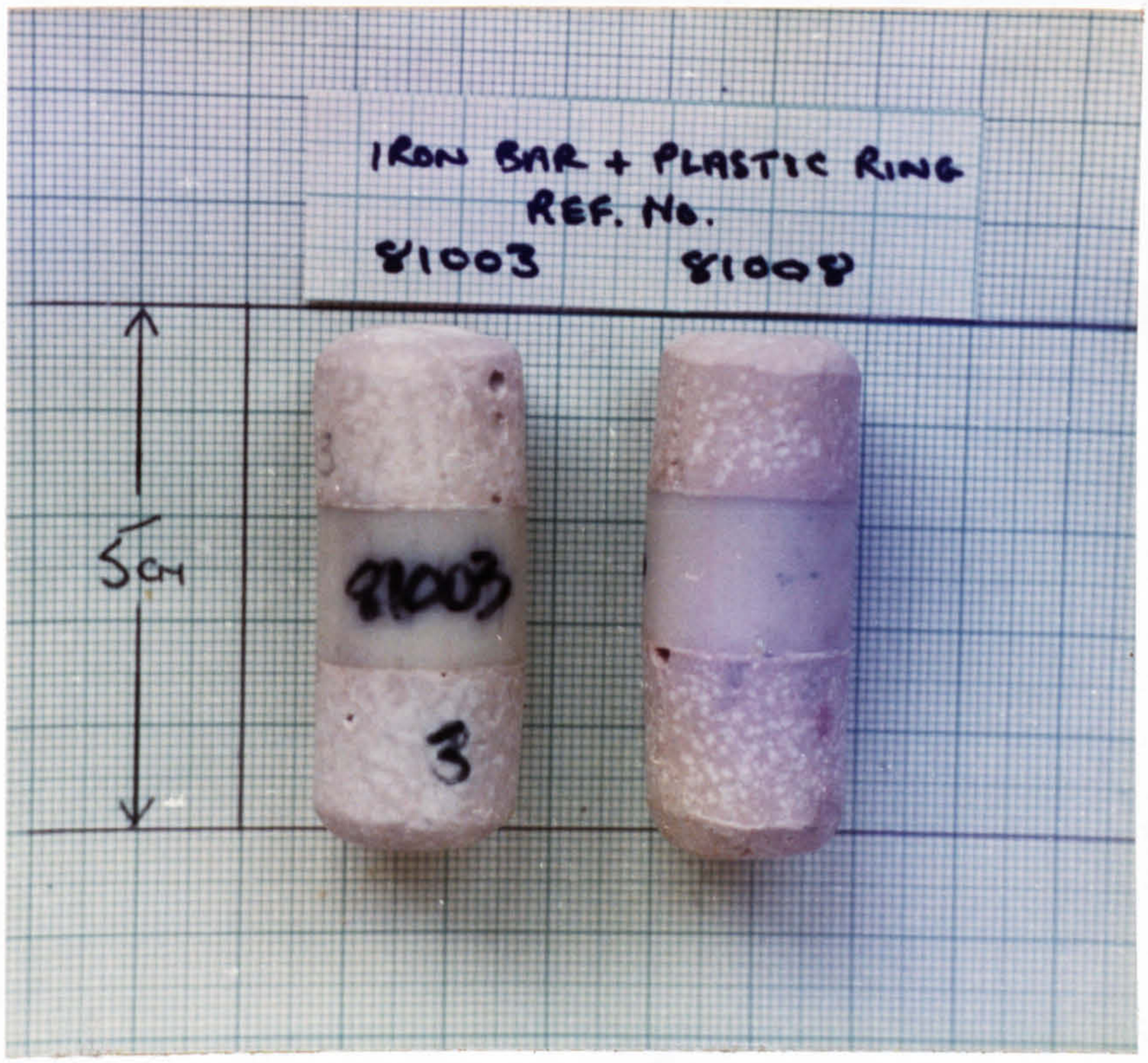
Group 4 - Iron bar, plastic ring and fibre reinforcing

Group 5 - Copper ring

The matrix included 30 per cent of the softener, a trace of an antioxidant and 2 per cent of an emulsifier.

Faecal samples were collected at intervals following treatment to monitor the drug output by the plate assay method.

PLATE 5 : <u>PRE-DOSE EXAMPLES OF BOLUSES FROM GROUP 3 (REF.81003) AND GROUP 4 (REF.81008).</u> Experiment 3.4.



The recovery results obtained from this series of boluses after a 28 to 31 day retention period are summarised in Table 18.

All the rubber rings were lost, iron bars only being recovered, except from one sample around which the green wax-like substance had been deposited as previously observed.

Copper and plastic ring combinations were retained and erosion/disintegration of the matrix was complete, down to the rings. Fibre reinforcement did not appear to aid the integrity of the bolus within the rumen.

The presence of drug in the faeces was still erratic but was more consistent than had been previously recorded from other experiments.

TABLE 18 : Summary of the recovery results of boluses incorporating structural aids. Experiment 3.4.

Group	Bolus type	Bolus weight (gms)	Density	Lamb No.	Recovery + 28 - 31 days post-treatment
1	Iron bar rubber ring	22.54	1.9	038	Iron bar only, no ring
		23.67	1.9	066	One end intact, covered in green wax-like deposit
		22.99	1.9	173	Iron bar only, no ring
2	Iron bar fibre reinforcing	23.05	1.8	097	Iron bars only
		22.69	1.9	142	
		22.94	1.9	654	
3	Iron bar plastic ring	23.74	2.0	165	No covering over ends of bars. Matrix remaining under rings
		24.42	1.9	168	
4	Iron bar plastic ring fibre reinforcing	25.20	1.8	252	Small portion of matrix on iron bar. Intact under ring.
5	Copper ring	22.89	1.9	281	All eroded down to the ring
		21.98	1.9	320	
		22.67	1.8	620	

3.5. Discussion

The first initial batch of boluses (Experiment 3.1.) rapidly disintegrated so retention was difficult to assess. The anthelmintic activity could probably be attributed to this fact, the curative effect being achieved when a high level of drug was released after the breakdown of the bolus. In group 3 where two half boluses were joined by a nylon thread, the regurgitation of both sections simultaneously was considered to have been unlikely. The 2 lambs in which the boluses were identified up to 26 days had each received a single bolus containing steel ball bearings. The weight and/or density of these may have contributed to their retention.

This observation was confirmed in the following experiment (3.2.). The boluses showed little signs of erosion (Plates 3 and 4) so their weight remained relatively stable while in the rumen, indicating a good retention using formulations with a density equal to or greater than 2.0.

In the third experiment (3.3.) all the boluses containing steel shot had disintegrated by 30 days (Table 17). From the faecal drug output results it appeared that they had broken up within 14 days. The boluses incorporating an iron bar core indicated a stronger matrix structure with signs of erosion and subsequent drug release occurring throughout the 30 day retention period. Since disintegration had occurred before the first rumenotomy was performed, an assessment on the effect on the drug release rate of incorporating extra softener in the matrix could not be made.

The integrity of the boluses in the last experiment (3.4.) was improved upon, erosion occurring at a more consistent rate as monitored by the amounts of excreted drug detected in the faecal plate assay. As

a whole, the boluses appeared to maintain their structure for longer periods. The samples that were recovered partially intact were still heavily cracked.

These initial experiments using the Peter Möller matrix gave a good indication as to the bolus density that will be required in future studies. However, because of the rapid disintegration that was occurring in the majority of the formulations, the fatty acid matrix did not appear to be the ideal medium in which to incorporate active ingredients for retention within the rumen unless perhaps a sturdier "carrier" to provide a more rigid backbone could be utilised.

4. THE 'DOBBIN'

Concurrently with the trials conducted in collaboration with Peter Möller, studies were also being undertaken at Ongar to formulate an intra-ruminal bolus.

A conventional slow release bolus (for the rumen) is formulated to provide a release of its active ingredient over a predetermined period. Concurrent with release is loss of weight and volume and eventually the reduced bolus is lost.

The 'Dobbin' was developed as a device for retaining a formulation in the rumen by providing a stable "carrier" on which a suitable matrix containing an active ingredient can be loaded and which will, when dosed by mouth, be retained in the rumen and allow the whole of the active ingredient to be slowly released.

Such a "carrier" must be cheap to produce, of a size and shape which is easily dosed by mouth, is atraumatic when lodged in the rumen and which will carry a sufficient dosage. As a guide, the dimensions of the magnesium bullet previously mentioned in the Introduction are:-

	Weight (gms)	Volume (mls)	Density	Diameter (cms)	Length (cms)
Sheep	33.5	12.0	2.8	2.0	4.7
Cattle	87.3	32.5	2.7	2.4	7.4

Based upon these figures a framework (the 'Dobbin') was prepared consisting of a rod 5.0 cms long and 0.4 cms in diameter with a flange (3.5 mm thick, 1.9 cm diameter) at either end.

Four types were manufactured* as illustrated in Plate 6.

*Kindly prepared by May & Baker's Workshops at Ongar Research Station.

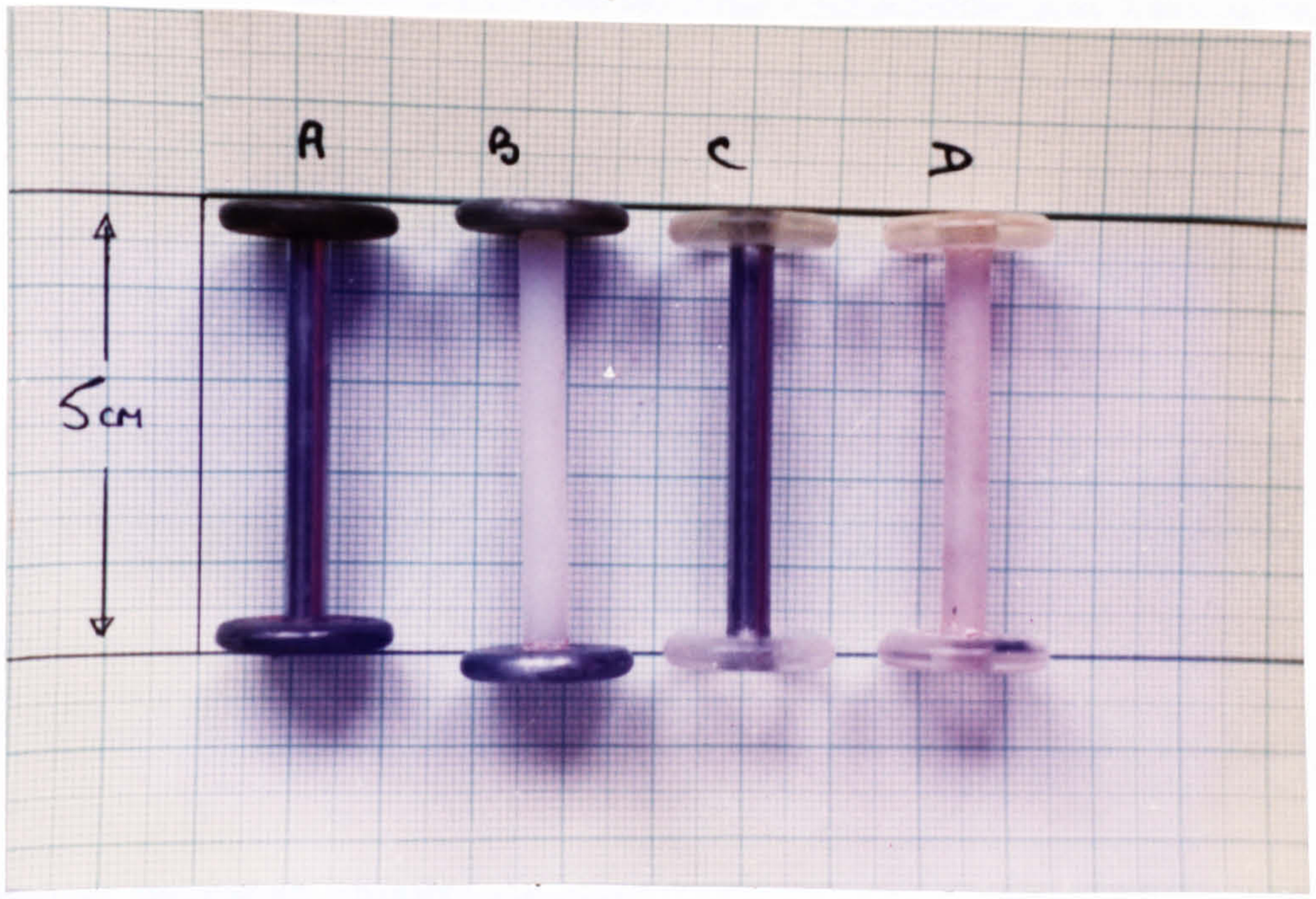


PLATE 6 : 'DOBBIN' TYPES MANUFACTURED

Type	Average Weight (gms)	Volume (mls)	Density
A. All metal	18.2	2.5	7.3
B. Nylon rod - metal flanges	12.5	2.0	6.2
C. Metal rod - nylon flanges	8.05	2.5	3.2
D. All nylon	3.1	2.8	1.1

The type of 'Dobbin' used in a trial depended upon the density of the load it was to carry keeping the final density equal to or greater than 2.0.

Preliminary retention trials eliminated the all nylon 'Dobbin' as the load itself had to be too dense for practical usage. Further studies were carried out using mainly type A or B, all metal or a nylon rod with metal flanges.

5. PRELIMINARY EXPERIMENTS INCORPORATING THE 'DOBBIN' WITH THE PETER MÖLLER MATRIX

Following the negative results obtained with the fatty acid matrix in the previous experiments, preliminary trials were conducted to try to improve upon the stability of this matrix by using a firmer "carrier" in the form of the 'Dobbin'.

5.1. Initial helminth activity study

Eleven lambs were each infected with H.contortus larvae and 21 days later 2 groups of 4 lambs each were treated with one bolus of the following formulations (Plate 7):-

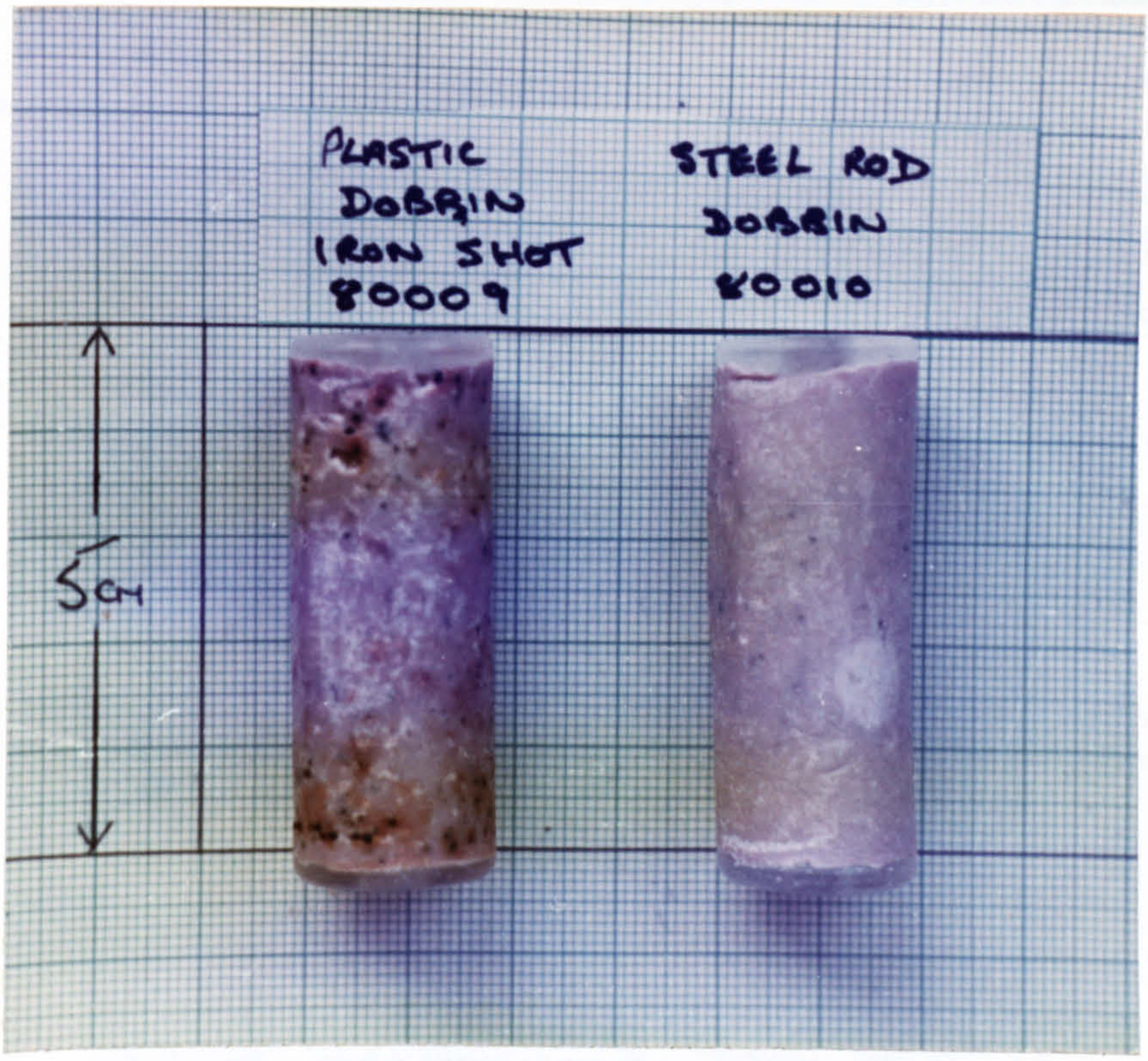
Group 1. All nylon 'Dobbin' loaded with 50 per cent w/w thiophanate in a 45 per cent fatty acid matrix containing steel shot plus 5 per cent softener.

Group 2. Metal rod-nylon flanged 'Dobbin' loaded with 50 per cent w/w thiophanate in a 40 per cent fatty acid matrix plus 10 per cent softener.

The average density of both types of 'Dobbin' was calculated to be 1.5.

PLATE 7 : <u>EXAMPLES OF PRE-DOSE 'DOBBINS' FROM GROUPS 1 AND 2</u>.

Experiment 5.1.



Three lambs were retained as untreated infected controls.

Four worm-free lambs were also treated (2 per formulation) and together with an untreated control, each was infected with 500 <u>H.contortus</u> larvae daily for 10 days from the start of medication.

Two infected sheep from each group (including controls) were turned out to graze. Two paddocks were used in rotation throughout the grazing period. A tracer lamb was used to monitor any overwintered infection present on the pasture.

Faecal samples were collected at intervals to assess the anthelmintic activity by egg count and egg viability and to monitor the drug release by the faecal plate assay. Two consecutive days samples were pooled together for each sample assayed.

'Dobbin' recovery 30 to 32 days following treatment is summarised in Table 19.

Of the 6 nylon 'Dobbins' dosed, 2 lost their load, one was regurgitated and the 3 that were recovered still loaded were considerably swollen and had significant crack formation (Plate 8.). Only 23.1 to 30.1 mg thiophanate per day had been released.

PLATE 8 : ALL NYLON 'DOBBINS' AT RECOVERY. Experiment 5.1.

Of the 6 metal rod-nylon flanged 'Dobbins' dosed, all were recovered loaded (Plate 9). Drug release ranged from 6.8 to 50.8 mg thiophanate per day. Two of the samples recovered, from lambs 281 and 1553, were coated with the green wax-like deposit as previously observed.

No consistent reduction in either nematode egg count (Figs. 5 and 6) or the percentage egg hatch (Figs. 7 and 8) was observed with either formulation.

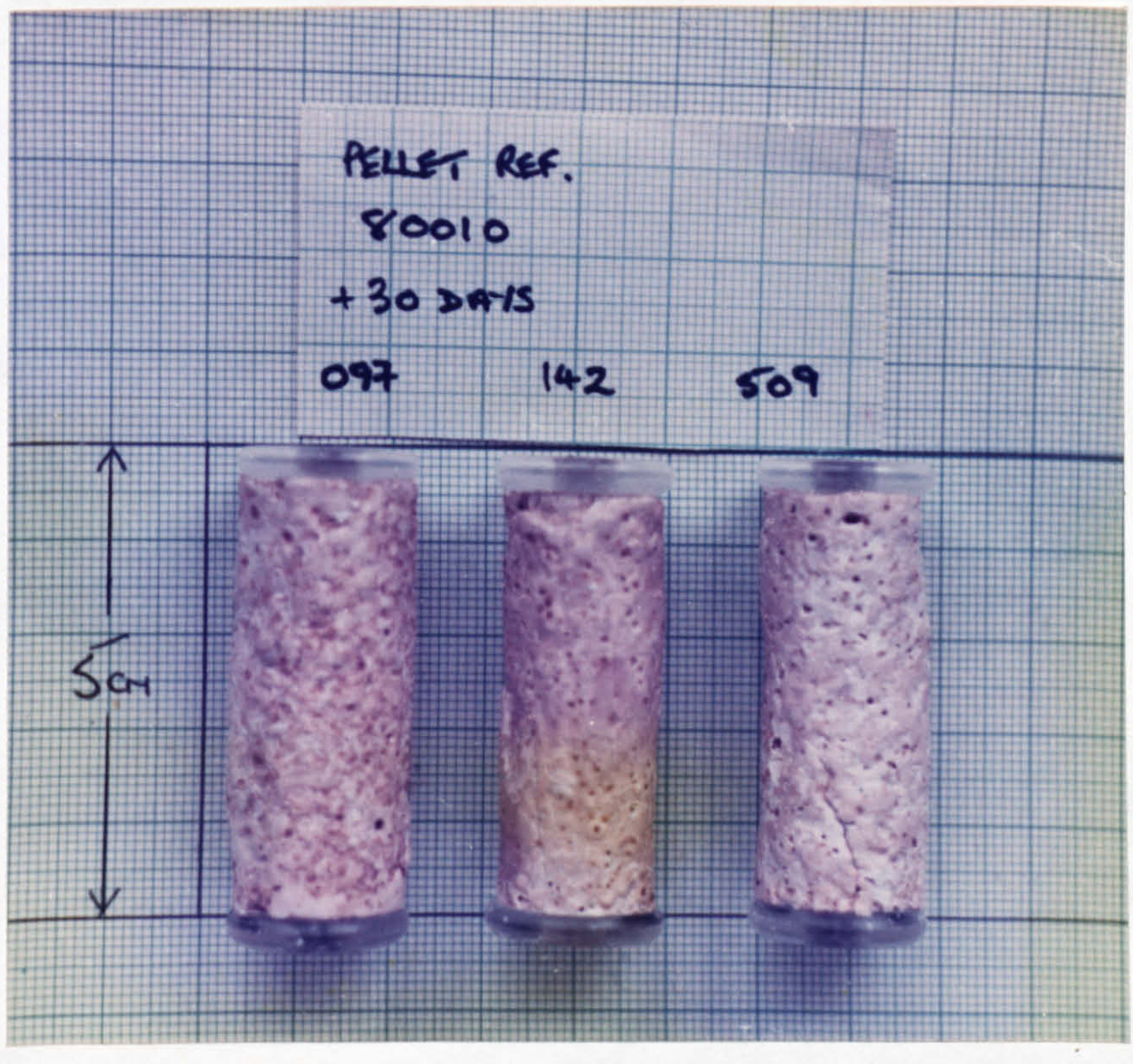
The trickle infected lambs had all recorded positive egg counts by 26 days post infection.

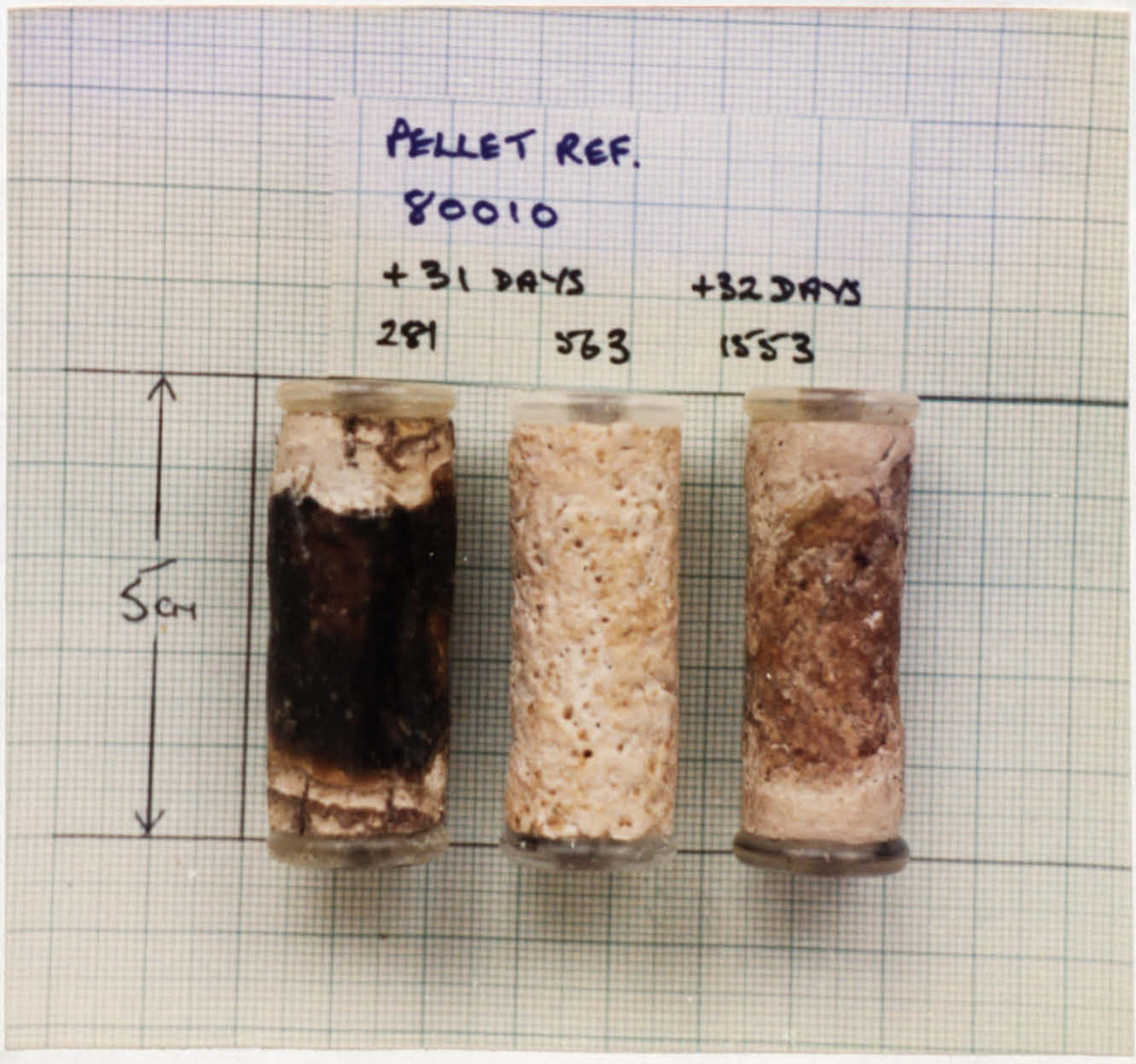
TABLE 19 : Summary of the recovery results of 'Dobbins' loaded with a fatty acid matrix. Experiment 5.1.

Group	'Dobbin' type	Bolus weight (gms)	Lamb No.	At recovery + 30 - 33 days				
				Comment	Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day
1	All nylon	24.01	038	Empty + 30 days				
		24.49	066	Loaded + 33 days	22.5	1.99	60.3	30.1
		24.14	254*	Empty + 33 days				
		25.65	1532*	Lost				
		23.95	252	Loaded + 31 days	22.49	1.46	47.1	23.5
		24.59	359	Loaded + 31 days	23.16	1.43	46.1	23.1
2	Metal rod nylon flanges	20.76	097	+ 30 days	19.08	1.68	56.0	28.0
		20.32	142	+ 30 days	17.27	3.05	101.7	50.8
		20.38	281*	All loaded + 31 days	19.96	0.42	13.5	6.8
		20.27	1553*	+ 32 days	17.72	2.55	79.7	39.8
		20.26	509	+ 30 days	18.01	2.25	75.0	37.5
		20.0	563	+ 31 days	17.7	2.3	74.2	37.1

*Lambs at pasture

PLATE 9 : <u>EXAMPLES AT RECOVERY OF THE METAL ROD-NYLON FLANGED 'DOBBINS' LOADED WITH THE FATTY ACID MATRIX</u>. Experiment 5.1.





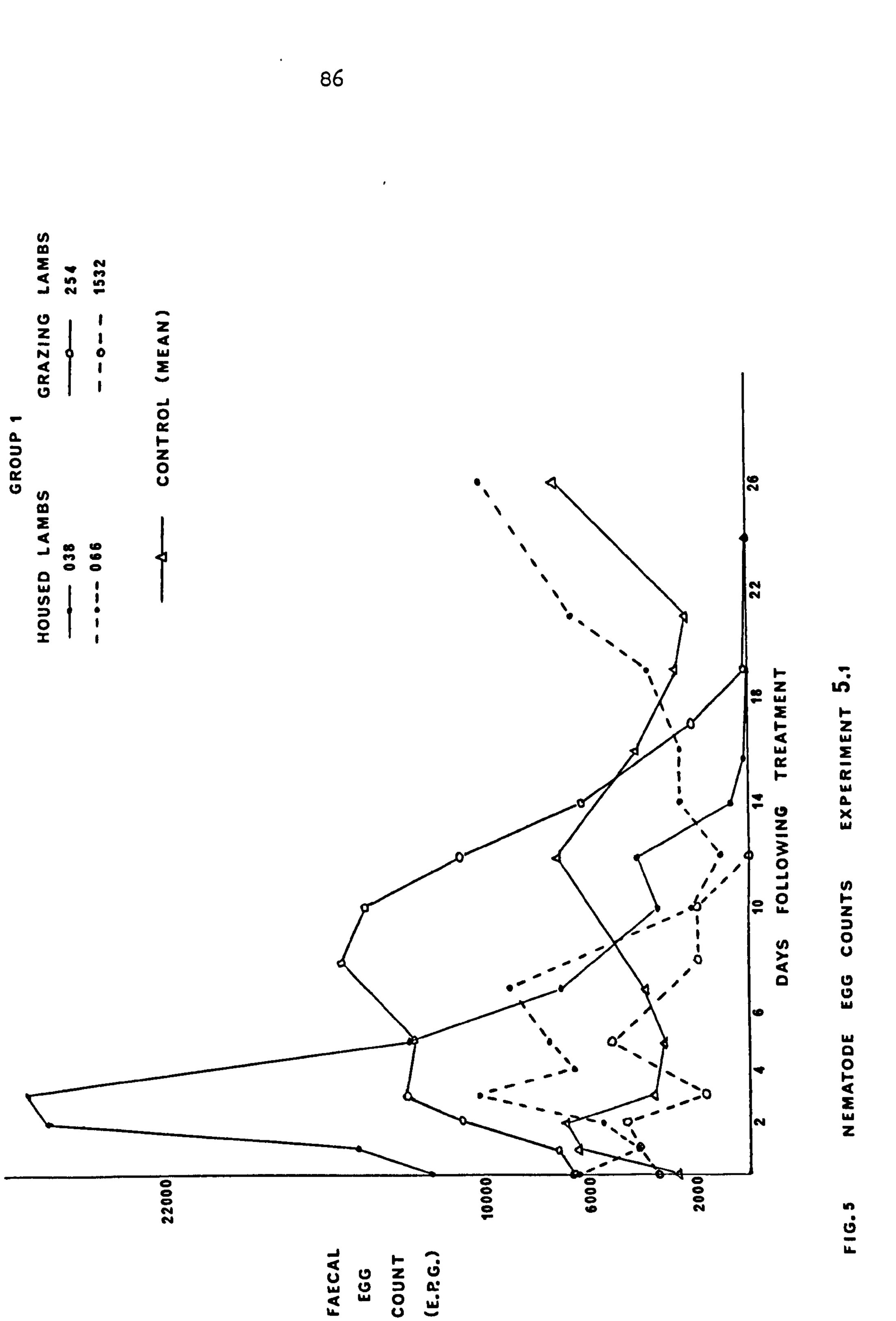


FIG. 5 NEMATODE EGG COUNTS EXPERIMENT 5.1

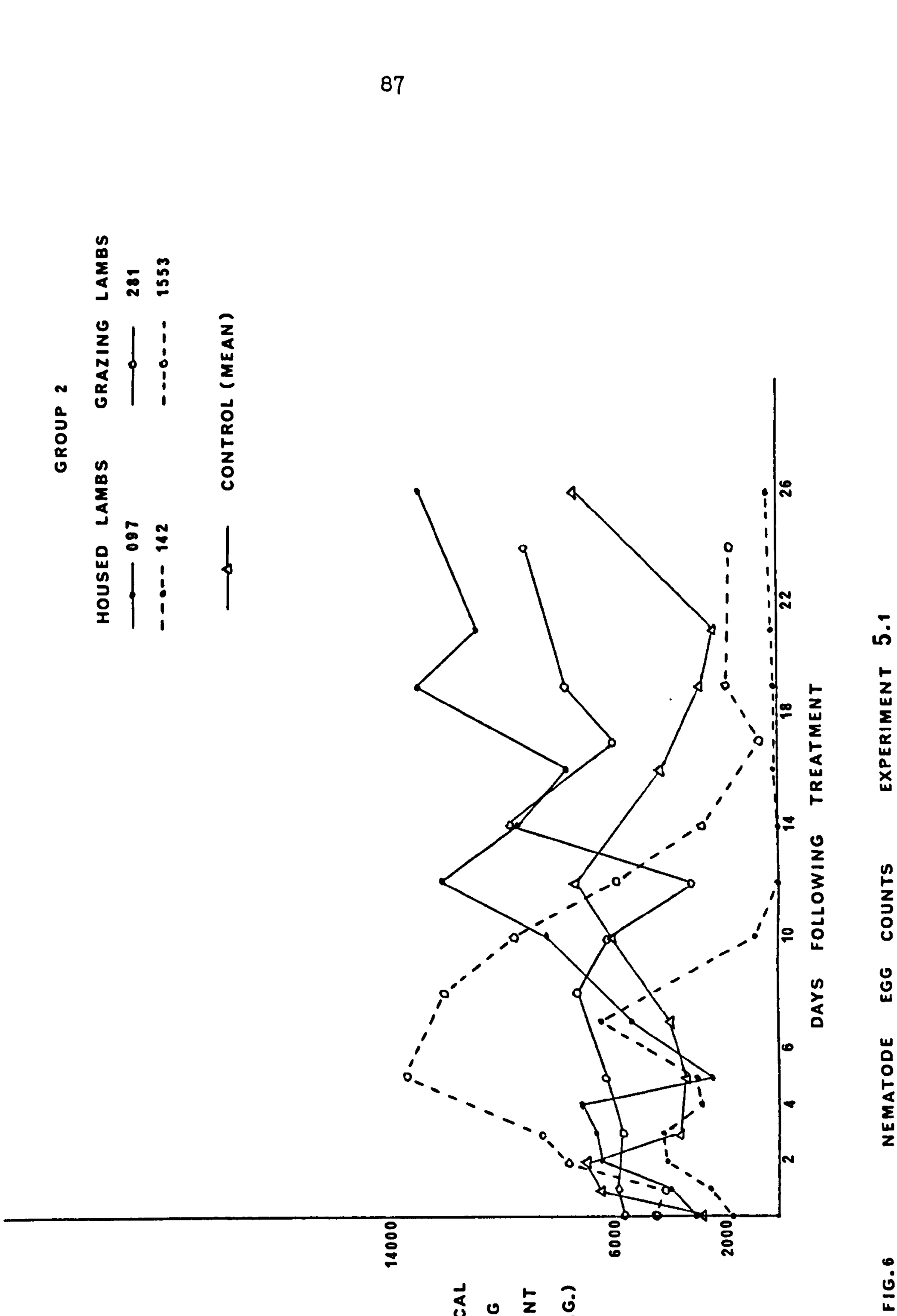

GROUP 2
HOUSED LAMBS
097
142
GRAZING LAMBS
281
1553
CONTROL (MEAN)
1400
600
200
0
0
0
FAECAL EGG COUNT (E.P.G.)
2
4
6
10
14
18
22
26
DAYS FOLLOWING TREATMENT
NEMATODE EGG COUNTS EXPERIMENT 5.1

FIG. 6

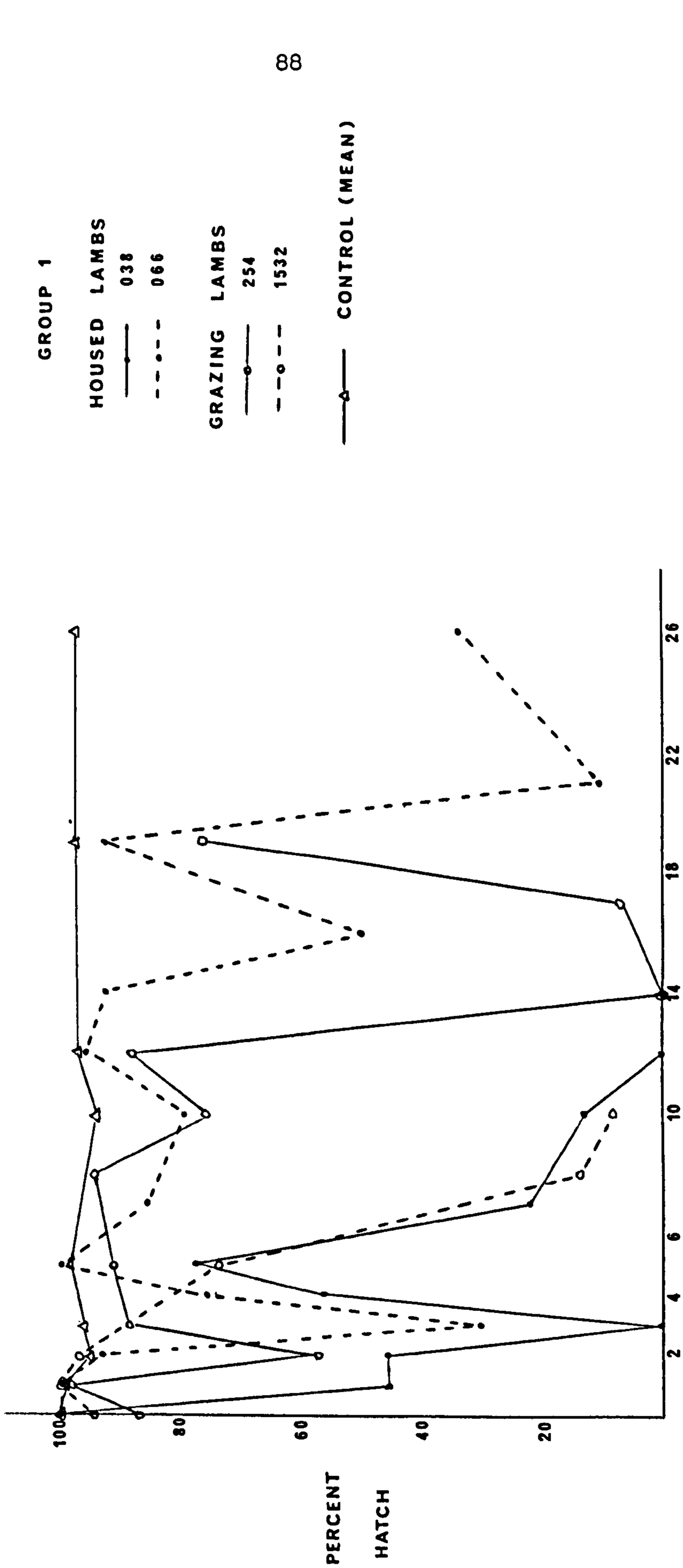


FIG.7 PERCENTAGE EGG HATCH EXPERIMENT 5.1

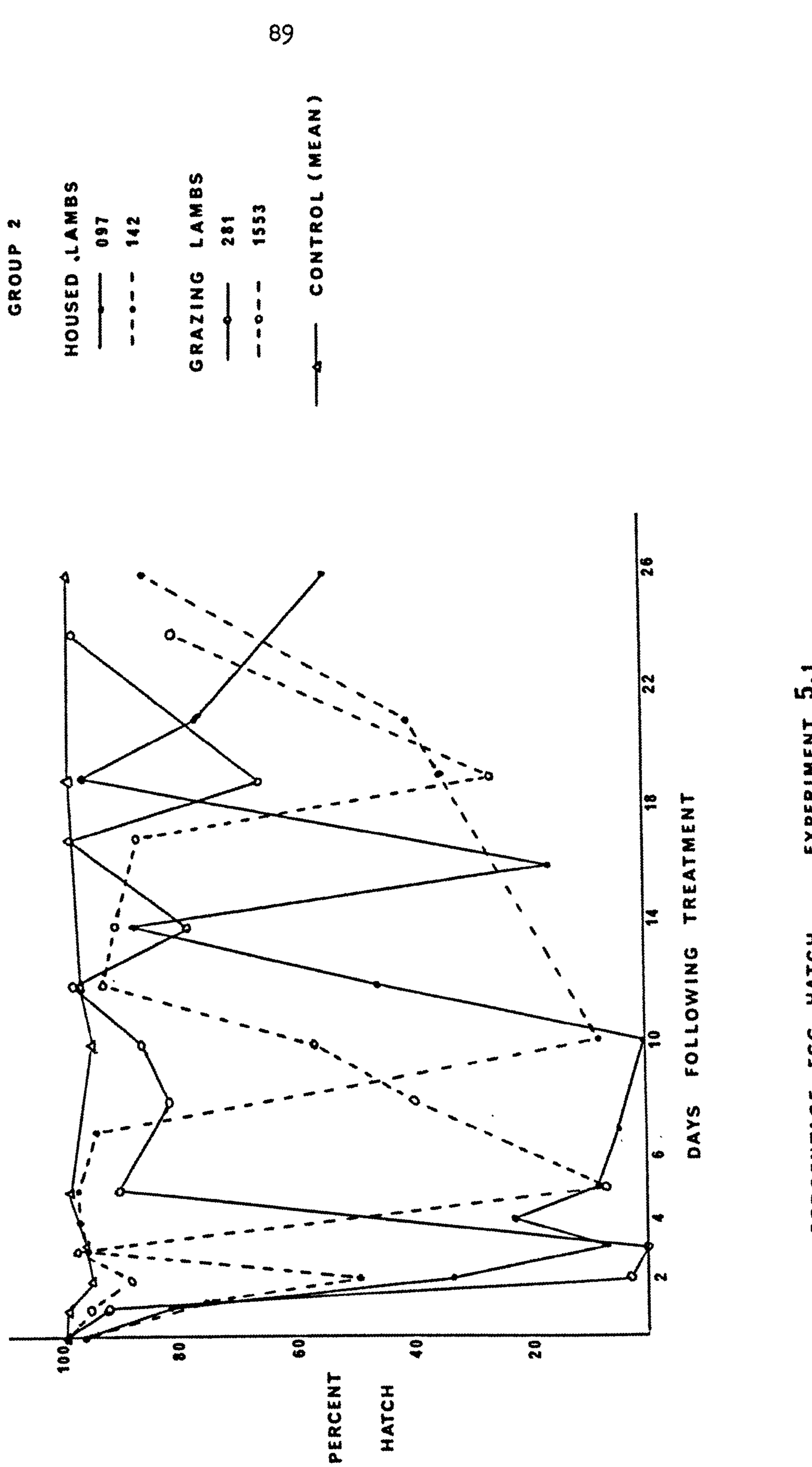


FIG. 5.8 EXPERIMENT 5.1

The tracer lamb remained negative throughout.

A steady faecal drug output was also not apparent and the levels that were recorded on the plate assay indicated a slow, spasmodic erosion rate.

5.2. Effects of additional softener incorporated into the matrix on the drug release rate

In the previous experiment (5.1.) the fatty acid matrix structure was retained when loaded onto a metal rod 'Dobbin' but the erosion rate was inadequate. The effect of varying amounts of softener added to the matrix were examined as previously described in Experiment 3.3. when the iron bar core was used.

Lambs were each dosed with a 'Dobbin' (metal rod, nylon flanges) loaded with the fatty acid matrix incorporating 20, 30 or 40 per cent softener. A sample of the 30 per cent softener matrix was also formulated to include short fibres for additional reinforcing.

The final loaded density was low, an average of 1.45, the bolus weights ranging from 19.7 to 20.75 gms.

Faecal samples were collected at intervals to monitor the drug release by the plate assay.

The 'Dobbin' recovery results 30 days following treatment are summarised in Table 20.

Of the 8 'Dobbins' dosed, 2 were regurgitated and only one was recovered intact (incorporating 20 per cent softener) having released an average of only 12.2 mg thiophanate per day over the 30 day period.

TABLE 20 : 'Dobbin' recovery results. Experiment 5.2.

Group	Per cent softener	Lamb No.	'Dobbin' weight (gms)	Recovery + 30 days
1	20	1532	20.73	Loaded*
		1535	20.32	Regurgitated
2	30	1553	20.51	Small fragment attached
		1587	19.77	Empty
3	30 + fibre reinforcing	1624	20.23	Empty
		489	19.70	Regurgitated
4	40	1560	20.75	Empty
		1561	20.11	Empty

* Weight = 20.0 gms

Loss = 0.73 gms 24.3 mg per day 12.2 mg thiophanate per day

The results showed that the additional softener at all the inclusion levels caused disintegration of the matrix with very little drug release, the matrix having broken away and passed through the rumen before the drug was released. The inclusion of the fibres provided some reinforcement as a more consistent faecal drug output was recorded in the plate assay.

5.3. Discussion

In the first experiment (5.1.) the iron shot incorporated in the matrix loaded onto the nylon 'Dobbin' appeared to act as a buffer on

the surface so preventing the normal erosive process and causing the matrix to crack and disintegrate (Plate 8). The low, variable levels of excreted drug monitored in the plate assay from the individual lambs were comparable with the 'Dobbin' recovery results, i.e. little or no drug release. The green wax-like deposit as previously observed (Experiments 3.3. and 3.4.) was also apparent in this study. A chemical reaction, possibly a soap formation, appeared to be occurring between the rumen contents and the fatty acid composition of the matrix. Calcium and magnesium soaps were possibly being formed by a reaction between the ions present in the rumen liquor and the fatty acids in the bolus. A white crystalline concretionary deposit of calcium phosphate masking a cobalt pellet has previously been reported (Dewey, Lee & Marston, 1958) but the cause was left undiscovered.

In the second experiment (5.2.) the matrix still appeared to disintegrate rather than slowly erode over an extended length of time, the added softener rapidly increasing the disintegration time.

The final densities of all the loaded 'Dobbins' was not high enough to achieve a 100 per cent retention confirming the observations that weight also contributed to the retention of a particle within the reticulo-rumen in Experiment 3.2. and by Byford, Riner & Hair (1980).

Even though the 'Dobbin' provided a firmer backbone, the inadequate erosion rate occurring where the matrix remained intact confirmed the previous conclusion that the fatty acid matrix may not be the ideal medium on which to base a slow release ruminal bolus.

From all the results obtained using this matrix, it was concluded not to be a suitable material for extended exposure to rumen mechanics and selection of another matrix for the remainder of the experimental work was considered necessary.

6. EXPERIMENTAL STUDIES TO SELECT A NEW MATRIX

As concluded from the previous work, before further trials were undertaken a new matrix had to be selected. The material of choice needed to be of a workable medium suitable for laboratory manufacture of the boluses, for example, a molten liquid which could be set in a mould. With such a substance, the melting point must be low enough not to cause degredation of the active ingredient. It must also be cheap to produce, non-toxic, undigested by rumen enzymes and compatible with any active ingredients likely to be incorporated with it.

Since the method of drug release from the ruminal bolus being studied here was by erosion rather than dissolution of the matrix, a wax material was considered. Two such substances were selected:-

Palmitic acid - $C_{15}H_{31}COOH$, a normal fatty acid. A white crystalline solid with a melting point of 63 - 64°C and a density of approximately 0.8.

Paraffin wax - Mixture of solid hydrocarbons. A white, translucent solid with a melting point of 50 - 59°C, and a density of approximately 0.9. The wax used in the studies reported in this thesis was a grade suitable for histological purposes with a melting point of 54 - 58°C.

6.1. Comparison of matrices incorporating various density agents

Twelve sheep were dosed with boluses incorporating either iron shot, iron powder (iron pin dust - 85 per cent ferrous iron) or coarse sand in matrices of the fatty acid blend, palmitic acid or paraffin wax and loaded onto iron bar cores which formed the main density agent.

The waxes were included in the ratios of 1 : 1, 1 : 2 and 1 : 3 (matrix : thiophanate plus iron or sand).

The densities ranged from 2.0 to 2.7, 1.8 to 2.7 and 1.9 to 2.7 for the fatty acid, palmitic acid and paraffin wax matrices respectively, depending on the density agent incorporated.

The boluses were administered in threes or fours per lamb to allow a higher number of formulations to be tested.

When recovered after the initial 32 day period, the boluses still intact were replaced into the rumen and recovered again after a further 24 days.

The recovery results for each of the 3 matrices are summarised in Tables 21, 22 and 23.

Fatty acid blend - Of 14 dosed, 4 were regurgitated, the remainder had disintegrated by 32 days. All the iron bars were retained. (Table 21).

Palmitic acid - Of 13 dosed, 6 were regurgitated, 3 disintegrated and 4 were retained intact for 56 days (Plate 10). An average of 11.1 to 95.0 mg thiophanate per day was released over this period (Table 22).

Paraffin wax - Of 13 dosed, 2 were regurgitated, 1 disintegrated and 10 were retained intact for 56 days (Plates 11 and 12). An average of 30.9 to 86.8 mg thiophanate per day was released over this period (Table 23).

TABLE 21 : Bolus recovery results - fatty acid blend matrix.

Group 1. Experiment 6.1.

Lamb No.	Bolus			Recovery + 32 days
	Type*	Density	Weight (gms)	
599	50 : 50	2.0	22.2	Regurgitated + 1 day
647		2.0	21.23	Regurgitated + 1 day
599	Sand 33 : 33 : 33	2.2	27.12	Broke at dosing
647		2.2	26.29	Regurgitated + 1 day
654	Sand 25 : 50 : 25	2.3	30.09	Iron bar only
682		2.2	29.92	Iron bar only
599	Iron powder 33 : 33 : 33	2.3	26.39	Regurgitated + 29 days
647		2.4	24.15	Iron bar only
654	Iron powder 25 : 50 : 25	2.7	32.57	Iron bar only
682		2.7	32.78	Iron bar only
599	Iron shot 33 : 33 : 33	2.5	27.01	Iron bar only
647		2.3	27.03	Broke at dosing
654	Iron shot 25 : 50 : 25	2.7	32.06	Iron bar only
682		2.6	31.84	Iron bar only

* Ratio = Per cent wax : thiophanate : density agent

TABLE 22 : Bolus recovery results and drug release rates - palmitic acid matrix. Group 2. Experiment 6.1.

Lamb No.	Bolus			Recovery + 32 days				Recovery + 56 days				+ 32 - + 56 days		
	Type*	D	Weight (gms)	Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Loss (gms)	Mg/day	Mg thiophanate per day
185	50 : 50	1.8	21.95	Regurgitated + 1 day										
252		1.8	21.64	Regurgitated + 7 days										
185	Sand 33 : 33 : 33	2.1	25.45	Regurgitated + 1 day										
252		2.1	25.93	Broke at dosing										
281	Sand 25 : 50 : 25	2.2	30.27	23.07	7.2	225.2	112.6	19.63	10.64	190.0	95.0	3.44	143.3	71.65
489		2.2	29.25	Regurgitated + 5 days										
185	Iron powder 33 : 33 : 33	2.3	25.84	Iron bar only										
252		2.3	25.71	23.73	1.98	61.8	20.4	21.04	4.67	83.4	27.5	2.69	112.1	37.0
281	Iron powder 25 : 50 : 25	2.7	31.40	Iron bar only										
489		2.7	32.28	Iron bar only										
185	Iron shot 33 : 33 : 33	2.4	26.20	Regurgitated + 18 days										
252		2.4	26.09	24.38	1.71	53.3	17.5	24.21	1.88	33.6	11.1	0.17	7.08	2.3
281	Iron shot 25 : 50 : 25	2.6	33.0	30.9	2.1	65.6	32.8	29.87	3.13	55.9	27.9	1.03	42.9	21.45
489		2.6	32.18	Regurgitated + 12 days										

* Ratio = Per cent wax : thiophanate : density agent

D = Density

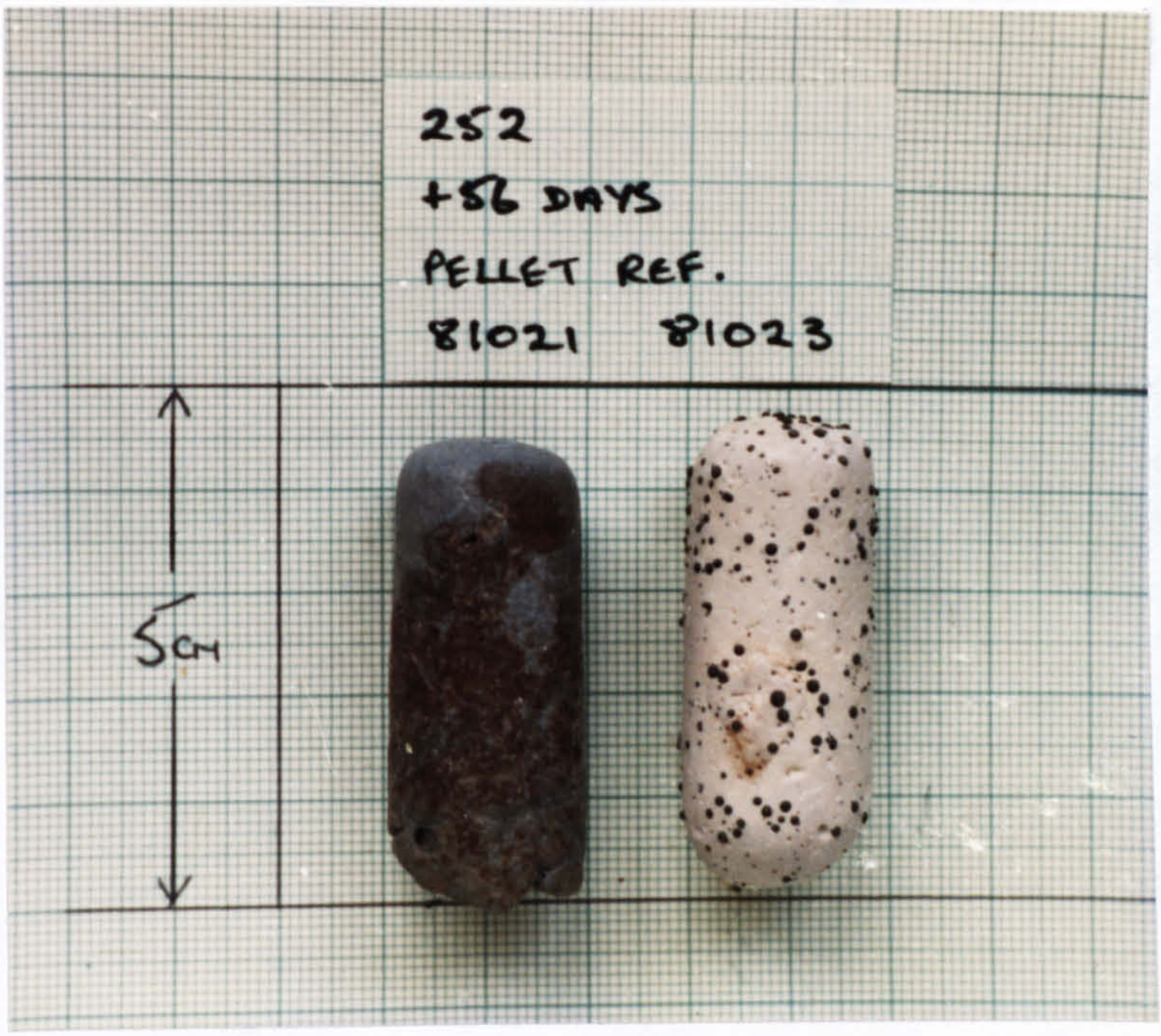
TABLE 23 : Recovery results and drug release rates - paraffin wax matrix. Group 3. Experiment 6.1.

Lamb No.	Bolus			Recovery + 32 days				Recovery + 56 days				+ 32 - + 56 days		
	Type*	D	Weight (gms)	Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Loss (gms)	Mg/day	Mg thiophanate per day
066	50 : 50	1.9	22.92	18.57	4.35	135.9	67.9	16.31	6.61	118.0	59.0	2.26	94.2	47.1
165		1.9	22.96	Regurgitated + 1 day										
066	Sand 33 : 33 : 33	2.0	27.0	22.33	4.67	145.9	48.1	20.01	6.89	124.8	41.2	2.32	96.7	31.9
165		2.1	26.56	Iron bar only										
168	Sand 25 : 50 : 25	2.2	29.76	23.91	5.85	182.8	91.4	20.71	9.05	161.6	80.8	3.2	133.3	66.65
173		2.2	28.75	Regurgitated + 1 day										
066	Iron powder 33 : 33 : 33	2.3	25.99	21.23	4.76	148.7	49.1	18.79	7.2	128.6	42.4	2.4	101.7	33.5
165		2.3	26.94	23.37	3.57	111.6	36.8	21.70	5.2	93.6	30.9	1.67	69.6	22.9
168	Iron powder 25 : 50 : 25	2.6	30.47	24.26	6.21	194.1	97.05	20.75	9.72	173.6	86.8	3.51	146.2	73.1
173		2.7	30.02	24.87	5.15	160.9	80.45	22.95	7.07	126.2	63.1	1.92	80.0	40.0
066	Iron shot 33 : 33 : 33	2.3	26.70	22.22	4.48	140.0	46.2	19.89	6.81	121.6	40.1	2.33	97.1	32.0
165		2.4	24.40	Broke at dosing										
168	Iron shot 25 : 50 : 25	2.6	32.41	27.69	4.72	147.5	73.75	25.06	7.35	131.2	65.6	2.63	109.6	54.8
173		2.6	32.62	28.39	4.23	132.2	66.1	26.21	6.41	114.5	57.25	2.18	90.8	45.4

* Ratio = Per cent wax : thiophanate : density agent

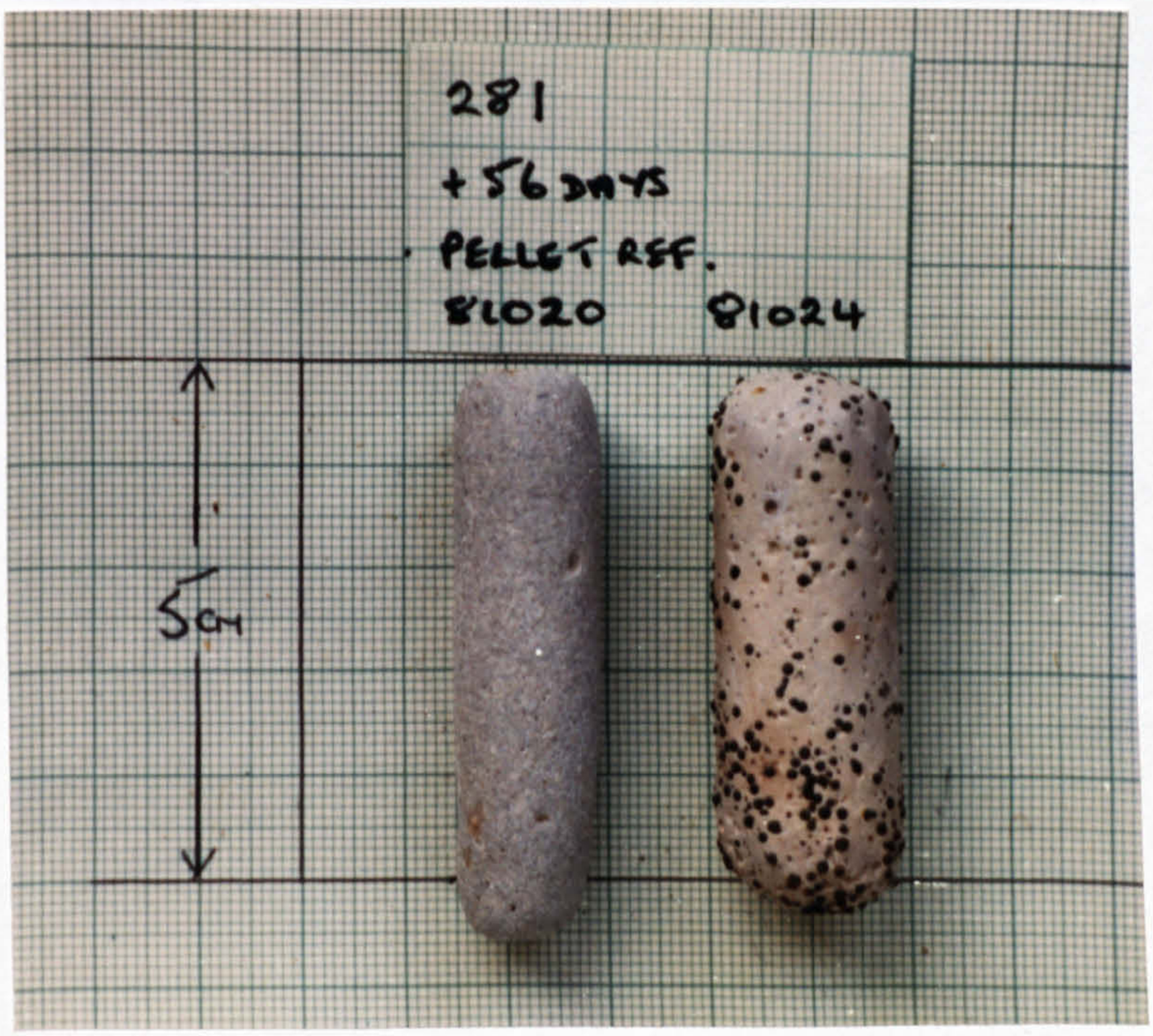
D = Density

PLATE 10 : PALMITIC ACID BOLUSES AT RECOVERY. Experiment 6.1.



Ref: 81021 33 : 33 : 33 Iron powder

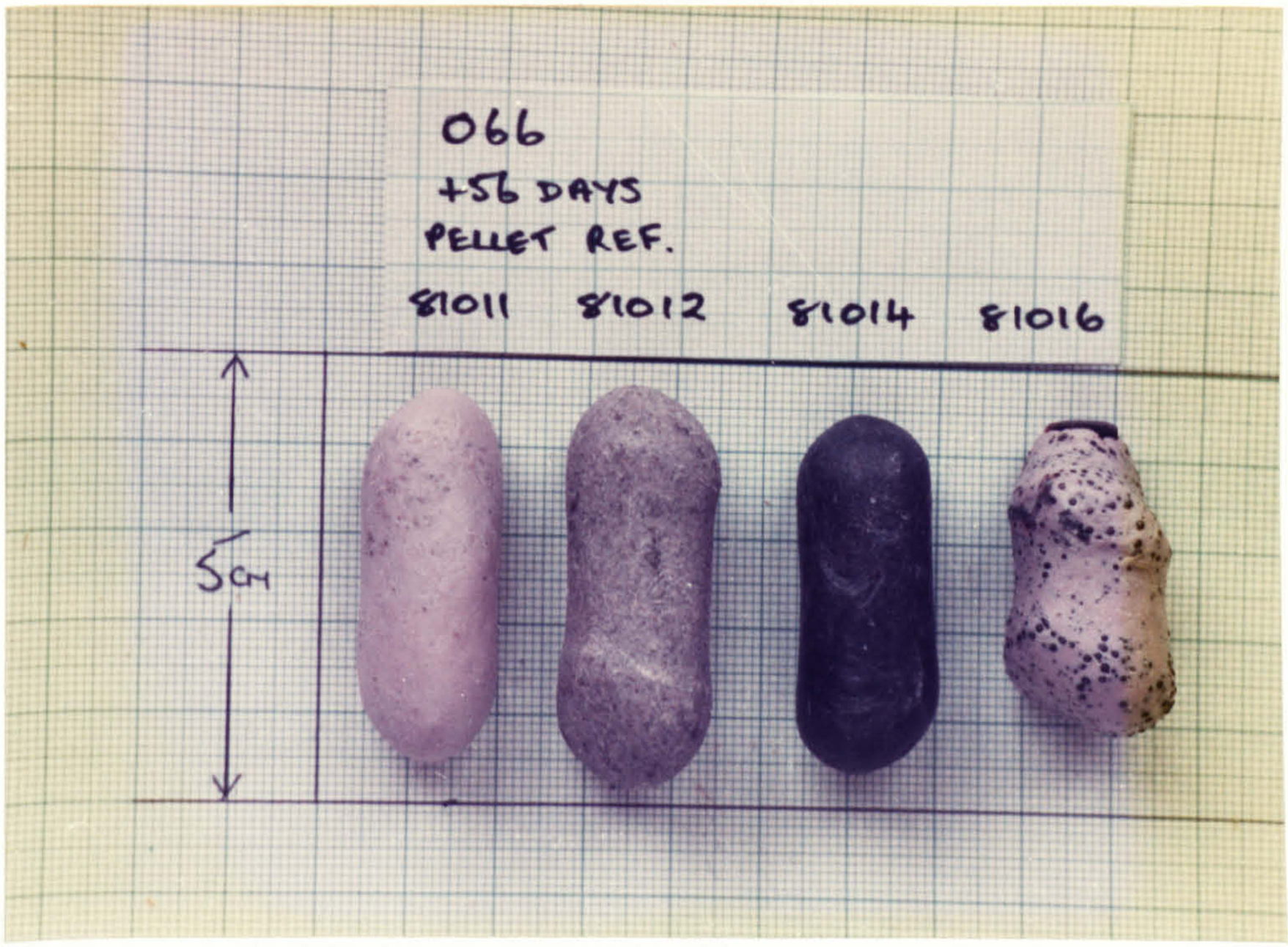
Ref: 81023 33 : 33 : 33 Iron shot

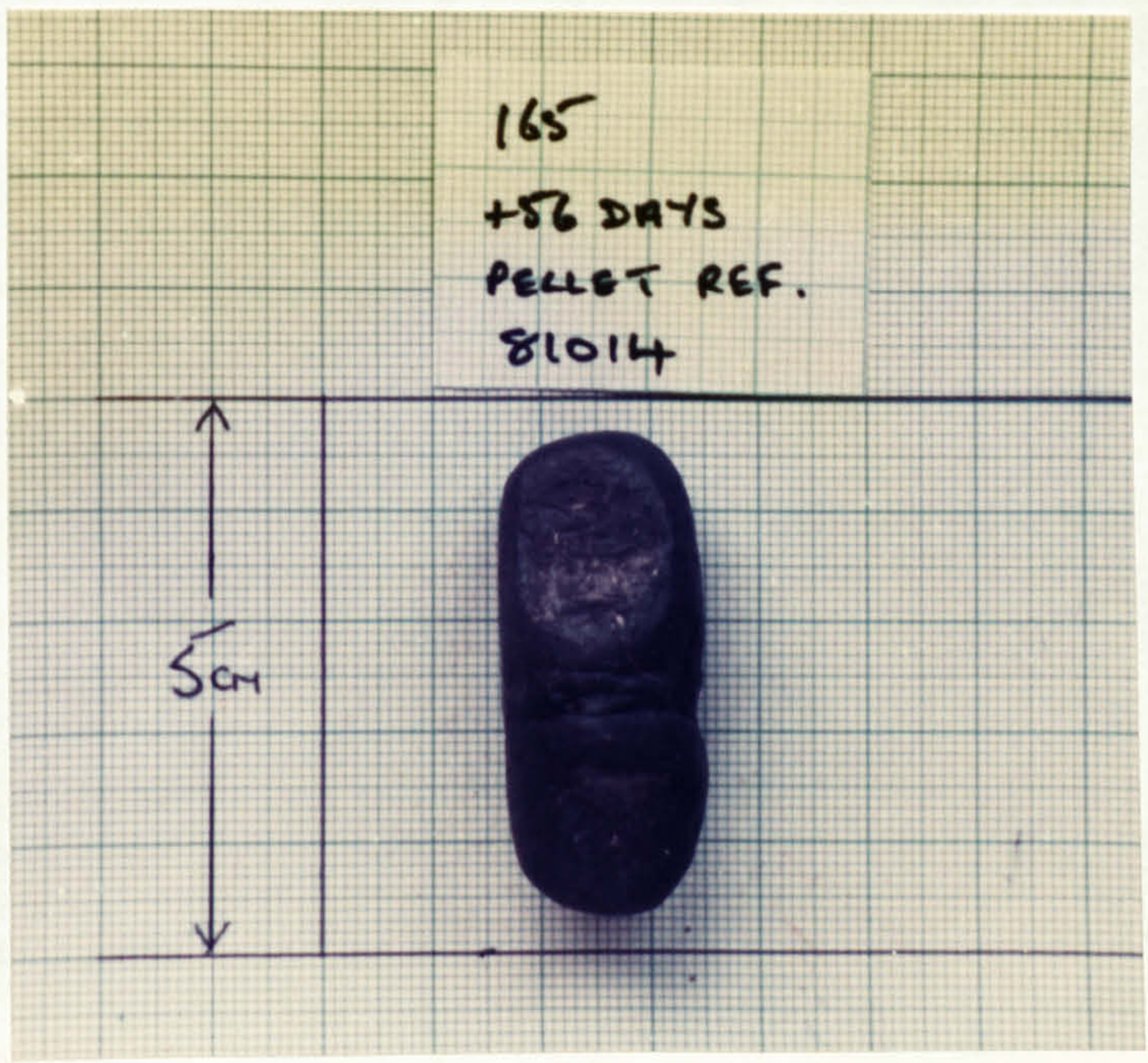


Ref: 81020 25 : 50 : 25 Sand

Ref: 81024 25 : 50 : 25 Iron shot

PLATE 11 : PARAFFIN WAX BOLUSES AT RECOVERY. Experiment 6.1.





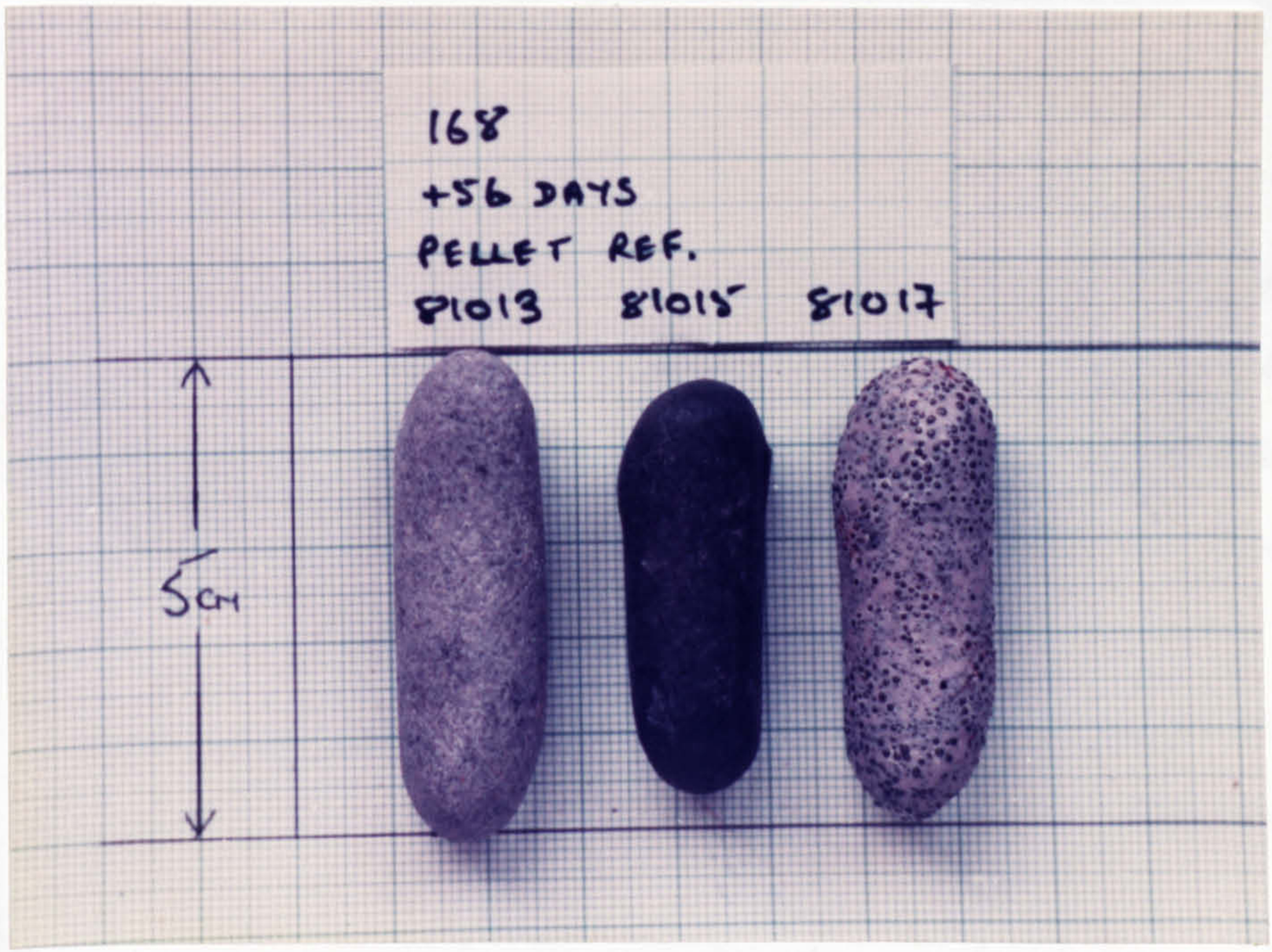
Ref: 81011 50 : 50

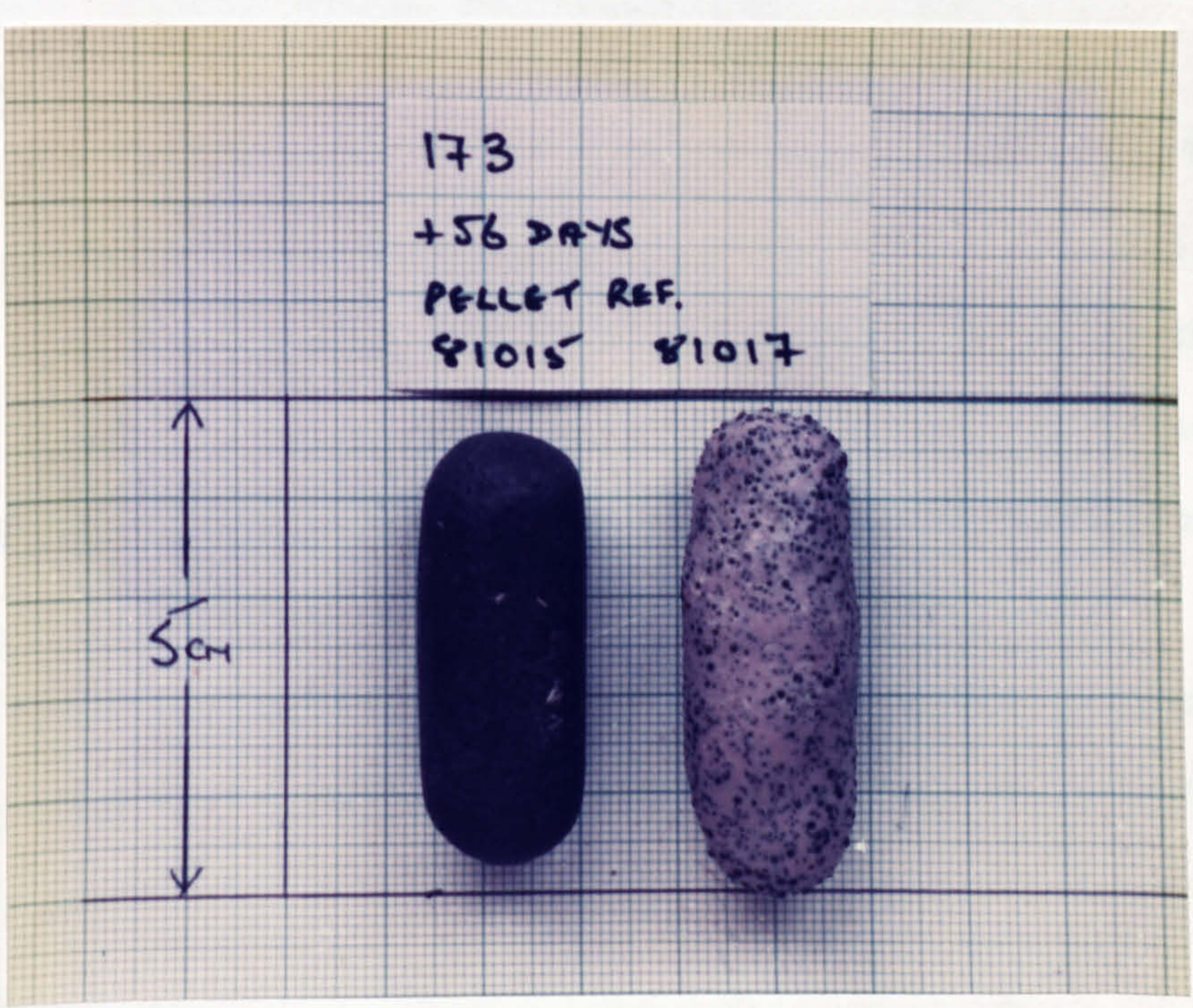
81012 33 : 33 : 33 Sand

81014 33 : 33 : 33 Iron powder

81016 33 : 33 : 33 Iron shot

PLATE 12 : <u>PARAFFIN WAX BOLUSES AT RECOVERY</u>. Experiment 6.1.





Ref: 81013 25 : 50 : 25 Sand
81015 25 : 50 : 25 Iron powder
81017 25 : 50 : 25 Iron shot

Of the 3 matrices tested, paraffin wax gave good retention and stability with improved drug release rates compared to the other two examined. This wax was, therefore, selected for further studies.

6.2. Effect on retention of increasing the iron bar core size

To try and improve upon the bolus retention figures using a paraffin wax matrix (15 per cent regurgitated in the previous study Experiment 6.1.), the weight of the iron bar core was increased from 11 to 16 gms. A preliminary assessment of the anthelmintic activity of this matrix was also undertaken.

Seventeen lambs were each infected with H.contortus larvae and 24 days later, 16 were dosed with one of the following formulations (8 lambs per group):-

Group 1 - 50 : 50 :: wax : thiophanate (Ref.81035)

Group 2 - 45 : 55 :: wax : thiophanate (Ref.81036)

The average bolus densities were 2.26 and 2.15 for Groups 1 and 2 respectively, the weights ranging from 26.8 to 29.0 gms.

One lamb was retained as an untreated infected control.

After recording the erosion rate 31 to 32 days following treatment, the boluses were replaced into the rumen, alone, or three together, and recovered again after a further 27 days.

Faecal samples were collected at intervals throughout the experiment for assessment of anthelmintic activity by nematode egg count and egg viability and to monitor the faecal drug output by the plate assay method.

The results are summarised in Tables 24 and 25.

All boluses were retained for the complete period of 58 to 59 days and recovered intact. The daily drug release was similar within each group, ranging from 25.2 to 33.9 and 25.5 to 39.2 mg thiophanate

TABLE 24 : Summary of the drug release rates achieved from boluses before and after joining in Group 1 -50 per cent thiophanate. Experiment 6.2.

Bolus No.	Lamb No.	Bolus weight (gms)	Recovery + 31/32 days					Recovery + 58/59 days				+ 31/32 - + 58/59 days		
			Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day		Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Loss (gms)	Mg/day	Mg thiophanate per day
1	3597	28.03	26.17	1.86	60.0	30.0	Joined in lamb 3617	25.13	2.9	49.1	24.6	1.04	37.1	18.5
8	3617	29.27	27.42	1.85	57.8	28.9		26.13	2.96	50.17	25.1	1.11	41.1	20.5
4	3641	27.40	25.70	1.7	53.1	26.6	Joined in lamb 3641	24.63	2.77	46.9	23.5	1.07	39.6	19.8
7	3599	28.33	26.68	1.65	51.6	25.8		25.53	2.8	47.5	23.7	1.15	42.6	21.3
2	3643	27.39	25.83	1.56	50.3	25.2		25.20	2.19	37.1	18.5	0.63	22.5	11.25
3	1663	26.86	25.28	1.58	50.9	25.5		24.5	2.36	40.6	20.3	0.78	28.9	14.4
5	3637	28.03	26.08	1.95	62.9	31.4		25.29	2.74	46.4	23.2	0.79	28.2	14.1
6	3651	28.89	26.79	2.1	67.7	33.9		25.6	3.29	56.7	28.3	1.19	44.1	22.0

TABLE 25 : Summary of the drug release rates achieved from boluses before and after joining in Group 2 - 55 per cent thiophanate. Experiment 6.2.

Bolus No.	Lamb No.	Bolus weight (gms)	Recovery + 31/32 days					Recovery + 58/59 days				+31/32 - + 58/59 days		
			Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day		Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Loss (gms)	Mg/day	Mg thiophanate per day
5	3635	29.03	27.24	1.79	55.9	30.7	Joined in lamb 3635	25.42	3.61	62.2	34.2	1.82	70.0	38.5
7	3624	28.77	26.96	1.81	58.4	32.1		25.05	3.72	64.1	35.3	1.91	70.7	38.9
8	3645	28.62	26.61	2.01	62.8	34.5		25.82	2.8	48.3	26.5	0.79	30.4	16.7
1	3606	28.53	26.32	2.21	71.3	39.2	Joined in lamb 3622	24.95	3.58	61.7	33.9	1.37	50.7	27.9
2	3622	27.38	25.80	1.58	50.9	28.0		24.51	2.87	49.5	27.2	1.29	47.8	26.3
3	3632	27.77	26.33	1.44	46.4	25.5		25.5	2.27	38.5	21.2	0.83	29.6	16.3
4	1664	28.09	26.56	1.53	49.3	27.1		25.75	2.34	40.3	22.2	0.81	30.0	16.5
6	3657	28.55	26.72	1.83	57.2	31.4		25.78	2.77	46.9	25.8	0.94	34.8	19.1

per day for Groups 1 and 2 respectively during the first 31 to 32 days. The actual daily dosage received per lamb varied from 0.66 to 1.37 mg thiophanate per kg bodyweight.

In Group 1 where boluses were joined for the second retention period, the individual bolus drug release rate did not increase, but a general decline was observed from all samples (Table 24). Examples of these boluses are illustrated in Plate 13.

In Group 2 the slightly higher drug to wax ratio maintained a steadier drug level after the boluses were joined together for the second retention period. Two boluses from the group of three in lamb 3635 showed an increase in their drug release rates. Examples of these boluses are illustrated in Plates 14 and 15. The single dosed boluses in this group showed a decline in drug release during the latter stages as observed in Group 1.

During the first 31 to 32 days, there was no effect on faecal egg count (Figs. 9 and 10) but a 50 per cent or more reduction in egg hatch (Figs. 11 and 12) occurred in both groups. After joining the boluses together in one lamb, the output of viable nematode eggs was prevented and the counts had dropped to <50 by the end of medication. (All lambs retaining a single bolus throughout the experiment were grouped within their respective groups and the mean figure graphed throughout).

Excreted drug was rarely registered in the faeces from lambs in either group during the first retention period of 31 to 32 days but after this period zones of drug activity were measured from the faecal samples collected from lambs dosed with the multiple boluses.

PLATE 13 : <u>BOLUS RECOVERY GROUP 1</u>. Experiment 6.2.

<u>50 per cent thiophanate</u>

PLATE 14 : SINGLE DOSED BOLUSES RECOVERED 31/32 DAYS AFTER DOSING

GROUP 2. Experiment 6.2.

55 per cent thiophanate

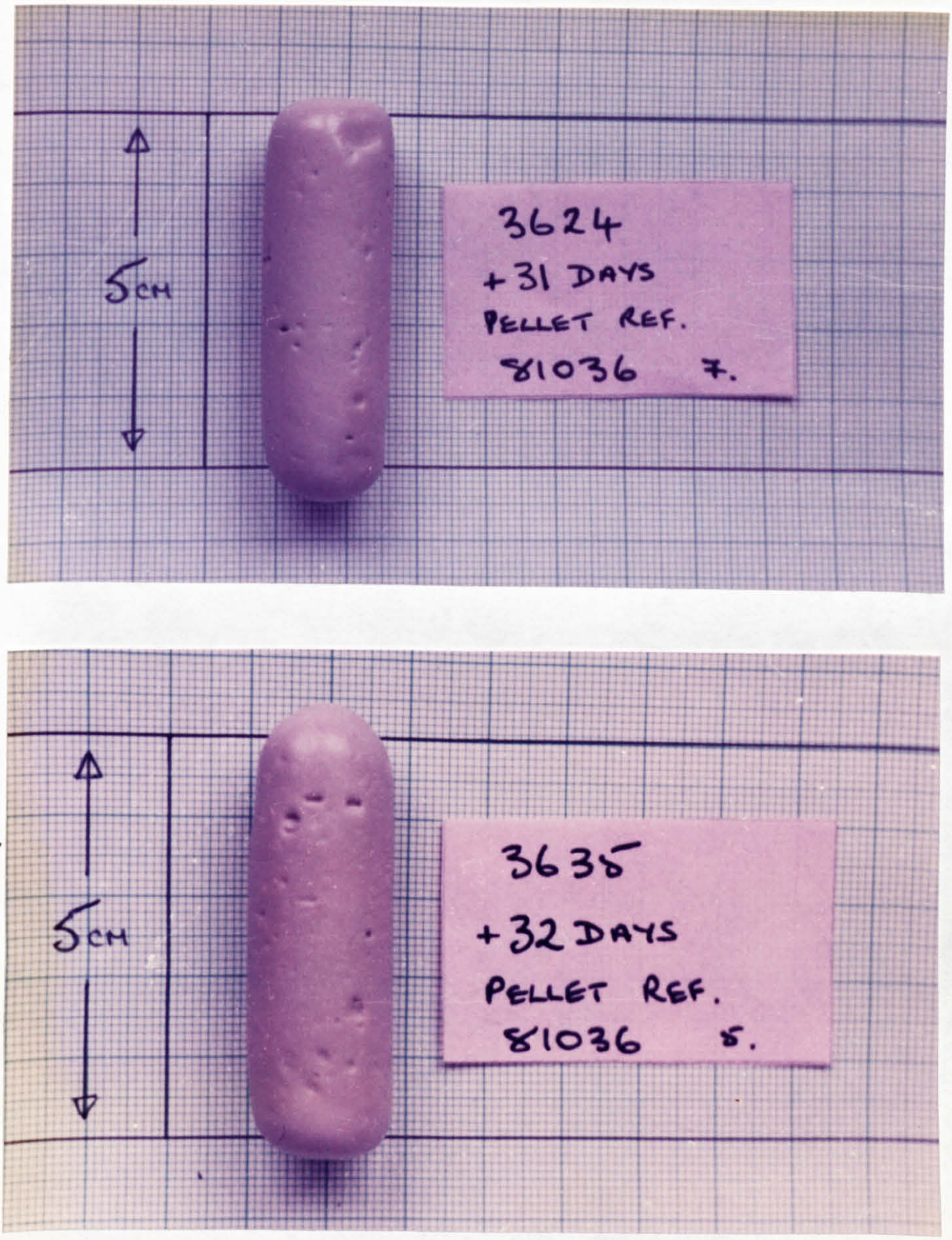


PLATE 15 : RECOVERY OF THREE BOLUSES DOSED TOGETHER

GROUP 2. Experiment 6.2.

55 per cent thiophanate

GROUP 1

TWO BOLUSES AFTER 32 DAYS

3617 — — • — —

3641 — — o — —

SINGLE BOLUS ——•——

CONTROL ——o——

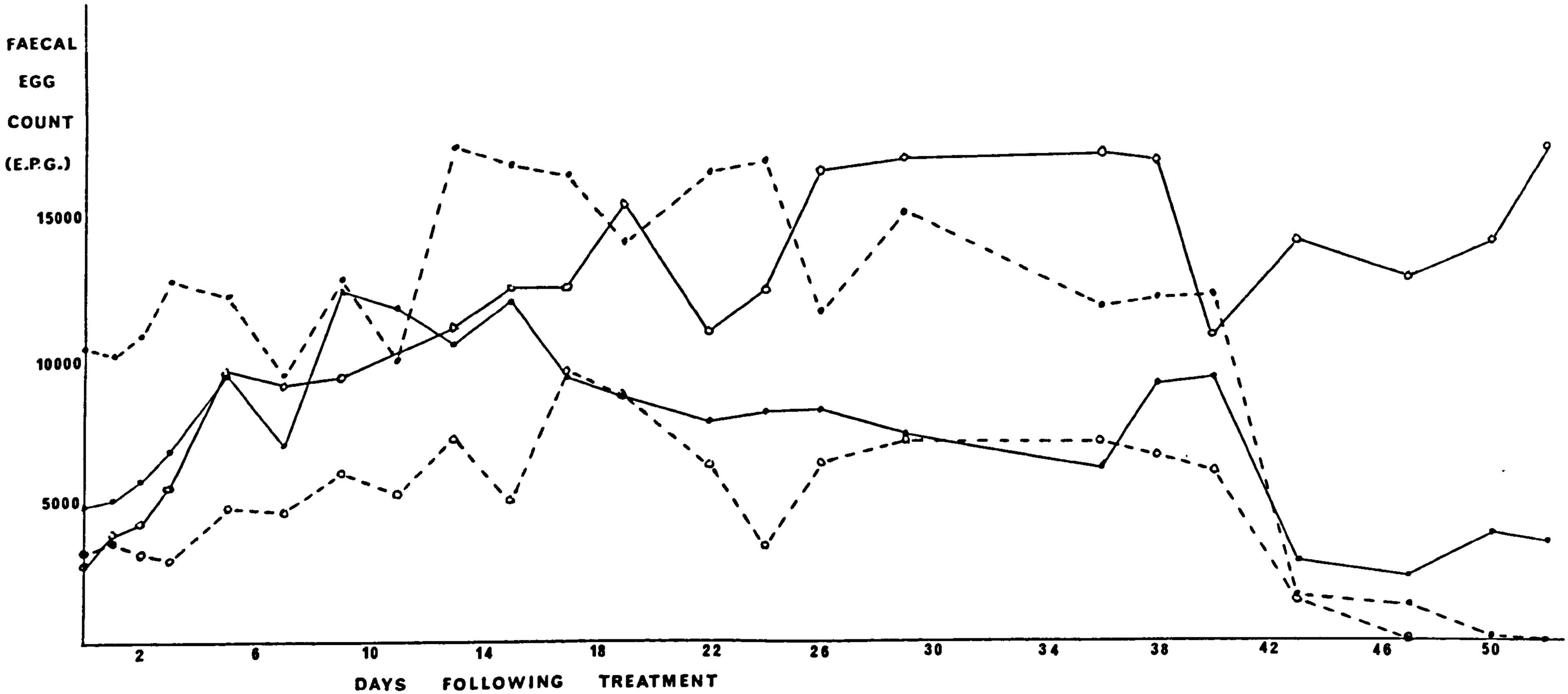


FIG. 9 NEMATODE EGG COUNTS EXPERIMENT 6.2

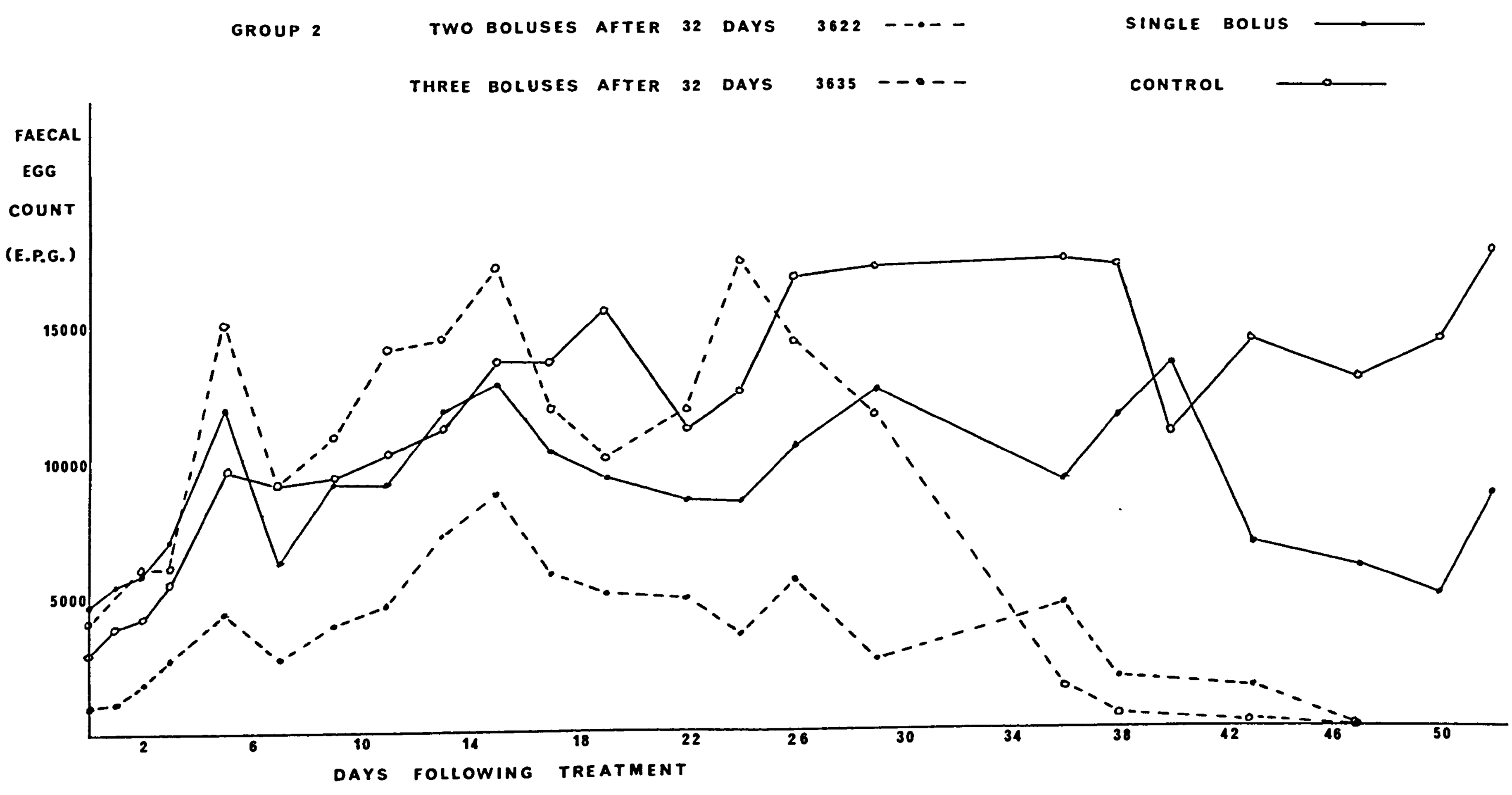


FIG.10 NEMATODE EGG COUNTS EXPERIMENT 6.2

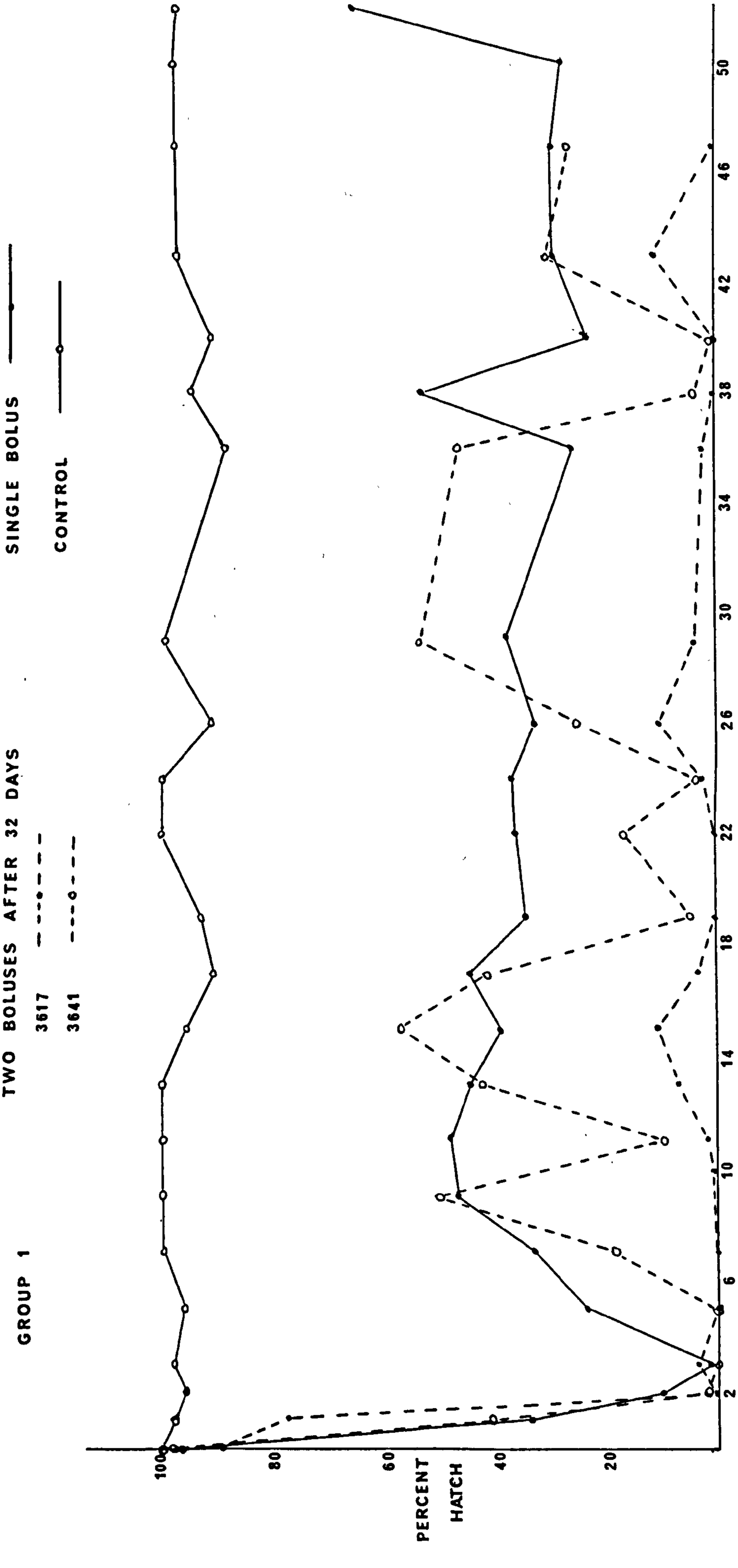

GROUP 1
TWO BOLUSES AFTER 32 DAYS
3617 ---•---
3641 ---o---
SINGLE BOLUS ——
CONTROL ——
PERCENT HATCH
100
80
60
40
20
2
6
10
14
18
22
26
30
34
38
42
46
50
DAYS FOLLOWING TREATMENT
PERCENTAGE EGG HATCH
EXPERIMENT 6.2

FIG. 11

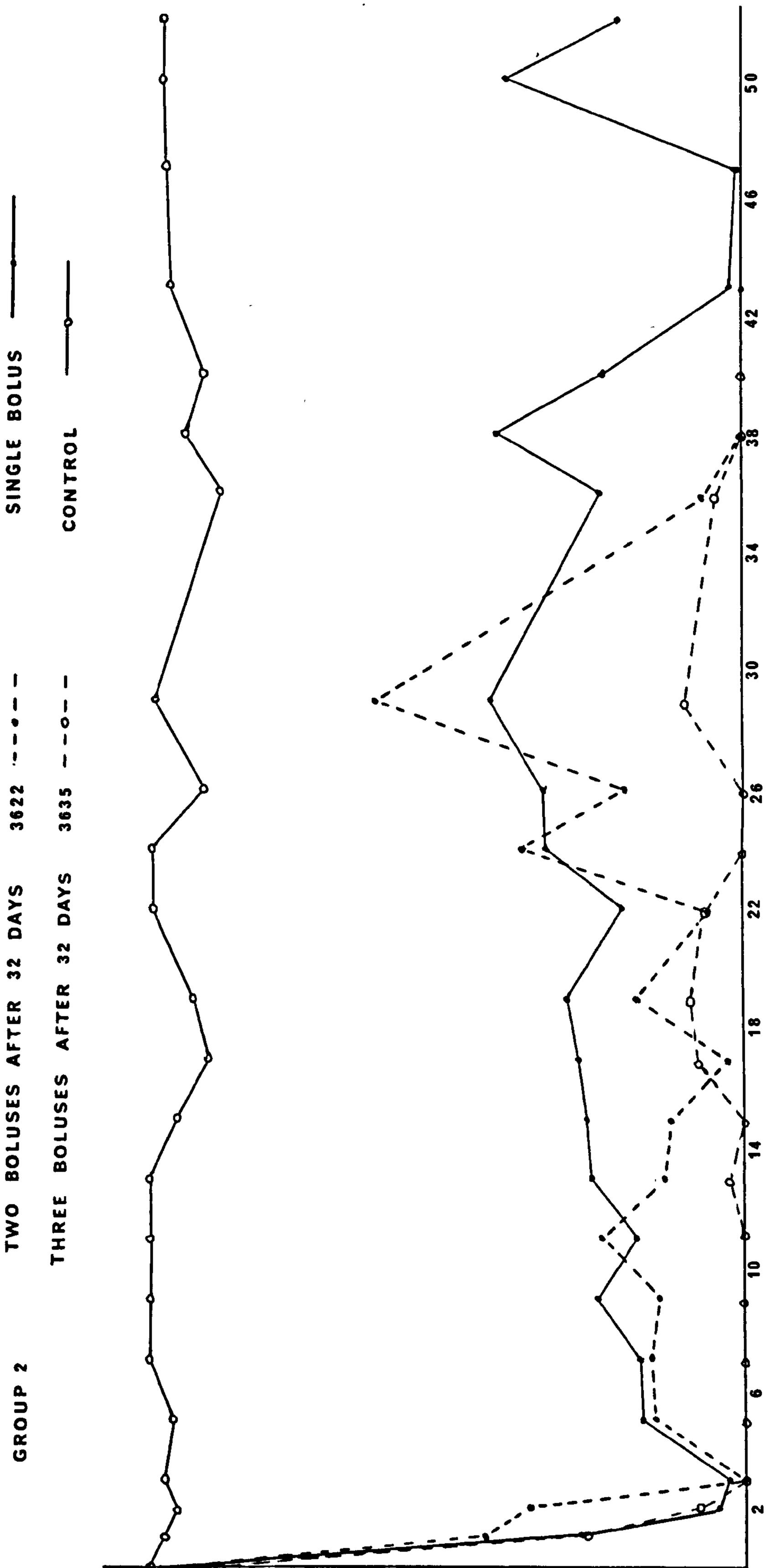

SINGLE BOLUS
CONTROL
TWO BOLUSES AFTER 32 DAYS 3622
THREE BOLUSES AFTER 32 DAYS 3635
GROUP 2
100
80
60
40
20
PERCENT HATCH
2
6
10
14
18
22
26
30
34
38
42
46
50
DAYS FOLLOWING TREATMENT
EXPERIMENT 6.2

FIG.12

6.3. Effect on the drug release rate of adding a releasing agent to the matrix.

To improve the release of drug from the bolus, two soluble releasing agents (cobalt sulphate heptahydrate and anhydrous glucose) were incorporated into a 50 : 50 :: wax : thiophanate matrix at levels of 1, 2, 4 or 8 per cent.

The bolus densities ranged from 2.25 to 2.43, the weights from 27.0 to 29.2 gms.

Lambs were dosed with a single bolus and rumenotomies were performed after 34 to 35 days. For a further 30 days, the boluses were replaced in pairs or kept as singles.

The results are summarised in Table 26.

No difference was recorded in the release rates during the first 34 to 35 days but a slight increase was observed from the paired boluses recovered after 63 to 64 days. As previously observed, the release of drug from the single boluses declined during the latter retention period.

An example of boluses from each group after recovery is illustrated in Plates 16 and 17.

TABLE 26 : Summary of the drug release rates achieved from boluses incorporating either cobalt sulphate heptahydrate (Group 1) or anhydrous glucose (Group 2).
Experiment 6.3.

Group	Per cent releasing agent	Lamb No.	Bolus weight (gms)	Recovery + 34/35 days					Recovery + 63/64 days				+ 34/35 - + 63/64 days		
				Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day		Weight (gms)	Loss (gms)	Mg/day	Mg thiophanate per day	Loss (gms)	Mg/day	Mg thiophanate per day
1	1	3599	27.0	25.51	1.49	43.8	21.5	Joined in lamb 3599	23.13	3.87	60.5	29.6	2.2	73.3	35.9
	2	3660	27.9	26.35	1.55	45.6	21.9		24.64	3.26	50.9	24.4	1.71	57.0	27.4
	4	1623	27.8	26.38	1.42	41.8	19.2	Joined in lamb 1623	24.05	3.75	58.6	26.9	2.33	77.7	35.7
	8	3640	27.5	25.64	1.86	54.7	23.0		23.11	4.39	68.6	28.8	2.53	84.3	35.4
2	1	3624	29.2	27.26	1.94	57.1	27.9	Joined in lamb 1661	25.39	3.81	60.5	29.6	1.87	64.5	31.6
	2	1661	28.15	26.25	1.9	55.9	26.8		23.37	4.78	75.9	36.4	2.88	99.3	47.7
	4	1662	27.7	26.13	1.57	44.8	20.6		25.17	2.53	40.1	18.5	0.96	34.3	15.8
	8	3612	28.0	26.39	1.61	46.0	19.3		25.65	2.35	37.3	15.7	0.74	26.4	11.1

PLATE 16 : BOLUS RECOVERY GROUP 1. Experiment 6.3.

Cobalt Sulphate Heptahydrate

1 per cent level

2 per cent level

PLATE 17 : BOLUS RECOVERY GROUP 2. Experiment 6.3.

Anhydrous glucose

1 per cent level

2 per cent level

6.4. Discussion

The first experiment (6.1.) again confirmed the previous results that the fatty acid blend was an unsuitable medium for a slow release bolus, complete disintegration occurring within a 30 day period (Table 21). A high percentage of the paraffin wax boluses were retained with improved erosion rates and this wax was therefore selected as preference. Even though 3 or 4 boluses were administered together and were also recovered together, the erosion rates and subsequent daily drug release levels did not reach the required level as evaluated in Section A, i.e. a minimum of 120 mg thiophanate per day. However, the drug level was increased and with a suitable formulation the use of multiple dosing to provide an extra erosive process to increase the drug release could be considered feasible.

In the second experiment (6.2.) where this concept was studied, the extra erosive process of the paired boluses was not apparent with the matrix used (Plates 13, 14 and 15). The individual drug release rates did not rise but the effect of multiple dosing on the total amount of drug continually received by the animal was reflected in the improved anthelmintic activity results (Figs. 9 - 12) and by the levels of excreted drug monitored. These indicated that with the right matrix, this method of medication could prevent a nematode infection. The higher percentage of drug incorporated in the bolus matrix of Group 2 gave a slight increase in the amount of drug released but the average bolus weight loss within the 2 groups was identical (56.8 and 56.5 mg per day) during the first 31/32 days. The sudden reduction in egg hatch that occurred during the first 3 days indicates an initial high drug release from the bolus. The increased weight of the iron bar core in this experiment improved the retention figures.

The releasing agents incorporated into the matrix in Experiment 6.3. appeared only to assist the drug release slightly after the boluses were paired, but there was no noticeable difference between the drug release rates observed in Experiments 6.2. and 6.3.

From the results observed in Experiment 6, it was concluded that paraffin wax provided a stable matrix within the rumen on which to base future studies. The problem of retention has been solved but the drug release rate is still inadequate.