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Studies on the pathophysiology

and immune response to

Ostertagia ostertagi in cattle

by

Quintin Archibald McKellar

A thesis submitted for the degree of Doctor of Philosophy

in the Faculty of Veterinary Medicine

of the University of Glasgow

Department of Veterinary Parasitology

September 1984

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Summary

SUMMARY

The studies reported in this thesis were undertaken to examine in detail several aspects of infection with Ostertagia ostertagi in cattle. The effect of age on the development of immunity was investigated and an attempt was made to immunise calves against O. ostertagi. More detailed work was concerned with the interaction of the parasite at the mucosal surface of the abomasum, together with the pathophysiological changes in the parasitised host.

Section I describes two experiments on the immune response of cattle to Ostertagia. The first was designed to ascertain whether immunity to ostertagiasis developed more quickly in 9-15 month old cattle than in calves. Although there was evidence of two manifestations of resistance, namely reduced worm size and fecundity, the study demonstrated that worm establishment was not markedly affected by the age of the host and suggested that natural age resistance is not significantly developed in 9-15 month old cattle.

In the second experiment, an attempt was made to immunise calves against O. ostertagi using a closely related parasite Ostertagia leptospicularis which has been shown to induce a marked reaction in the abomasum of calves. Two doses of either O. ostertagi, O. leptospicularis or a combination of O. ostertagi and O. leptospicularis were given to young calves prior to challenge with O. ostertagi but none of these immunising regimens conferred protection and similar challenge burdens were recovered from all of the calves at post mortem examination.

The major part of this thesis was concerned with studies on the biochemical and hormonal changes which occur in the host as a result of infection with larval and adult O. ostertagi and these are described in Section 2. In Experiment 3, calves were infected with 50,000 O. ostertagi L_3 and serum pepsinogen and gastrin levels measured; these rose markedly from day 15 after infection and peaked between days 20 and 30. Experiments 4, 5 and 6 were designed to investigate the role of the adult parasite in the pathophysiological changes in the host. Serum pepsinogen levels rose consistently when animals were exposed to adult parasites. However, serum gastrin levels only rose in response to large numbers of adult worms and the association of these changes with altered abomasal pH are discussed. Lesions were observed in the abomasa of calves which were infected with adult parasites only and a further experiment (Experiment 7) was carried out to determine whether the intimate association of the parasites with the mucosal surface was responsible for the pathological and physiological changes which took place. During this study, it was apparent that adult Ostertagia were quickly killed when restricted and suspended in chambers in the abomasum and it was then concluded that the parasites require the microenvironment close to the epithelium for their survival. No pathophysiological changes were observed probably due to the early mortality of the adult parasites in this experiment.

The two remaining experiments in Section 2 were designed to investigate changes in pepsinogen and gastrin levels which occur in naturally acquired Ostertagia infections in the field. Animals in Experiment 8 were kept for two seasons under typical

husbandry conditions and plasma pepsinogen and gastrin levels were recorded regularly. Values for pepsinogen and gastrin rose to two peaks as Type I and Type II ostertagiasis developed and subsequently fell as the animals recovered. The pepsinogen levels did not return to pre-infection values either during the pre-Type II period, or during the second grazing season when immunity had developed.

The aetiology of the raised pepsinogen values observed during the second grazing season were further investigated in Experiment 9. Immune adult dairy cows which had been housed for the winter period were challenged with Ostertagia larvae and blood was collected routinely for the estimation of serum pepsinogen and gastrin. Pepsinogen levels rose to about 3.0 i.U. Tyrosine within 3 days of challenge while gastrin levels remained at pre-infection levels. It was concluded that Ostertagia larvae may induce a hypersensitive response in the abomasa of immune cattle with a consequent leakage of pepsinogen into the blood.

General Introduction

INTRODUCTION

The nematode parasite Ostertagia ostertagi belongs to the family Trichostrongylidae which is included in the superfamily Trichostrongyloidea of the order Strongylida.

Ostertag described the parasite in 1890 and named it Strongylus convolutus. It has since been renamed Strongylus ostertagi Stiles, 1892 and S. cervicornis Gilruth, 1899. The present name was assigned by Ransom in 1907.

Following the first report on the pathogenic effects of O. ostertagi in cattle in the U.K. (Gardener, 1911) there have been a plethora of reports published on its significance and it is now regarded as the most pathogenic and economically important gastrointestinal parasitism of cattle in the temperate areas of the world (Armour, 1970).

Life Cycle

Eggs measuring 70-84 μ long and 40-50 μ wide are deposited by adult female O. ostertagi in the abomasum of the host (Threlkeld, 1946). These pass out in the faeces at the morula stage and hatch in 24 hours into active first stage larvae which feed on coliform bacteria in the faeces (Smyth, 1976). These larvae then undergo two moults within the dung pat to become infective third stage larvae. Preparasitic development is influenced by temperature, moisture and aeration (Rose, 1961). During the second ecdysis the cuticle of the second stage larva is retained (Taylor, 1930) and the third stage larva is thus

rendered highly resistant to conditions of drought and extremes of temperature. Embryonated eggs may survive for a short time under dry conditions but non-embryonated eggs and first and second stage larvae are extremely susceptible to desiccation (Rose, 1961).

Infective third stage larvae (L_3) display random movement which requires a water medium (Crofton, 1954) and the migration of these larvae onto herbage is favoured after rainfall which softens the faecal pats (Rose, 1961). Free living L_3 average 700 μ in length (Rose, 1969) have a small buccal cavity and a claviform oesophagus. The intestine is made up of 16 nucleated cells tapering posteriorly into a slender rectum. The excretory system is located in the anterior part of the body and an oval-shaped genital primordium can be seen.

Third stage trichostrongyloid larvae have been induced to exsheath in solutions of dissolved gaseous carbon dioxide at 38°C (Rogers, 1966). After ingestion L_3 are thought to exsheath in the rumen (Armour, 1970) but exsheathed larvae have been observed in ingesta collected from sheep fitted with an oesophageal fistula (Bairden, personal communication) and it may be that the mastication or salivation of the host also provides a stimulus for exsheathment.

Exsheathed L_3 have been demonstrated in the gastric glands of calves within six hours of oral infection (Osborne, Batte and Bell, 1960). Threlkeld (1946) necropsied animals at various times after oral administration of infective larvae and observed the length and morphology of the parasitic stages. In the course of this work he described two ecdysis within the host. The

parasitic stages of O. ostertagi have since been classified as third stage, third moult, fourth stage, fourth moult, fifth stage and adult (Douvres, 1956).

During a subsequent study on the development of the parasitic stages of O. ostertagi, Rose (1969) infected calves on one or two occasions with 20,000 O. ostertagi L_3. The calves were necropsied at intervals and the worms examined microscopically while alive and after fixation. Worms which were from one to 16 days old and mature adults which were 22 days old were described.

On the first day after oral infection, third stage larvae had lost their outer cuticle and by day 3 post-infection had undergone the third moult. At this stage the sexes could be differentiated by the distance of the genital primordium from the tip of the tail which ranges from 120-175 μ in the female and 250-380 μ in the male. The third ecdysis was complete by day 4 and development of the fourth stage larvae continued for 8 days. On the tenth day post infection the larvae were enclosed in a loose cuticular sheath and in the males the bursa with its supporting rays had formed and unpigmented spicules were present. In the females there were tubular uteri and the ovaries consisted of rounded cells. The pars haustrix and pars ejectrix of the ovejector had developed muscle fibres and the lips of the vulva were positioned around the vulval orifice.

The fourth ecdysis was completed by day 12 and by day 13 the vulval flap was formed in the young adult females. Immature ova were apparent in the anterior end of the uterus by day 14 and

these had matured and passed to the ovejector by day 16. In the males, spermatogenesis was complete by day 15.

The fifth stage larvae had triangular mouth parts with three lips and in both sexes there were cervical papillae. In the males there were in addition a pair of papillae anterior to the bursa.

Disease Classification and Clinical Signs

A series of field investigations into parasitic gastritis due to O. ostertagi in the West of Scotland led Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965b) to classify the syndrome into three phases; Type I, pre-Type II and Type II.

Type I ostertagiasis affected mainly calves on grass for the first time and usually occurred between late July and the end of the grazing season. It resulted from the ingestion and the maturation of infective larvae in the expected period of three weeks. Stewart and Crofton (1941) had previously described calves with parasitic gastritis as diarrhoeic, wasting and thirsty, with some loss of appetite and a dry hidebound skin. Anderson et al (1965b) observed that the diarrhoea was bright green in colour and that as the disease progressed, the eyes became sunken and the coat had a harsh staring appearance.

The pre-Type II phase when animals harboured large numbers of larvae arrested as early L_4 was not clinically evident but there was a history of animals grazing infected pasture in the late autumn. They generally appeared healthy but some were ill-thriven. During this stage, in many cases, large populations of over 100,000 O. ostertagi were present of which over 80% were

inhibited in the early fourth stage of development. Where the adult worm numbers are large, a mild diarrhoea and stunting has been reported (Armour, 1970).

Although the Type II syndrome occasionally occurred in out-wintered animals, it generally affected animals which had been housed at about the beginning of November often with no history of diarrhoea or weight loss during the grazing season. Three weeks to six months after housing the animals started to lose weight and developed profuse watery diarrhoea. The appearance of these clinical signs coincided with the development to maturity of large numbers of inhibited larvae. Animals suffering from Type II ostertagiasis were sometimes well grown but more often they were poorly grown and dull with a scurfy coat caked with faeces around the tail. Submandibular oedema was described in housed cattle with atypical parasitic gastritis by Martin, Thomas and Urquhart (1957). Anderson et al (1965b) noted that oedema was transitory and reported weight loss, decreased appetite, thirst and intermittent diarrhoea. In some cases moderate anaemia was present.

After a period of diarrhoea lasting 7-10 days, the majority of Type II cases became extremely weak, were unable to stand and had sunken eyes. Some animals stopped eating or drinking two to three days prior to death.

Pathology

Early work on the pathogenicity of O. ostertagi by Threlkeld and Johnson (1948) described inflammation of the abomasum and the

presence of small white ulcers on the mucosal surface associated with infection. Microscopically there was erosion of the glandular epithelium, extending from the orifice to the base of the gastric glands, which often contained coiled larvae. An increased amount of mucus was present and there was an increase in the number of tissue eosinophils. Pathological changes have been studied using different levels of infection (Osborne, Batte and Bell, 1960) and comparisons made between single and double infections (Ross, 1963). Also, detailed studies of the pathogenesis of O. ostertagi have been undertaken by Ross and Dow (1965b) and Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart (1966). In both of the later experiments calves were given a single dose of 100,000 O. ostertagi and necropsied at intervals thereafter.

The results of these studies showed that two days post-infection small white nodules, about 1 mm in diameter, were present in the abomasal mucosa and in the first foot of duodenum. The nodules had an umbilicated centre where the gastric gland containing the larva had become dilated. By day four, larvae had grown so that the nodules were 1-2 mm in diameter and the occupied glands protruded above the general mucosal surface. Nodules were most numerous on the fundic mucosa and where infection was severe, nodules coalesced giving the mucosa a corrugated (Ross and Dow, 1965b) or morrocco-leather (Ritchie et al, 1966) appearance. By day 21 most worms were found in the abomasal lumen. At this stage, cytolysis and sloughing of the superficial epithelial cells enhanced the umbilicated appearance

of the nodules. Where superficial mucosal erosions became confluent "thumbprint" lesions developed (Ritchie et al, 1966).

Histopathological changes were apparent from day 2 when the glands containing larvae became dilated. There was loss of differentiation of the parietal and zymogen cells which were replaced by irregular cuboidal epithelium. Some granulocytes and mononuclear cells were often seen between the cells of the occupied glands. Continued growth of the larvae between 8 and 16 days post infection caused the glands to lengthen and they became completely lined with mucous cells. There was increased cellularity of the lamina propria and stromal oedema was often present around the necks of the occupied glands. Most of the surrounding glands were still normal at day 16. As the young adults started to emerge around day 21, neighbouring glands began to lose much of their cellular differentiation and became lined by cuboidal cells while previously occupied glands were still easily recognisable by their high mucous cell lining. By day 28 few glands contained larvae and globule leukocytes became prominent, especially between the epithelial cells in the superficial areas of the glands. By 60 or 90 days post infection previously occupied glands could still be recognised by their mucous cell lining, but most of the epithelium was otherwise normal in appearance. Occasional globular leukocytes and foci of plasma cells remained.

Lesions in pre-Type II ostertagiasis are minimal, inhibited larvae occupying glands which become lined by undifferentiated epithelium (Anderson et al, 1965b). Changes are restricted to the occupied gland and there is no cytolysis or umbilication of

the area superficial to the larvae.

The pathology associated with the Type II disease relates to the sequential development of large numbers of previously inhibited larvae. All the changes seen in Type I ostertagiasis occur simultaneously and superficial diphtheresis and oedema are regular features (Anderson _et al_, 1965b).

In France a syndrome of oedematous ostertagiasis has been described in two to three year old cattle (Raynaud and Bouchet, 1976). The abomasum becomes very much enlarged and the abomasal folds show watery oedema. There may only be a few parasites associated with this condition and it has been suggested that it may be the result of an allergic reaction to the parasites.

Biochemical changes occur both locally in the abomasum and in the blood of calves suffering from ostertagiasis. Hydrochloric acid and pepsin concentrations of the abomasal contents are reduced (Ross, Todd and Dow, 1963) while the pepsinogen concentration in the blood becomes elevated (Anderson, Armour, Jennings and Ritchie, 1964).

Ross and Todd (1965) measured the pH and pepsin concentration of abomasal contents from calves with clinical and subclinical ostertagiasis: those calves most heavily infected had consistently high pH and low pepsin, while in those only moderately infected the changes were less marked. Experimental work by Jennings, Armour, Lawson and Roberts (1966) using calves fitted with abomasal cannulas, clearly demonstrated the sequential biochemical changes. In two calves infected with 300,000 _O. ostertagi_ larvae, plasma pepsinogen values stayed at

Pre-infection levels of approximately 0.4 i.U. until day 5 post-infection when they rose to approximately 1.0 i.U. No further change occurred until day 15 but they then rose rapidly to a peak of approximately 6.0 i.U. on day 22. In one surviving calf, plasma pepsinogen levels remained above 4.0 i.U. until necropsy at day 62. Also observed was a rapid rise in pH of the abomasal fluid to more than 7.0 between days 20 and 22 but this had returned to near normal in the surviving calf by day 61. The level of pepsin in the abomasal fluid fell after day 21 and fluctuated markedly at lower levels until the pH dropped to below 6.0. An increase in sodium and a decrease in potassium and chloride concentrations was also apparent between days 20 and 23.

The rise in plasma pepsinogen has been attributed to a loss of integrity of the junctions of the abomasal epithelial cells associated with the hyperplastic changes in the mucosa (Jennings et al, 1966). As the cells of the gastric glands become less well differentiated, parietal and zymogen cell secretion is reduced and the consequent rise in pH means that less pepsinogen is converted into pepsin. This pepsinogen is then allowed to leak into the circulation through the defective cell junctions. The elevated pH also allows the number of bacteria in the abomasum to increase which may be a factor in the development of diarrhoea (Jennings et al, 1966).

Hypoalbuminaemia (Martin et al, 1957) and raised gamma globulins (Andersen, Graff, Hammond, Fitzgerald and Miner, 1960) are consistent findings during ostertagiasis. The decrease in the serum albumin and increase in gamma globulins occur concurrently, approximately 3 weeks after experimental infection

(Mahrt, Hammond and Miner, 1964; Ross and Todd, 1965).

Using ^{131}I labelled polyvinyl pyrrolidone (PVP), Mulligan, Dalton and Anderson (1963) were able to demonstrate that, after intravenous injection, a much higher percentage of the radioiodinated polymer appeared in the faeces of clinical cases of ostertagiasis than in the faeces of unaffected controls. They also demonstrated a shorter half life of labelled serum albumin in clinical cases and concluded that there was a loss of albumin into the gastrointestinal tract. Increased albumin catabolism has also been demonstrated in naturally occuring cases of parasitic gastroenteritis (Cornelius, Baker, Kaneko and Douglas, 1962) and in Type II ostertagiasis (Halliday, Mulligan and Dalton, 1968).

The precise mechanism underlying the hypoalbuminaemia has been the subject of some controversy and although associated with a leakage of albumin into the gastrointestinal tract by Halliday et al (1968), Stringfellow and Madden (1979) refuted this "leak" theory, because they could not demonstrate any rise in the level of albumin in the abomasa of calves infected with 250,000 O. ostertagi L_3 between 22-30 days after infection. Using horseradish peroxidase as a tracer for vascular leakage, they obtained similar values in the abomasal contents of uninfected and infected calves.

Epidemiology

Host and environmental factors contributing to the propagation of O. ostertagi and to the development of clinical

ostertagiasis in cattle have been extensively investigated.

Seasonal patterns in pasture availability of O. ostertagi in the U.K. were described by Anderson, Armour, Jennings, Ritchie and Urquhart (1969) and Michel (1969a). Anderson et al (1969) assessed the intake and fate of infective larvae in "tracer" and "permanent" calves on farms where ostertagiasis was endemic, while Michel (1969a) used pasture larval counts to demonstrate the seasonal pattern of infestation on the herbage. In temperate areas it is known that the number of infective larvae recovered from herbage in the spring falls as a result of both the adverse effect of changes in temperature on larval survival and the dilution effect of grass growth. Calves turned out for the first time in May, therefore, usually ingest relatively few overwintered L_3 but occasionally the herbage contamination in the spring may be sufficient to cause clinical Type I ostertagiasis (Taylor, Cawthorne, Kenny and Regan, 1973; Tharaldsen, 1976). Infections acquired at this time become patent in 3 weeks and eggs deposited on the pasture towards the end of May together with any eggs which may have overwintered take 4-5 weeks to develop into infective larvae. The time taken for development from egg to L_3 decreases as the season advances and by the second week in July this development may be complete within as little as one week (Rose, 1970). Large numbers of infective larvae may therefore be present by early July and provided conditions suitable for their migration from the dung pat to the herbage prevail (Rose, 1962), clinical disease may develop in susceptible calves by the end of July.

Calves in their first season at grass, which usually only experience a light infection in the spring, are not immune, and often develop clinical Type I ostertagiasis in the late summer. During August and September, larvae reaching the herbage are derived from eggs recently deposited but these do not normally cause any further rise in herbage infestation only replacing larvae which have died or been ingested. Towards the end of the season, mortality of pre-infective stages is high and eggs deposited on pasture after the middle of September do not develop into infective larvae (Michel, 1969a). This study suggested that most infective larvae on herbage between July and the spring of the following year were derived from eggs passed before July and that in the U.K., O. ostertagi generally only completes one generation each year. Where the climate is warm and humid, however, it is likely that several generations occur (Donald, 1964).

It is now known that many larvae ingested in the autumn become inhibited in their development. Early reports described the presence of fourth stage larvae in the abomasum weeks or months after cattle were last exposed to infection (Porter and Cauthen, 1946; Threlkeld and Johnson, 1948; Martin et al, 1957). Where many early L_4 accumulate without obvious clinical signs, Anderson et al (1965b) classified the condition as pre-Type II ostertagiasis. This is most likely to arise after a dry summer followed by rain in autumn which allows a build up of L_3 on pasture at a time when they will be climatically conditioned to inhibit (Armour, 1970; Chiejina and Clegg, 1978). Pre-Type II ostertagiasis is the necessary prelude to the Type II disease

which develops when sufficient numbers of inhibited larvae in the abomasum resume their development to the adult stage en masse.

The occurrence of clinical ostertagiasis depends upon the existence of a suitable infecting mass on the pasture and the susceptibility of the grazing stock (Armour, 1980). Clinical Type I ostertagiasis is most often seen in segregated dairy or dairy-cross calves grazing permanent calf paddocks at high stocking rates (Armour, 1970).

Gastrointestinal nematode infections in single suckled beef calves have been investigated by Michel, Lancaster and Hong (1972) who showed that the occurrence of significant pasture L_3 infestations depended on the season at which the calves were born. Autumn and winter born calves turned out with their dams in the spring can acquire infection from overwintered larvae. While they graze with their mothers however contamination of the pasture is diluted by the relatively clean faeces of the adults but when the calves are weaned in the summer and grazed alone there may be a build up of potentially dangerous pasture infestation. Spring and summer born calves on the other hand do not begin to graze until overwintered pasture infestation has declined to a low level and only a light herbage infestation is likely to result. Although a slight rise in faecal egg output of cows at parturition has been observed (Corticelli and Lai, 1960) this is not considered an important source of pasture larval infection.

Pasture contamination from sources other than the direct deposition of Ostertagia eggs in infected faeces has been

reported. For example Downey and Moore (1977) found trichostrongylid eggs in slurry from weanling cattle throughout the storage period and where this slurry was spread on fields, herbage larval counts were higher than on control plots. These continued to be higher until the following spring and during the grazing season tracer calves picked up seven times as many Ostertagia and Cooperia worms as those grazed in the control plots. A clinical outbreak of parasitic gastroenteritis occurred in early June on the plot contaminated with slurry.

There is less risk of recycling nematode parasites with slurry produced by older immune cattle and where straw or sawdust are used to compost manure, aerobic microbial breakdown elevates the temperature to 60-70°C and thus reduces larval survival (Persson, 1974). Covering of liquid manure tanks for 5 months in the winter and 2 months in the summer before disposal, together with aeration by bubbling air through the tanks and the addition of lime, have also been recommended (Nansen and Jorgensen, 1977).

Outbreaks of bovine ostertagiasis have been reported on silage aftermath in fields not grazed since the previous autumn (Bairden, Parkins and Armour, 1979). Emergence of overwintered O. ostertagi L_3 from the soil were considered to be the most likely source of infection and later the presence of such larvae in soil was confirmed (Armour, Al Saqur, Bairden, Duncan and Urquhart, 1980). It was suggested that these had either migrated to the soil surface independently or with the aid of transport hosts such as dung beetles or earthworms. Fincher (1973) demonstrated the remarkable ability of the dung beetle to bury faeces, while Gronvold (1979) recovered viable O. ostertagi L_3

from the gut of earthworms and pointed out that where these worms were eaten by predators there could be horizontal and between farm dissemination of larvae as well as vertical migration through the soil.

The role of dipteran flies (Jacobs, Tod, Dunn and Walker, 1968) and the sporangiophores of Pilobolus (Robinson, 1962) in the dissemination of the nematodes responsible for porcine oesophagostomiasis and bovine dictyocauliasis respectively have been investigated but their significance in the epidemiology of ostertagiasis is unknown.

Inhibition

Inhibited or arrested larval development has been defined as the temporary cessation of development of a nematode at a precise point in its early parasitic life. Inhibition of O. ostertagi generally occurs at the early fourth larval stage during periods when environmental conditions are unsuitable for the survival or development of the free living stages.

A number of factors have been considered important in the development of inhibition including host resistance and physiological changes in the parasite.

Martin et al (1957) speculated that a massive challenge of infective larvae over a short period of time could cause their inhibition. Ross (1963) on the other hand demonstrated that an initial infection with the parasite considerably increased the numbers of retarded fourth stages from a second larval infection while Michel (1963) related the accumulation of inhibited larvae to changes in the turnover of the adult worm burden in the

abomasum.

A physiological change in the host or the parasite was first suggested as a cause of inhibition by Anderson, Armour, Jennings, Ritchie and Urquhart (1965a) and later confirmed by Armour, Jennings and Urquhart (1969a). Using tracer calves they demonstrated a ten-fold increase in the number of larvae inhibited at the early fourth stage in calves which grazed in late autumn, compared with those grazed during summer. In the animals grazed in the autumn approximately 70% of the total worm burden consisted of inhibited L_4 and it was shown that the percentage inhibition was independent of either the number of days grazed or the magnitude of the burden established. It was also shown that inhibition was not transient, similar proportions of early fourth stage larvae being recovered from calves necropsied 4 and 27 days after removal from pasture. In a subsequent study (Armour, Jennings and Urquhart, 1969b) paddocks contaminated with either a laboratory culture of O. ostertagi L_3 or with L_3 derived from a natural field infection, were grazed in late autumn by tracer calves. At necropsy a higher proportion of inhibited larvae were found in the calves which had grazed on the paddocks contaminated with the field strain. In addition, few inhibited larvae were found at necropsy of calves grazing parasite free paddocks which were orally infected with the laboratory strain of O. ostertagi.

The combination of innate strain susceptibility and environmental circumstances of late autumn were considered responsible for larval inhibition and a phenomenon comparable to

diapause in insects was suggested. The environmental trigger for inhibition was further investigated by Armour (1970) using larvae conditioned in a climatic chamber. The results suggested that a percentage of the population of O. ostertagi were prone to inhibit while others were not and that the relative availability of the inhibition-prone larvae increased during the winter, but fell in the spring due to ingestion, selective mortality or hatching of fresh larvae from overwintered eggs. These results were in agreement with earlier work by Armour, Jennings and Urquhart (1967) and with the suggestion of Sollod (1967) but later Michel, Lancaster and Hong (1974) failed to demonstrate any change in the relative numbers of inhibition-prone larvae on pasture. Recent work by Michel, Lancaster and Hong (1979) has demonstrated that age and previous experience of infection can also be significant in increasing inhibition. For example, in previously infected 20 month-old cattle 86% of the worms resulting from a single experimental challenge infection were inhibited as early fourth stage larvae. The possibility therefore exists for the accumulation of inhibited larvae at times of the year other than autumn.

In parts of Australia (Hotson, 1967) and America (Craig, 1979) O. ostertagi larvae which are picked up in the spring become inhibited. Michel (1974) suggested that the change in time of onset of inhibition is due to the operation of a different selection pressure, possibly the reduced survival of worms reproducing during the hot dry summer.

Maturation of inhibited larvae has been observed by Armour and Bruce (1974), in calves experimentally infected with larvae

conditioned by chilling, 16-18 weeks after the onset of inhibition, and by Michel, Lancaster and Hong (1976) in naturally infected calves during March and April. Under natural grazing conditions resumption of development coincides with the end of winter in the United Kingdom, when conditions would be suitable for the development of free living stages. Resumed development of inhibited O. ostertagi 'en masse' is responsible for the development of clinical Type II ostertagiasis (Martin et al, 1957; Anderson et al, 1965b) and this in turn may result in increased contamination of pastures during the late winter and spring if the cattle are outwintered (Kistner, Wyse and Averkin, 1979).

The mechanism underlying resumption of development is still unclear. The work of Armour and Bruce (1974) indicates a spontaneous and synchronous development resembling diapause in insects while Michel (1974) suggests that a regular turnover of inhibited O. ostertagi proceeds in the host, regulated by the number of adults present: when large numbers of fourth stage larvae develop in April it is evident that the population of adult worms is turned over rapidly.

Changing hormonal status of the host may trigger the resumption of larval development. Improved performance has been observed in cattle treated with a combination of luteinising hormone and anthelmintic (Cawley and Lewis, 1975). There is, however, doubt whether the improvement reflected a reduction in worm burden. Treatment with pregnant mares serum, luteinising hormone and stilboestrol did not render inhibited larvae

susceptible to anthelmintics in trials by Armour, Jennings, Reid and Selman (1975) nor did the luteinising hormone-like activity of human chorionic gonadotrophin (Cummins and Callinan,1979).

A rise in circulating oestrogens in adult cows near parturition has been associated with relaxation of immunity and maturation of inhibited larvae (Wedderburn, 1972), however it appears unlikely that a depression of immunity alone is responsible for the maturation of inhibited fourth stage larvae, since Armour (1967) and Prichard, Donald and Hennessy (1974) could not demonstrate resumed development of inhibited larvae in corticosteroid treated calves.

Host Resistance

An effective immunity to infection with O. ostertagi develops slowly. It has been shown that calves exposed to natural infection at pasture are still susceptible to challenge mid-way through their first grazing season (Ross and Dow, 1965a) while prolonged daily exposure over five to eight months duration whether by natural acquisition (Ross and Dow, 1965a) or by experimental infection (Michel, Lancaster and Hong, 1973) confers protection.

Michel et al (1973) infected calves with 1000 O. ostertagi L_3 daily for 250 days then challenged with 30,000 L_3. Ninety-five percent fewer worms established in the previously infected calves than in uninfected controls.

Armour (1967) demonstrated that immunity, established after the first grazing season, wanes during the winter housing and observations on young cattle grazing over two seasons (Armour,

Bairden, Duncan, Jennings and Parkins, 1979) suggest that small infections become established early in the second grazing period, which may be significant in the epidemiology of ostertagiasis. There is no marked age immunity in ostertagiasis and outbreaks of acute (Selman, Reid, Armour and Jennings, 1976) and chronic (Cawley and Lewis, 1976) disease have been reported in older cattle. Yearlings are however better able to withstand the effects of gastrointestinal parasitism than younger animals and reduced establishment, stunting of worms and lower egg outputs have been reported in 15 month-old yearlings compared with three month-old calves exposed to mixed trichostrongyle infections (Smith, 1970).

Immunity to Ostertagia is acquired more rapidly in adults than in calves (Michel et al, 1979). This, however, may be lost in heifers during early lactation and outbreaks of clinical Type II ostertagiasis have been reported in cows and heifers at parturition (Wedderburn, 1970; Petrie, Armour and Stevenson, 1984). Previously infected adult cattle have been shown to acquire large burdens of inhibited larvae (Michel et al, 1979) and although the mechanism which stimulates their maturation is at present unknown it may be associated with a relaxation of immunity around calving: a rise in oestrogens during the late stages of parturition have been incriminated (Wedderburn, 1972).

Attempts to immunise against O. ostertagi (including the use of irradiated larvae) have met with little success (Armour, 1967; Burger, Eckert,Chevalier, Rahman and Konigsmann, 1968). It has been shown that various regimens of single or multiple oral doses

of larvae may elicit resistance in that reduced establishment, lower fecundity and morphological changes were present in challenge inoculations, but no significant protection against the pathogenic effects of ostertagiasis was evident (Herlich, 1976).

Herlich and Douvres (1979) inoculated calves, intraperitoneally with parasitic larvae grown *in vitro*, and intraperitoneally and intravenously with exoantigens of *O. ostertagi* but could demonstrate no immunity to subsequent oral challenge with L_3.

The mechanisms of resistance to ostertagiasis remain unknown. Treatment with cortisone has been shown to influence the length of worms and the incidence of females without vulvar flaps (Michel and Sinclair, 1969). Female worms in cortisone treated calves tended to be longer and had better developed vulvar flaps than those recovered from untreated control calves. In cortisone treated calves, faecal egg counts remained higher and worm expulsion was slower than in untreated controls and although corticosteroids do not appear to affect the development of inhibition (Michel and Sinclair, 1969) or the redevelopment of inhibited larvae (Prichard, Donald and Hennessy, 1974), they may inhibit the natural rejection of emergent larvae. Although elevated levels of serum IgG, IgM and IgA have been demonstrated in ostertagiasis (Jensen and Nansen, 1978), the relative importance of humoral and cell mediated components of the immune response has not yet been determined.

Treatment and Control

A wide range of anthelmintics are available for the

treatment of Type I ostertagiasis and the most commonly used belong to four chemical groupings i.e. the benzimidazoles, probenzimidazoles, imidazothiazoles and avermectins (Armour and Bogan, 1982). Of these only the less soluble benzimidazoles, which remain longer in the gut lumen at effective concentrations, and the avermectins are effective against inhibited larvae.

The uptake of anthelmintic by inhibited larvae was originally thought to be restricted by their reduced metabolic rate (Armour, 1967) but more recent in vivo observations on the incorporation of ^{14}C-thiabendazole by adult worms and inhibited fourth stage larvae have indicated that the uptake by inhibited larvae is not reduced (Prichard, Donald, Dash and Hennessy, 1978). Rather it seems that the duration of exposure effects the anthelmintic tolerance of inhibited O. ostertagi suggesting that inhibited larvae can withstand a temporary energy deprivation as a result of treatment but not a sustained one (Prichard et al, 1978).

The benzimidazoles of proven efficacy against inhibited larvae include fenbendazole (Duncan, Armour and Bairden, 1978), oxfendazole (Armour, Duncan and Reid, 1978; Kistner et al, 1979) and albendazole (Williams, Knox, Sheehan and Fuselier, 1977; Downey, 1978). However, variations in efficacy of fenbendazole (Elliott, 1977; Lancaster and Hong, 1977; Lancaster, Hong and Michel, 1981; Williams, Knox, Baumann, Snider and Hoerner, 1981b) and albendazole (Williams, Knox, Baumann, Snider and Hoerner, 1981a) have been reported.

Duncan, Armour, Bairden, Jennings and Urquhart (1977)

suggested several factors which could account for the conflicting data. These include seasonal differences in the depth of inhibition, the aetiology of the inhibited development and the route and rate of passage through the upper alimentary tract. By treating cattle with pre-Type II ostertagiasis both in the autumn and in winter Duncan et al (1978) were able to demonstrate that the efficacy of fenbendazole was not affected by seasonality. Although the oesophageal groove reflex may divert orally administered anthelmintics directly into the abomasum in a proportion of animals (McEwan and Oakley, 1978) it seems unlikely that rumen bypass greatly affects drug efficacy (Anderson,1979; Marriner and Bogan, 1979). However it is apparent that the susceptibility of inhibited burdens of Ostertagia to fenbendazole can vary (Lancaster et al, 1981) but the reasons for this variation remain unexplained.

Recently a new group of anti-parasitic drugs, the avermectins have been produced. These are the fermentation metabolites of the actinomycete, Streptomyces avermitilis (Burg, Miller, Baker, Birnbaum, Currie, Hartman, Kong, Monaghan, Olson, Putter, Tunac, Wallick, Stapley, Oiwa and Omura, 1979). The natural B_{1A} component, ivermectin, has more than 90% efficacy against all stages of Ostertagia as well as broad spectrum activity against other cattle parasites (Armour, Bairden and Preston, 1980; Elliott and Julian, 1981; Yazwinski, Greenway and Williams, 1981).

In general, control measures are designed to prevent the occurrence of disease, complete eradication of O. ostertagi being impossible (Michel, 1969b); the majority of control programmes

are also designed to allow sufficient exposure of young stock to *Ostertagia* infection to allow the acquisition of immunity.

Michel(1967a) used the seasonal pattern of herbage infectivity to devise a control system commonly referred to as the Weybridge 'dose and move' system. This relies on the fact that herbage larval levels are relatively low until July. Before the annual rise in pasture larvae which generally occurs in late July the animals are moved to aftermath or to pasture not grazed by other cattle that year. Where this move is accompanied by anthelmintic treatment it is unlikely that the new pasture will become heavily contaminated.

Unfortunately the dose and move system does not always allow the most economic utilisation of available herbage and other control systems have been developed. These include rotational grazing, alternate grazing with different hosts and integrated rotational grazing with different groups of cattle of varying immune status. Simple rotational grazing has proven to be unsuccessful (Levine and Clark, 1961; Michel, 1969b)mainly because the animals have ultimately returned to graze paddocks which they had earlier contaminated. The annual alternation of sheep and cattle depends on a low degree of cross contamination of parasites between hosts and has been shown to provide good control (Southcott and Barger, 1975). Where grassland management is suitable another area may be set aside for crops such as hay or silage thus extending the rotation to three years (Rutter, 1975).

Integrated rotational grazing systems have been shown to

improve the productivity of young susceptible stock (Leaver, 1970; Nagle, Brophy, Caffrey and O'Nuallain, 1980). Basically susceptible animals are grazed in rotation ahead of immune adult cattle. Thus calves consume the upper relatively uncontaminated echelons of the herbage while the immune animals graze the lower more heavily contaminated strata.

Where young stock must be set stocked for management reasons, anthelmintic treatment on several occasions in the early part of the grazing season has been shown to reduce pasture contamination later in the year (Armour, 1978). Such treatment regimens may be superseded with the advent of sustained release devices which provide the daily release of anthelmintic for the first 2-3 months of the grazing season. In the U.K. for example the administration of a morantel device once during spring obviates the need for further control of gastrointestinal parasites during that grazing season (Anderson, Laby, Prichard and Hennessy, 1980; Armour, Bairden, Duncan, Jones and Bliss, 1981; Jacobs, Fox, Walker, Jones and Bliss, 1981; Jones, 1981).

The work reported in this thesis was undertaken to examine in detail several aspects of infection with _O. ostertagi_ in cattle. The effect of age on the immune status was investigated and an attempt was made to immunise calves against _O. ostertagi_ using a related parasite _Ostertagia leptospicularis_. The major part of the work however is concerned with the interaction of the parasite at the mucosal surface of the abomasum. Consequent biochemical and hormonal changes within the host are studied and compared in parasite naive and previously exposed animals.

General Material and Methods

GENERAL MATERIALS AND METHODS

Animals

All animals were born and reared indoors. Friesian and Ayrshire calves, approximately 4 months of age, were used in all the experiments with the exception of Experiment 1 when parasite-naive Friesian cattle aged 9-15 months old were obtained from the West of Scotland Agricultural College, Auchincruive, Ayrshire, and Experiment 9 when adult dairy cows from a commercial herd were used. During indoor trials, the animals were housed in concrete pens which were cleaned thoroughly three times per week and were bedded with fresh straw daily. 1-3 Kg concentrates (Calf rearing pencils, BOCM Silcock) were offered daily together with hay and water _ad lib._

Weighing Procedure

A calf crate (Precision Weighers, Reading, England) was used to weigh the smaller animals and a cattle weighing tape (Dalton Supplies, Nettlebed, Henley-on-Thames, Oxon, England) used to estimate the weight of larger animals.

Necropsy Technique

Animals were starved for 24 hours before slaughter. A captive bolt pistol was used to shoot the larger calves, and the smaller calves were killed by intravenous injection of pentobarbitone sodium*.

* ['Euthatal' - May and Baker Ltd, Dagenham]

The abdomen was opened and a ligature tied at the abomasal/duodenal junction before the abomasum was separated and removed together with the omasum.

Abomasum

After careful removal of the omasum, the abomasum was opened along its greater curvature and the contents collected. A 30 ml sample was taken for estimation of pH and sections of mucosa were taken for histology. The mucosal surface was then washed under slow running water and the contents made up to 4 litres. After thorough mixing, duplicate 200 ml samples were transferred into labelled containers, to which 10 ml of 40% formalin was added. The abomasal mucosa was scraped off with a boning knife, weighed and incubated for 6 hours with a pepsin-hydrochloric acid mixture at 42°C. The digest was then made up to 4 litres with cold water and duplicate 200 ml samples taken and formalised as above.

The pepsin-hydrochloric acid solution was made up by dissolving 80 grams of pepsin A, (British Drug Houses, Poole, Dorset, England) in 4 litres of lukewarm water, 250 ml concentrated HCl was added and the total volume made up to 8 litres with water.

Worm Counting and Identification

A few drops of iodine (45% solution) were added to the samples of abomasal washing or digests. After thorough mixing, 4 ml was withdrawn using a wide bore pipette and transferred to a lined petri dish. The sample was then decolourised with sodium thiosulphate solution and the parasites counted and identified

under a dissecting microscope (Model M.5., Wild, Heerbrugg, Switzerland). Ten 4 ml aliquots were counted and classified as adult male or female parasites, developing larval stages or early fourth stage larvae (L4). The extent of development in the bursal or vulvar regions and the presence of a sheath as well as size characteristics were used for larval differentiation.

Worm Measurement

One hundred adult worms of mixed sex were withdrawn from the duplicate 200 ml samples of abomasal contents or digests of each animal. The worms were mounted in glycerin jelly and projected onto the glass screen of a Projectina microscope (Projectina Co. Ltd, Heerbrugg, Switzerland) using the X 10 objective. The image of each worm was drawn onto tracing paper and measured using a drafting map measurer (H.B., Paris, France). The result was divided by 10 to give the length of the worm in centimetres.

Blood

Collection and Storage

Blood samples were taken from the jugular vein. A 20 gauge 1 inch needle and vacutainer holder (Becton-Dickinson, Rutherford, New Jersey, U.S.A.) were used and blood collected in an evacuated 15 ml test tube without coagulant. Tubes were stored overnight at room temperature and then the clot was removed using fine forceps. The serum was subsequently centrifuged at 3000 rpm for 15 minutes in a MSE Mistral centrifuge (Measuring Scientific Equipment, London, England) and

decanted into flat bottomed plastic tubes (Luckham Ltd, Victoria Gardens, Sussex). These were stored at -20°C.

In Experiment 8, blood was collected in 10 ml heparinised vacutainer tubes (Lithium Heparin 143 U.S.P. units). These were centrifuged without delay at 3000 rpm for 30 minutes and plasma was stored as above.

Serum Pepsinogen

Edwards, Jepson and Wood (1960) described a technique for the estimation of plasma pepsinogen in man. A similar method was used to measure serum pepsinogen levels in experimental animals. Serum was incubated at 37°C with bovine serum albumin substrate (Fraction V, Sigma Chemical Co., Kingston-upon-Thames, England) adjusted to pH 2. After 24 hours, protein was precipitated with 4% trichloroacetic acid (TCA) and the liberated phenolic amine acids (tyrosine) non-precipitable with TCA were estimated with Folin-Ciocalteau reagent (British Drug Houses, Poole, England). Readings were carried out using a spectrophotometer (Unicam, Cambridge, England). Corrections were made for the release of tyrosine from bovine serum albumin when incubated alone. The enzyme activity was expressed as international units (i.U.) Tyrosine (μ mols tyrosine released per 1000 ml serum per minute). An identical procedure was used for the estimation of plasma pepsinogen levels in Experiment 8.

Gastrin Assay

Gastrin levels in serum were assayed using a commercially available diagnostic kit (^{125}I Gastrin Radioimmunoassay Kit,

Cambridge Medical Diagnostics Inc., Mass., U.S.A.). The gastrin assay utilised ^{125}I labelled gastrin and a specific anti-gastrin antiserum to determine the levels of gastrin in serum specimens by the double antibody/polyethylene glycol precipitation technique.

The principle of the procedure is outlined in the kit protocol and is based on the ability of a limited quantity of antibody to bind a fixed amount of radiolabelled antigen and the inhibition of this reaction by an unlabelled antigen. The percentage of bound radiolabelled antigen decreases as a function of the increasing concentration of unlabelled antigen in the test sample. Separation of the bound and free radiolabelled antigen is necessary in order to determine the quantity of unlabelled antigen. This is accomplished by insolubilization of the antigen-antibody complexes by a combination of chemical means e.g. polyethylene glycol precipitation and by the addition of a second antibody directed towards the immunoglobulin present in the original antiserum. The quantity of unlabelled antigen in an unknown sample is then determined by comparing the radioactivity of the precipitate after centrifugation with values established using known standards in the same assay system.

The reagents and assay procedure used in this thesis are briefly outlined. Plasma was used instead of serum for the test samples in Experiment 8.

^{125}I Gastrin Radioimmunoassay

Reagents:

^{125}I Gastrin; CNR-115:

^{125}I-labelled human synthetic gastrin and non-immune rabbit serum. Supplied in lyophilised form. Contains approximately 2.0 μCi ^{125}I.

Precipitating Reagents; CNR-645:

Goat anti-rabbit gamma globulin and 2% polyethylene glycol in borate buffer containing preservative.

Gastrin (human synthetic) Standard; CNR-315:

Synthetic human gastrin in Assay Buffer, at the following approximate concentrations: 50, 100, 175, 300, 500 and 1000 pg/ml.

Gastrin Controls; CNR-515 and CNR-516:

Human synthetic gastrin in human plasma plus preservative supplied in lyophilised form.

Reconstitution of Lyophilised Reagents

The vial was tapped gently to dislodge any large particles that were trapped on the stopper or the side of the vial and then the rubber stopper was carefully removed and placed inverted onto a clean surface. The appropriate reconstitution volume was carefully added to each vial, the stopper replaced and the solid matter allowed to dissolve at room temperature for 20 minutes.

After 20 minutes the vial was inverted gently a few times to wash off any particles that may have adhered to the septum, then

the vial was allowed to stand for a few more minutes to achieve a complete solution. After visually checking for complete reconstitution, the vial was gently swirled to obtain a homogenous solution. Vigorous agitation and foaming was avoided.

Assay Procedure

The standard level points and samples were run in duplicate. A standard curve was run with each set of clinical specimens.

The capped reagent bottles and vials were brought to room temperature (18–30°C) before use and returned to recommended storage conditions immediately thereafter to minimize deterioration.

a. 12 x 75 mm plastic tubes were labelled as follows:
   Total counts (TC), tubes 1 and 2
   Non specific binding (NSB), tubes 3 and 4
   Maximum binding (B_o), tubes 5 and 6
   Standard (A to F), tubes 7 to 18
   Control (Low and High), tubes 19 to 22
   Starting with tube 23, each pair of sample test tubes were numbered consecutively.

b. 200 µl of assay buffer was added to the NSB tubes, numbers 3 and 4.

c. 100 µl of assay buffer was added to the B_o tubes, numbers 5 and 6.

d. 100 µl of gastrin standards (A to F), controls low and high and test samples were added to the appropriate tubes.

e. 100 µl ^{125}I gastrin solution was added to all tubes.

f. 100 µl gastrin antiserum was added to all tubes except TC and NSB tubes.

g. All the tubes were shaken gently within the test tube rack, covered and incubated for 60 minutes at room temperature.

h. After shaking the bottle of precipitating reagent well to ensure an even suspension, 1 ml of reagent was added to all tubes except the TC tubes.

i. Tubes were mixed (Vortex mixer) and incubated for 10 minutes at room temperature.

j. The tubes were then centrifuged at 1500 g for 10 minutes.

k. The supernatant of each tube (except the TC tubes) was decanted very carefully to avoid dislodging the pellet and the rim of each tube blotted.

l. The radioactivity in all tubes was counted for 30 seconds. This was sufficient to remove counting statistics as an important source of variability.

Calculation of Results

1. Determine the average counts for each set of duplicate assay tubes.
2. Subtract the average NSB counts (tubes 3 and 4) from the average B_o, standards, controls and test samples to determine the average net counts for each sample.
3. Divide the average net B_o counts (tubes 5 and 6) into the average net counts of the standards, controls and test samples. Multiply this number by 100 to yield the % B/B_o for each sample.

$$\%B/B_o = \frac{cpm_X - cpm_{NSB}}{cpm_{Bo} - cpm_{NSB}} \times 100$$

4. Plot $\%B/B_o$ for each standard versus the standard concentrations in pg/ml on semi-log graph paper. The concentration of gastrin in the test and control samples may be read directly from this standard curve.

Faecal Egg Counting Techinque

Faecal samples collected per rectum were examined by a modified McMaster technique (Gordon and Whitlock, 1939). Three grams of faeces were homogenised in 42 ml of water and the suspension passed through a 250 micron sieve (Endecotts Ltd, London, England). After mixing, 15 ml of the filtrate was withdrawn into a flat bottomed tube and centrifuged for three minutes at 2000 revolutions per minute (rpm). The supernatant was poured off and the pellet agitated (Vortex Jr. Mixer, Scientific Industries Inc., Queens Village, New York, U.S.A.). The sediment was resuspended in saturated salt (NaCl) solution, inverted six times and sufficient suspension to fill both chambers transferred by pipette to a McMaster worm egg counting slide (Gelman Hawksley Ltd, Harrowden, Northampton). The number of eggs under both grids (volume 0.15 ml) were counted and the result multiplied by 50 to give the number of eggs per gram.

In Experiment 9 where negative results were obtained by the modified McMaster technique, the remainder of the saturated salt solution, containing the resuspended faecal sediment was topped up until a positive meniscus formed and a coverslip placed on

top. The coverslip was removed after two minutes, care being taken to hold it horizontally. Eggs which had risen in the salt solution and stuck onto the coverslip could now be counted under the microscope.

Preparation of Larval Inocula

Experimentally infected male calves with positive faecal egg counts 21 days post infection were fitted with a harness and collecting bag. Faeces recovered twice daily were mixed with vermiculite and approximately 100 g samples placed in screw top honey jars which were then incubated at 23°C for 14 days. In order to harvest the larvae, the jars were filled with lukewarm water and left for 2-3 hours to allow larvae to migrate into the water. The jars were then emptied through a course sieve, which retained the faecal debris, and the filtrates pooled. This suspension was then passed through a Buchner apparatus, larvae being retained by two 8 inch milk filters (Maxa Filters, A. McCaskie, Stirling). Larvae were recovered from the filters using a Baerman apparatus which consisted of a glass funnel closed at the neck with rubber tubing and a clip, filled with lukewarm tap water. The filters were supported by a metal sieve (150 microns) placed on the wide end of the funnel just touching the surface of the water. Motile larvae which migrated into the water were collected from the neck of the funnel after 6 hours. For all counting procedures the suspension was thoroughly agitated to reduce clumping of larvae.

An estimate of the larval concentration was made by counting 40 X 0.025 ml aliquots (1 ml) of the suspension. A minimum of

400 larvae were counted and to facilitate counting the suspension was so diluted that the number of L_3 per 0.025 ml did not exceed 30. When the number of larvae per ml was known, the volume necessary to provide the required inoculum could be calculated. Individual doses were then pipetted out and made up to a standard 20 ml with tap water.

Abomasal Cannulae

The abomasal cannulae were made of solid plastic (Polypenco), similar in design to those used by Armour (1967), except that they had a wider base which was either flat or gutter shaped (Fig.I and Fig.II).

Insertion of Cannula

Calves were starved for 24 hours prior to surgery. Under general anaesthesia with pentobarbitone sodium B.P. (Vet.) 60 mg/ml*, the cannula was inserted in the greater curvature of the abomasum as near as possible to the fundic-pyloric junction and exteriorised on the right side approximately 8 cm from the last rib (Fig.III and Fig.IV). Calves were treated with procaine penicillin** for three days after surgery.

* [Sagatal, May and Baker Ltd, Dagenham, England]

** [Duphapen, Duphar Veterinary Ltd, Southampton, England]

Fig. I Assembled plastic cannula

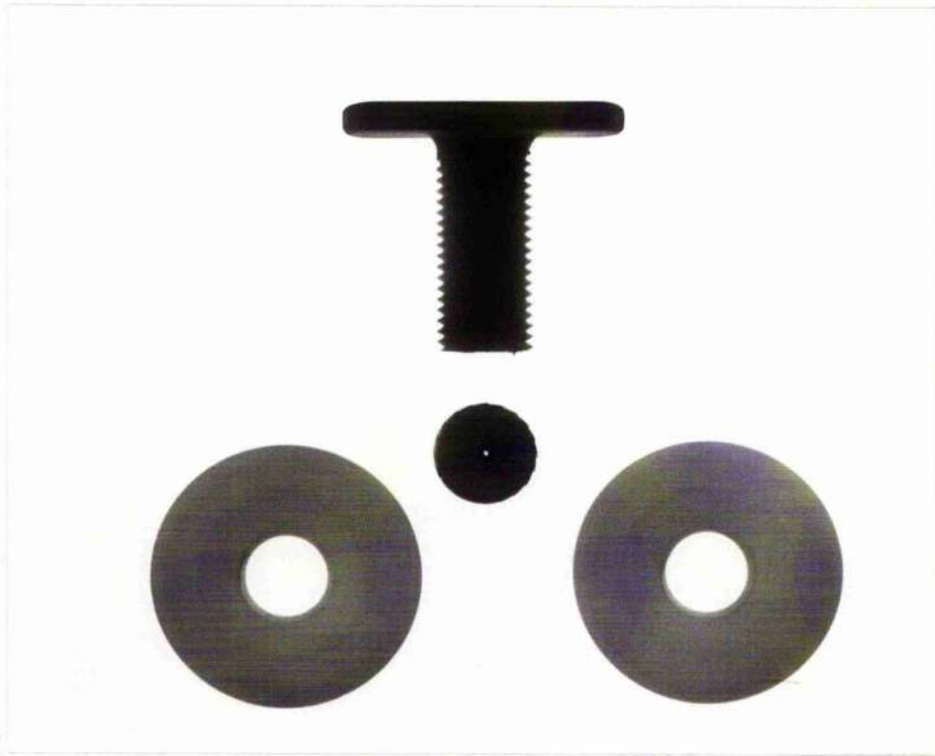

Fig. II Components of plastic cannula

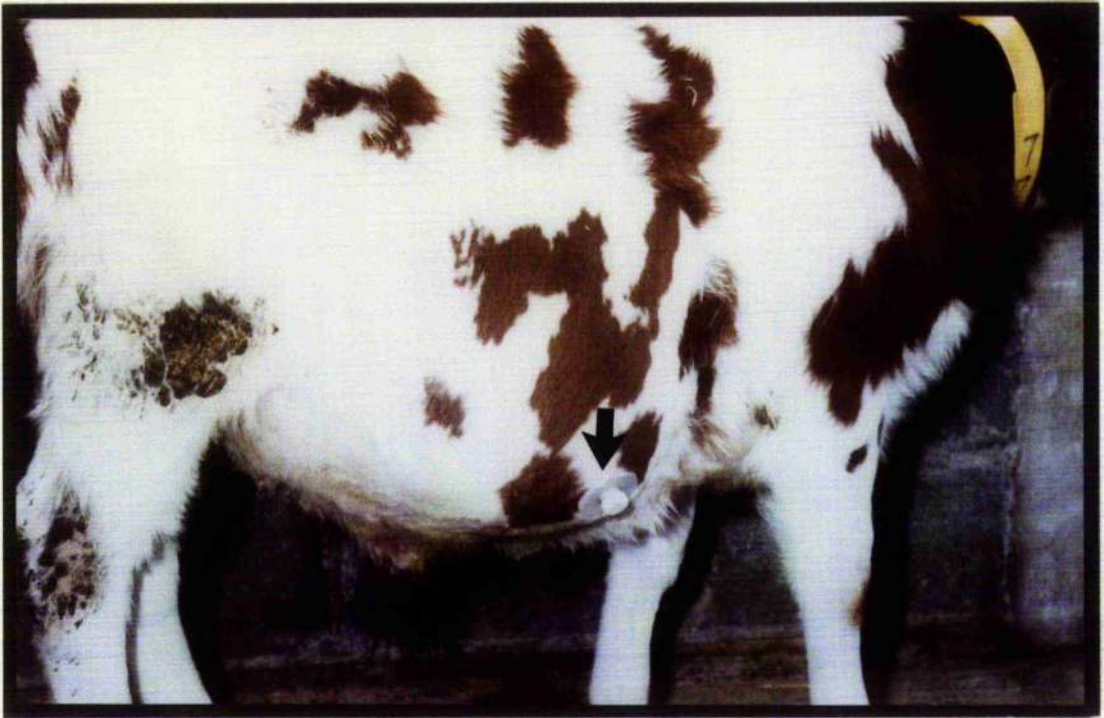

Fig. 111 Cannula in situ

Fig. IV Close up-cannula in calf

Collection of Samples

A wide bore plastic pipette 18 cm long was inserted into the cannula to facilitate collection of abomasal fluid. Withdrawal of the pippette and replacement of the cannula cap required considerable care, to avoid excessive loss of fluid. Samples were taken in plastic containers.

For endoscopy and biopsy procedures, a rubber stopper was inserted into the cannula, reducing the external diameter such that there was minimal loss of abomasal fluid.

Histological Methods

Collection of Samples

Biopsy material was taken using a flexible cable with serrated scissor attachment, through the appropriate aperture of a fibre optic endoscope (Pentax, Asahi Optical Co., Japan) or was taken blind using a solid biopsy instrument (Stort, Germany).

At post mortem examination abomasal sections were taken from the fundic and pyloric areas of all animals.

Preparation of Sections

Tissue samples were fixed in either 10% formalin for routine microscopic study of the histological structure and eosinophils, or in Carnoy's fluid for mast cells and mucus. Sections were transferred from Carnoy's fluid into 70% alcohol after six hours. Formalin-fixed tissues were processed in a standard 24 hour histokinette cycle and vacuum embedded in paraffin wax. Sections cut at 6 μ were examined after staining with haematoxylin and

eosin. Carnoy-fixed tissues were dehydrated and cleared in 3 changes each of 100% methanol and xylene at 4°C and vacuum embedded in wax. Sections cut at 6 μ were examined after staining with astra blue/safrannin O and alcian blue/periodic acid schiff (PAS).

pH of Abomasal Contents

The pH of abomasal contents was determined using a pH meter (Radiometer pH meter type PHM 266, Electronic Measuring Instruments Ltd, Copenhagan, Denmark). All the measurements were made within 10 minutes of removal of the fluid from the abomasum and the meter was regularly checked against standard phosphate buffer pH 6.5.

Pepsin Concentration

Abomasal contents were centrifuged for 15 minutes at 15,000 g and the supernatant fluid removed for analysis of peptic activity.

Pepsin concentration was estimated by incubation for 2 hours (37°C) of 0.2 ml abomasal contents with 5.0 ml of 2% bovine serum albumin substrate previously adjusted to pH 2.0 with 2N HCl. After precipitation with 2.0 ml 40% trichloroacetic acid, the liberated tyrosine was reacted with Folin-Ciocalteau's reagent and estimated with a spectrophotometer (Unicam SP600) at 680 mu. Results were expressed in international units tyrosine (μ mols tyrosine released per 1000 ml serum per minute).

Collection of Adult Parasites

Donor calves were starved for 24 hours prior to necropsy. Calves were killed by intravenous injection of pentobarbitone sodium. The abdomen was immediately opened and a ligature tied around the abomasal/duodenal junction. The abomasum was removed together with the omasum and was placed in a warm bucket where it was separated from the omasum. The greater curvature was opened and the mucosal surface washed under a gentle stream of warmed phosphate buffered saline (PBS) each litre of which had been supplemented with 2 grams of glucose. The contents were then made up to 2 litres with warm PBS and gently agitated prior to removal of 200 ml for the estimation of worm burden. The remainder was divided evenly and transferred into two recipient calves (Fig.V).

Anthelmintics

Fenbendazole 10% w/v solution (Panacur 10% suspension, Hoechst U.K., Middlesex, England) administered orally or intrarumenally at a dosage rate of 7.5 mg/kg body weight.

Thiabendazole 17.6% w/v solution (Thiabenzole suspension, MSD AGVET Division of Merck, Sharp and Dohme Ltd., Hertfordshire, England) administered orally at a dosage rate of 66 mg/kg body weight.

Levamisole Hydrochloride 1.5% w/v solution (Nilverm, Imperial Chemical Industries plc, Cheshire England) administered orally at a dosage rate of 7.5 mg/kg body weight.

Diethylcarbamazine Citrate 40% w/v solution (Caritrol, May and Baker Ltd., Dagenham, Essex, England) administered by

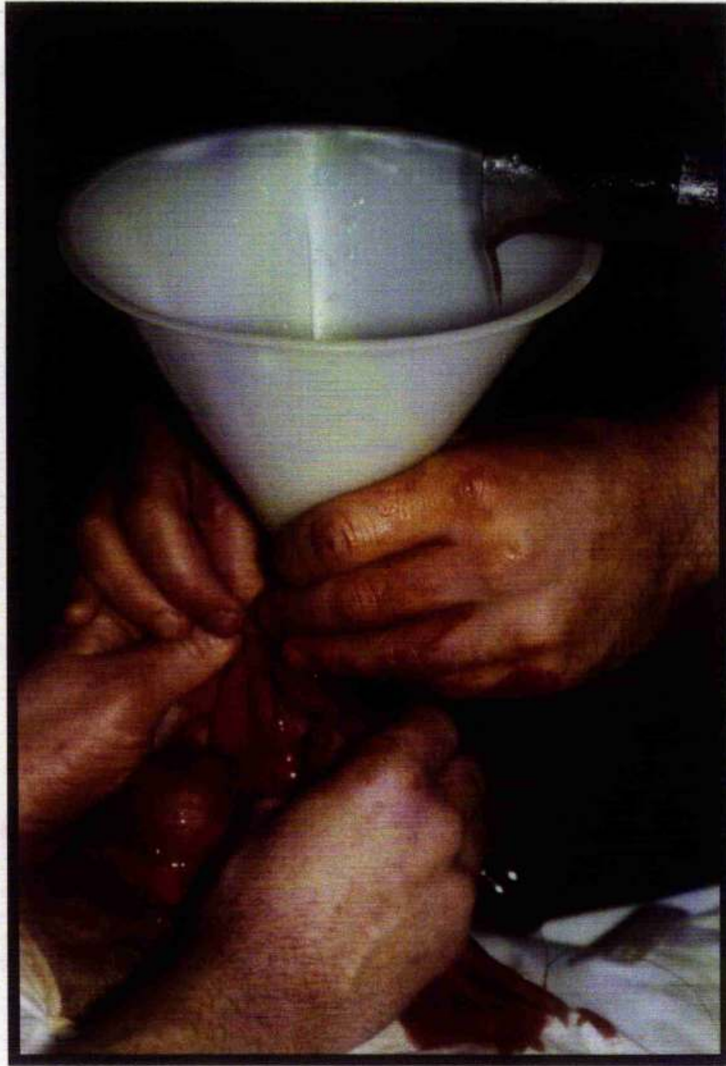

Fig. V Transfer of adult parasites into recipient calves

intramuscular injection at a dosage rate of 20 mg/kg body weight for three successive days.

Section 1

Some aspects of Immunity to *Ostertagia ostertagi* infection in cattle

INTRODUCTION

Resistance to infection by nematodes of the gastrointestinal tract is poorly understood. Most of the recent information on immunity to such infections has come from the study of laboratory host-parasite systems and there are a number of reviews of interest on the subject (Soulsby, 1957; Urquhart, Jarrett and Mulligan, 1962; Kelly, 1973; Ogilvie and Jones, 1973; Wakelin, 1978; Befus and Bienenstock, 1982). From these it is apparent that immune phenomena in helminth infections are extremely complex, and that an absolute sterile immunity to reinfection is rare. Natural resistance to certain infections however may be observed in animals which have not been previously exposed. For example, where a species of parasite is ingested by an incompatible host it is unlikely to establish (Miller, 1978b). Also age resistance to infection, which may reflect biochemical or metabolic incompatibilities between host and parasite, has been demonstrated in lambs which became refractive to the effects of infection with Nematodirus spp. when more that six months old (Gibson, 1959) and in adult dogs, resistant to Ancylostoma caninum (Miller, 1965). Parasite contact often stimulates a non-specific inflammatory process which represents a major component of the hosts natural defence mechanism thus an acute inflammatory response to a gastrointestinal nematode is likely to result in changes in the gut mucosa which will be deleterious to the establishment of the parasite.

Acquired immunity to most gastrointestinal parasites requires exposure to live parasites undergoing at least part of their life cycle. Both humoral and cell mediated arms of the immune system are thought to be involved and these may act on the parasite independently or in concert.

Passive immunity has been demonstrated by the transfer of immune serum in certain infections including Haemonchus contortus in sheep (Luisenko,1956) but such immunity is generally weaker than that stimulated by active infection. In other host parasite systems, such as Trichostrongylus colubriformis in the guinea pig, no such passive protection could be demonstrated (Wagland and Dineen, 1965). Although raised IgM immunoglobulin levels have been reported in many host parasite systems including sheep infected with Ostertagia circumcincta (Anderson, Curtain, Johnson and Simons, 1972 - cited Kelly, 1973) there is little evidence to suggest that these immunoglobulins are important in the defence of the host. However IgG antibody fractions from immune sera have been shown to confer protection when passively transferred to rats infected with Nippostrongylus brasiliensis (Jones, Edwards and Ogilvie, 1970). While reaginic antibodies, both specific and non-specific, are commonly produced in response to intestinal nematode infections (Sadun, 1972), a protective role has yet to be demonstrated for IgE (Jarrett, 1973). It is known that specific IgE mediates mast cell and basophil degranulation in the presence of parasite antigens resulting in the release of vasoactive amines (Wilson and Bloch, 1968) and it is possible that this may play an important part in the "self cure" phenomenon observed in some gastrointestinal nematode infections

such as *H. contortus* in sheep (Stoll, 1929).

Similarly, although IgA is the major immunoglobulin of mucosal secretions a functional role for IgA in immunity to nematode infection has still to be clearly established (Befus and Bienenstock, 1982).

There have been a number of reports on the importance of cell mediated immunity in resistance to nematodiases. Protective immunity against *T. colubriformis* has been demonstrated in guinea pigs with the transfer of immune mesenteric lymph node cells (Wagland and Dineen, 1965); also in guinea pigs, combined neonatal thymectomy and long-term lymph drainage has resulted in reduced resistance to *T. colubriformis* infection (Dineen and Adams, 1970). A number of other studies have shown that T cells are involved in the immune response of sheep to *O. circumcincta* (Anderson *et al*, 1972 - cited Kelly, 1973) and of rats to *N. brasiliensis* (Kelly and Dineen, 1972).

Non-lymphoid effector cells have also been implicated in worm expulsion. The release of vasoactive substances by mast cells in the intestine cause increased permeability of the mucosa (Murray, Jarrett and Jennings, 1971) and this may have an effect on the worms (Dineen and Kelly, 1973), either by rendering the environment unsuitable for parasite survival (Mulligan, Urquhart, Jennings and Neilson, 1965) or by allowing specific antiworm antibodies to reach the worms (Barth, Jarrett and Urquhart, 1966). The release of enzymes by eosinophils, which have been shown to attach to the surface of intestinal nematodes (McLaren, Mackenzie and Ramalho-Pinto, 1977), may also affect worm

establishment and expulsion.

Host resistance may affect parasite populations in a number of ways. For example a spectacular response has been observed in sheep infected with H. contortus when an existing adult worm burden is rapidly expelled in response to a superimposed larval challenge (Stewart, 1950). However there is also evidence to suggest that this expulsion or self cure may occur independently of larval challenge and may be mediated by dietary factors (Allonby and Urquhart, 1973). Whether larval and dietary stimuli trigger a common pathway resulting in expulsion of Haemonchus adults remains to be proven.

Less dramatically a reduction in the number of parasites becoming established has been observed in cattle immune to Dictyocaulus viviparus (Michel, 1962) and retardation of development has been demonstrated in a calf given repeated infections of O. ostertagi over a nine month period (Threlkeld and Johnson, 1948). Also morphological characteristics of some parasites may be affected by host immunity. In the case of O. ostertagi the development of the vulval flap is greatly reduced in resistant animals (Michel,1967b). Where acquired immunity to certain nematodes has developed, the biotic potential of mature female worms may be affected and suppression of egg production has been noted in the case of O. ostertagi infection in cattle (Michel, 1963) and in Nematodirus spathiger in sheep (Donald, Dineen, Turner and Wagland, 1964).

The present experiments were designed to study aspects of immunity to O. ostertagi in cattle.

EXPERIMENT 1

Studies on the development of immunity to O. ostertagi in young cattle.

Introduction

Acquired resistance has been shown to develop to many gastrointestinal parasites of cattle during their first 18 months at pasture and has been shown to develop more quickly to Cooperia than to Ostertagia (Roberts, O'Sullivan and Riek, 1952; Ross and Dow, 1965a). Clinical ostertagiasis has however been observed in adult cattle. Most of the reports describe a syndrome similar to Type II ostertagiasis of young stock (Hotson, 1967; Wedderburn, 1970; Selman et al, 1976) but there have been a number of outbreaks attributed to the development of parasites within the normal three week period (Andrews, Jones and Sippel, 1953; Bailey and Herlich, 1953; Beckland, 1962; Smith and Jones, 1962). It is unlikely, in view of these reports, that an effective age immunity operates in O. ostertagi infection.

A number of conflicting reports have been published on the effect of age on experimental infection with O. ostertagi. Herlich (1960) failed to demonstrate any change in prepatent period, worm size or egg output in worm free cattle of 18-25 months old experimentally infected with Ostertagia. The same author has since suggested that cattle up to two years old are equally susceptible to infection and reinfection with O. ostertagi in so far as establishment of worms is concerned, but that two year old cattle are better able to withstand the pathogenic effects of the parasites (Herlich, 1980). In contrast

stunting of worms, with inhibition of egg production and reduced faecal egg counts, were demonstrated after Ostertagia infection in 15 month old cattle compared with 3-4 month old calves (Smith and Archibald, 1968; Smith, 1970). In cattle more than 30 months old, a reduced clinical response to experimental Ostertagia infection was observed and attributed to a retardation in development, since the parasites had not commenced emergence by day 28 after infection (Armour, 1967). More recently, resistance has been shown to develop more quickly in 20 month old heifers than in young calves and this was unaffected by pregnancy. However a lowered resistance to infection was observed during lactation (Michel et al, 1979) and it was also demonstrated that age alone and previous experience could cause significant inhibition of an experimental infection. Worms which did not become inhibited grew more rapidly in calves and lactating heifers than in empty heifers or those in mid-pregnancy.

The object of this experiment was to investigate the effect of the age of the host on resistance to ostertagiasis, and to see whether previous infection with two doses of 50,000 O.ostertagia L_3 affected the establishment of a challenge infection in older cattle.

Experimental Design

Six Friesian cattle aged between 9 and 15 months were divided by weight into two Groups, 1 and 2. A third Group consisted of two worm-free Friesian calves aged 5 months (Group 3).

Animals in Group 1 were given two oral inoculations of 50,000 O. ostertagi L_3 28 days apart. Twenty-one days after the second dose of larvae all eight experimental animals were treated with fenbendazole and eight days later challenged with 100,000 O. ostertagi L_3. All animals were necropsied 21 days after challenge.

Observations

Animals were examined daily throughout. Faeces and serum samples were obtained twice weekly at which time animals were weighed. At necropsy the abomasa were processed as described previously: abomasal worm burdens and pH were recorded. A representative sample of 100 worms from each animal were mounted and measured and the egg numbers in the female worms were recorded.

Statistical Methods

Data were analysed using one-way analysis of varience. In the event of a significant difference ($p < 0.05$) further analyses were undertaken using the two sample t-test.

Results

The average weight of animals in each group increased throughout the experiment (Appendix 1a) and none of the animals became clinically ill although the two smaller calves of Group 3 passed soft faeces on the two days prior to necropsy.

Egg Counts

Individual faecal egg counts are shown in Appendix 1b. In Group 1, low faecal egg counts were recorded from two animals on day 9 and one animal on day 13 after the first inoculation of 50,000 O. ostertagi L_3. From day 22-43 after the first infection, positive counts were recorded from all three animals but individual counts never rose above 300 epg and returned to zero two days after fenbendazole treatment. Egg counts remained negative in all groups after challenge until the day before necropsy when low positive counts were recorded from Groups 2 and 3, Group 1 remaining negative.

Worm Burdens

The O. ostertagi worm counts for the individual animals are shown in Table 1a.

Small numbers of immature worms were present in the abomasa of both the older groups of animals. There were no immature stages found in the calves of Group 3. The mean worm burdens of all three groups were not significantly different and represented 33.3%, 37.5% and 42.8% of the challenge dose in Groups 1, 2 and 3 respectively.

Table 1a

Post mortem worm burdens in three groups of animals 21 days after challenge with 100,000 _O. ostertagi_ L_3.

Group	Animal No.	Fourth Larval Stage	Fourth Moult	Adults	Total
Group 1	21	800	0	23,600	24,400
	37	0	200	39,000	39,200
	55	0	3000	33,200	36,200
Mean					33,267
Group 2	56	0	0	38,400	38,400
	57	400	2600	38,800	41,800
	59	0	3200	29,000	32,200
Mean					37,467
Group 3	38	0	0	44,400	44,400
	39	0	0	41,200	41,200
Mean					42,800

Group 1 9-15 month-old animals previously infected with two doses of 50,000 _O. ostertagi_ L_3 and subsequently treated with fenbendazole.

Group 2 9-15 month-old animals maintained parasite naive.

Group 3 5 month-old animals maintained parasite naive.

Analysis of Varience

Source of variation	df	SS	MS	F
Among groups	2	10934.1	5467.05	1.56
Within groups	5	17493.4	3498.68	
Total	7	28427.5		

$$F_{0.20(2,5)} = 2.3$$

df - degrees of freedom

SS - sum of squares

MS - mean of squares

F - Variance ratio

Conclusion: There is not a significant added variance component among groups for the number of worms recovered at post mortem.

Worm Lengths

The percentage distribution of worms according to length are shown in Figs.1a, 1b and 1c. A table of the percentage distribution is given in Appendix 1c and 1d. The range of worm lengths in the Group 1 animals was much greater than in either of the other groups and in all three of these animals there were females less than 0.45 cm long and males less than 0.40 cm long. There were few females more than 0.75 cm long and a few males more than 0.60 cm long.

The Group 2 animals had a closer distribution, none of the females were less than 0.45 cm long and none of the males were less than 0.40 cm long. Small numbers of females more than 0.75 cm were found and there were considerable numbers of males more than 0.60 cm long.

The female worms in the Group 3 animals were all greater than 0.55 cm long and more than 35% of these were greater than 0.75 cm long. The males were all more than 0.45 cm long, although relatively few were more than 0.65 cm long.

Eggs in Female Worms

The number of eggs in the uteri of all the female worms mounted for measurement were estimated. Mean numbers of eggs in the worms of indivdiual animals are shown in Table 1b. Lower mean egg numbers were present in the worms from the Group 1 animals (3.02) than the Group 2 animals (6.37) while the worms from the Group 3 animals had more eggs (14.33) than either ofthe other groups.

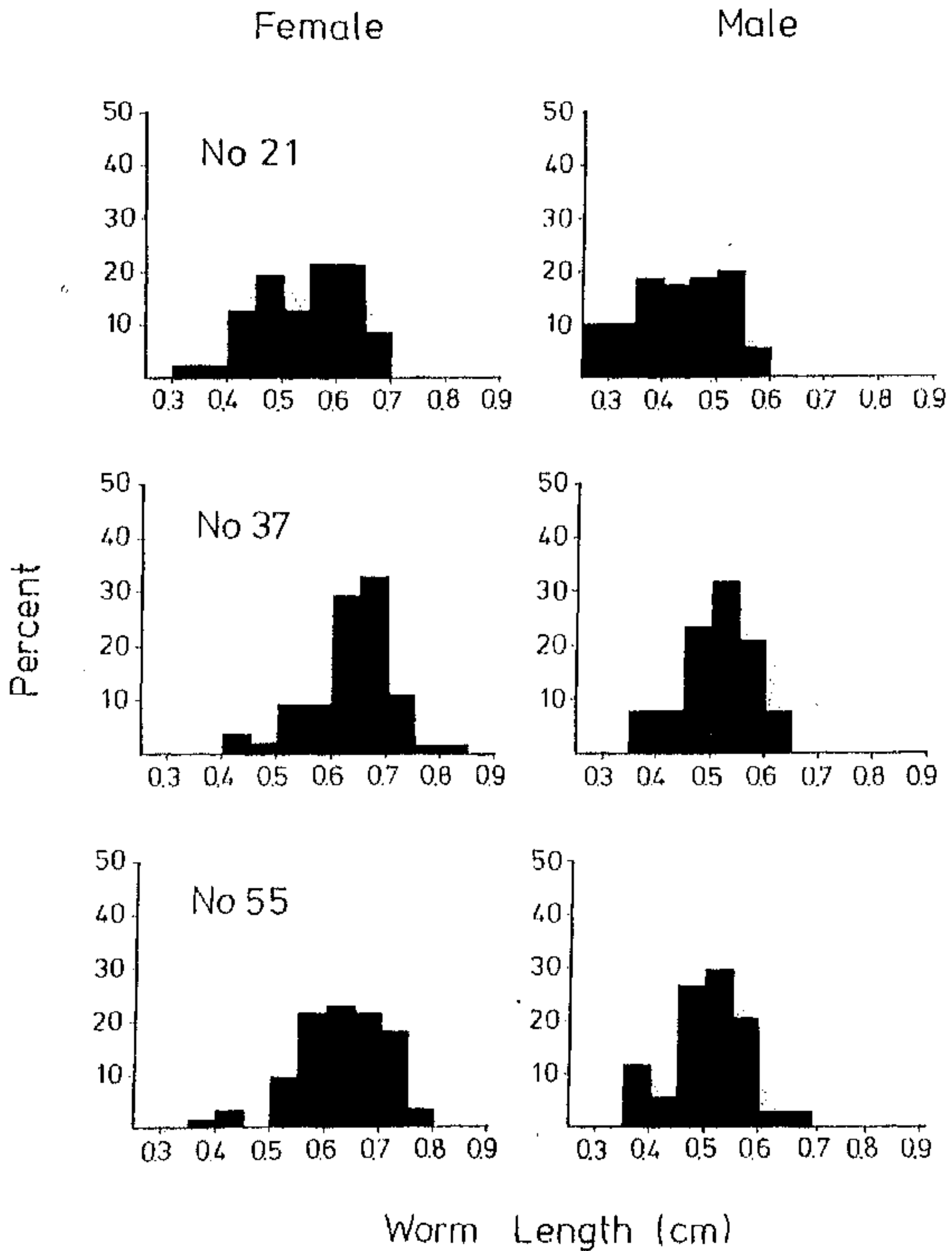

Fig. 1a Percentage distribution of worms according to length after challenge with 100,000 _O. ostertagi_ L_3

Group 1: 9-15 month old animals previously infected with two doses of 50,000 _O. ostertagi_ and subsequently treated with fenbendazole.

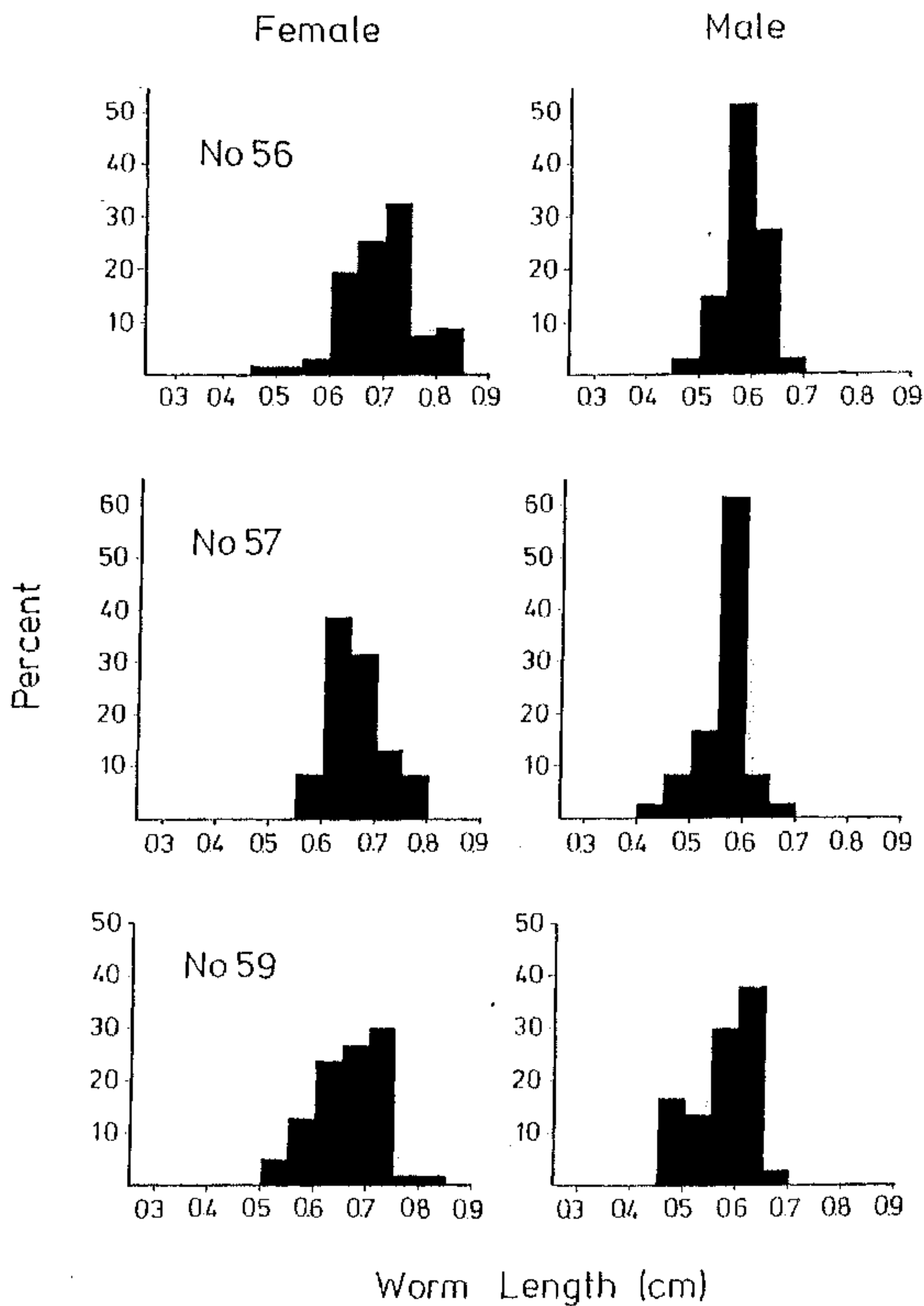

Fig. 1b Percentage distribution of worms according to length after challenge with 100,000 O. ostertagi L_3

Group 2: 9-15 month old animals maintained parasite-naive

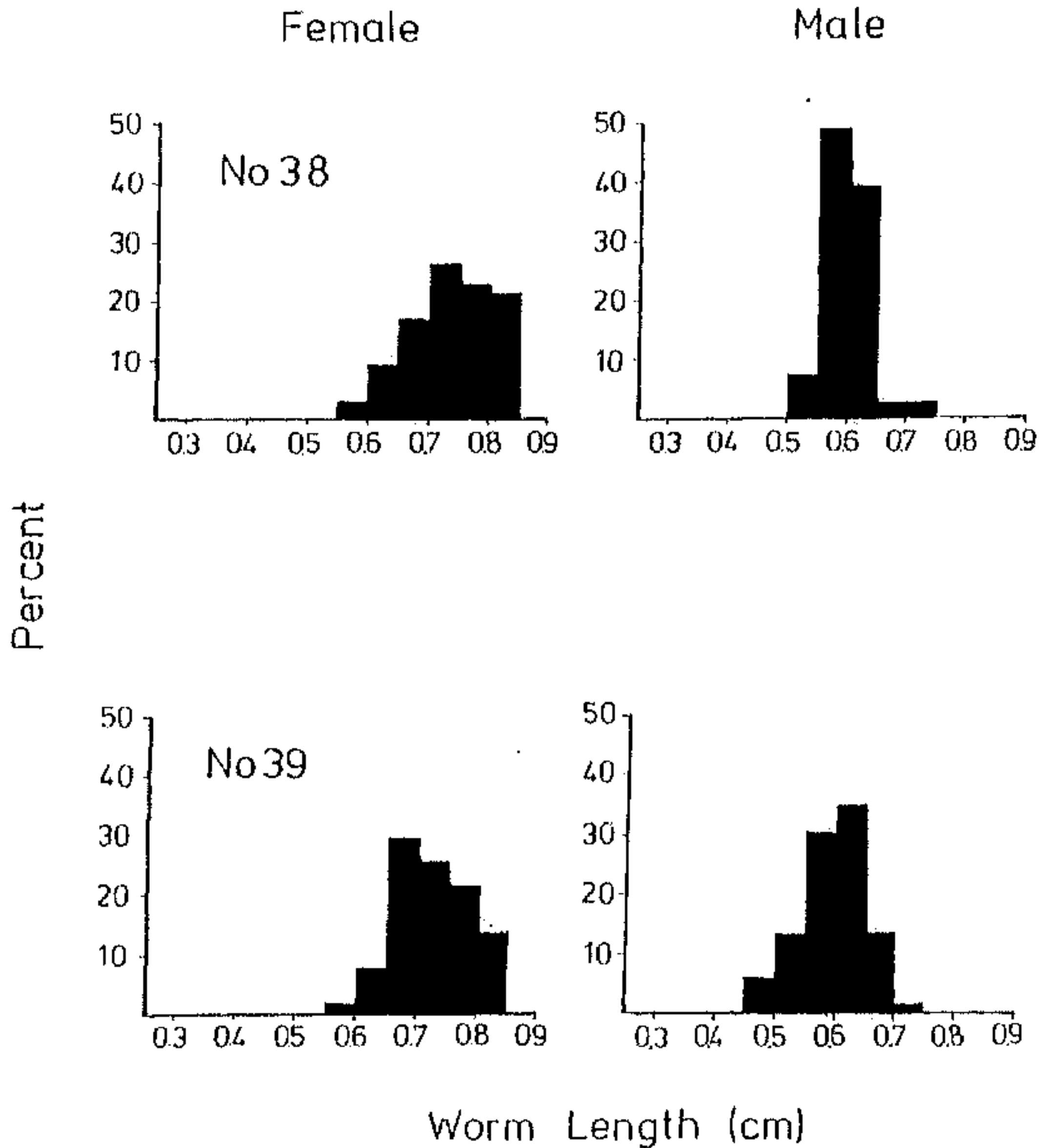

Fig. 1c Percentage distribution of worms according to length after challenge with 100,000 O. ostertagi L_3

Group 3: 5 month old animals maintained parasite-naive

Table 1b

Mean number of discernable eggs within the uteri of the female worms from three groups of animals 21 days after challenge with 100,000 _O. ostertagi_ L_3.

Group	Animal No.	Mean No. of eggs per female worm
Group 1	21	0.15
	37	5.29
	55	3.63
Mean		3.02
Group 2	56	6.37
	57	6.53
	59	6.20
Mean		6.37
Group 3	38	10.41
	39	18.25
Mean		14.33

Group 1 9-15 month-old animals previously infected with two doses of 50,000 _O. ostertagi_ L_3 and subsequently treated with fenbendazole.

Group 2 9-15 month-old animals maintained parasite naive.

Group 3 5 month-old calves maintained parasite naive.

Analysis of Variance

Source of variation	df	SS	MS	F
Among groups	2	156.01	78.01	8.76
Within groups	5	44.55	8.91	
Total	7	200.56		

$F_{0.05(2,5)} = 5.8 \quad F_{0.01(2,5)} = 13.3$

Conclusion: There is a significant ($p < 0.05$) added variance component among groups for the number of eggs in the uteri of the female worms. Further analyses were undertaken using the two sample t-test.

Between groups 1 and 2 t= 1.37430 Not significant

groups 1 and 3 t= 4.15640 $p < 0.01$

groups 2 and 3 t= 2.9272 $p < 0.05$

Abomasal pH

The individual pH values of abomasal fluid at necropsy are shown in Table 1c.

The abomasal pH at necropsy was significantly raised in the three animals of Group 1, normal in the animals of Group 2, and elevated in the two control calves.

Table 1c

Individual pH values of abomasal fluid at necropsy in three groups of animals 21 days after challenge with 100,000 *O. ostertagi* L_3.

Group	Animal No.	Abomasal pH
Group 1	21	3.8
	37	4.2
	55	3.5
Group 2	56	2.1
	57	2.4
	59	2.6
Group 3	38	5.4
	39	4.2

Group 1 9-15 month-old animals previously infected with two doses of 50,000 *O. ostertagi* L_3 and subsequently treated with fenbendazole.

Group 2 9-15 month-old animals maintained parasite naive.

Group 3 5 month-old animals maintained parasite naive.

Analysis of Variance

Source of Variation	df	SS	MS	F
Among groups	2	7.55	3.78	17.18
Within groups	5	1.10	0.22	
Total	7	8.65		

$F_{0.01(2,5)} = 13.3 \quad F_{0.001(2,5)} = 37.1$

Conclusion: There is a significant ($p < 0.01$) added variance component among groups for pH values at post mortem. Further analyses were undertaken using the two sample t-test.

Between groups 1 and 2 t= 3.84 $p < 0.02$

groups 1 and 3 t= 2.26 not significant

groups 2 and 3 t= 5.65 $p < 0.01$

Serum Pepsinogen Levels

Individual serum pepsinogen levels are given in Appendix le. Mean serum pepsinogen levels rose to a peak of 4.0 i.U. in Group 1 twenty one days after the second larval inoculation but levels quickly fell to around 1.5 i.U. after the fenbendazole treatment. Mean pepsinogen levels rose to 1.8, 2.0 and 4.2 i.U. in Groups 1, 2 and 3 respectively on day 21 after the challenge inoculation.

Discussion

The very low positive faecal egg counts in animals 21 and 37 from Group 1 on day 9 after infection suggests that despite the efforts made to maintain these animals worm free, they had in fact picked up a low worm burden prior to their first experimental exposure. A number of observations can however be made from the results. Firstly in all three groups worms became established and there was no significant difference in the numbers established in older animals, whether previously infected with two doses of 50,000 O. ostertagi L_3 or not. Nor did these differ significantly from the numbers of worms which established in the control calves. It is however interesting that the lowest worm burden recovered was from the largest of the previously infected animals (No.21). It may be therefore that age could confer some natural immunity against O. ostertagi but that such immunity had not yet developed in the animals used in this experiment. Alternatively it is possible that older animals attain an acquired resistance more rapidly than younger animals but that the double dose of 50,000 O. ostertagi L_3 was not quite sufficient to induce immunity even in this age of animal.

A further point, apparent from the worm burdens, is that only a very small proportion of the total population remained as fourth stage larvae and inhibition of development demonstrated by Michel et al (1979) in 20 month old cattle was not observed in these animals.

The major differences between the three groups of animals were apparent on examination of the worm lengths and the number of eggs in the uteri of female worms. From this data, age and previous exposure were shown to affect the length and apparent fecundity of the worms. All the worms in the control calves (Group 3) were larger than 0.45 cm and a much larger proportion of the population of worms in the Group 1 animals was less than 0.45 cm compared with the Group 2 animals. There were significantly fewer eggs in the uteri of female worms from both older groups of animals than from the younger calves. However previous exposure of the older animals did not result in a significant reduction in fecundity when compared to parasite-naive animals of the same age given the same challenge.

It is interesting that the Group 2 animals which were 9-15 months old and maintained parasite-naive until challenge had significantly lower abomasal pH at post mortem then either of the other groups and it may be that because of the retarded development of worms in this group they had not reached emergent stages by day 21 post infection in sufficient numbers to cause parietal cell damage and elevated pH. If this were the case then the elevated pH observed in the Group 1 animals in which the worm burdens were also retarded could have been a consequence of the previous infections with Ostertagia larvae.

It would appear from this study therefore that the age of an animal can influence the development of a worm population, in the older animals of Group 2 worms were retarded both in length and egg production compared with the Group 3 calves. Also in the older cattle of Group 1 a double exposure inoculation of 50,000 O. ostertagi L_3 was sufficient to produce a greater degree of retardation in a challenge burden compared with animals of a similar age (Group 2) but parasite naive. These results agree with those of Armour (1967) who demonstrated a retardation of maturation of parasites after their final moult in worm-free animals aged two and a half years old and Smith and Archibald (1968) who demonstrated stunting of worms and inhibition of egg production in worm-free animals 15 months-old after exposure to natural infection. Markedly lower worm burdens, as demonstrated by Smith (1970) in 15 month-old animals were not characteristic of the animals in this experiment. Herlich (1960) observed no noticable size difference between worms recovered from calves and adult cattle, but did suggest that age conferred greater resistance to the debilitating effects of gastrointestinal nematodes. More recently Herlich (1980) has demonstrated that cattle from 2 to 24 months old were equally susceptible to infection and reinfection with O. ostertagi in so far as establishment of worms is concerned but the 24 month old cattle were able to withstand the pathogenic effects of the parasites.

It may be therefore that the morphological changes observed in parasites from adult cattle occur concurrently with changes in pathogenicity, accounting for reduced debilitating effects.

Certainly the reduced egg production by parasites in these animals must be considered where faecal egg counts are used in diagnosis and where pasture contamination is being related to the epidemiology of ostertagiasis.

EXPERIMENT 2

A study of the potential immunisation of calves against *O. ostertagi* using an intergeneric species *O. leptospicularis*.

Introduction

The development of immunity to *O. ostertagi* has been shown to require prolonged exposure to the parasite both under natural (Ross and Dow, 1965a) and experimental (Michel *et al*, 1973) conditions. It is not surprising therefore that attempts to immunise against this parasite using live larvae have met with little success (Ross, 1963; Ross and Dow, 1964; Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart, 1967). Herlich (1976) did however demonstrate a lower rate of establishment and morphological changes in the worms resulting from challenge, after immunisation with different larval regimens, although the challenge resulted in similar pathological changes in both immunised and non-immmunised calves. Similar poor results have been obtained in attempts to immunise calves with intraperitoneal injections of larvae and intravenous and intraperitoneal injections of exoantigens, derived from media used to grow Ostertagia *in vitro* (Herlich and Douvres, 1979).

Attempts to immunise against a number of other parasites have been more successful and vaccines have been developed against the tissue migrating nematodes *D. viviparis* (Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart, 1959) and *A. caninum* (Miller, 1978a). It has been suggested that the intimate exposure to the host's reticuloendothelial system is

responsible for the strong acquired immunity in these systems (Miller, 1978b).

There have also been attempts to immunise against parasitic nematodes using closely related and unrelated species. For example, vaccination of sheep with _T. colubriformis_ gave 81% protection against challenge with _Trichostrongylus vitrinus_. No protection was achieved against a challenge of the genetically unrelated parasite _N. spathiger_ but simultaneous challenge of vaccinated sheep with _T. colubriformis_ and _N. spathiger_ produced 98-100% protection against both parasites (Dineen, Gregg, Windon, Donald and Kelly, 1977). These results suggest that the final effector mechanism for nematode expulsion is not immunologically specific, but that a specific antigenic trigger is required to provoke an appropriate response. Cross-protection has been demonstrated for several other nematode species. Intake of _H. contortus_ larvae in naturally infected sheep has been shown to induce the self cure reaction not only to _H. contortus_ but also to _O. circumcincta_ and _Trichostrongylus axei_ infections (Stewart, 1955). Also simultaneous experimental infections of _H. contortus_ and _O. circumcincta_ have been shown to reduce the numbers of both species becoming established and concurrent infection with _T. axei_ and either _H. contortus_ or _O. circumcincta_ has been shown to be detrimental to the establishment of _Haemonchus_ or _Ostertagia_, but not to _T. axei_ (Turner, Kates and Wilson, 1962). Immunisation with different strains of a species has been studied, for example using a parasite adapted to a different host. Partial immunity has been induced in sheep using isolates of _Haemonchus_ from the pronghorn antelope. It was found that the

antelope strain produced a lesser degree of anaemia in sheep but stimulated an immune response comparable to that produced by the sheep strain (Allen, Samson and Wilson, 1970).

Although O. ostertagi is considered to be the most important and most frequently identified abomasal nematode of cattle in the United Kingdom (Michel, 1976; Armour, 1980), recently it has been identified together with an intergeneric species O. leptospicularis (Al Saqur, Armour, Bairden, Dunn and Jennings, 1980) in outbreaks of disease. Subsequent experimental studies showed that an isolate containing 30% O. leptospicularis and 70% O. ostertagi was highly infective and produced severe pathological changes (Al Saqur, Armour, Bairden, Dunn, Jennings and Murray, 1982). Significantly, mature female worms and eggs were found in the gastric glands and eggs were present in the surrounding lamina propria, in the submucosa and in submucosal lymphatics. Lesions were accompanied by diffuse infiltration of mononuclear cells, predominantly plasma cells, notably in the upper part of the lamina propria. Because of the proximity of O. leptospicularis to components of the host reticuloendothelial system it was considered possible that this parasite might be more potent than O. ostertagi in stimulating an immune response. Accordingly, an experiment was designed to attempt to immunise calves against O. ostertagi using the related species O. leptospicularis.

Experimental Design (Table 2a).

Fourteen Friesian calves were reared parasite free from birth. When five months old they were divided into three groups of four (Groups 1-3) and one group of two calves (Group 4). Group 1 were inoculated orally with two doses of 50,000 O. leptospicularis L_3, Group 2 were inoculated twice with 50,000 O. ostertagi L_3 while Group 3 received two doses which consisted of a mixture of 25,000 O. leptospicularis L_3 and 25,000 O. ostertagi L_3; twenty seven days separated the larval doses. Twenty-one days after the second larval inoculation all of the calves, including the two control calves (Group 4), were treated with fenbendazole orally and, eight days later, challenged with 100,000 O. ostertagi L_3. All of the calves were necropsied 21 days after challenge.

Animals were examined closely throughout the experiment. Faeces and serum samples were collected and animals were weighed twice weekly. At autopsy the abomasa were processed as described previously and abomasal worm burdens, mucosal weights and pH estimated.

Results

Clinical Observations

Three calves from Group 2 and one calf from Group 1 developed severe pneumonia during the experiment and despite treatment the condition of two animals in Group 2 necessitated their early euthanasia. Otherwise animals remained bright and healthy, although all the calves passed soft faeces on the two

Table 2a A study of the potential immunisation of calves against O. ostertagi using an intergeneric species O. leptospicularis - Experimental Design.

Day		D- 56 First Inoculation	D- 29 Second Inoculation	D-8 Treatment	D 0 Challenge	D 21 Necropsy
	No. of calves					
Group 1	4	50,000 0.1	50,000 0.1	FBZ	100,000 0.o	+
Group 2	4	50,000 0.o	50,000 0.o	FBZ	100,000 0.o	+
Group 3	4	25,000 0.o	25,000 0.o			
		25,000 0.1	25,000 0.1	FBZ	100,000 0.o	+
Group 4	2	-	-	FBZ	100,000 0.o	+

0.1 - Ostertagia leptospicularis

0.o - Ostertagia ostertagi

FBZ - Fenbendazole (7.5 mg/kg)

\+ - Necropsy

days prior to necropsy. The weights of the individual calves are shown in Appendix 2a.

Faecal Egg Counts

Mean faecal egg counts for each group are shown in Fig.2a and individual values are given in Appendix 2b.

Faecal egg counts were positive in calves of Groups 1, 2 and 3, nineteen days after the first larval inoculation. Counts returned to zero after fenbendazole treatment and were positive in calves of all four groups twenty days after the challenge infection.

Post Mortem Worm Burdens

The post mortem worm burdens of individual animals are shown in Table 2b. Animals 45 and 48 which were necropsied prematurely had worm burdens of 20,100 and 40,700 respectively. In all the other animals the majority of worms were adults. The mean worm burdens were 41,425 for Group 1, 45,200 for Group 2 (excluding calves 45 and 48) and 36,400 for Group 3.

The mean worm burden for the two control calves was 42,800. This represents a range of establishment of the challenge infection of 36.4% - 45.2%.

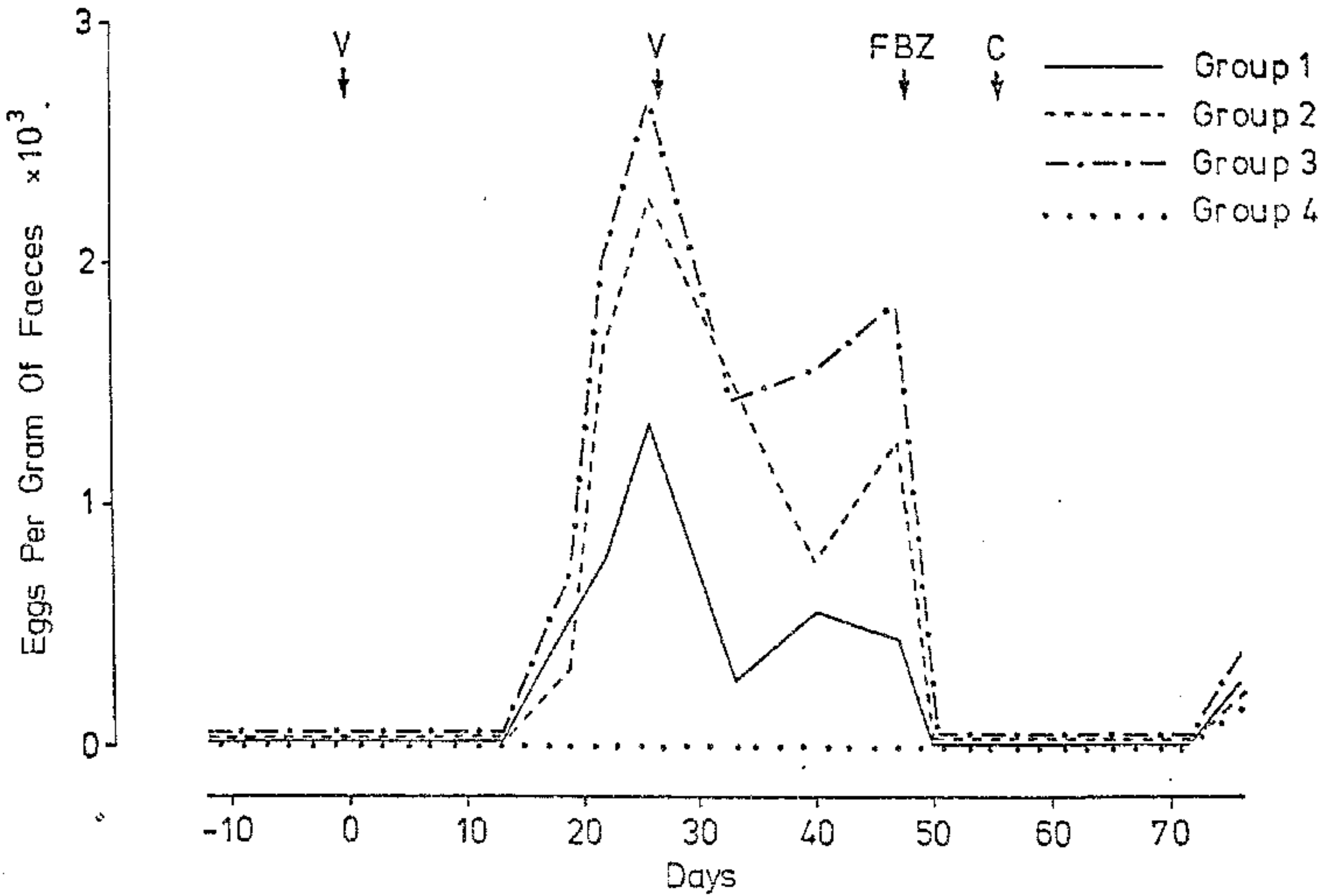

Fig. 2a Mean faecal egg counts (epg) of calves vaccinated with *O. leptospicularis* (Group 1), *O. ostertagi* (Group 2) or a mixture of *O. leptospicularis* and *O. ostertagi* (Group 3) and control calves (Group 4).

Table 2b

Post mortem worm burdens of calves vaccinated with either <u>O. leptospicularis</u> (Group 1), <u>O. ostertagi</u> (Group 2) or a mixture of <u>O. leptospicularis</u> and <u>O. ostertagi</u> (Group 3) on day 21 after challenge with 100,000 <u>O. ostertagi</u>

Group	Animal No.	Fourth Larval Stage	Fourth Moult	Adults	Total
Group 1	41	0	0	44,800	44,800
	42	1000	600	36,800	38,400
	43	0	200	34,400	34,600
	44	200	0	47,700	47,900
Mean					41,425
Group 2	46	0	200	43,800	44,000
	49	200	0	46,200	46,400
Mean					45,200
Group 3	50	0	0	31,800	31,800
	52	0	200	34,600	34,800
	53	0	0	37,200	37,200
	54	0	0	41,800	41,800
Mean					36,400
Group 4 Control Calves	38	0	0	44,400	44,400
	39	0	0	41,200	41,200
Mean					42,800

Abomasal pH and Mucosal Weight at Necropsy

The abomasal pH values of the individual animals at necrospy are shown in Table 2c.

The mean pH for each group was raised and ranged from 3.8 in the Group 3 animals to 4.8 in the control calves of Group 4. At necropsy the abomasal mucosa of each animal was weighed and the results are shown in Table 2c. Although the mucosal weight varied, according to the size of the abomasum, the mean abomasal mucosal weight of calves in Groups 1, 2 (excluding calves 45 and 48) and 3 were all greater than the mean mucosal weight of the control calves in Group 4.

Table 2c

Abomasal pH and abomasal mucosal weight at necropsy of calves vaccinated with either O. leptospicularis (Group 1), O. ostertagi (Group 2) or a mixture of O. leptospicularis and O. ostertagi (Group 3) on day 21 after challenge with 100,000 O. ostertagi L_3

Group	Animal No.	Abomasal Mucosal Weight (gm)	Abomasal pH
Group 1	41	376	3.9
	42	559	3.8
	43	373	4.3
	44	440	4.5
Group 2	46	580	4.0
	49	406	5.1
Group 3	50	N.S.*	3.9
	52	550	2.5
	53	409	4.2
	54	493	4.7
Group 4	38	353	5.4
Control calves	39	380	4.2

*N.S. - no sample taken

Serum Pepsinogen Levels

Mean serum pepsinogen levels for each group are shown in Fig.2b and individual values are given in Appendix 2c.

The mean pepsinogen levels of calves in Groups 1, 2 and 3 rose from day 15 after the primary infection. There was a further rise in each of these groups between 13 and 20 days after the second larval dose but pepsinogen levels then fell steeply following anthelmintic treatment. Pepsinogen levels rose in all four groups after challenge.

Discussion

From the post mortem worm burdens it is apparent that none of the immunisation regimens conferred a significant degree of protection against the establishment of a subsequent challenge dose of *O. ostertagi*. One point of interest which arose from this study concerned the serum pepsinogen levels of the three groups of calves after the two immunising infections. The animals which were inoculated twice with *O. ostertagi* larvae had a much higher pepsinogen response, up to almost 7.0 i.U., than two groups which were immunised with either *O. leptospicularis* or a combination of *O. ostertagi* + *O. leptospicularis*. In these the serum pepsinogen values never rose above 3.0 i.U. This is contrary to the findings of Al Saqur *et al* (1982) who demonstrated similar pepsinogen changes in three groups of calves, two of which were infected with different strains of *O. ostertagi* and a third was infected with a mixture of *O. ostertagi* and *O. leptospicularis*.

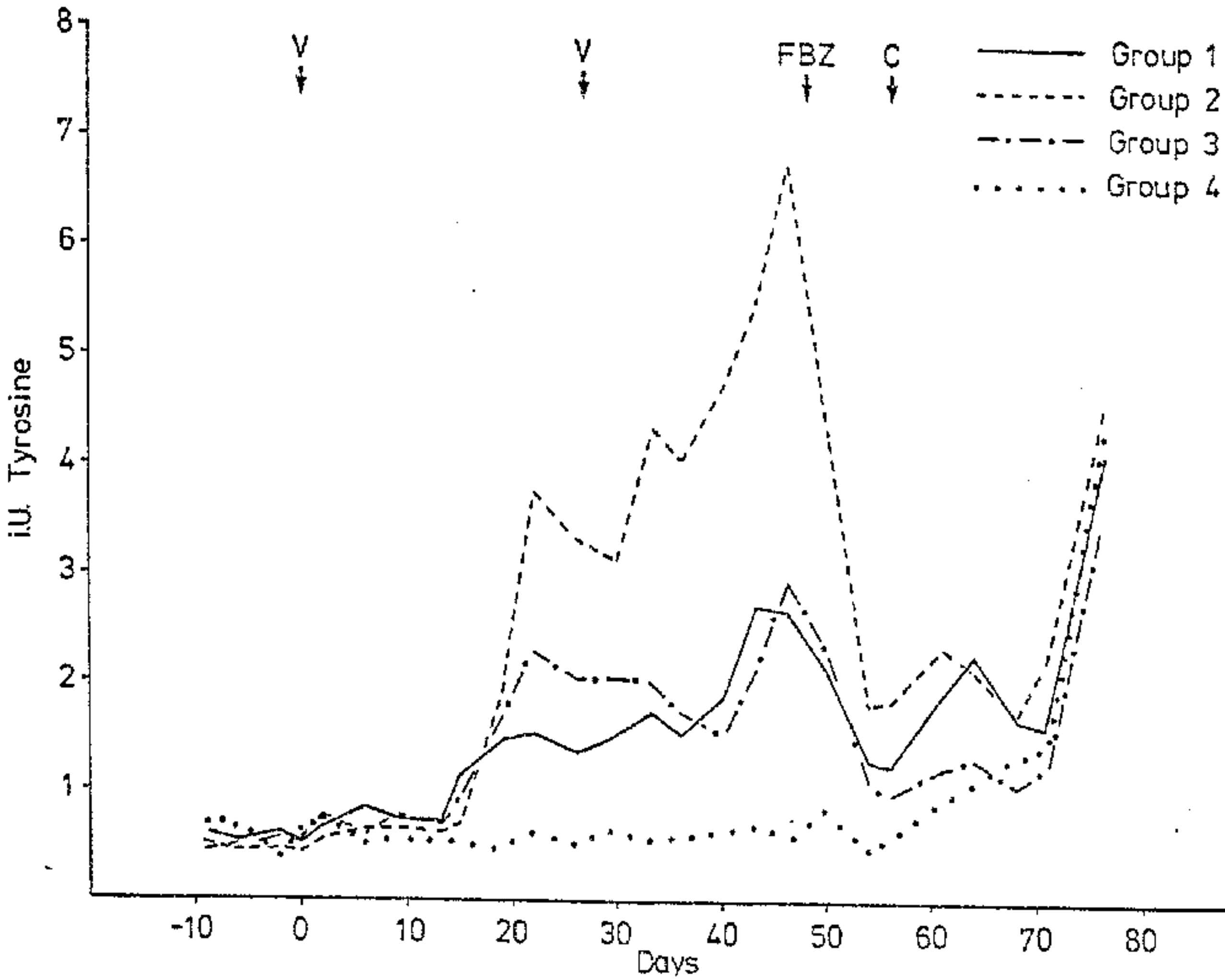

Fig. 2b Mean serum pepsinogen levels (i.U. Tyrosine) of calves vaccinated with _O. leptospicularis_ (Group 1), _O. ostertagi_ (Group 2) or a mixture of _O. leptospicularis_ and _O. ostertagi_ (Group 3) and control calves (Group 4).

General Discussion

Reduced fecundity and stunting of parasite growth may be a consequence of host resistance, although reduced fecudity in at least one parasite infection, A. caninum in the dog, has been attributed to overcrowding in the intestine (Krupp, 1961). Modification of parasite characteristics have been demonstrated in calves repeatedly infected with O. ostertagi by Michel (1963) who concluded that worms which developed from later infections failed to become as fertile and did not achieve the same length as the worms which developed earlier; in this study it appeared that inhibition of ovulation was distinct from stunting as it was not necessarily the smallest worms which had the fewest eggs.

Worms have also been shown to adapt as their hosts become resistant. For example, in N. brasiliensis infections in rats, egg-laying worms ceased ovulation from about day 12 post infection (Ogilvie and Jones, 1971) and populations of adult Strongyloides ratti from rats given a single challenge were smaller 26 days post challenge than those found on day 11 of infection (Moqbel and Denham, 1977). Host immunity has been confirmed as a cause of worm modification in Trichinella spiralis infection in mice by the transfer of immune mesenteric lymph node cells from infected donor animals to infected irradiated (400-850 rads) recipient mice. This resulted in reduced fecundity of T. spiralis without worm expulsion (Wakelin and Wilson, 1980). In addition, 'shrinking' of adult S. ratti worms without elimination has been induced in rats which received low numbers of immune mesenteric lymph node cells (Moqbel and Wakelin, 1981)

and the passive transfer of immune serum has produced stunting of worms in Nematospiroides dubius infected mice (Dobson, 1982).

The stunting and inhibition of egg laying, manifest in older cattle which do not show resistance to the establishment of O. ostertagi infection (Smith and Archibald, 1968) has been confirmed in this study. It also appears that in adult cattle two infections of 50,000 O. ostertagi L_3 are sufficient to stimulate a degree of immunity which is manifest by a reduction in worm size after challenge although this is insufficient to cause any marked reduction in worm establishment.

The mechanisms responsible for the development of such phenomena indicative of resistance remain unknown; but it appears that the cellular and humoral immunity demonstrated in many laboratory host/parasite systems already mentioned with the transfer of immune mesenteric lymph node cells and immune serum are also important in the immune response of cattle to Ostertagia. The more rapid acquisition of immunity to gastrointestinal parasites in older animals has been previously demonstrated in cattle and sheep. Michel et al (1979) found that an acquired resistance to the establishment of worms developed more rapidly in 20 month old heifers than in calves and it has been shown in 10 month old sheep made immune to O. circumcincta by repeated infection that there is a secondary type response to challenge consisting of large increases in output of IgA antibody and IgA blast cells (Smith, Jackson, Jackson, Dawson and Burrells, 1981). When the latter experiment was repeated in younger lambs (4-5 months) the animals were found to be less immune to challenge and their ability to mount this local

response was markedly less well developed (Smith - cited Miller, 1984).

Although the acquisition and development of immunity to O. ostertagi is more rapid in older cattle, these animals may also have a greater natural resistance to the pathogenic effects of the parasites (Herlich, 1980). Certainly the mean pepsinogen levels in the control calves of Experiment 1 were more than double the values of the two groups of older cattle on day 21 after the challenge inoculation and this would suggest much greater damage to the mucosal epithelium in the younger calves after challenge.

In this study the attempt to stimulate immunity against O. ostertagi using the related species, O. leptospicularis proved unsuccessful. This was disappointing in view of the marked stimulation of components of the immune system demonstrated by Al Saqur et al (1982) in calves infected with O. leptospicularis. In retrospect it would have been interesting if a further group of calves had been available which could have been vaccinated and challenged with O. leptospicularis to see if immunity develops more quickly to this particular parasite alone. The low pepsinogen response following immunisation with O. leptospicularis alone and with a mixture of O. leptospicularis and O. ostertagi is contrary to the rise in pepsinogen values observed by Al Saqur et al (1980) after O. leptospicularis infection. One probable explanation for this is that there had been an alteration in the pathogenicity of the laboratory maintained strain of O. leptospicularis with repeated passage

through experimental cattle. Ross and Purcell (1969) demonstrated a similar alteration in the pathogenicity of T. axei for calves, after several passages through sheep. From recent experimental work (Maciel, 1984 - personal communication) it would seem that where a mixed population of O. leptospicularis and O. ostertagi are passaged for a number of generations, the percentage of each isolate changes, and an optimal proportion of each, for the development of severe pathological lesions may be reached after several generations.

Section 2

Studies on the pathophysiological changes associated with *Ostertagia ostertagi* infection in calves

INTRODUCTION

A number of pathological and physiological changes accompany O. ostertagi infection in cattle. The gross and histological alterations in the abomasal mucosa have been described by Ritchie et al (1966) and include loss of cellular differentiation, glandular hyperplasia and, following parasite emergence, cytolysis of the mucosal surface. Ultrastructural examination of the parasitised gastric mucosa has revealed dilated intercellular spaces between epithelial cells with separation of the zonulae occludentes, which is the likely cause of the enhanced permeability of the bowel wall to macromolecules (Murray, 1969). Coincident with the structural alterations, there are various biochemical changes in the abomasal fluid and blood. The concentration of sodium ions in the abomasal contents increases, while that of hydrogen, potassium and chloride ions decreases. At the same time there is an increase in plasma pepsinogen levels (Jennings et al, 1966).

Pepsinogen is thought to leak into the plasma through the separated junctional complexes of the damaged epithelium (Jennings, Armour, Kirkpatrick and Murray, 1967). Evidence for the leak theory has been provided by the previously mentioned ultrastructural studies of Murray (1969) and by Mulligan et al (1963) who demonstrated an increased loss of plasma protein into the alimentary tract of animals with clinical ostertagiasis.

Although albumin turnover rate has been shown to decline between days 14-22 after infection with O. circumcincta in sheep, during this period serum pepsinogen remained elevated and could

not be directly related to continuing plasma leak (Holmes and Maclean, 1971). Also, plasma proteins were found to be reduced in the abomasal contents of calves infected with O. ostertagi and it has been shown that horseradish peroxidase, which is considered to be a suitable marker for pepsinogen on the basis of their similar molecular weight, leaked from the circulation into gastric contents to a similar degree in infected and non-infected calves (Stringfellow and Madden, 1979).

This work suggested that pepsinogen, secreted by zymogen cells, was released directly into the circulation rather than leaked from the abomasal contents through damaged epithelium, and that it was retained in the circulation at high levels between 22-30 days after infection, even when zymogen cells were denuded of pepsinogen granules.

The elevated abomasal pH noted on day 20 after infection with O. ostertagi was associated with the rapid replacement of parietal cells by undifferentiated cells as parasites emerged from the gastric glands (Jennings et al, 1966). Although Stringfellow and Madden (1979) identified parietal cells in the fundus, even at 30 days after infection, they were sparse in the immediate vicinity of infected gastric glands and carbonic anhydrase activity was negligible throughout the tissue from day 26 onwards, suggesting that these parietal cells were inactive.

From experiments in rats where an injected extract of homogenised O. ostertagi has been shown to elevate the pH of gastric secretion, it has been suggested that the hypochlorhydria found during ostertagiasis may be mediated partially by a chemical released from the parasite (Eiler, Baber, Lyke and

Scholtens, 1981). A possible chemical mediator has also been suggested for the gastric dysfunction observed in sheep infected with the intestinal nematode T. colubriformis (Barker and Titchen, 1982). During the course of T. colubriformis infection, parietal cells lost prominence, had ultrastructural features of inactivity, and abomasal acidification was reduced. A gastric inhibitory factor was considered to be released from the parasitized small intestine and it was suggested that the observed changes could be explained by interactions of gastrointestinal hormones.

The interactions of parasite factors and hormones have been studied in sheep infected with O. circumcincta (McLeay, Anderson, Bingley and Titchen, 1973). Sheep surgically prepared with separated fundic pouches were orally infected with O. circumcincta. An increased volume of secretion and increased acid output was obtained from the pouch four days after infection and parietal cells of the pouch had the appearance ultrastructurally of cells subjected to strong secretory stimuli. Coincident with pouch changes, acid secretion from the infected part of the abomasum decreased and parietal cells had features of inactivity. Two different mechanisms were thought to be operating. First, a factor released locally from the parasites was considered responsible for inhibition of parietal cell function and second, it was suggested that increased levels of circulating gastrin might account for the increased secretion of HCl from the pouches.

Anderson, Blake and Titchen (1976) have since shown that

previously infected sheep maintain the capacity to acidify their abomasal contents after infection and they speculated that either an immunoglobulin produced locally as a result of repeated infection inactivates the inhibiting factor, or that the worms had adapted to the immune environment and were no longer capable of producing the inhibiting factor.

Elevated serum levels of the gastrointestinal hormone gastrin have been confirmed by immunoassay in sheep infected with O. circumcincta (Anderson, Hansky and Titchen, 1975). Gastrin levels rose most rapidly from 11-20 days after infection and reached peak values between days 20-35. The rise in gastrin appeared to occur slightly in advance of the elevation of abomasal pH and did not therefore appear to be initiated by the reduced acidity of abomasal contents. The distribution of cells in the sheep digestive tract having immunoreactivity to gastrin antiserum has been demonstrated by Bunnett and Harrison (1979). These cells occurred throughout the mucosa of the abomasal antrum and pylorus and on the basis of their distribution were assumed to be gastrin secretory cells. The fact that fundic pouch hypersecretion was not observed in sheep infected with O. circumcincta after surgical removal of the abomasal antrum, further suggests that the hypergastrinaemia is of antral origin (Anderson et al, 1975).

Endocrine changes have been implicated in the pathophysiology of a number of other parasitic infections. For example, male lambs are more susceptible than females to Oesophagostomum columbianum but gonadectomy of the females removes the difference by increasing their susceptibility to the

parasite (Dobson, 1964). These worms have also been shown to survive better in thyroidectomised lambs (Dobson, 1964). T. colubriformis infected sheep have markedly elevated corticosteroid levels and reduced thyroxine levels which could be expected to have a catabolic effect on muscle protein and to depress wool production (Ferguson, Wallace and Lindner, 1960). It has also been suggested that increased corticosteroid levels could lower the animals immunity, thus preventing expulsion of the parasites (Prichard, Hennessy and Griffiths, 1974).

Endocrine and paracrine secretions from specialised cells of the gastrointestinal tract have profound effects on their immediate environment and may have more general regulatory roles. Secretion, motility and absorption within the gut are all influenced by gastrointestinal peptide hormones. In addition they may exert trophic effects necessary for the maintainance of mucosal mass and may influence the regulation of food intake (Glass, 1980). Plasma gastrin has been shown to become elevated not only during infection with the abomasal parasite O. circumcincta (Anderson et al, 1975) but also in two intestinal parasitisms, Strongyloides ransomi in the pig (Enigk and Dey Hazra, 1978) and T. spiralis in the rat (Castro, Copeland, Dudrick and Johnson, 1976). The mechanism by which gastrin becomes elevated in enteric parasitism is unknown, although comparison of T. spiralis and Hymenolepis diminuta infection led Castro et al (1976) to suggest that invasiveness of the parasite may be an important factor as serum gastrin only rose in the T. spiralis infection. Hypergastrinaemia has also been

demonstrated in rats infected with the intermediate stage of the cat tapeworm Taenia taeniaeformis (Cysticercus fasciolaris) which occurs in the rat liver. Gastrin levels become markedly elevated only after about 50 days of infection and it may be that hypergastrinaemia in the case of C. fasciolaris is a product of a compromised hepatic metabolism (Cook, Williams and Lichtenberger, 1981).

Other gut hormones which may be altered during parasitisms include secretin, which appears to be decreased during the intestinal phase of T. spiralis in dogs (Dembinski, Johnson and Castro, 1979) and somatostatin which becomes increased in sheep experimentally infected with T. colubriformis (Symons and Titchen, cited; Titchen, 1982). Plasma concentration of cholecystokinin (CCK) have also been shown to rise in sheep infected with T. colubriformis, the rise coinciding with a fall in food consumption. Reduced food consumption was demonstrated in uninfected sheep after intravenous infusion of CCK and it was concluded that the anorexia associated with some intestinal nematode infections could be mediated by higher concentrations of CCK (Symons and Hennessy, 1981)

Obviously endocrine responses of the host to infection with more than one parasite species may result in complex hormonal interactions. For example, in a lamb with a separated fundic pouch given dual larval infections of O. circumcincta per os. and T. colubriformis by enterotomy (Barker and Titchen, 1982) the acidity and volume of secretion from the pouch was depressed, characteristic of T. colubriformis infection rather than the hypersecretion typical of O. circumcincta infection. It was

suggested that hormones originating from the parasitised small intestine might block gastrin induced hypersecretion, or abnormal amounts of analogous hormone might directly inhibit this secretion (Barker and Titchen, 1982).

The endocrine status of the host is undoubtedly affected by parasitism but whether many of the observed changes are to the host's benefit or detriment remain to be established, also the exact pathophysiological responses to known hormonal changes require investigation.

The work presented here has been carried out to study the biochemical and hormonal changes which occur in the host as a result of infection with larval and adult O. ostertagi and to compare these changes in naive and previously exposed animals.

EXPERIMENT 3

Observations on serum pepsinogen and serum gastrin levels in calves infected with *O. ostertagi* larvae

Introduction

Serum pepsinogen levels are known to rise in bovine ostertagiasis (Anderson *et al*, 1964). Although elevated pepsinogen values have been used for many years as a diagnostic parameter for the disease there is still dispute over the mechanism responsible for the rise. Since serum gastrin has been shown to rise in sheep infected with *O. circumcincta* (Anderson *et al*, 1975) and as gastrin is a known stimulator of zymogen cell secretion (Emas and Grossman, 1967) it was suggested that elevated gastrin could play a role in the pepsinogen response to bovine ostertagiasis. This experiment was designed therefore to follow the sequential changes in serum pepsinogen and serum gastrin in previously worm free calves following a single experimental infection of *O. ostertagi* (Table 3a).

Table 3a

Observations on serum pepsinogen and serum gastrin levels in calves infected with O. ostertagi larvae - Experimental Design

Group	No. of animals	Infection Day 0	D0 - D30
1	5	-	Weekly faecal samples and
2	5	50,000 O. ostertagi Larvae	Twice weekly serum samples

Faecal Egg Counts

Faecal egg counts for individual animals are given in Appendix 3a. These remained negative in Group 1 but all animals in Group 2 had low positive counts from day 21 onwards.

Serum Pepsinogen Values

The mean pepsinogen levels for both groups are shown in Figure 3a and individual values are given in Appendix 3b.

Mean pepsinogen levels in Group 1 did not rise above 1.0 i.U. Tyrosine throughout the experiment. Levels recorded from the calves in Group 2 were slightly elevated on day 6, however a marked rise was not apparent until day 16. Between days 16 and 23, mean pepsinogen values rose dramatically to a peak of almost 6.0 i.U. Tyrosine and remained at about this level until the end of the experiment.

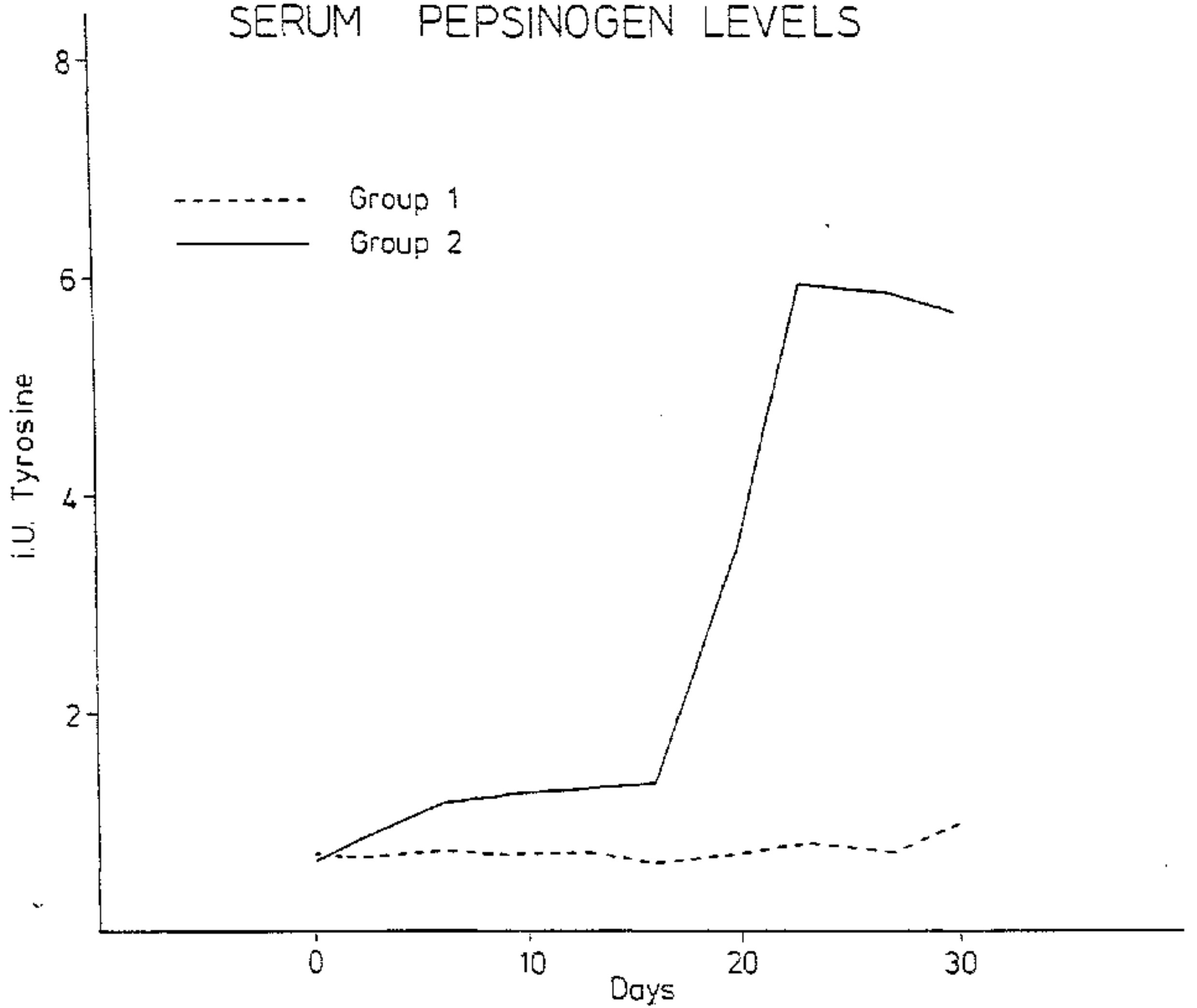

Fig. 3a Mean serum pepsinogen levels (i.U. Tyrosine) of calves. Group 1 were maintained parasite free and Group 2 were infected with 50,000 <u>O. ostertagi</u> L_3 on day 0.

Serum Gastrin Levels

Mean serum gastrin levels for both groups are shown in Figure 3b and the individual values are given in Appendix 3c.

Mean serum gastrin levels in Group 1 remained below 200 pg/ml throughout the experiment while those of Group 2 rose above 200 pg/ml on day 20 and reached a peak of 552 pg/ml 27 days after infection.

Discussion

The results of this study indicate that serum gastrin levels do become elevated in experimental infections of calves with *O. ostertagi*; the gastrin response occurred after day 13 and reached peak levels on day 27. Serum pepsinogen levels rose most steeply between days 16 and 23. Although the changes in both of these parameters occurred at approximately the same time, that is at the time when fifth stage larvae were likely to be emerging from the gastric glands, very much more detailed investigation is necessary before gastrin can be implicated as a factor involved in the development of the elevated pepsinogen levels of ostertagiasis.

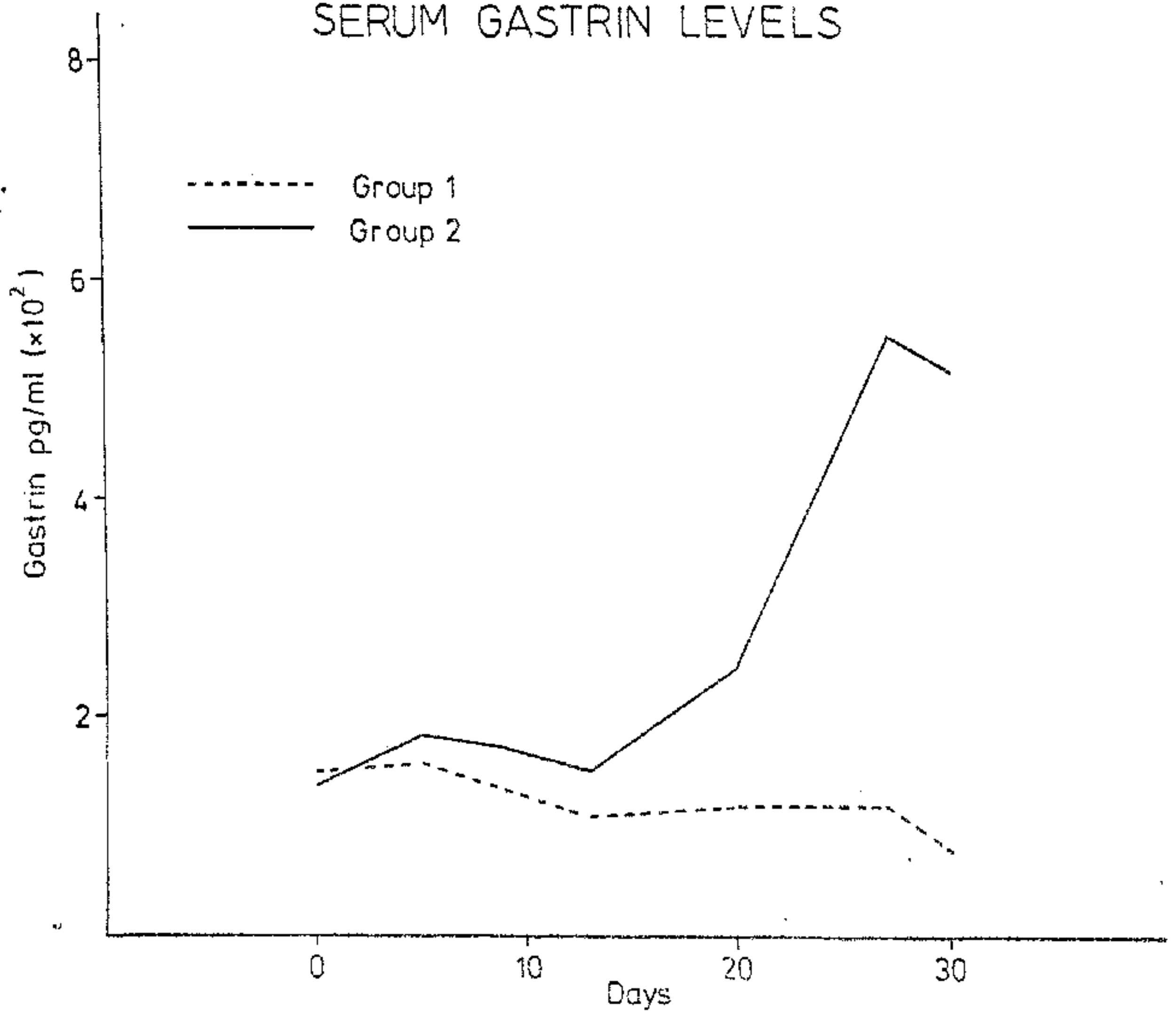

Fig. 3b Mean serum gastrin levels (pg/ml) of calves. Group 1 were maintained parasite free and Group 2 were infected with 50,000 _O. ostertagi_ L_3 on day 0.

EXPERIMENT 4

Observations on serum pepsinogen levels in response to transplanted adult O. ostertagi in calves.

Introduction

The major biochemical changes associated with O. ostertagi occur after the fifteenth day of a primary infection. For example, abomasal pH and serum pepsinogen values reach peak levels from day 22 onwards and may remain elevated for a further 40 days (Jennings et al, 1966). Although the major histopathological changes of the disease have been attributed to the emergence of fifth stage larvae from the gastric glands, the long duration of altered biochemical values would suggest that adult parasites contribute to the disease syndrome.

The objective of this experiment was to study the role of the adult parasite in the pathogenesis of ostertagiasis. The histopathological and serum pepsinogen changes were studied after the implantation of adult parasites in previously naive calves. Adult parasites were also implanted in the abomasa of calves which had been previously infected with O. ostertagi larvae and then treated with anthelmintic, where the abomasal environment would more closely resemble that in a natural infection.

Experimental Design

The design of the experiment is given in Table 4a. Twelve four month-old male calves were divided into three groups of four calves. Group 1 consisted of donor calves which were given a

Table 4a Observations on serum pepsinogen levels in response to transplanted adult *O. ostertagi* in calves – Experimental design

Day	D-25 to -23	D-4 to -2	D 0	D4	D11	D17	D21
Group 1	DONOR INF.		+				
Group 2			TR	+	+	+	+
Group 3	RECIPIENT INF.	LEV.	TR	+	+	+	+

DONOR INF - 300,000 *O. ostertagi* L_3

RECIPIENT INF - 10,000 *O. ostertagi* L_3 on alternate days for 20 days

TR - Transplant

+ - Necropsy

LEV - Levamisole (7.5 mg/kg)

single dose of 300,000 *O. ostertagi* L_3. Group 2 were maintained parasite free and Group 3 each received 10,000 *O. ostertagi* L_3 every second day for 20 days.

The Group 3 calves were treated with levamisole on day 21 and together with Group 2 calves prepared for laparotomy and transfer of adult *O. ostertagi* obtained from the donors.

Donor animals from Group 1 were killed 23-25 days after infection and their adult parasite worm burden implanted surgically into the abomasa of Group 2 and Group 3 calves. The contents of each donor calf were split equally and transferred immediately to two recipients, one from the naive group and one from the previously exposed group. The time required to perform surgery necessitated carrying out the transplants over 3 days.

After transfer, one calf from each of the recipient groups was killed at 4, 11, 17 and 21 days and total worm burdens estimated. Throughout the experiment faecal egg counts and serum pepsinogen levels were recorded.

Results

Faecal Egg Counts

Faecal egg counts for individual animals are given in Appendix 4a. Samples remained negative in Group 2 until transplant. With the exception of number 52, these became positive on day 1 and remained so until they were killed. Eggs were at no time detected from faecal samples of calf number 52. Group 3 faecal egg counts fell to zero after levamisole treatment

and rose again one day after transplant. These animals continued to pass eggs until the end of the experiment.

Post Mortem Worm Burdens

Post mortem worm burdens are shown in Table 4b. The mean worm burdens recovered from the previously infected recipients, Group 3, were very much greater than from the naive recipients Group 2. Larval stages were only recovered from one previously infected animal (No. 57) which had 1600 fourth stage and 2100 early fifth stage *O. ostertagi* in its abomasum.

Table 4b

Abomasal pH and post mortem worm burdens after transplantation of adult *O. ostertagi* into parasite-naive and previously infected recipients.

Group 2 — Naive Recipients

Day of Kill	Calf No.	Total *O. ostertagi*	Abomasal pH
4	50	2600	4.3
11	56	6000	4.6
17	55	1100	3.5
21	52	0	2.65

Group 3 — Previously infected recipients

Day of Kill	Calf No.	Total *O. ostertagi*	Abomasal pH
4	57	17900	3.1
11	59	16700	5.2
17	42	25100	5.4
21	43	17300	4.4

Pathology

Group 2

On gross examination of number 52 the abomasal mucosa had a normal appearance. In the other three calves of the previously naive group, abomasal damage was present.

Hyperplasia of the mucosa was evident and large thumbprint lesions were seen in both fundus and pylorus (Fig. 4a); between the fundic folds small nodules were apparent, some of which had depressed centres.

Group 3

At post mortem examination, the abomasal mucosa of all previously infected calves showed obvious pathological changes typical of ostertagiasis (Fig.4b).

Abomasal pH

Abomasal pH values at post mortem are shown in Table 4b. The abomasal pH was greater than 3.0 in all animals except No. 52.

Pepsinogen Levels

Mean serum pepsinogens are shown in Fig.4c. Individual values are given from the day of transplant in Appendix 4b. In the Group 2 animals, pepsinogen levels remained within the normal range until after transplant when levels rose in all animals except No. 52. Mean pepsinogens in Group 2 rose to 2.5 i.U. Tyrosine five days after transplant and remained above 1.5 i.U. until day 11. In Group 3 which were previously exposed,

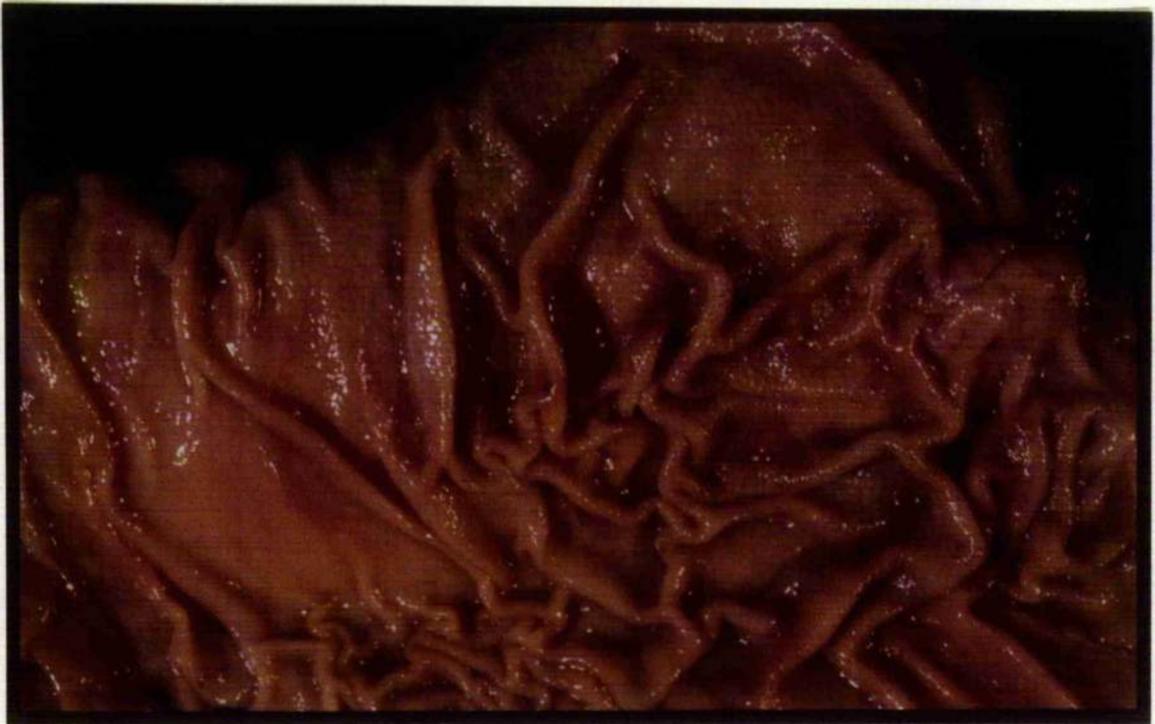

Fig. 4a 'Thumbprint' lesions on the abomasal mucosa of animal no. 55 from the naive recipient group (Group 2).

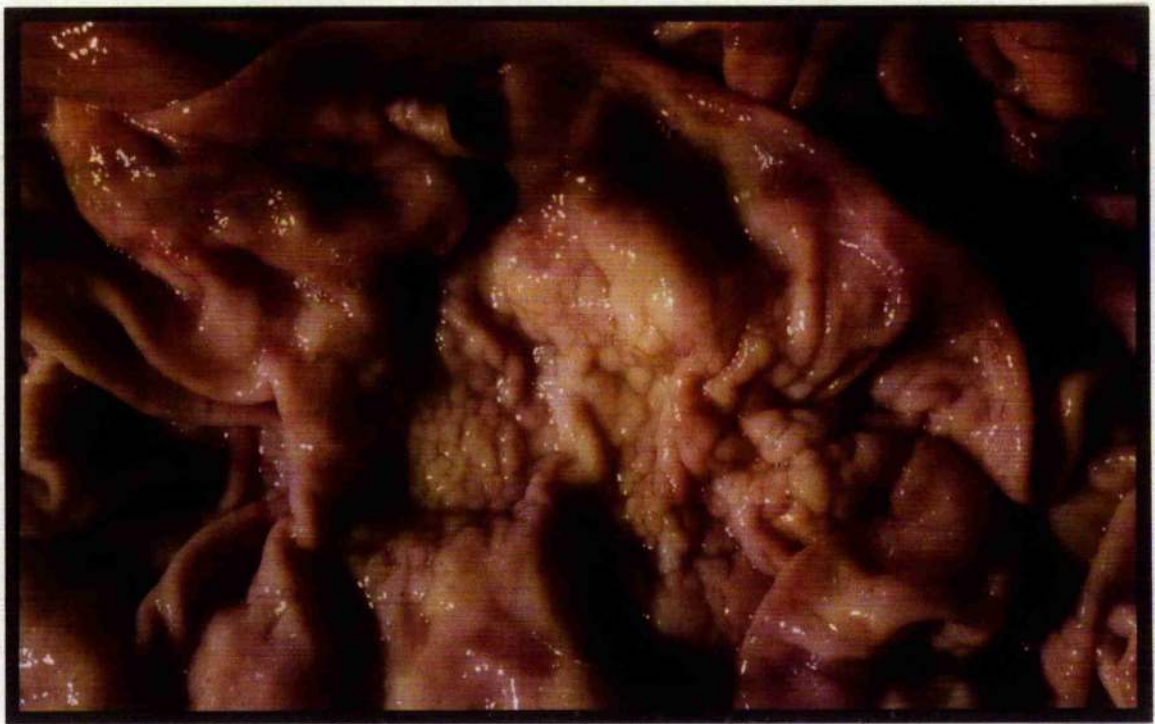

Fig. 4b Hyperplastic response, typical of ostertagiasis of the abomasal mucosa of animal no. 43 from the previously infected group (Group 3).

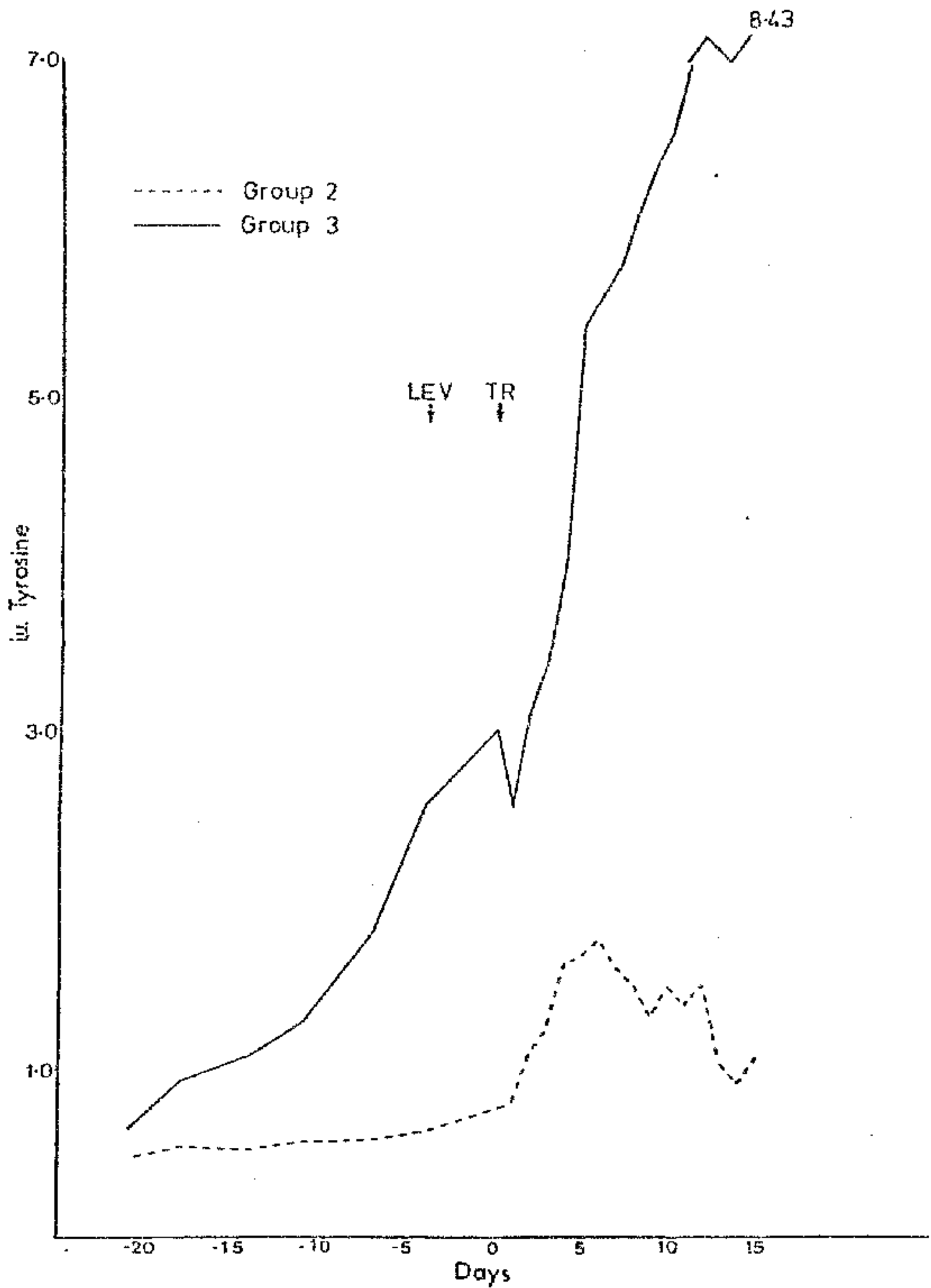

Fig. 4c Mean serum pepsinogen levels of the previously parasite naive (Group 2) and previously infected (Group 3) recipient calves.

the pepsinogen levels averaged 3.0 i.U. Tyrosine at the time of transplant. These subsequently rose markedly to above 8.0 i.U. Tyrosine on day 15 after transplantation of the adult worms.

Discussion

Parasites became established in all recipient calves except No. 52. The reason for the failure of establishment of transplanted worms in this animal is not known but it was fortuitous in that it served as a surgical control and indicated that the pathological changes observed were not a result of operative manipulations.

Examination of the post mortem worm burdens showed that very many more parasites were present in the Group 3 calves than in the Group 2 calves although an approximately similar number of adult O. ostertagi worms were transplanted into paired individuals of both groups. It is possible that some of the worms present in the Group 3 calves originated from the repeated infections with O. ostertagi larvae and had survived the levamisole treatment. This was confirmed by the finding of immature larvae which could not have originated from transplant in animal No. 57 which was the calf from Group 3 killed 4 days after transplant.

Even allowing for a 30% establishment of worms derived from the larval infections there is still a considerable discrepancy between the number of worms in Groups 2 and 3. The reason for this is not known but a possible explanation is that the damaged mucosa in the previously infected recipients of Group 3 provided a better environment for the worms to become established.

However the major object of the experiment was to assess the pathogenic effects of adult worms. Transplanted adults in the naive calves of Group 2 were responsible for gross pathological changes in the abomasal mucosa. In addition the serum pepsinogen levels of these calves increased to a peak of 2.5 i.U. Tyrosine five days after adult worm transplant. Also, despite the larger number of worms in the previously exposed Group 3 calves, the rise in serum pepsinogens following transplant was disproportionately high, for example calf No. 42 at 17 days post transplant had serum pepsinogen levels of nearly 10.0 i.U. Tyrosine. It is interesting that in a field study, Anderson et al (1965) associated natural disease with upwards of 40,000 adult parasites and in the same study recorded mean plasma pepsinogen levels of 2.9 i.U. Tyrosine from calves with clinical Type I ostertagiasis.

EXPERIMENT 5

Further studies on the response to transplanted adult *O. ostertagi* in calves

Introduction

This experiment was carried out to confirm the results of the preliminary adult *O. ostertagi* transplant experiment. More detailed investigations of the pathological and serum pepsinogen changes were undertaken and an assay performed to study the effect of transplanted adult *O. ostertagi* on serum gastrin levels.

Experimental Design

The design of the experiment is given in Table 5a. The source of adult parasites for the transplants were a group of six calves each infected with 250,000 *O. ostertagi* L_3. Donor calves were killed 22-24 days after infection. The abomasum of each donor calf was opened immediately into a warmed bucket and the mucosal surface was gently washed under a stream of warm phosphate buffered saline (PBS). The volume of fluid was estimated and after gentle agitation a 100 ml sample was taken for estimation of the total donor worm burden. The remaining contents were divided equally and kept warm until transfer to two recipient calves. An estimate of the number of worms obtained from each donor is given in Table 5b.

Table 5a Further studies on the response to transplanted adult *O. ostertagi* in calves - Experimental Design.

									Necropsy	
Day	D-42	D-28	D-21	D-7	D 0	D 4	D10	D11	D17	D21
Group 1					TR	+		+	+	+
Group 2		INF		FBZ	TR	+		+	+	+
Group 3	INF		FBZ		TR	+		+	+	+
Group 4					SHAM TR					+
Group 5			INF		FBZ		+			

INF - 10,000 *O. ostertagi* L_3 on alternate days for 21 days

FBZ - Intraruminal fenbendazole at 7.5 mg/kg.

TR - Transplant of adult *O. ostertagi*

\+ - Necropsy

Table 5b

Number of adult O. ostertagi recovered from donor calves given a single infection of 250,000 O. ostertagi L_3

Donor Calf No.	Estimated Adult O. ostertagi Burdens
R99	24,600
R100	52,300
B59	9,550
B60	33,300
Y49	31,450
Y50	52,300

Three experimental groups, each of four calves, were treated as follows.

Group 1 calves were maintained parasite free until transplanted with adult O. ostertagi.

Group 2 were infected with 10,000 O. ostertagi L_3 on alternate days for 21 days then treated with fenbendazole and transplanted with adult O. ostertagi seven days later.

Group 3 calves received the same larval infection regimen as Group 2 but were transplanted with adult O. ostertagi 21 days after anthelmintic treatment.

One calf from each of Groups 1, 2 and 3 were killed 4, 11, 17 and 21 days after transplant. A surgical control group of two calves (Group 4) was maintained parasite-naive and received only sterile water at laparotomy. A further two calves (Group 5)

given the same larval infection regimen as Group 2 and 3 were killed 10 days after anthelmintic treatment to assess anthelmintic efficiency. Throughout the experiment, faecal egg counts and serum pepsinogen levels were recorded. Serum gastrin levels were measured in the Group 1 calves and in the surgical controls (Group 4). At post mortem abomasal pH and total worm burdens were estimated and sections were taken for histology.

Results

Faecal Egg Counts

Mean faecal egg counts (epg) are shown for Groups 1, 2 and 3 in Fig. 5a and individual counts are given for all the recipient animals in Appendix 5a. Ostertagia eggs were present in the faeces of Groups 1, 2 and 3 immediately after transplant confirming establishment of the adult parasites. In Group 1 these increased rapidly and were positive until day 21 after transplant. A small rise in faecal egg count occurred in the Group 2 calves towards the end of the period of larval infections. This fell to zero after fenbendazole treatment and rose again after transplant. Group 3 showed a similar peak after exposure to larvae, which fell after anthelmintic therapy and increased again when the adult parasites established. Samples from Group 4 remained negative throughout while those from group 5 showed a slight rise after larval infection and returned to zero when given fenbendazole.

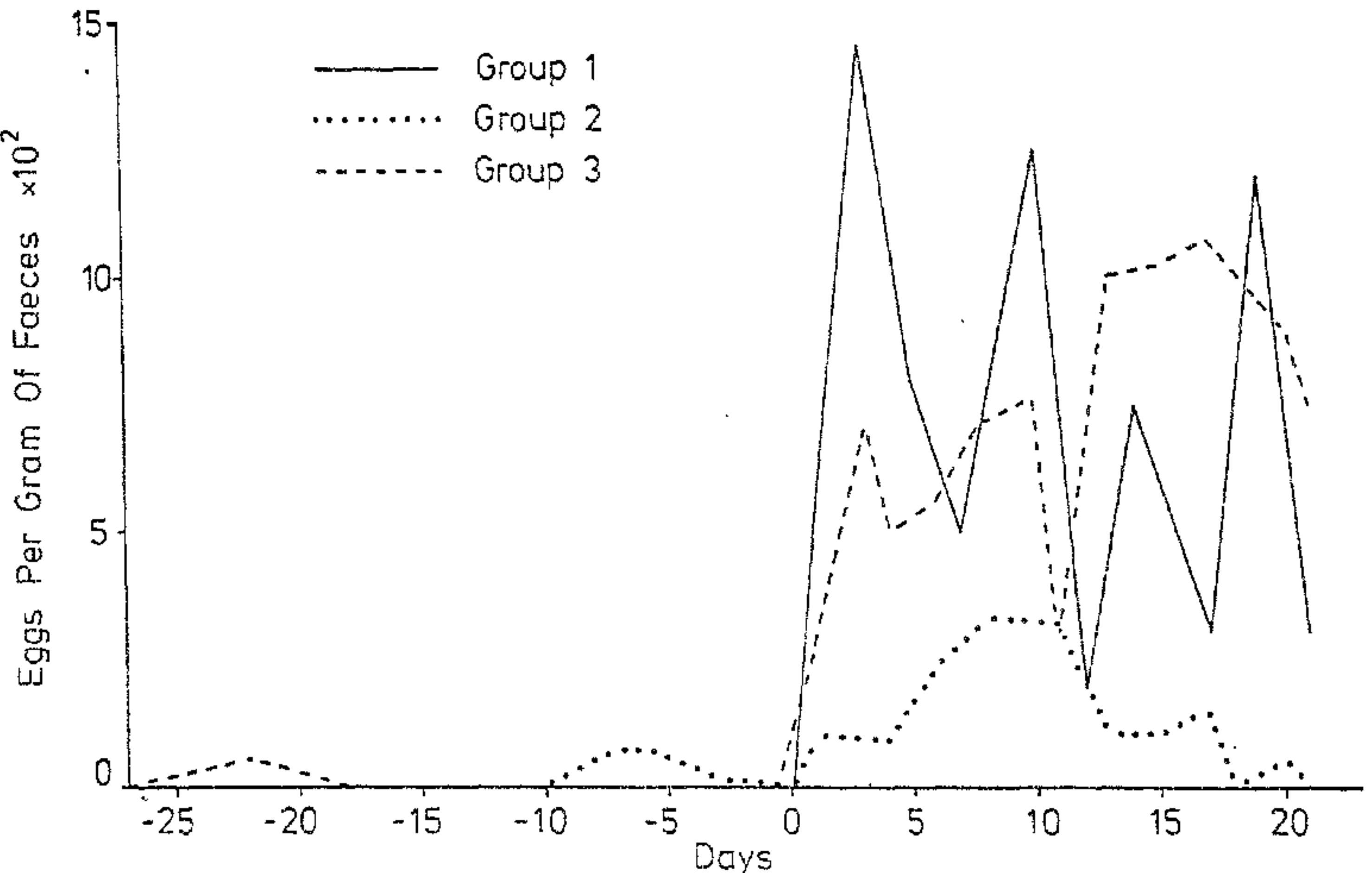

Fig. 5a Mean faecal egg counts (epg) for three groups of calves transplanted with adult O. ostertagi on day 0. Group 1 calves were maintained parasite free until transplant. Group 2 were infected with 10,000 O. ostertagi L_3 on alternate days for 21 days then were treated with fenbendazole seven days before transplant. Group 3 were infected with 10,000 O. ostertagi L_3 on alternate days for 21 days then were treated with fenbendazole 21 days before transplant.

Post Mortem Worm Burdens

Individual worm counts at post mortem are shown in Table 5c. There was little difference in the mean numbers of adult parasites recovered from the three major groups with the exception of two animals from Group 2 which had low post mortem worm counts. This was probably a reflection on the low worm burdens of the donor calf (No. 59) used to provide worms for transplant into these two calves. Low worm burdens were found at necropsy of the two anthelmintic controls confirming the high efficiency of fenbendazole (>99%).

Table 5c

Post mortem worm burdens and abomasal pH of calves transplanted with adult *O. ostertagi* on Day 0.

Group	Day of Necropsy	Total *O. ostertagi*	Abomasal pH
1 Parasite naive recipients	4	14,800	6.7
	11	11,000	2.1
	17	12,900	2.8
	21	18,300	4.1
2 Previously infected recipients	4	22,300	5.6
	11	17,100	4.1
	17	1,900	2.2
FBZ D-7	21	200	2.0
3 Previously infected recipients	4	8,000	5.1
	11	17,800	2.2
	17	11,000	3.1
FBZ D-21	21	8,800	2.4
4 Surgical controls	21	0	2.0
	21	0	2.3
5 Anthelmintic controls	10	1,000	2.4
	10	300	3.1

Pathology

Gross examination of the abomasal mucosa of the Group 1 calves revealed small umbilicated nodules scattered over the fundus (Fig.5b). The previously infected calves (Group 2 and 3) all showed pathological changes typical of O. ostertagi infection.

Group 4, the surgical controls, had apparently normal abomasal mucosa while in the treated calves of Group 5, Ostertagia nodules could still be seen.

Histopathology

In abomasal sections from the calves in Group 1, some dilated gastric glands were evident throughout the lamina propria and these were lined by columnar cells. Many eosinophils and lymphocytes could be seen in the inter glandular tissue. In the Group 2 animals similar dilated glands were present which occasionally contained parasites while in sections from animals from Group 3, the parietal and zymogen cells of the glands were well differentiated. Surgical control animals had normal glandular morphology and although some expanded empty glands were noted in the anthelmintic controls, the tissue between such glands appeared normal.

Abomasal pH

Individual abomasal pH values at post mortem are shown in Table 5c. The mean abomasal pH values recorded from Groups 1, 2 and 3 were similar. In each of these groups the animal killed on day 4 had the most markedly elevated pH.

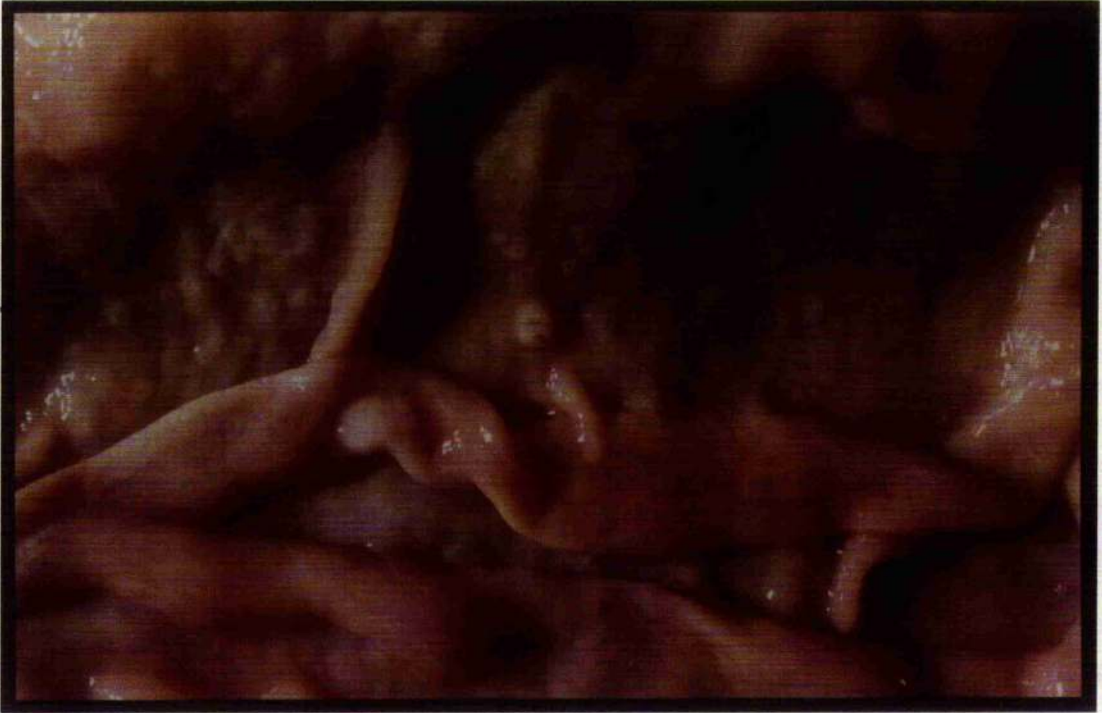

Fig. 5b Umbilicated nodules on the abomasal fundus of animal no. R11 from the previously parasite-naive group (Group 1).

Pepsinogens

Serum pepsinogen levels are shown for individual animals in Figs. 5c - 5f.

Group 1 (Fig.5c). A sharp rise in serum pepsinogen to about 4 i.U. Tyrosine occurred in all animals within 24 hours after transplant of the adult parasites and these remained elevated until slaughter.

Group 2 (Fig.5d). Serum pepsinogen values in two of these animals rose steeply after transplant while the other two animals showed a lesser pepsinogen response. The donor animal (B59) which acted as the source of parasites for the latter two calves was diarrhoeic prior to transplant and when killed, the abomasum had markedly diphtheritic lesions. It was estimated that only 4000 parasites were transplanted from this donor into each recipient; from the post mortem worm counts it is obvious that very few worms had established.

Group 3 (Fig.5e). These calves showed a similar pattern in serum pepsinogen levels to those observed in Group 1.

Group 4 (Fig.5f). In the surgical controls, pepsinogen levels remained below 0.9 i.U. Tyrosine throughout.

Group 5 (Fig.5f). In Group 5 a moderate rise in serum pepsinogen in response to larval infection was followed by a return to near normal levels after fenbendazole treatment.

Serum Gastrin

Mean serum gastrin levels for Groups 1 and 4 are shown in Fig.5g. Individual gastrin values for animals in these groups are given in Appendix 5b. There was a rapid rise in serum

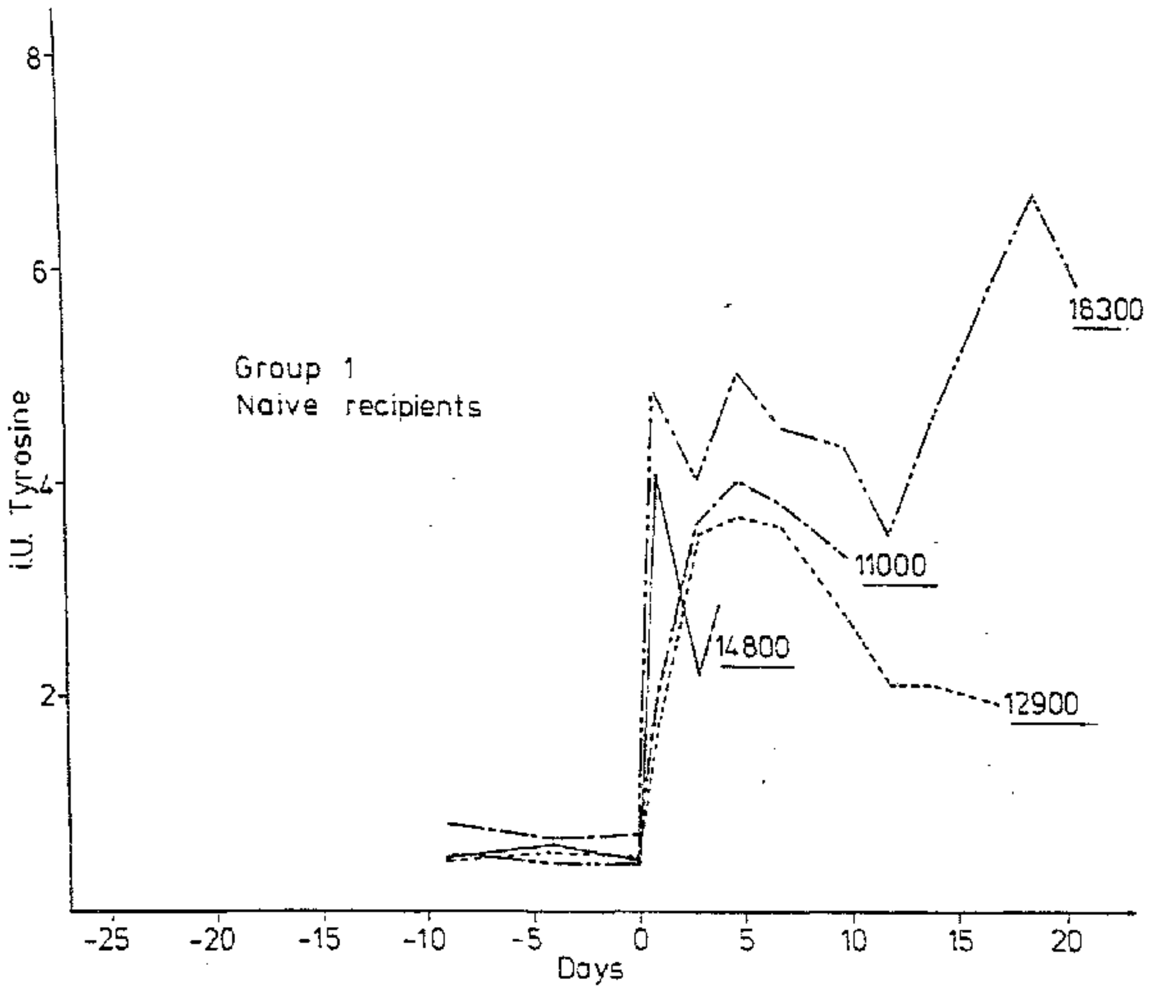

Fig. 5c Individual serum pepsinogen levels (i.U. Tyrosine) of Group 1 calves maintained parasite free until transplanted with adult _O. ostertagi_.

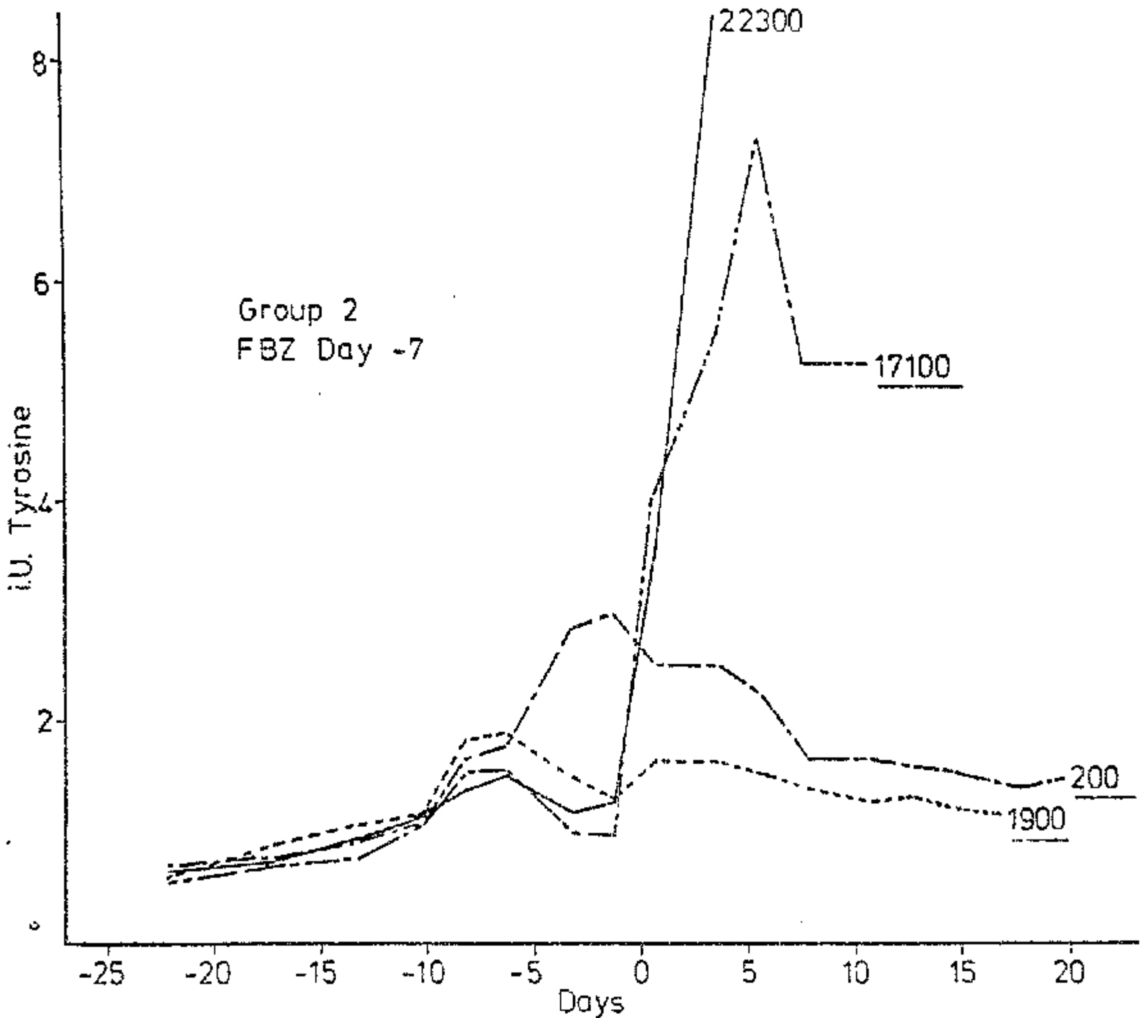

Fig. 5d Individual serum pepsinogen levels (i.U. Tyrosine) of Group 2 calves previously infected with _O. ostertagi_ larvae, treated with fenbendazole on day -7 then transplanted with adult _O. ostertagi_ on day 0.

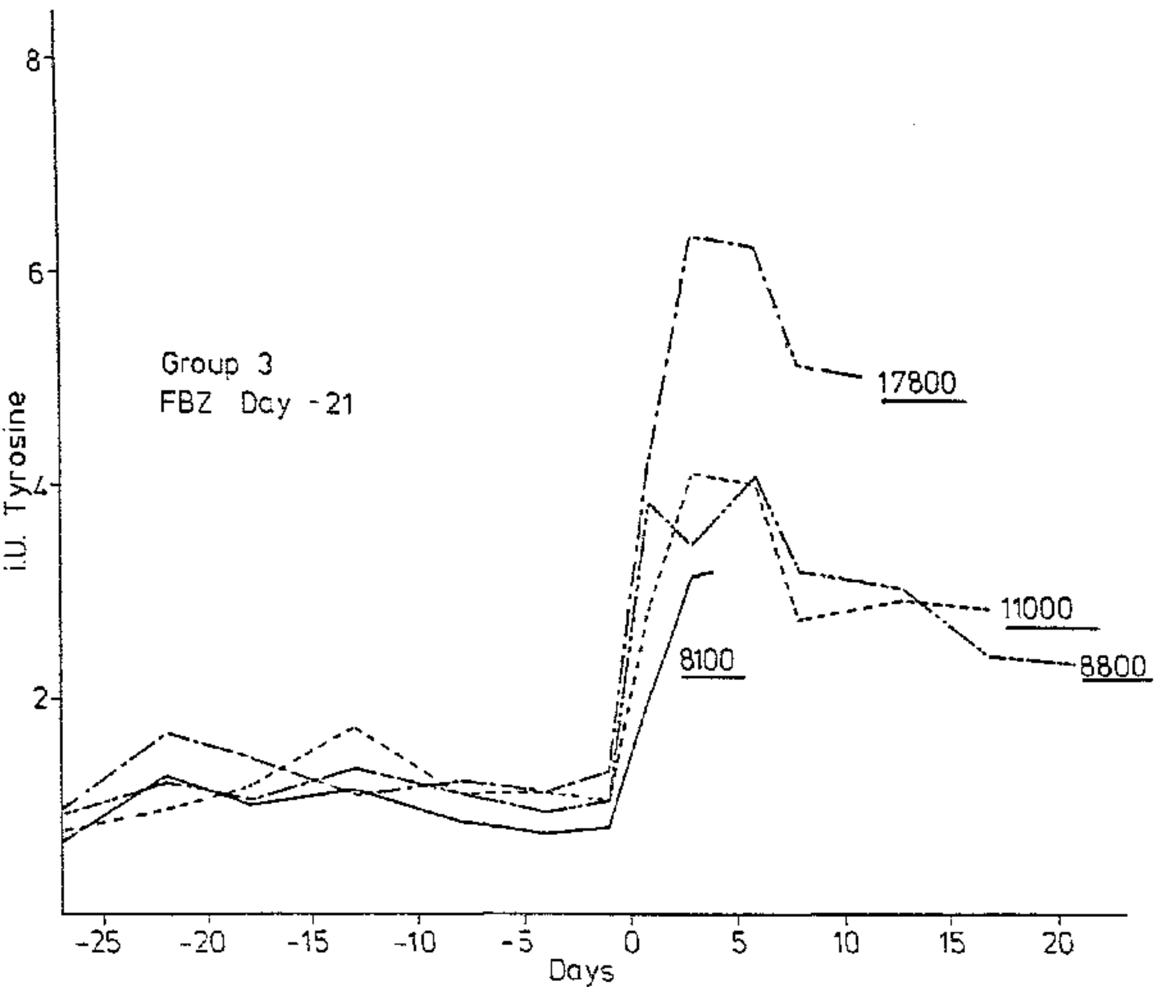

Fig. 5e Individual serum pepsinogen levels (i.U. Tyrosine) of Group 3 calves, previously infected with O. ostertagi larvae, treated with fenbendazole on day -21 then transplanted with adult O. ostertagi on day 0.

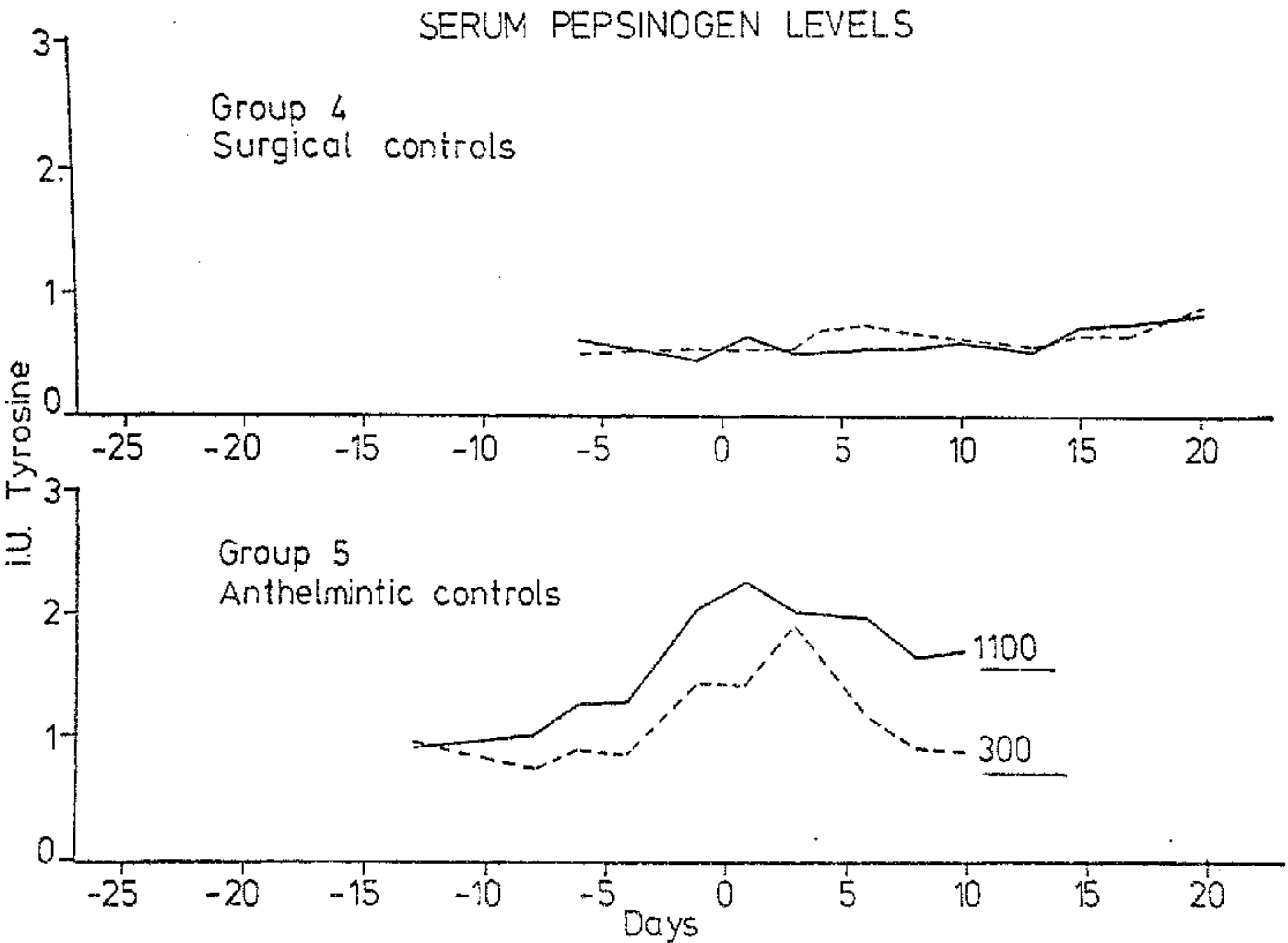

Fig. 5f Individual serum pepsinogen levels (i.U.Tyrosine) of Group 4, the surgical control calves and Group 5, the anthelmintic control calves.

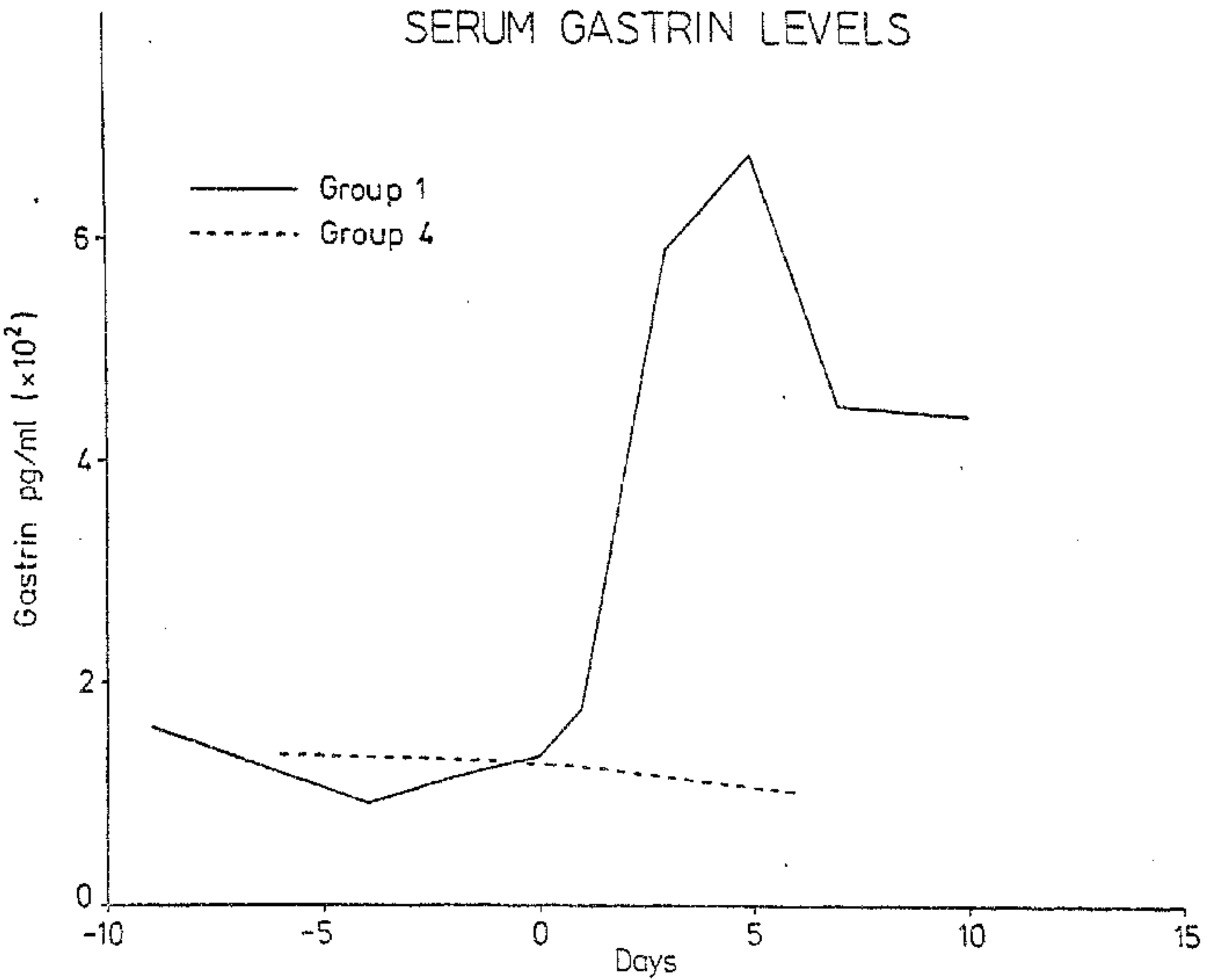

Fig. 5g Mean serum gastrin levels of calves in Group 1, maintained parasite free until transplanted with adult _O. ostertagi_ and Group 4 which were surgical controls.

gastrin levels of the naive recipients almost immediately after transplant of adult worms which was maintained over a period of 10 days while the levels recorded from the surgical controls remained within normal limits.

Discussion

It is apparent from these results that adult O. ostertagi per se can elicit significant pathological changes in the abomasum. Thus serum pepsinogen and gastrin levels were elevated and gastric acidity was altered.

Changes were observed following the transplant of adult O. ostertagi worms into both parasite naive calves and those which had received previous larval infections subsequently terminated by anthelmintic treatment. The fact that pepsinogen levels were increased within 24 hours of transplanting worms to the naive calves indicates a positive role for the mature worm in initiating a pepsinogen response. This was a surprising result since current opinion suggests that the elevation in pepsinogen levels in ostertagiasis is associated with the emergence of 5th larval stages from the gastric glands at which time the glands are stretched and dilated and become lined with rapidly dividing undifferentiated epithelial cells between which pepsinogen is thought to leak into the blood (Jennings et al, 1966).

There was little difference in the survival of adult Ostertagia in parasite naive calves and in those previously subjected to repeated larval infection prior to anthelmintic treatment. Furthermore surgery was not responsible for any of the biochemical or pathological changes. Parasitic burdens in

previously naive and previously infected groups contrast with the results of Experiment 4 where very many more parasites were recovered from the previously infected recipient calves than the previously naive recipient calves.

The aetiology of the nodular response in the Group 1 calves remains uncertain. Although there was some dilation of the gastric glands with a reduction in cellular differentiation, parasites were not seen within the glands of Group 1.

It is possible, therefore, that the hyperplastic response could be initiated by a mechanism other than the physical stretching of the glands by growing parasites. Hormonal or inflammatory mediators may be involved and the observed rise in serum gastrin provides some evidence for hormonal involvement.

EXPERIMENT 6

Comparison of the local and systemic response to transplanted adult O. ostertagi in calves prepared with abomasal cannulae.

Introduction

The results of experiments 4 and 5 indicated that adult O. ostertagi alone could be responsible for considerable pathophysiological changes in the abomasum of infected calves and the observed rise in serum gastrin levels suggested that some of these changes could be hormonally mediated. A further experiment was therefore carried out to confirm and expand these earlier observations.

Cannulated calves were used so that local changes in the abomasum could be compared with serum gastrin and pepsinogen values. This experiment was designed to follow in detail the sequence of events over the first 36 hours after adult worms were implanted and subsequently daily for about 10 days (Table 6a).

Table 6a

Comparison of the local and systemic responses to transplanted adult *O. ostertagi* in calves prepared with abomasal cannulae – Experimental Design.

Day	D -35	D -14	D -7	D 0	D 8 or D 13
Group 1	INF.	FBZ	Cannulation	TR	+
Group 2	–	–	Cannulation	TR	+

INF. 10,000 *O. ostertagi* L_3 on alternate days for 21 days

FBZ Intrarumenal 7.5 mg/kg

TR Transplant of adult *O. ostertagi*

\+ Necropsy

The source of adult parasites for transplantation were a group of 3 donor calves each infected with 250,000 *O. ostertagi* L_3 and killed 23-28 days later. Eight recipient calves were divided into two groups of four. Group 1 were infected with 10,000 *O. ostertagi* L_3 on alternate days for 21 days then treated with fenbendazole intrarumenally, seven days prior to insertion of abomasal cannulae. Group 2 were maintained parasite naive until they were cannulated. Seven days after cannulation a known number of adult *Ostertagia* were introduced to the abomasa of three calves from each group. The remaining calf from each group acted as a control being given only sterile water via the cannula. Blood and faecal samples, together with samples of abomasal contents were collected once every two or three hours for the first 36 hours after transplant, then twice daily.

Pepsin and pH estimations were carried out on the abomasal fluid. Abomasal biopsies were taken at regular intervals throughout the experiment. Calves were killed on day 8 or 13 after transplant and abomasal worm burdens estimated.

Results

Clinical Observations

Wound breakdown necessitated the early euthanasia of the uninfected control from Group 2. Otherwise all calves remained bright until necropsy. The three recipient calves from the previously infected group (Group 1) passed soft faeces for two days after transplant of the adult parasites; faeces from the control calf of this group and from the calves of Group 2 were normal.

Faecal Egg Counts

Individual faecal egg counts are shown in Appendix 6a. *Ostertagia* eggs were present in the faeces of all calves which were transplanted with adult parasites. Low faecal egg counts of 50 e.p.g. were recorded from the control calf of the previously infected group, indicating that anthelmintic treatment had not been 100% effective; faeces from the uninfected control animal remained negative until necropsy.

Post Mortem Worm Burdens

Post mortem worm burdens are shown in Table 6b. The estimated worm burdens of the donor calves which were used in the

Table 6b

Post mortem worm burdens of donor and recipient calves.

Group 1 - previously infected recipients
Group 2 - previously naive recipients

Group	Animal No.	L_4	Adult Male	Adult Female	Total	Donor which provided worms for transplant
Donors	B81	0	3,700	15,000	18,700	
	B95	0	17,750	20,800	38,550	
	72	0	10,100	25,100	35,200	
Group 1	74	0	5,300	5,400	10,700	B95
	76	0	3,400	7,600	11,000	72
	77	0	5,300	6.400	11,700	72
	73	200	1,500	2,200	2,700	Control
Group 2	64	0	600	2,600	3,200	B81
	67	0	800	3,300	4,100	B81
	71	0	5,200	3,000	8,200	B95
	68	0	0	0	0	Control

transplants are also shown and the source of worms for each recipient is indicated.

Worms were recovered from all recipient calves at necropsy. The control calf from the previously infected group had a low post mortem worm burden, approximately five percent of which were fourth stage larvae. No worms were recovered from the control calf from Group 2.

Gross Pathology

At post mortem all the abomasa of the Group 1 calves had lesions typical of ostertagiasis. There was no significant difference in the lesions observed in the three transplanted calves and the control calf in this group. The Group 2 calves had little or no obvious gross pathological changes, although small areas of mucosal erosion could be seen in the pylorus of one animal (No. 71). There were no gross lesions in the Group 2 control calf (No. 68).

Histopathology

Group 1: The three previously infected recipient calves had similar lesions in the abomasal mucosa. In sections from the pylorus there were clumps of eosinophils and areas of lymphoid hyperplasia; in some sections dilated, empty glands could be seen lined by undifferentiated epithelium. In many cases, worms were present in close association with the mucosa (Fig.6a) or in the mucus and debris on the mucosal surface (Fig.6b). In the fundic mucosa, empty glands could be seen and there were many lymphoid

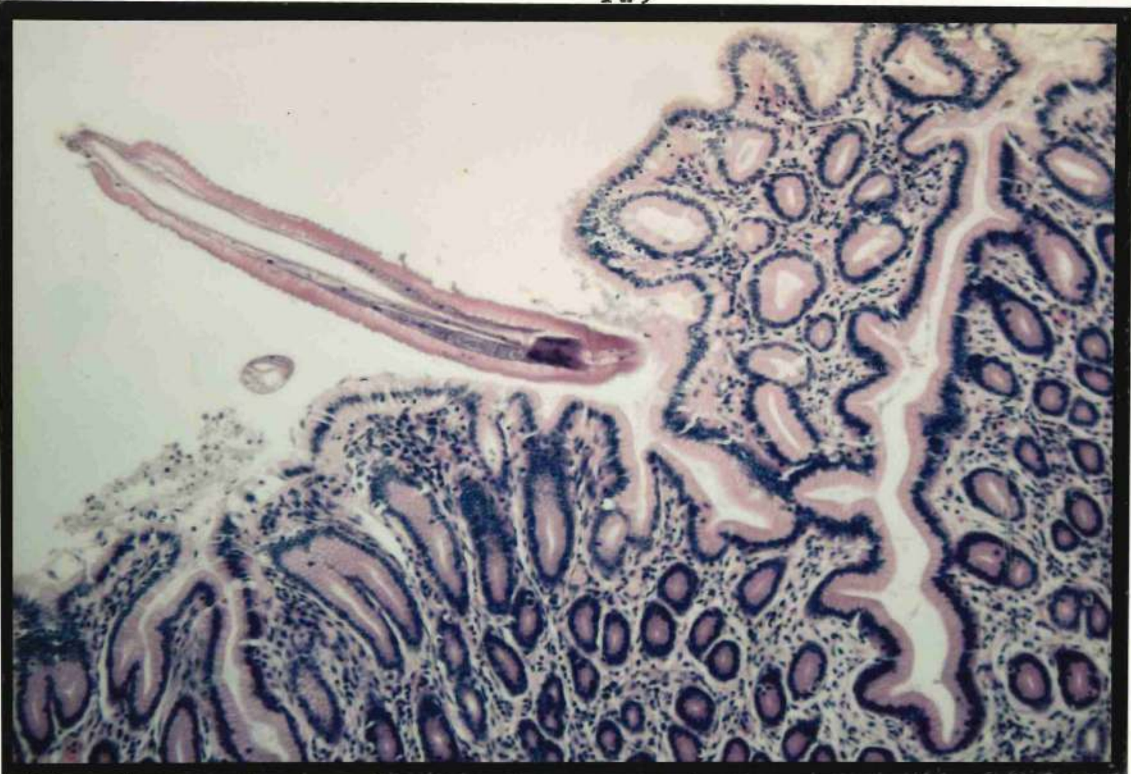

Fig. 6a A parasite in close association with the mucosal surface of calf no. 77 (Group 1). Haematoxylin and Eosin.

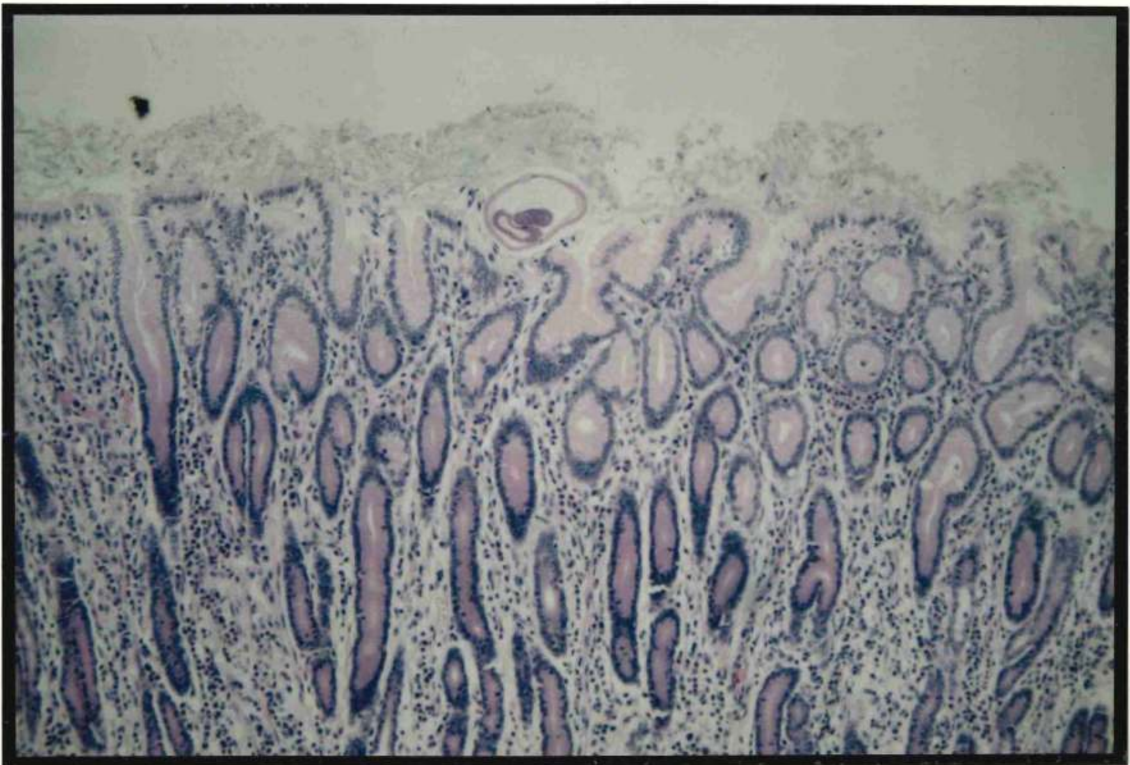

Fig. 6b A parasite in the abomasal mucus layer of calf no. 77 (Group 1). Haematoxylin and Eosin.

aggregates and eosinophils. However there was clear differentiation of the parietal and zymogen cells and in general there was an obvious transition from the columnar mucus secreting cells of the crypts through to the pepsinogen and acid secreting cells of the gland body.

In the Group 1 control (No. 73), lymphoid hyperplasia with eosinophil infiltration was marked and in one section, parasites could be seen both on the surface and deep in the mucosa.

Group 2: In sections from two animals (Nos. 71 and 64) which were maintained parasite-naive until transplanted, adult worms were observed close to the mucosal surface (Fig.6c) and in one of these a cross-section of a parasite was present within the mucosal tissue (Fig.6d). In sections from the pylorus of one animal (No. 71) clumps of lymphocytes and eosinophils could be seen and there were occasional dilated empty glands. In all sections from the remaining animals of this group, there was good differentiation of cells within the glands. In the uninfected control of this group (No. 68) the mucosa had a normal appearance.

Abomasal pH

The pH values of the abomasal contents of individual animals are shown in Figs.6e and 6f. The abomasal pH of animals in Group 1 did not rise above 4 at any time after transplant and in most samples the pH was below 3. The majority of samples from the Group 2 controls also remained within the normal range although the pH from one animal (No. 64) rose to a peak of 4.7, 14 hours after transplant.

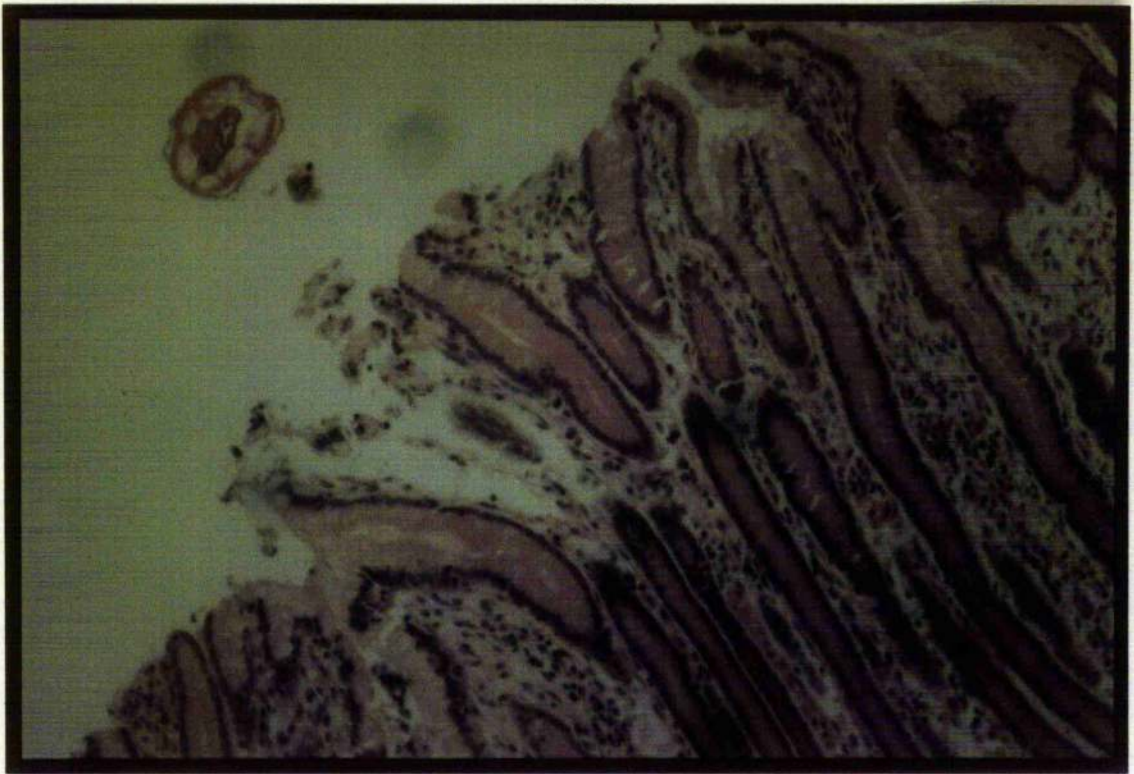

Fig. 6c An adult parasite in the abomasal lumen of animal no. 71 (Group 2). Haematoxylin and Eosin.

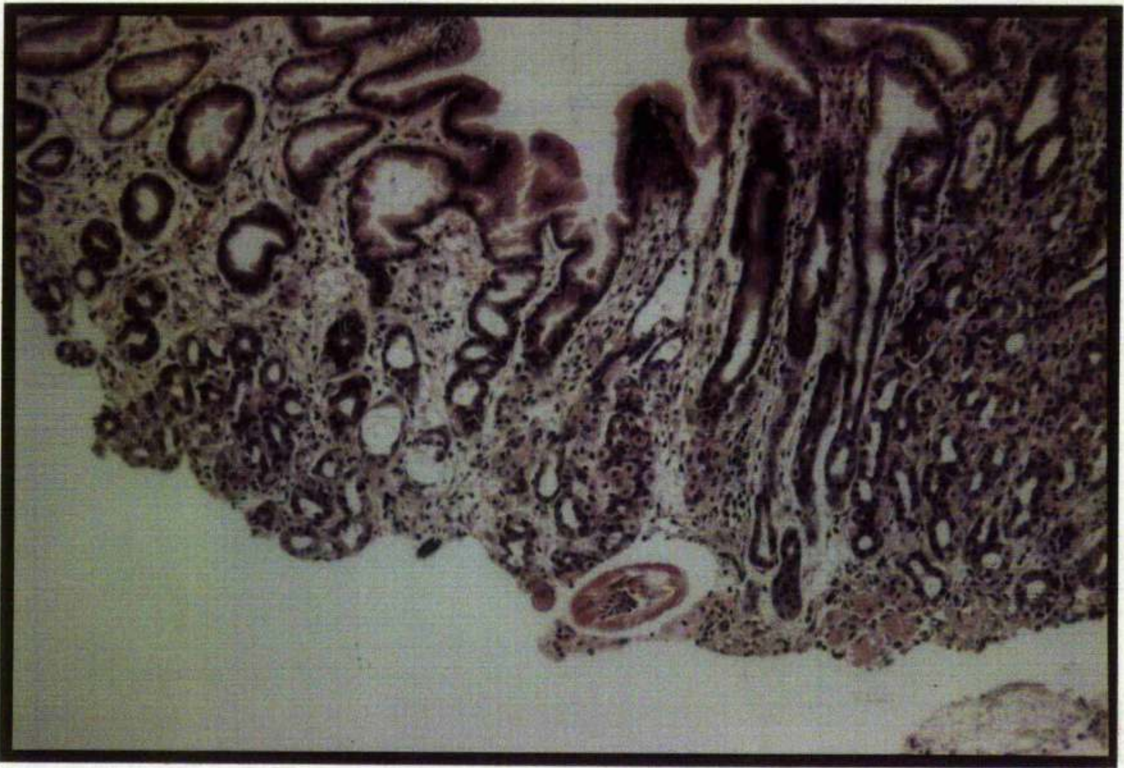

Fig. 6d A cross section of a parasite within the abomasal mucosa of animal no. 64 (Group 2). Haematoxylin and Eosin.

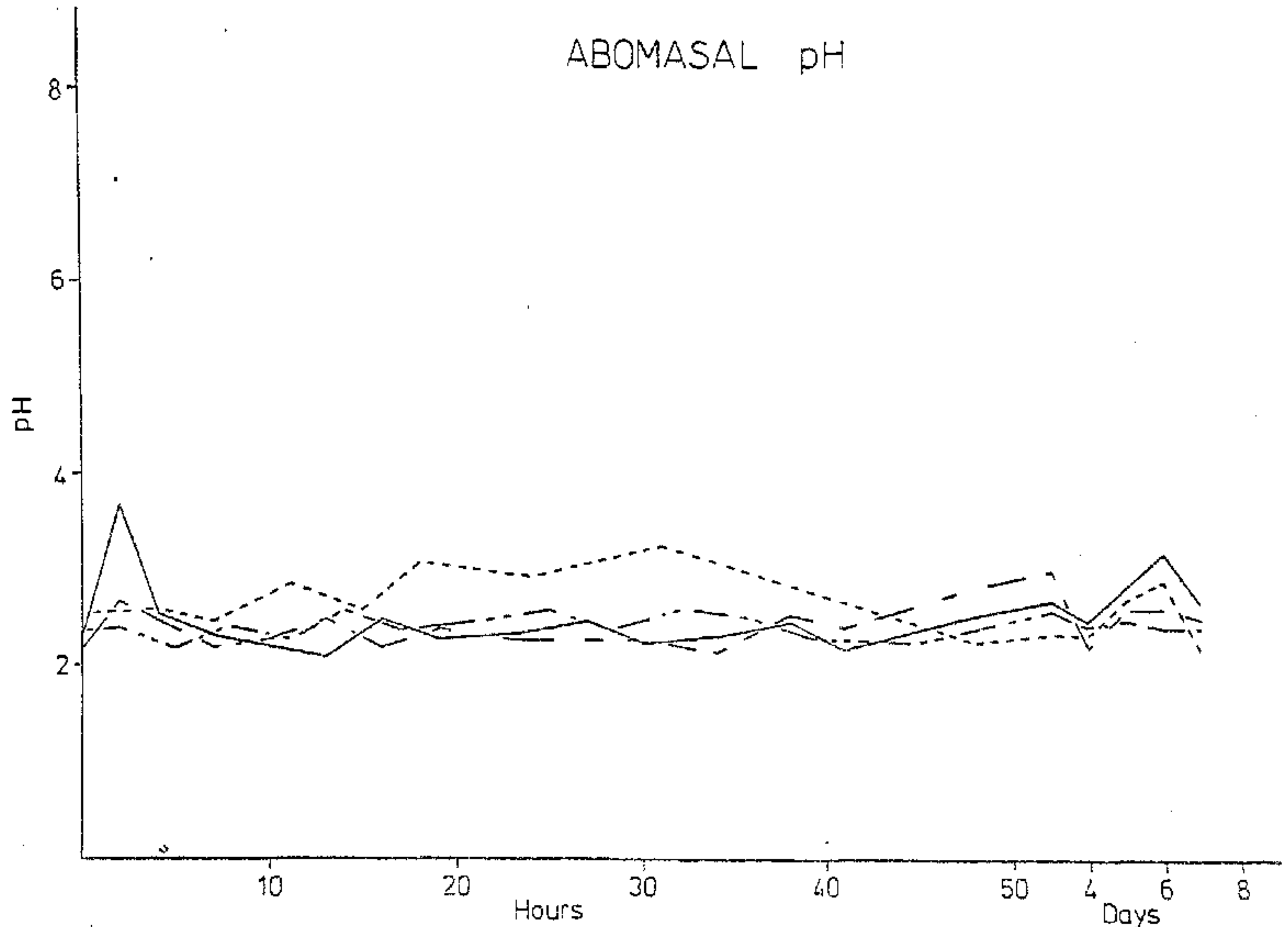

Fig. 6e The abomasal pH values of individual animals of Group 1, which were previously infected with <u>Ostertagia</u> larvae and treated with fenbendazole prior to transplantation with adult parasites at 0 hours

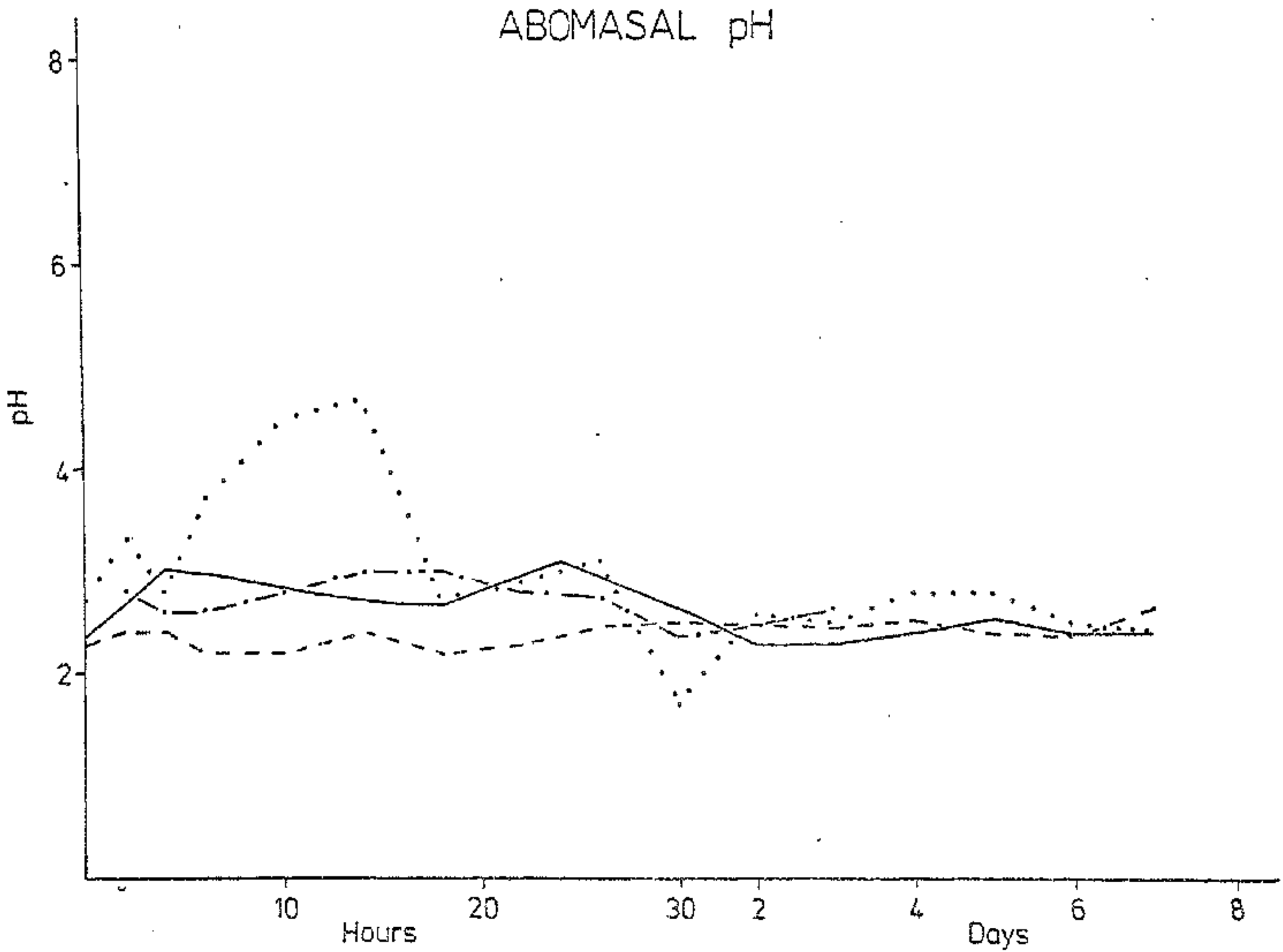

Fig. 6f The abomasal pH values of individual animals of Group 2 which were maintained parasite free until transplantation with adult parasites at 0 hours.

Abomasal Pepsin

The mean abomasal pepsin values of the three recipient calves of each group are shown in Fig.6g with individual values in Appendix 6b.

There was considerable individual variation in abomasal pepsin levels throughout, although the mean level for Group 1, which were the previously infected recipients, was always higher than that of the naive recipients of Group 2.

Serum Pepsinogen Levels

Individual serum pepsinogen values for Group 1 animals are shown in Fig.6h and for Group 2 animals in Fig.6i.

Group 1: The serum pepsinogen levels of the calves in the previously infected group all rose after transplant of the adult parasites. The two calves which received the greatest number of parasites had the highest levels of pepsinogen; these rose to peak values of greater than 5 i.U. Tyrosine between 3-4 days after transplant and although they subsequently declined they were still above 3.0 i.U. Tyrosine ten days after transplant. The remaining recipient which had fewer worms at necropsy (No. 74) had considerably lower pepsinogen levels. The control animal from this group (No. 73) had low pepsinogen values throughout.

Group 2: Pepsinogen levels recorded from the naive recipient calves were all lower than those of the previously exposed recipients. However the highest value of 1.9 i.U.Tyrosine for calf No. 71 was only slightly lower than for calf No. 74 of the previously infected group, both of these calves receiving their

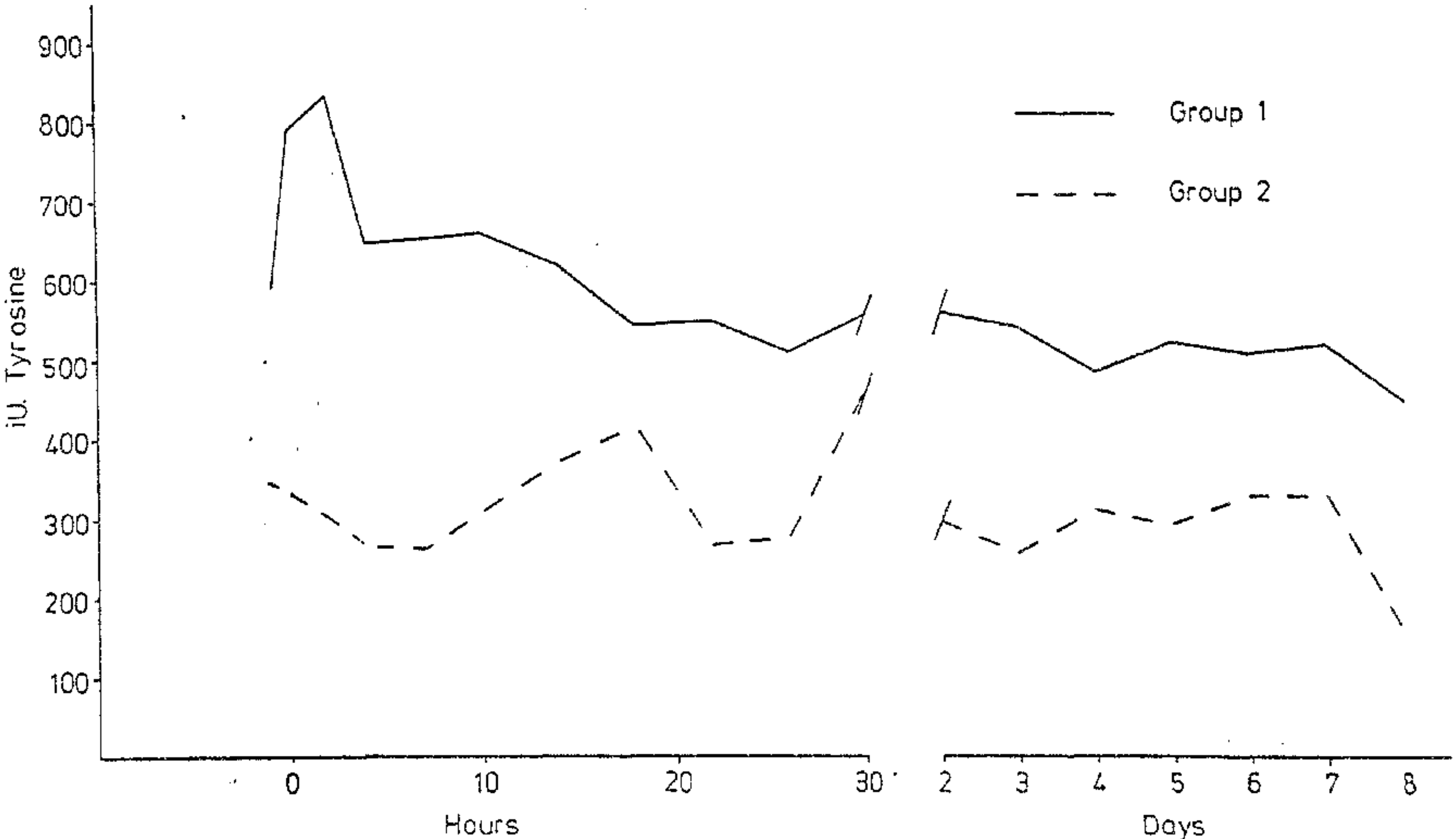

Fig. 6g The mean abomasal pepsin values of the three recipient calves of each group. Group 1 were previously infected with _Ostertagia_ larvae and treated with fenbendazole prior to transplantation with adult parasites at 0 hours. Group 2 were maintained parasite free until transplantation with adult parasites at 0 hours.

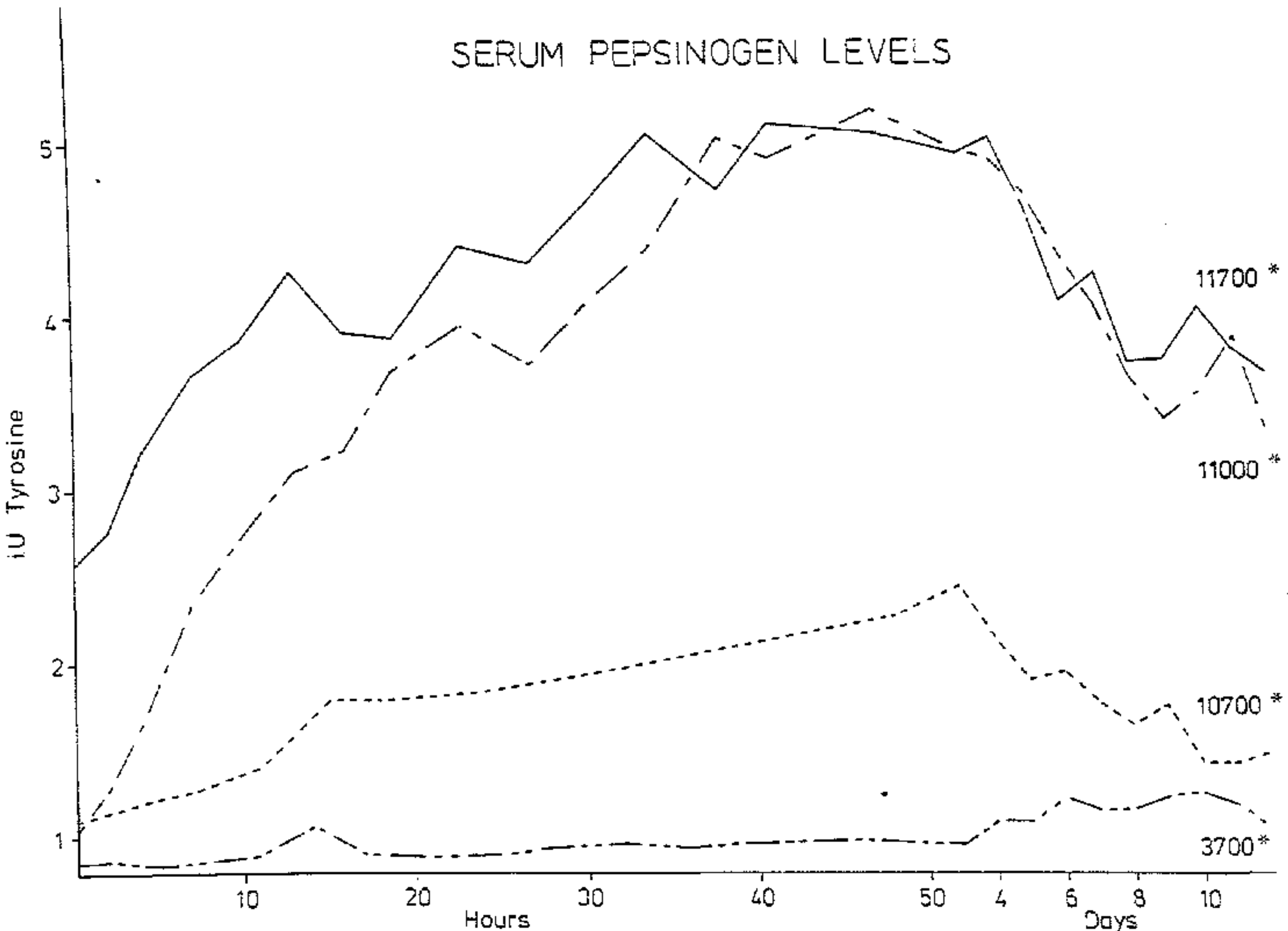

Fig. 6h The serum pepsinogen values of individual animals of Group 1 which were previously infected with <u>Ostertagia</u> larvae and treated with fenbendazole prior to transplantation with adult parasites at 0 hours.

* Worm burdens of individual animals

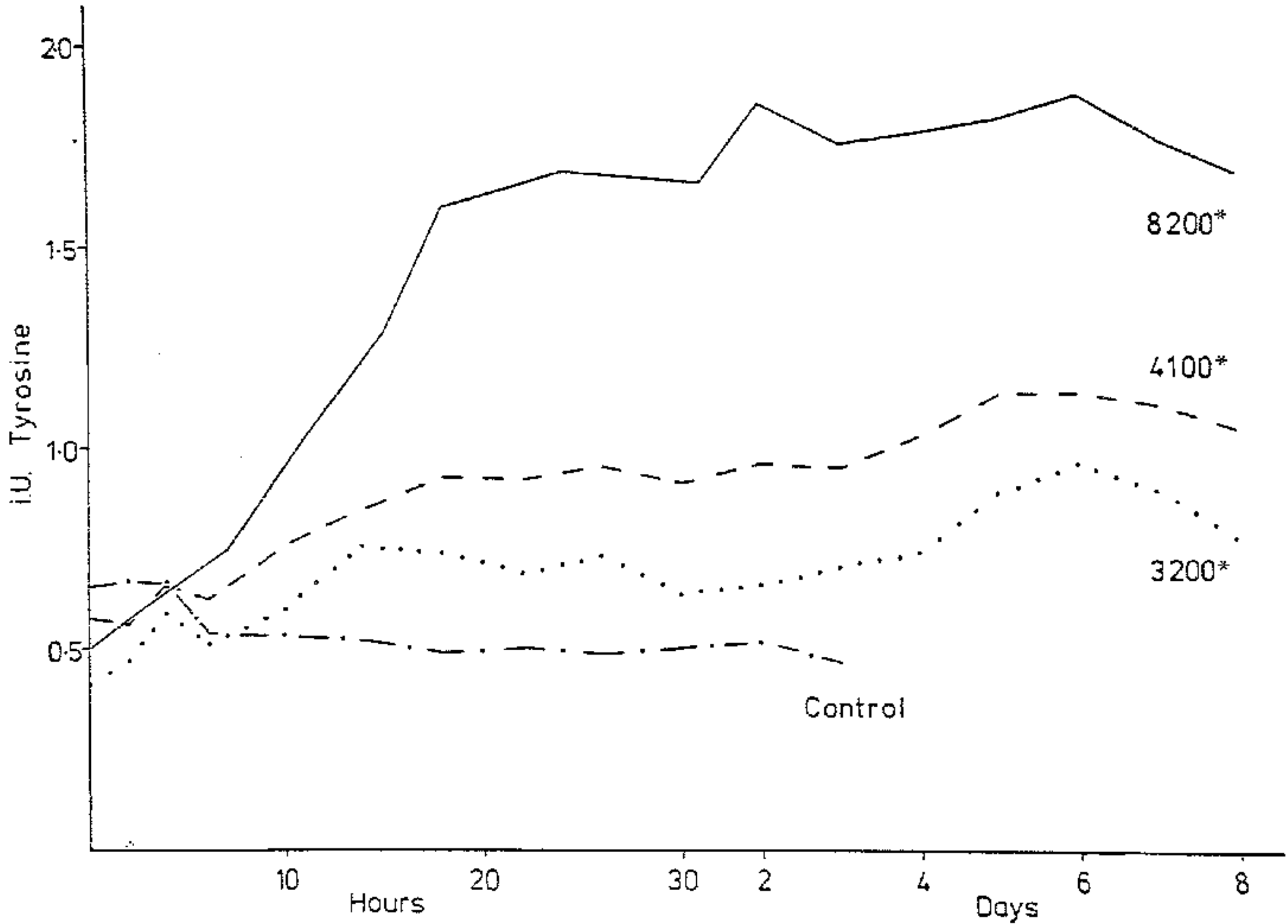

Fig. 6i The serum pepsinogen values of individual animals of Group 2 which were maintained parasite free until transplantation with adult parasites at 0 hours.

* Worm burdens of individual animals

adult worm burden from the same donor and both having similar post mortem worm burdens. The other naive recipient calves (No. 67 and 64) which had the lowest worm burdens also had the lowest pepsinogen values, rising to peak levels of only 1.4 i.U and 1.0 i.U. Tyrosine respectively. No significant change was observed in the Group 2 control calf (No. 68) which had serum pepsinogen values of approximately 0.5 i.U. Tyrosine throughout.

Serum Gastrin Levels

Serum gastrin levels for individual animals of Group 1 are shown in Fig.6j and for individual animals of Group 2 in Fig. 6k.

Group 1: After transplant, gastrin levels in the three recipient calves of this group were always higher than in the control calf. Serum gastrin levels of the two most heavily infected calves rose slowly throughout although levels in both animals only exceeded 400 pg/ml on one sampling occasion. The previously infected recipient animal with the lowest post mortem worm count (No. 74) had a higher serum gastrin level at the time of transplant but did not show any consistent upward trend over the subsequent sampling period. At no time did serum gastrin levels exceed 400 pg/ml in this animal. The control calf of this group had low gastrin values of below 200 pg/ml which remained fairly constant until necropsy.

Group 2: Values fluctuated in all animals of this group but never exceeded 350 pg/ml on any occasion. No upward or downward trend was apparent and the control animal did not appear to differ from the infected animals in this group.

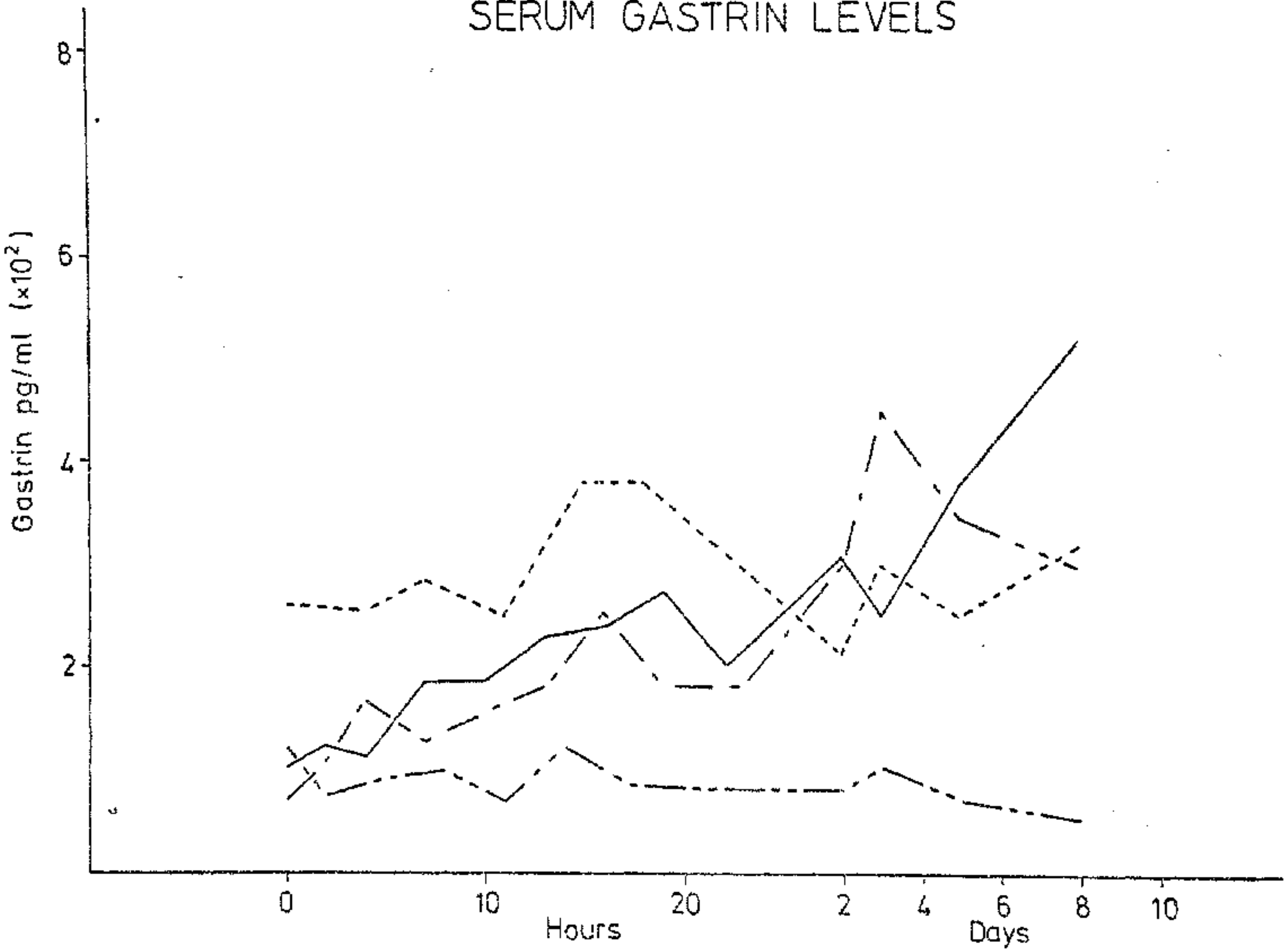

Fig. 6j The serum gastrin levels of individual animals in Group 1 which were previously infected with _Ostertagia_ larvae and treated with fenbendazole prior to transplantation with adult parasites at 0 hours.

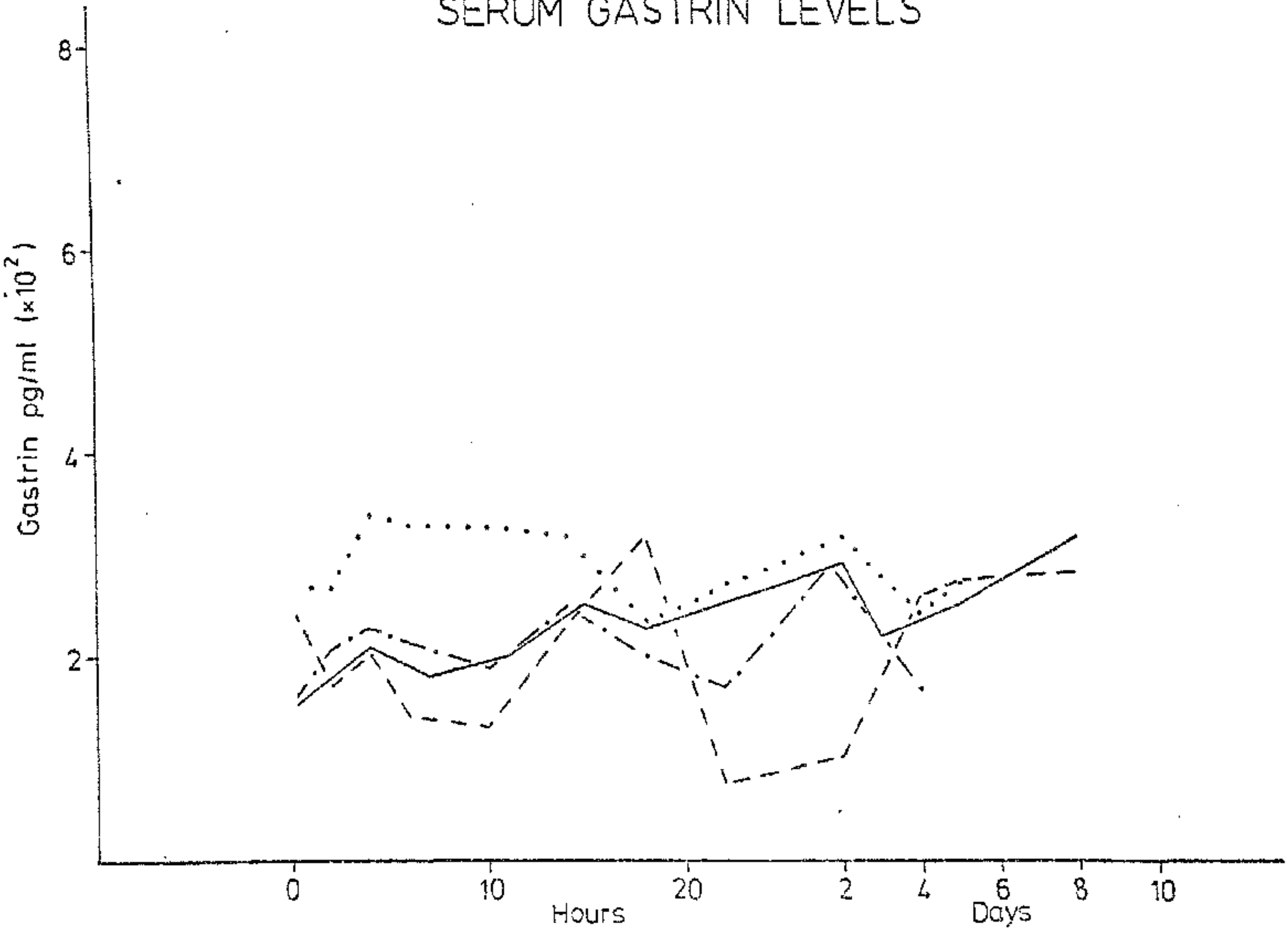

Fig. 6k The serum gastrin levels of individual animals in Group 2 which were maintained parasite free until transplantation with adult parasites at 0 hours.

Discussion

The results of this experiment confirm that adult O. ostertagi are responsible for pathophysiological changes in their hosts. The most remarkable change was in the serum pepsinogen levels of both the previously exposed Group 1 recipient calves and the parasite-naive recipient calves of Group 2. The increased pepsinogen levels were closely related to the number of adult parasites which became established and where one calf from each group received similar numbers of parasites from the same donor, the magnitude of the pepsinogen response was similar (e.g. calves 71 and 74). The low pepsinogen levels of 1.2i.U. Tyrosine 17 days after fenbendazole treatment in the control calf of Group 1, reflects the low number of parasites which survived treatment.

The worm burdens which established in the recipient calves reflected the donor worm burdens. If it is assumed that 3,700 (from calf 73) parasites survived the anthelmintic treatment in each of the Group 1 calves, then 36.3%, 41.5% and 45.5% of the worms transplanted became established in calves 74, 76 and 77 respectively. This compares with 34.2%, 43.9% and 42.5% establishment in calves 64, 67 and 71 which were not previously exposed. The close relationship between the two groups indicates that previous exposure does not affect the percentage establishment of the adult parasites.

The rapid rise in serum gastrin levels in response to the transplanted adult parasites which was demonstrated in Experiment 5 was not confirmed in this study. However the mean post mortem worm burden of the calves in Group 1 of Experiment 5 was 14,250

O. ostertagi which was higher than the total worm burden of any animal in Experiment 6. It is possible therefore that a minimum threshold number of worms may be necessary to cause elevated gastrin levels. In Experiments 5 and 6, parasites were obtained 23 days and up to 28 days after infection of the donor calves respectively. The age of the parasite therefore may also have an influence on gastrin stimulation; certainly in experiment 3 where serum gastrin was examined following infection with O. ostertagi L_3 there was a significant rise in gastrin between days 20 and 27, with a subsequent slight fall by day 30 post infection.

An unexpected feature of this study was that the calves retained the ability to acidify their abomasal contents, which was not compromised by the presence of adult parasites. This observation is of considerable interest for a number of reasons. First, it provides further evidence for the theory proposed by Jennings et al (1966) that parietal cells lose their function only as the maturing parasites emerge from the gastric glands, and that it is as a consequence of this emergence that the abomasal pH becomes elevated in ostertagiasis.

Also, it is apparent when the post mortem worm counts are considered that adult Ostertagia can survive for at least 10 days in a highly acidic environment. This is contrary to the observations of Eiler et al (1981) who demonstrated a high mortality rate for these parasites at a low pH. It may be that in the abomasum the worms maintain such a close association with the mucus layer on the mucosa that they avoid the acidity of the

lumen fluid. Those parasites which were observed in histological sections were certainly always found in very close proximity to the mucosal surface.

A further point of interest concerning the abomasal acidity is its possible relationship with serum gastrin levels. In Experiment 3, serum gastrin rose when elevated abomasal pH would be expected (Jennings _et al_, 1966). In this experiment, it is interesting that there was no change in pH or in serum gastrin. The work of Anderson _et al_ (1975) suggests that in sheep the serum gastrin changes of ostertagiasis are partially but not wholly associated with changes in abomasal pH. The reason for the slight rise in abomasal pH in animal No.64 is unknown, but in view of its small parasite burden it seems unlikely that the worms were responsible.

The differences in abomasal pepsin between the two groups may reflect an increase in the zymogen cell mass or cell secretion of the Group 1 calves in response to their earlier larval infection but the relatively low abomasal pepsin levels of the control calf in this group would suggest otherwise. However because such large variations in individual abomasal pepsin levels occurred in the limited number of animals used in this experiment it is impossible to draw any firm conclusions from these observations.

Biopsy material obtained using the fibre optic endoscope was of better quality than that taken blind although in both cases only the superficial mucosa was recovered.

Parasites were seen in sections of tissue from the control animal in Group 1 (No. 73), confirming that the anthelmintic had

not been 100% effective and the similarity of pathological changes in this control animal and the other calves of Group 1 suggests that the majority of lesions were a consequence of the larval infection. The limited cellular response within the mucosa of animals in Group 2 suggests a relatively minor role for the adult parasite in the pathological changes of ostertagiasis. However the thumbprint lesions and small nodules seen in the abomasa of calves from Experiment 4 and 5 indicate otherwise, and it may be that severe pathological changes only occur in the presence of large numbers of adult parasites.

EXPERIMENT 7

A preliminary investigation of possible chemically mediated responses in calves with adult *O. ostertagi* in abomasal chambers.

Introduction

The hypochlorhydria associated with *O. ostertagi* infection of cattle has been considered to be partially mediated by a chemical released by the parasite (Eiler *et al*, 1981) and a locally released parasite factor has also been implicated in the inhibition of parietal cell function associated with *O. circumcincta* infection of sheep (McLeay *et al*, 1973).

In the present studies (Experiments 4, 5, 6) serum pepsinogen levels showed a significant rise in calves infected with adult *O. ostertagi* alone suggesting that the tissue damage associated with developing larval stages of the parasite is not solely responsible for the pepsinogen changes observed during the course of a natural infection (Anderson *et al*, 1965).

A small experiment was therefore designed to investigate whether factors released by adult *Ostertagia* have a direct effect on serum pepsinogen and abomasal pH. This was done by confining adult *Ostertagia* to chambers subsequently suspended in the abomasum of parasite-naive calves.

Experimental Design

Three four month old Ayrshire calves were used. One donor calf (No. 10) was infected with 150,000 *O. ostertagi* L_3 to provide worms for transplantation. The two remaining calves were

surgically prepared with abomasal cannulae. The donor calf was killed 24 days after infection and its abomasal contents collected in a warmed bucket. The contents were divided into a number of small samples which were strained through double thickness gauze. Worms and particulate material retained by the gauze were immediately suspended in beakers of warmed phosphate buffered saline supplemented with 1 gm of glucose per litre (Fig.7a). Worms migrated quickly through this modified Baerman apparatus and a clean preparation of parasites was collected.

The worms were divided roughly into two equal batches and were placed in small permeable chambers (Fig.7b) still submerged in warmed PBS. Each chamber was transferred quickly into the abomasum of one recipient calf prepared with the cannula, a retaining cord being attached to prevent expulsion of the chamber from the abomasum. The chambers had a mesh aperture size of 24 microns, sufficiently small to retain the parasites but large enough to permit passage of abomasal fluid. Faecal samples were taken at the beginning and end of the experiment. Abomasal pH and serum pepsinogens were recorded twice daily for two days and daily for a further eight days. At necropsy the abomasum and the chambers were examined for parasites.

RESULTS

Faecal Egg Counts

Faecal egg counts remained negative throughout in both animals.

Fig. 7a A modified baermann apparatus for recovery of adult parasites from abomasal contents.

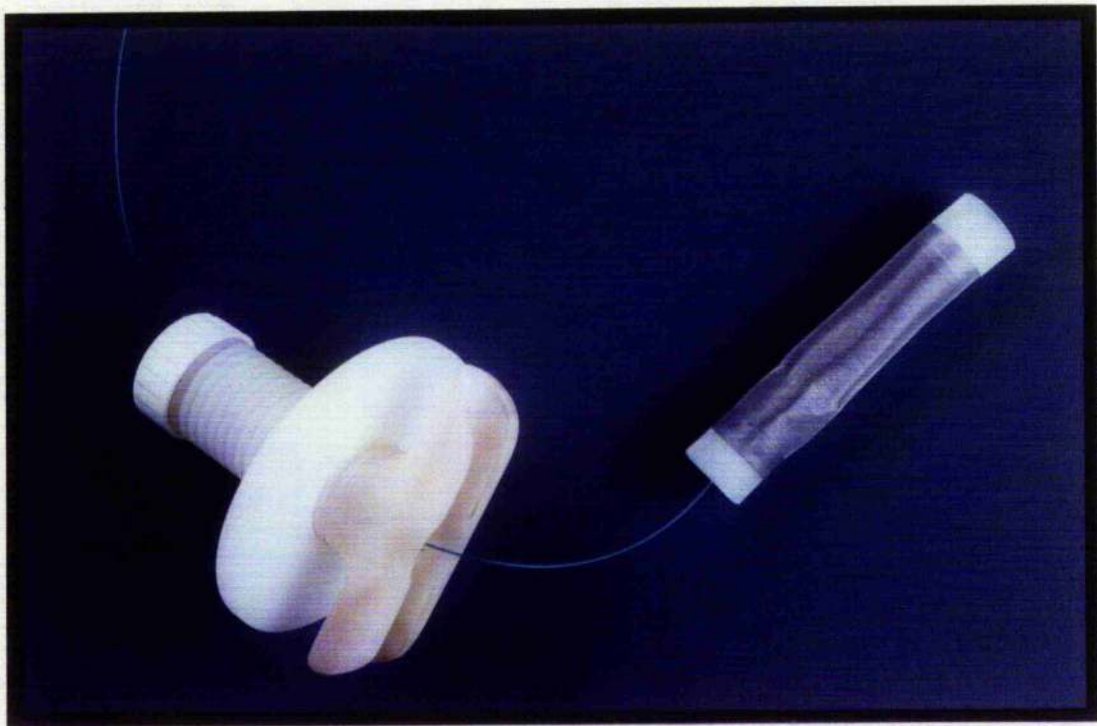

Fig. 7b A permeable chamber used to suspend adult O. ostertagi in the abomasum.

Worm Burdens

The post mortem worm burdens are shown in Table 7a. No worms were recovered from the abomasal lumen or the mucosal digest of either of the recipient calves. A total of 102 worms were recovered from the chamber in animal no. 20 and 45 from animal no. 58. Most of these worms were damaged and were in various stages of disintegration.

TABLE 7a

Post Mortem Worm Recoveries from the abomasa and chambers of recipient calves

	Animal No.	Adult Male	Adult Female	Total
Abomasal lumen	20	0	0	0
or digest	58	0	0	0
Chamber	20	78	24	102
	58	29	16	45

Abomasal pH

Abomasal pH values for the individual recipients are shown in Table 7b. The pH of animal No.20 was 3.4 on the second day after insertion of the chamber but had returned to 2.5 on the following day. The abomasal pH of animal No. 58 never rose above 2.5 throughout the experiment.

TABLE 7b

Abomasal pH values of individual animals after insertion of chambers containing adult Ostertagia into the abomasum.

Date	Day Relative to Insertion of Chamber	Animal Number 20	58
16.12.83	-3	2.7	2.5
19.12.83 a.m.	0	2.2	2.4
p.m.		2.6	2.0
20.12.83 a.m.	1	2.4	2.1
p.m.		3.1	2.3
21.12.83	2	3.4	2.3
22.12.83	3	2.5	2.0
23.12.83	4	2.8	2.3
24.12.83	5	2.8	2.2
25.12.83	6	2.3	2.2
26.12.83	7	2.3	2.1
27.12.83	8	2.6	2.1
28.12.83	9	3.1	2.1

Serum Pepsinogen

Serum pepsinogen levels are shown for individual animals in Table 7c. Pepsinogen levels remained below 0.8 i.U. Tyrosine in both animals on all sampling occasions.

TABLE 7c

Serum pepsinogen levels of individual animals (i.U. Tyrosine) after insertion of chambers containing adult _Ostertagia_ into the abomasum.

Date	Days after insertion of Chamber	Animal Number 20	58
16.12.83	-3	0.65	0.61
19.12.83 a.m.	0	0.41	0.53
p.m.		0.35	0.49
20.12.83 a.m.	1	0.36	0.44
p.m.		0.39	0.56
21.12.83	2	0.45	0.56
22.12.83	3	0.42	0.56
23.12.83	4	0.45	0.57
24.12.83	5	0.45	0.63
25.12.83	6	0.44	0.72
26.12.83	7	0.36	0.70
27.12.83	8	0.43	0.75
28.12.83	9	0.46	0.78

DISCUSSION

There were no significant changes in abomasal pH or serum pepsinogen in either calf after insertion of the chamber containing adult _O. ostertagi_. This may reflect a failure of the adult parasites to produce chemical substances which might

mediate changes in abomasal pH or serum pepsinogen, but it is possible that close contact between the parasites and the abomasal mucosa is required to provoke such changes.

Alternatively, failure of the worms in the chambers to affect abomasal pH or serum pepsinogen levels might have been related to their altered metabolism or early mortality. Certainly only small numbers of recognisable worms were recovered from the chambers at necropsy, eight days after implantation.

There are several possible reasons for the high mortality rate of the worms in the chambers. The abomasal pH may have been too low for worm survival since Eiler et al (1981) demonstrated that O. ostertagi survived better at pH 7.0 than at pH 2.2. However, in a later study presented in this thesis (Experiment 6) it was found that adult parasites survived perfectly well in the abomasum of calves when the pH of the contents remained below 3.0 on most sampling occasions. It is possible that adult parasites under natural conditions remain within the mucus layer, in close apposition to the abomasal mucosa, thus creating their own microenvironment which could be quite different from the highly acidic fluid of the lumen proper to which the worms in the chambers in this study were exposed. Inadequate nutrition or overcrowding or a combination of these factors may also have contributed to the high mortality of worms in the chambers.

EXPERIMENT 8

Observations on serum pepsinogen and serum gastrin levels in calves kept over two grazing seasons.

Introduction

A series of field investigations into parasitic gastritis led to the classification of the disease syndrome into three phases or types; Type I, pre-Type II and Type II (Anderson *et al*, 1965). However a comprehensive study of the sequential development of Type I and Type II ostertagiasis in young cattle over a two year period has only recently been completed (Entrocasso, 1984). Metabolism, production and carcass appraisal studies were made on these animals and were compared with animals receiving a morantel sustained release bolus at the start of each grazing season. Plasma samples collected routinely from five of the untreated control animals in this study were made available and pepsinogen and gastrin levels were followed over a period of 18 months.

Experimental Design

A group of five castrated male Friesian calves reared parasite free were turned out onto pasture known to be infected with *O. ostertagi* in May 1982. The animals were about 4 months old and 140 kg at turnout and were kept at a stocking density of six calves per hectare. All calves were housed for the winter on the 10th of October 1982 and were fed hay and a commercial concentrate ration which was gradually increased up to a maximum

of 3 kg per animal. The animals returned to their original grazing plot on the 15th May 1983 for a second period at grass and were finally housed on the 20th September 1983 for fattening. Observations were made regularly for clinical signs of parasitism and plasma samples were obtained routinely throughout the first and second grazing period and through the first winter housing period.

Results

Clinical Findings

During the first grazing season, animals remained in good health until August when some were noticed to be coughing. Towards the end of August, the coughing became more severe and most of the animals had loose faeces. At this time lungworm larvae were found in the faeces and all animals were treated on three consecutive days with diethylcarbamazine citrate. Further loss of condition with clinical signs of parasitic gastroenteritis necessitated treatment of all animals with a therapeutic dose of levamisole in mid-September. Levamisole was used to remove a large proportion of adults and developing larvae but not the inhibited 4th stage O. ostertagi larvae (Reid, Armour, Jennings and Urquhart, 1968). The clinical condition of the animals improved and animals appeared healthy until February 1983 when they became dull and diarrhoeic and clinical Type II ostertagiasis was diagnosed. Although the most severely affected animals had a reduced appetite until the first week after turn out in mid-May 1983, they did not require treatment and for the remainder of the second grazing season they appeared clinically

normal.

Plasma pepsinogen levels

The mean plasma pepsinogen levels for the five animals throughout the study are shown in Fig.8a and the mean values are given in Table 8a.

Pepsinogen levels rose dramatically from the end of July in the first grazing period to more than 6 i.U. and although there was a slight fall after the mid-September levamisole treatment, pepsinogen levels were above 6 i.U. at housing in October. Between October and early December, pepsinogen levels fell to below 3 i.U. and did not rise again until early February when Type II ostertagiasis was clinically diagnosed. The plasma pepsinogen levels rose to almost 6 i.U. in April and peaked at 7 i.U. around mid-May, shortly after turn out. During the early part of the second grazing season, the plasma pepsinogen levels fell sharply so that by mid-August they were below 3 i.U. From then until the end of the study there was a very gradual decrease in pepsinogen levels, but they remained above 2.5 i.U. until the 18th October which was the last sampling date.

Plasma Gastrin

Plasma gastrin levels for the five animals are shown over the two grazing seasons in Fig.8a and are given in Table 8a.

There was a small rise in plasma gastrin just after turnout and then a much larger rise during August, reaching a peak of 1212 pg/ml on 1st September 1982. Gastrin levels subsequently fell and had returned to about 300 pg/ml in mid-December 1982.

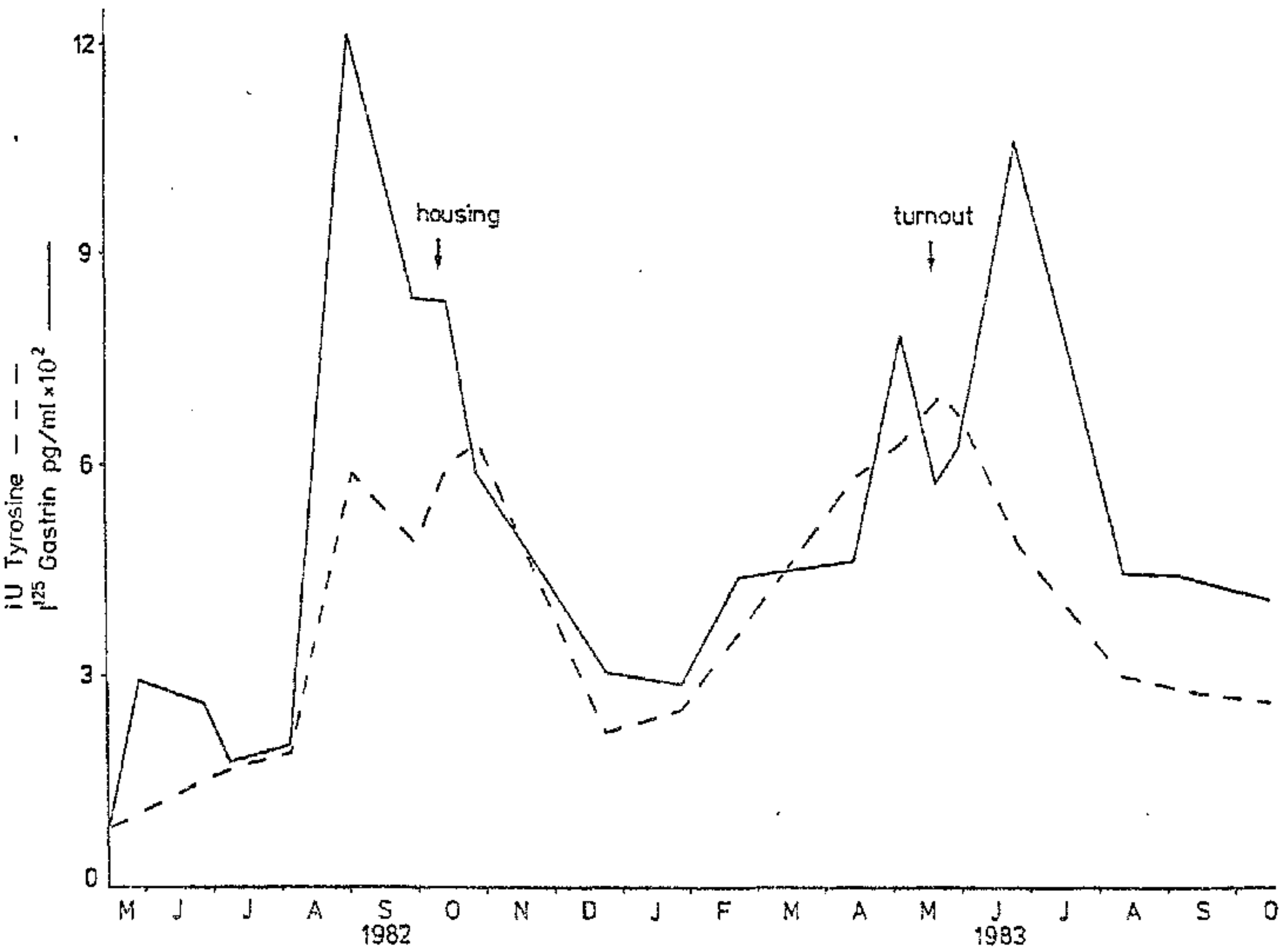

Fig. 8a The mean plasma pepsinogen (i.U. Tyrosine) and gastrin (pg/ml) levels for 5 calves over two seasons.

Table 8a Mean plasma pepsinogen (i.U.Tyrosine) and gastrin (pg/ml) levels (± SE) for five calves over two seasons

Date	Gastrin (pg/ml)	Pepsinogen (i.U. Tyrosine)
13.5.82	93 ± 8.4	0.9 ± 0.10
26.5.82	296 ± 12.1	1.0 ± 0.06
24.6.82	259 ± 11.4	1.5 ± 0.07
7.7.82	178 ± 17.9	1.7 ± 0.08
3.8.82	202 ± 26.7	1.9 ± 0.10
1.9.82	1212 ± 286.3	5.9 ± 0.50
29.9.82	836 ± 351.7	4.9 ± 0.40
13.10.82	832 ± 95.4	6.0 ± 0.40
27.10.82	590 ± 112.4	6.3 ± 0.52
22.12.82	304 ± 68.1	2.2 ± 0.26
26.1.83	292 ± 78.3	2.5 ± 0.25
24.2.83	446 ± 83.4	3.6 ± 0.26
13.4.83	464 ± 108.5	5.8 ± 0.49
4.5.83	784 ± 363.8	6.3 ± 0.60
18.5.83	573 ± 98.1	7.0 ± 0.60
31.5.83	626 ± 161.3	6.7 ± 0.48
27.6.83	1064 ± 171.8	4.9 ± 0.57
9.8.83	450 ± 72.7	3.0 ± 0.33
5.9.83	448 ± 43.3	2.8 ± 0.27
18.10.83	410 ± 54.0	2.6 ± 0.25

From the end of January 1983, plasma gastrin gradually rose to a peak of 1064 pg/ml at the end of June 1983 and then fell to about 450 pg/ml in August. From August until the end of the study, gastrin levels remained at just above 400 pg/ml.

Discussion

The sequential changes in serum pepsinogen in these animals exposed to natural O. ostertagi infection over two grazing seasons are similar to those previously described for cattle kept under similar husbandry conditions (Armour, Bairden, Duncan, Jennings and Parkins, 1979). Of particular interest were the pepsinogen levels during December and January of the winter housing period and from August until the end of the second grazing period. During December and January of the winter housing period, plasma pepsinogen levels remained higher than 2.0 i.U.. This level is considerably higher than the pre-infection level of 0.9 i.U. and the level of 1.0 i.U. considered to be normal for non-parasitised calves (Anderson et al, 1965) yet at this time the animals were not being exposed to infective larvae, nor were they displaying any clinical signs of ostertagiasis. The development of clinical Type II disease with markedly elevated plasma pepsinogen values in the following spring, confirmed that these controls were harbouring large numbers of Ostertagia larvae inhibited in their development at the early fourth stage. It is known that a small but significant proportion of inhibited larvae mature into adult parasites during the pre-Type II period (Michel, 1963; Ross, 1965; Michel et al, 1976) and it seem likely that this maturation to the adult stage

is responsible for the elevated pepsinogen levels at this time.

The mechanism by which the pepsinogen levels are maintained may involve either leakage of pepsinogen between damaged epithelial cells as the maturing parasites emerge or the adult parasites may directly stimulate the zymogen cells to secrete more pepsinogen into the blood.

During the second grazing period from August to October, the animals were being exposed to large numbers of Ostertagia larvae on the pasture but they displayed no signs of clinical ostertagiasis due to immunity acquired during the previous seasons exposure. However while the animals were at grass, pepsinogen levels remained above 2.0 i.U. and it seems possible that in the immune animal, exposure to larvae alone may be sufficient to stimulate elevated serum pepsinogen. These results lend support to the suggestion by Armour et al (1979) that a hypersensitive reaction in the mucosa could increase the gut permeability to macromolecules and allow leakage of pepsinogen into the plasma.

It is also apparent from this study that plasma gastrin levels become elevated during both Type I and Type II ostertagiasis. Large peaks in plasma gastrin occurred during August and September of the first grazing season when the animals were suffering from clinical Type I ostertagiasis and in April at the end of the winter housing period when clinical Type II ostertagiasis was apparent.

Gastrin levels returned to near normal during the pre-Type II period of December and January. It is interesting that a peak

in gastrin levels occurred just after turnout in May 1982 with a much larger peak of 1064 pg/ml at the end of June 1983, shortly after the animals had been turned out for their second grazing season. These peaks in gastrin levels could be explained by the change in diet when the animals were turned on to grass, or by exposure of the animals to infective larvae on the pasture which may stimulate gastrin production. Another possibility for the high gastrin response in June 1983 might be related to the recent episode of clinical Type II ostertagiasis since during June 1983, larvae from the inhibited population picked up in the autumn of 1982 may still have been maturing and emerging from the gastric glands. Emergent fifth stage larvae are known to damage parietal cells with consequent elevated abomasal pH (Jennings et al, 1966) and reduction in the acid inhibition of gastrin producing cells in the abomasal antrum could therefore be responsible for an increased gastrin output by these cells and an elevated plasma gastrin concentration.

It is interesting that plasma pepsinogen levels were falling at the time of peak gastrin levels during the second season, possibly reflecting the development of sufficient immunity against the adult parasites resulting in their rapid expulsion after emergence thus limiting the stimulus to zymogen cells to secrete pepsinogen. It is likely that the gastrin levels in the plasma only returned to near normal when all the parasites from the pre-Type II burden had emerged and when sufficient immunity had developed so that infective larvae picked up from the pasture in the second grazing season did not develop to the emergent stages.

Although gastrin levels at the end of the grazing period never returned below 400 pg/ml, this may reflect chronic or permanent damage to the parietal cell mass, which could result in a long term change in the abomasal pH. Another possibility is that infective larvae ingested by the animals at this time were capable of stimulating the gastrin cells even when immunity was sufficient to prevent their establishment.

EXPERIMENT 9

An investigation into the serum pepsinogen and serum gastrin responses of adult dairy cows after challenge with O. ostertagi larvae

Introduction

Serum pepsinogen levels have been shown to be of great value as an aid to diagnosis in cases of ostertagiasis in young cattle (Anderson et al, 1965; Ford, 1976; Jorgensen, Henriksen, Sejrsen and Nansen, 1976). There have, however, been a number of reports which suggest that in cases of ostertagiasis in adult cattle the determination of serum pepsinogen is of limited value (Mylrea and Hotson, 1969; Chiejina, 1977). Also, low pepsinogen levels have been obtained from moribund animals in which subsequent post mortem findings have confirmed the diagnosis of ostertagiasis (Clements, Hamilton and Redahan, 1977; Richardson, 1977). In these cases it was suggested that mucosal damage with loss of zymogen cell mass so reduces the volume of pepsinogen produced that despite leakage into the serum pool, blood levels remain low.

The finding of pepsinogen levels up to 4 i.U. Tyrosine in adult cattle with low faecal egg counts and pepsinogen levels of up to 5 i.U. in steers with fewer than 5,000 adult Ostertagia at post mortem are more difficult to explain (Mylrea and Hotson, 1969). Because of the variability of serum pepsinogens in adult cattle, an experiment was carried out to investigate the effect of O. ostertagi larval challenge on the pepsinogen response in

adult dairy cows.

Experimental Design

The design of the experiment is shown in Table 9a.

Twelve Friesian cows, more than two years of age were divided into three groups of four. Group 1 were given 20,000 O. ostertagi L_3 on five consecutive days, Group 2 were given 100,000 O. ostertagi L_3 once only and Group 3 were maintained as uninfected controls. Groups 1 and 2 were treated with thiabendazole on day 28. Thiabendazole was chosen since there is no milk withdrawal period after treatment.

Faecal samples were taken at the beginning of the experiment and 23 days after the first larval infection. Serum was collected three times weekly for pepsinogen and gastrin estimation and milk yields were recorded regularly throughout. The experiment was begun two weeks after the animals had been housed for the winter. All animals were at approximately the same stage of lactation and were located on a farm in the West of Scotland where ostertagiasis is endemic. It was assumed therefore that all of the cattle had experienced infection with O. ostertagi as calves or heifers.

Results

Clinical Observations

All cows used in this experiment remained bright and healthy throughout. The consistency of the faeces did not vary markedly between groups, although on occasions animals from all groups including the controls, passed soft faeces.

Table 9a An investigation into the serum pepsinogen and serum gastrin responses of adult dairy cows after challenge with O. ostertagi - Experimental Design

	Nos. of O. ostertagi administered per os					
Day	1	2	3	4	5	28
Group 1	20,000	20,000	20,000	20,000	20,000	Thiabendazole
Group 2	100,000					Thiabendazole
Group 3						-

Faecal Egg Counts

Faecal samples were examined by a technique designed to detect low numbers of eggs but samples were negative in all animals both at the beginning and throughout the experiment.

Milk Yields

Average milk yields for each group are shown in Fig.9a and individual yields are given in Appendix 7a.

Both infected groups had similar yields throughout. The uninfected group had a higher mean milk yield at the start of the experiment but production in this group declined more rapidly than in the infected animals. One month after the first larval infections the cows in Group 3 were producing on average 2 kg/day less milk than those in Group 2 and almost 3 kg/day less than the animals in Group 1.

Serum Pepsinogen Levels

Mean serum pepsinogen levels of Groups 1, 2 and 3 are given in Fig.9b. Individual levels are shown in Appendix 7b.

The mean pepsinogen value for Group 1 animals rose steeply over the first five days of the experiment during which cows were being challenged with 20,000 O. ostertagi L_3 daily. A peak of 2.4 i.U. Tyrosine was recorded on day 6 after the first infection. From day 6 levels fell slowly until on day 27 post infection when the thiabendazole was given, the mean pepsinogen level for this group was 1.4 i.U. Tyrosine. A further serum pepsinogen estimation was carried out 17 days after the thiabendazole treatment, by which time the pepsinogen was 1.2

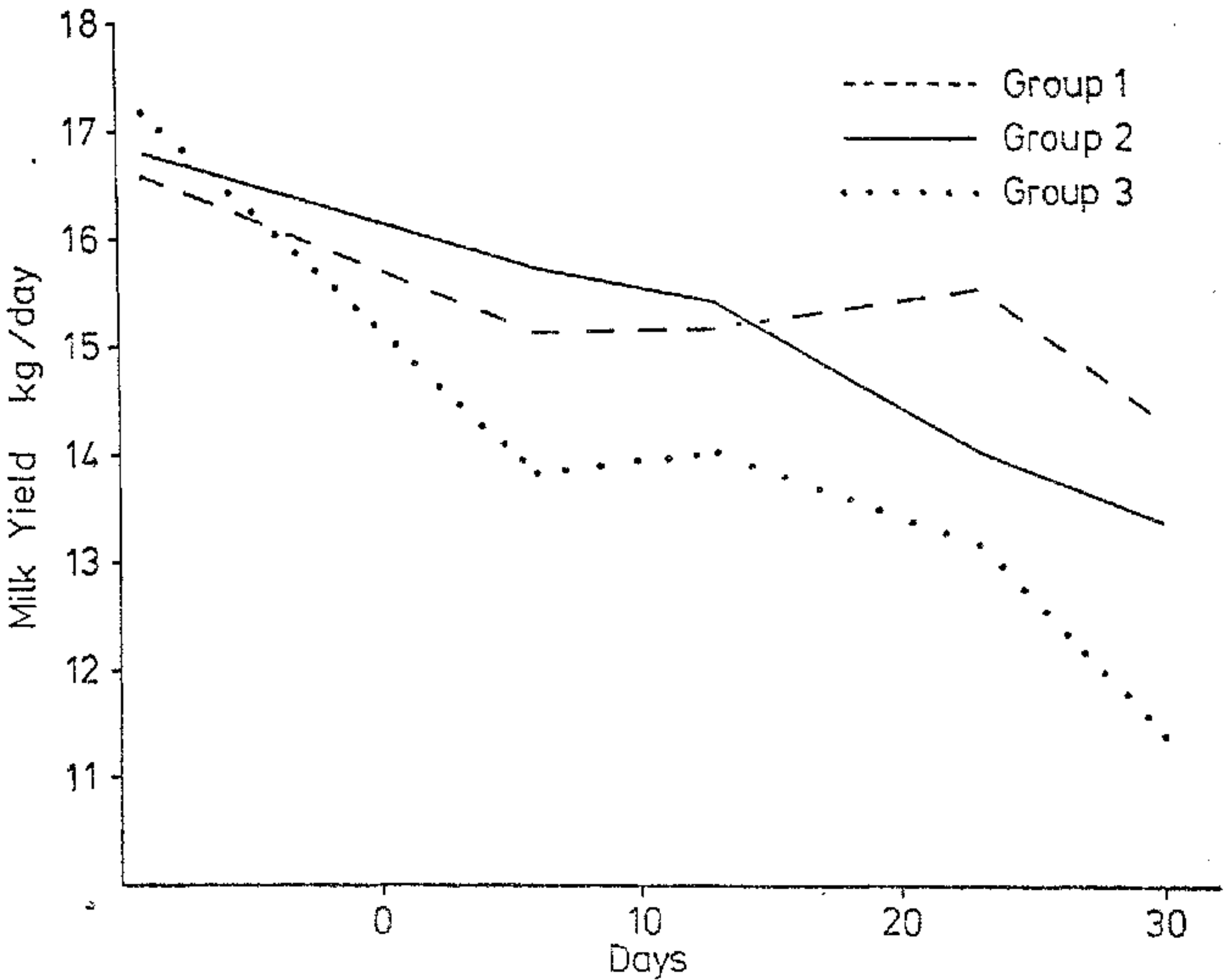

Fig. 9a The average milk yields of 3 groups of adult dairy cows
Group 1 - infected with 20,000 _O. ostertagi_ L_3 on each of 5 consecutive days
Group 2 - infected with 100,000 _O. ostertagi_ L_3 once only.
Groups 3 - control animals

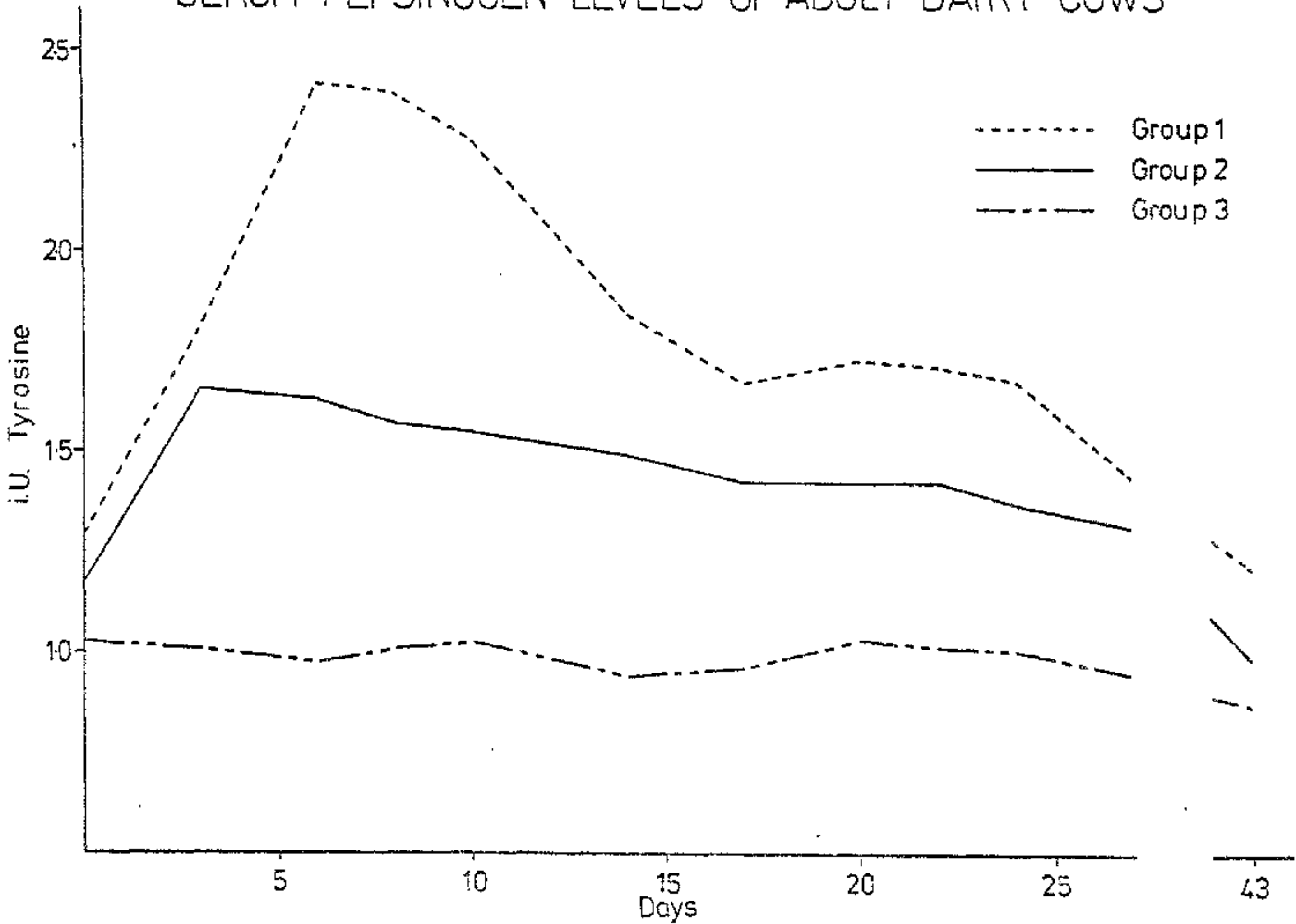

Fig. 9b The mean serum pepsinogen levels (I.U. Tyrosine) of 3 groups of adult dairy cows
Group 1 - infected with 20,000 _O. ostertagi_ L_3 on each of 5 consecutive days.
Group 2 - infected with 100,000 _O. ostertagi_ L_3 once only.
Group 3 - control animals

i.U. Tyrosine, that is, lower than the preinfection level of 1.3 i.U. Tyrosine.

The mean pepsinogen levels of the Group 2 animals which were infected once with 100,000 O. ostertagi L_3 rose to a peak of 1.7 i.U. Tyrosine, three days after infection, from which time the pepsinogen levels fell slowly to 1.3 i.U. Tyrosine on day 27 and 1.0 i.U. Tyrosine on day 44 after infection. Serum pepsinogen levels of the uninfected Group 3 animals never rose above 1.1 i.U. Tyrosine throughout.

Serum Gastrin Levels

Mean serum gastrin levels for each group are shown in Fig. 9c and individual values are given in Appendix 7c.

Mean serum gastrins of each group remained fairly constant. Levels for Groups 1 and 2 were always higher than those of Group 3, however mean values for all groups remained between 190 pg/ml and 430 pg/ml throughout.

Discussion

Because of the farm husbandry, the ubiquitous nature of O. ostertagi and the age of the cattle the assumption was made that the animals had an acquired immunity to O. ostertagi before the experiment began. The failure of any cows to develop clinical signs and the negative faecal egg counts 23 days after the large larval challenge given to Groups 1 and 2 confirmed that this was the case.

The most remarkable observation was the rapid rise in serum

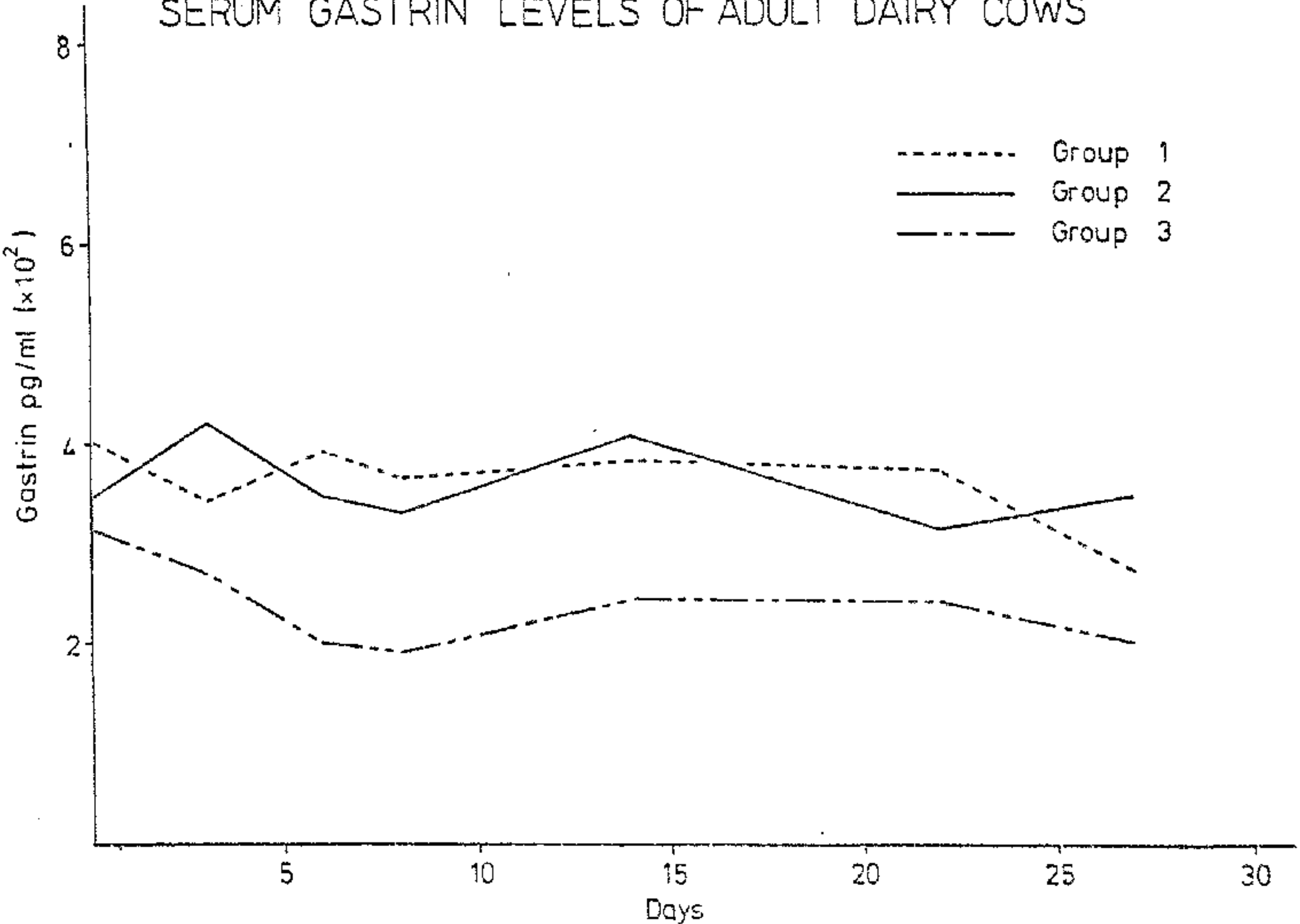

Fig. 9c The mean serum gastrin levels (pg/ml) of 3 groups of adult dairy cows.
Group 1 - infected with 20,000 _O. ostertagi_ L_3 on each of 5 consecutive days.
Group 2 - infected with 100,000 _O. ostertagi_ L_3 once only.
Group 3 - control animals.

pepsinogen which occurred in the two groups which were infected with O. ostertagi larvae. Serum pepsinogen levels in the Group 1 animals continued to rise while they were being exposed to the larval challenge infections. A peak level of 2.4 i.U. Tyrosine was reached on the day after the final larval inoculation (Day 6). Thereafter, pepsinogen levels plateaud and declined rapidly from day 10 to day 17. In the Group 2 cows given the larger dose of 100,000 O. ostertagi L_3 on one occasion, pepsinogen levels reached a peak of 1.6 i.U. Tyrosine three days after infection and then fell slowly over the subsequent fortnight. Although a small rise in pepsinogen has been noted in parasite naive calves five days after oral administration of O. ostertagi larvae, pepsinogen does not generally rise much above 1.0 i.U. Tyrosine until day 15 when parasites are beginning to emerge (Jennings et al, 1966).

A relationship has been recognised between serum pepsinogen levels and the availability of infective larvae on pasture in immune grazing sheep (Anderson, 1972) and cattle (Armour et al, 1979). Pepsinogen levels have also been shown to rise in immune sheep after housing and subsequent exposure to daily inoculation with trichostrongyle larvae (Yakoob, Holmes and Armour, 1983). The kinetics of the pepsinogen response in the housed dairy cows of the present experiment however precluded the possibility that the elevation in pepsinogen could be associated with the pathological changes which occur as O. ostertagi mature and emerge from the abomasal mucosa. It was considered more likely that the observed rapid increase in serum pepsinogen was related to an immune reaction of the mucosa provoked by the

administration of *O. ostertagi* larvae. Such a reaction has been proposed by Anderson (1973) and Armour *et al* (1979) to explain the responses observed in immune sheep and cattle respectively to the ingestion of infective larvae from pasture.

The precise mechanism whereby serum pepsinogen can become elevated due to a local hypersensitive reaction of the abomasum remains to be established although increased mucosal permeability of the gut has been demonstrated during the self-cure reaction of rats immune to *N. brasiliensis* (Barth *et al*, 1966). Such lesions could cause a leakage of pepsinogen into the serum in a manner similar to that suggested by Jennings *et al* (1966) to explain the raised serum pepsinogen levels associated with Type I ostertagiasis.

Alternatively the products of a hypersensitive reaction could themselves mediate an increased production of pepsinogen from the zymogen cells, which could be secreted directly into the bloodstream (Stringfellow and Madden, 1979) or leaked into the blood from the abomasal lumen through separated epithelial junctions (Jennings *et al*, 1966). For example, histamine could have a direct effect since it has been shown to stimulate pepsin secretion in a number of species (Samloff, 1971). Also many of the gastrointestinal peptide hormones are known to stimulate pepsin secretion (Samloff, 1971) although in the present study one such hormone, gastrin was not found to be elevated in any of the serum samples assayed.

The intake of infective trichostrongyle larvae by immune sheep has been suggested by Anderson (1973) to have a detrimental

effect on production. In the present experiment however there were no critical observations made on production: although milk yields fell in all groups this did not apparently reflect parasite infection since the drop in yield was most marked in the uninfected controls.

General Discussion

GENERAL DISCUSSION

One of the most remarkable observations in this study involved the alteration in serum pepsinogen concentration of cattle in response to O. ostertagi infection. Serum pepsinogen levels were shown to rise in a number of different circumstances. For example, in parasite naive calves, serum pepsinogen was elevated between sixteen and twenty days after oral infection with third stage larvae and within one day after surgical implantation of adult parasites into the abomasum. In adult dairy cows, considered to be immune to ostertagiasis, serum pepsinogen levels rose within three days of oral exposure to infective third stage larvae. It seems likely therefore that different pathogenic mechanisms were responsible for the pepsinogen response in each situation.

In parasite naive calves, pepsinogen is always detectable to some degree in the blood and it is possible that the serum pepsinogen concentration is proportional to the amount of pepsinogen in the stomach (Spencer, Stern and Thayer, 1966). In ostertagiasis the increased serum pepsinogen concentration may be a reflection of an increased abomasal pepsinogen production as proposed by Ross, Purcell and Todd (1969) to explain the increased serum pepsinogen levels observed in calves infected with T. axei. Also it has been demonstrated that in sheep pepsin secretion in separated abomasal pouches was maintained or increased, when the abomasum proper was infected with O. circumcincta. However in the latter situation the pepsin secretion by the fundic mucosa of the infected part of the

abomasum was not measured and in this region cells appeared to be non-secreting on ultrastructural examination (McLeay et al, 1973).

Several studies in calves infected with O. ostertagi L_3 have indicated that gastric pepsin concentration may drop to very low values from day 22 post infection (Ross and Todd, 1965; Jennings et al, 1966; Stringfellow and Madden, 1979). It is interesting that in the present study there was no apparent increase in abomasal pepsin in calves which received adult Ostertagia by surgical implantation while serum pepsinogen levels were markedly elevated. These results however are based on pepsin concentration and not on total pepsin output. If total gastric secretion was increased or if the amount of ingesta in the abomasum increased, the concentration of abomasal pepsin might consequently have decreased, while production was in fact maintained or increased. Since inappetence is a common feature of ostertagiasis from day 18 after infection (Jennings et al, 1966) and the histological and histochemical appearance of the abomasal mucosa suggests a failure rather than an increased secretion of pepsinogen after day 21 (Murray, Jennings and Armour, 1970; Stringfellow and Madden, 1979), interpreting reduced pepsin concentration as an indication of total pepsin output probably gives a conservative estimation of the true reduction in pepsinogen production.

A widely accepted explanation for the elevated blood pepsinogen levels which occur in bovine ostertagiasis was first proposed by Jennings et al (1966). They suggested that leakage

of pepsinogen into the blood followed an increase in mucosal permeability as young adult parasites emerged from the gastric glands. Support for this theory was provided by studies on plasma albumin loss (Mulligan et al, 1963), histological changes (Richie et al, 1966) and ultrastructural observations (Murray, 1969) in calves infected with O. ostertagi. The sequential changes have been outlined by Armour et al (1979): In non parasitised cattle pepsinogen produced by zymogen cells in the gastric glands is largely converted immediately into pepsin within the glands by hydrochloric acid produced by the adjacent parietal cells. In ostertagiasis these parietal cells, lining both the parasitised and surrounding glands, are replaced by rapidly dividing undifferentiated cells and hydrochloric acid is not produced; thus pepsinogen is not converted to pepsin and relatively large amounts enter the circulation and increased permeability of the mucosa to macromolecules exacerbates the leakage into the circulation. However, it has been shown that plasma protein loss into the gut does not parallel pepsinogen loss into the blood (Holmes and McLean, 1971) and that the mucosal permeability to horseradish peroxidase does not necessarily increase when calves are infected with O. ostertagi (Stringfellow and Madden, 1979). An alternative explanation that zymogen cell pepsinogen is released directly into the circulation rather than taken up from the gastric contents through a damaged epithelium has therefore been proposed (Stringfellow and Madden, 1979). Observations in this thesis suggest that neither explanation provides the complete answer. For example serum pepsinogen levels rose dramatically in animals infected with

adult parasites but in these animals there was no elevation in abomasal pH and it may be assumed therefore that pepsinogen produced by cells of the abomasal mucosa would be immediately converted into pepsin when secreted into the acidic environment of the abomasal lumen (Piper and Fenton, 1965). Thus there would be a limited amount of pepsinogen available to leak into the serum through the separated junctional complexes and it now seems likely that the positive correlations observed between elevated abomasal pH and increased serum pepsinogen in calves with ostertagiasis are coincidental (Anderson et al, 1965b; Allen, Sweasey, Berrett, Herbert and Patterson, 1970).

Direct secretion of pepsinogen into the circulation was suggested by Stringfellow and Madden (1979) on the basis of their observations on the leakage of horseradish peroxidase into the abomasum to an equal degree in parasitised and control calves. However the results of the experiment on adult cows reported in this thesis indicate that other factors are likely to be involved since in these animals the pepsinogen response occurred within three days of oral infection with Ostertagia larvae. This is more rapid than the typical response at day 16 after infection of young susceptible calves and suggests that in older immune animals zymogen cells may exhibit a greater secretory response to stimulation by Ostertagia larvae. An alternative and more likely explanation proposed by Armour et al (1979) is that changes in the abomasum of immune animals to challenge with Ostertagia larvae increases mucosal permeability due to a local hypersensitive reaction of the abomasum, similar to that

demonstrated in immune rats challenged with *N. brasiliensis* (Barth *et al*, 1966) and allows pepsinogen to leak into the serum.

If pepsinogen is secreted directly into the circulation by zymogen cells, then during infection with *Ostertagia* there must be a stimulus to this secretion. The polypeptide hormone gastrin has been suggested as a possible stimulant to peptic cells in sheep infected with *H. contortus* (Dakkak, Bueno and Fioramonti, 1981) and has been shown to increase in the blood during parasitic gastritis in sheep (Anderson *et al*, 1975). Although hypergastrinaemia was demonstrated in calves from day 20 post infection with *O. ostertagi* L_3 in the studies reported in this thesis, there was no elevation in serum gastrin after transplantation with adult *Ostertagia* directly into the abomasum despite a marked rise in serum pepsinogen levels in these animals. Thus it appears that although gastrin may rise during the course of ostertagiasis, it is not necessarily responsible for the elevated serum pepsinogen levels.

It is possible that elevated serum pepsinogen levels have multifactorial aetiology, no single mechanism being entirely responsible but several contributing to different degrees at different stages of infection.

In a primary infection with *Ostertagia* larvae, pepsinogen levels begin to rise at day 16; at this stage pepsinogen may be secreted by the zymogen cells directly into the circulation or may be leaked through a damaged mucosal epithelium into the blood. As adult parasites emerge it is possible that they exert a strong stimulus on the zymogen cells to secrete pepsinogen,

whether this is a direct effect of an excretory/secretory antigen or whether it is mediated by hormones remains unknown. Excretory/secretory antigens include both enzymes and metabolic products and have been shown to be involved in the maintainance behaviour of parasites, including the hold fast for adult worms and interference with host cell secretion (Miller, 1984). Although pepsinogen was shown to rise in the serum of calves without hypergastrinaemia, it may be that when gastrin is elevated, in a primary *Ostertagia* infection, it compounds the pepsinogen response by providing a further stimulus to the zymogen cells. It is also likely that whether or not pepsinogen is secreted directly into the circulation, it will also leak through the separated junctional complexes of a damaged mucosal epithelium. Although it was demonstrated that serum pepsinogen levels could still be elevated in calves with normal abomasal pH, it may be that when the pH is elevated, more pepsinogen is available for secretion into the blood.

The magnitude of the pepsinogen response to adult parasites alone was surprising and was disproportionately high for the number of worms present. This was clearly demonstrated in Experiment 5 where pepsinogens rose to more than 6 i.U. in a calf with an adult *Ostertagia* burden of only 18,300 which compares with mean plasma pepsinogen levels of 2.9 i.U. from calves with clinical Type I ostertagiasis and upward of 40,000 adult parasites at post mortem (Anderson *et al*,1965b). There is thus further evidence that adult parasites provide a stimulus to zymogen cell secretion and the difference between serum

pepsinogen levels in adult and larval infections could reflect the degree of damage inflicted by emergent stages of the parasite on zymogen cells so that despite stimulation, the total cell mass in the larval infection is reduced such that the pepsinogen response is lower.

Results presented in this thesis suggest that care must be exercised when interpreting pepsinogen values as an aid in the diagnosis of ostertagiasis and that it is especially important to consider the age of animals involved and their grazing history. In young calves exposed for the first time to infective _Ostertagia_ larvae, levels of more than 1.5 i.U. Tyrosine are suggestive of abomasal parasitism and levels of greater than 3.0 i.U. Tyrosine are virtually diagnostic of the disease. During winter housing after one season at grass, pepsinogen levels of up to 3.0 i.U. Tyrosine may reflect a small number of adult parasites in the abomasa, but the animals may appear clinically normal. However at this time they may be in the pre-Type II stage and as large numbers of inhibited larvae resume their development in the late winter or early spring, clinical Type II disease may develop and pepsinogen values of above 3.0 i.U. may be recorded.

Results from animals in their second grazing season and from adult dairy cows suggest that where a significant degree of immunity to ostertagiasis has developed, ingested larvae may cause elevated pepsinogen levels of up to 3.0 i.U., but are unlikely to develop to the emergent stages. The low levels of serum pepsinogen recorded from immune adult dairy cows which were not exposed to _Ostertagia_ larvae indicates that age _per se_ is not

responsible for raised values as proposed by Mylrea and Hotson (1969) and the hypersensitive mechanism already discussed provides a more satisfactory explanation.

The hypochlorhydria associated with ostertagiasis is well documented and has been associated with damage to parietal cells as young adult parasites emerge from the gastric glands (Jennings et al, 1966) or with a chemical mediator derived from parasite extract (Eiler et al, 1981) or locally released by the parasite (McLeay et al, 1973). The present study supports the work of Jennings et al (1966) as there was no consistent elevation of pH in calves in which an adult parasite burden was surgically implanted into the abomasum, or after exposure to adult parasites in chambers suspended in the abomasum.

The major changes in serum gastrin appear to occur concurrently with pH changes. Increased abomasal acidity has been shown to inhibit gastrin secretion (Nilsson, 1980) and it may be that during the course of a primary larval infection with O. ostertagi, gastrin becomes elevated as the pH rises in the abomasum and there is no longer acid inhibition of gastrin cells. It is interesting that during studies on the effect of O. circumcincta larval infection of sheep, gastrin became elevated in advance of elevated pH (Anderson et al, 1975) and in this situation the larval invasion or development in the mucosa may have directly stimulated gastrin producing cells. It may be that the differences observed between O. circumcincta in sheep and O. ostertagi in cattle reflect a different localisation of the species within the abomasum. It is possible that the

invasiveness of the parasite is responsible for hypergastrinaemia or that a specific minimum number of parasites are necessary to elicit a gastrin response. Castro et al (1976) first suggested that parasite invasiveness could be an important factor in the elevation of serum gastrin. Using two unrelated parasites, T. spiralis which is invasive and H. diminuta which is not, he demonstrated hypergastrinaemia only in rats infected with T. spiralis. In the present study, hypergastrinaemia was demonstrated in calves infected orally with Ostertagia larvae, but not in one experiment where groups of calves were surgically implanted with adult parasites alone (Experiment 6). However, contrary results were obtained in another experiment (Experiment 5) where hypergastrinaemia was evident in a group of calves infected only with adult parasites. Post mortem worm burdens showed that many more parasites were present in the calves in which gastrin levels rose and it was interesting that the two calves with the largest post mortem worm burdens also had an elevated abomasal pH.

Whatever the aetiology of the gastrin response, it is likely that its effects in ostertagiasis are signficant. The effects of gastrin on stimulation of hydrochloric acid and other secretions and on gastrointestinal motility and absorption have been reviewed by Gregory (1974) and of particular interest are its trophic effects on the gastrointestinal mucosa (Johnson, 1980). This could account for the nodular response seen in the abomasa of calves in Experiment 5 which were infected with the presumably non-invasive adult stages of Ostertagia and which had a demonstrable hypergastrinaemia.

The responses to and effects of abomasal parasitism are obviously very complex. In this thesis only some of the biochemical and hormonal responses have been examined but other mediators of inflammatory reactions such as prostaglandins, histamine or serotonin may also be released and since these may mediate changes in intestinal myoelectric activity (Schanbacher, Nations, Weisbrodt and Castro, 1978), gastric and pancreatic fluid secretion (Castro, Hessel and Whalen, 1979) and aborption (Brasitus, 1979), it is apparent that a great deal of work is still required before the pathophysiological and immunopathological reactions of the host to parasitism are fully understood.

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Appendices

Appendix 1a Individual weights (Kg) of 3 groups of animals challenged with 100,000 O. ostertagi L_3.

Group 1. 9-15 month old animals previously infected with 2 doses of 50,000 O. ostertagi L_3 and subsequently treated with fenbendazole.

Group 2. 9-15 month old animals maintained parasite naive

Group 3. 5 month old animals maintained parasite naive.

Date	Day after first infection	Group 1			Group 2			Group 3	
		Animal No.			Animal No.			Animal No.	
		21	37	55	56	57	59	38	39
13.10.82	0	373	256	249	260	249	332	71	89
19.10.82	6	354	249	253	249	249	346	67	93
26.10.82	13	373	273	260	277	260	373	74	99
1.11.82	19	354	265	265	269	265	346	75	102
11.11.82	29	421	295	298	280	256	373	79	100
18.11.82	36	386	277	290	265	269	354	82	102
25.11,82	43	373	273	308	285	290	367	83	104
1.12.82	49	421	280	280	280	277	373	83	102
9.12.82	57	393	290	320	280	277	338	85	103
20.12.82	68	403	317	299	280	299	341	87	98

Appendix 1b Individual faecal egg counts (epg) of 3 groups of animals challenged with 100,000 _O. ostertagi_ L_3.

Group 1. 9-15 month old animals previously infected with 2 doses of 50,000 _O. ostertagi_ L_3 and subsequently treated with fenbendazole.

Group 2. 9-15 month old animals maintained parasite naive.

Group 3. 5 month old animals maintained parasite naive

Date	Day after first Infection	Group 1			Group 2			Group 3	
		Animal No.			Animal No.			Animal No.	
		21	37	55	56	57	59	38	39
11.10.82	-2	0	0	0	0	0	0	0	0
13.10.82	0	0	0	0	0	0	0	0	0
19.10.82	6	0	0	0	NS*	0	0	0	0
22.10.82	9	100	50	0	0	0	0	0	0
26.10.82	13	100	0	0	0	0	0	0	0
1.11.82	19	0	0	0	0	0	0	0	0
4.11:82	22	150	0	300	0	0	50	50	0
11.11.82	29	50	100	150	0	0	0	0	0
18.11.82	36	100	100	100	0	0	0	0	0
25.11.82	43	100	100	200	0	0	0	0	0
1.12.82	49	0	0	0	0	0	0	0	0
6.12.82	54	0	0	0	0	0	0	0	0
9.12.82	57	0	0	0	0	0	0	0	0
13.12.82	61	0	0	0	0	0	0	0	0
20.12.82	68	0	0	0	0	0	0	0	0
23.12.82	71	0	0	0	0	0	0	0	0
28.12.82	76	0	0	0	100	50	50	250	100

*NS - no sample taken

Appendix 1c Percentage distribution of worms according to length from individual animals.

Group 1. 9-15 month old animals previously infected with 2 doses of 50,000 _O. ostertagi_ L_3 and subsequently treated with fenbendazole.

Group 2. 9-15 month old animals maintained parasite naive

Group 3. 5 month old animals maintained parasite naive.

Female Worms

Worm Size (cm)	Group 1 Animal No.			Group 2 Animal No.			Group 3 Animal No.	
	21	37	55	56	57	59	38	39
0.30 - 0.34	2.13	0	0	0	0	0	0	0
0.35 - 0.39	2.13	0	1.52	0	0	0	0	0
0.40 - 0.44	12.77	3.64	3.03	0	0	0	0	0
0.45 - 0.49	19.15	1.82	0	1.49	0	0	0	0
0.50 - 0.54	12.77	9.09	9.09	1.49	0	4.69	0	0
0.55 - 0.59	21.28	9.09	21.21	2.99	8.57	12.50	3.08	1.96
0.60 - 0.64	21.28	29.09	22.73	19.40	38.57	23.44	9.23	7.84
0.65 - 0.69	8.51	32.73	21.21	25.37	31.43	26.56	16.92	29.41
0.70 - 0.74	0	10.91	18.18	32.84	12.86	29.69	26.15	25.49
0.75 - 0.79	0	1.82	3.03	7.46	8.57	1.56	23.08	21.57
0.80 - 0.84	0	1.82	0	8.96	0	1.56	21.54	13.73

Appendix 1d Percentage distribution of worms according to length from individual animals

Group 1. 9-15 month old animals previously infected with 2 doses of 50,000 O. ostertagi L_3 and subsequently treated with fenbendazole.

Group 2. 9-15 month old animals maintained parasite naive.

Group 3. 5 month old animals maintained parasite naive.

Male Worms

Worm size (cm)	Group 1			Group 2			Group 3	
	Animal No.			Animal No.			Animal No.	
	21	37	55	56	57	59	38	39
0.25 - 0.29	10.00	0	0	0	0	0	0	0
0.30 - 0.34	10.0	0	0	0	0	0	0	0
0.35 - 0.39	18.57	7.89	11.76	0	0	0	0	0
0.40 - 0.44	17.14	7.89	5.88	0	2.77	0	0	0
0.45 - 0.49	18.57	23.68	26.47	3.03	8.33	16.22	0	5.77
0.50 - 0.54	20.00	31.58	29.41	15.15	16.66	13.51	7.31	13.46
0.55 - 0.59	5.71	21.05	20.59	51.52	61.11	29.73	48.78	30.77
0.60 - 0.64	0	7.89	2.94	27.27	8.33	27.84	39.02	34.62
0.65 - 0.69	0	0	2.94	3.03	2.77	2.70	2.44	13.46
0.70 - 0.74	0	0	0	0	0	0	2.44	1.92

Appendix 1e Individual serum pepsinogen levels (i.U. Tyrosine) of 3 groups of animals challenged with 100,000 O. ostertagi L_3.

Group 1. 9-15 month old animals previously infected with 2 doses of 50,000 O. ostertagi L_3 and subsequently treated with fenbendazole.

Group 2. 9-15 month old animals maintained parasite naive.

Group 3. 5 month old animals maintained parasite naive.

Date	Days after first Infection	Group 1 Animal No.			Group 2 Animal No.			Group 3 Animal No.	
		21	37	55	56	57	59	38	39
11.10.82	-2	0.75	0.72	0.80	0.70	0.75	0.92	NS*	0.39
13.10.82	0	0.79	0.71	0.79	0.65	0.72	0.87	0.72	0.52
15.10.82	2	1.23	1.26	1.00	0.71	0.79	0.84	0.79	0.63
19.10.82	6	1.45	1.37	1.14	0.62	0.63	NS	0.59	0.43
22.10.82	9	1.39	1.41	1.23	0.63	0.72	NS	0.76	0.37
26.10.82	13	1.22	1.14	1.01	0.60	0.64	0.80	0.65	0.47
1.11.82	19	1.17	1.25	1.19	0.65	0.64	0.76	0.53	0.53
4.11.82	22	NS	2.13	2.38	0.68	0.63	0.77	0.64	0.59
11.11.82	29	2.86	2.69	3.13	0.65	0.75	0.79	0.67	0.61

Continued overleaf

Appendix 1e (continued)

Date	Days after first Infection	Group 1			Group 2			Group 3	
		Animal No.			Animal No.			Animal No.	
		21	37	55	56	57	59	38	39
18.11.82	36	2.68	2.42	2.81	0.68	0.66	0.80	0.70	0.49
25.11.82	43	NS	2.54	3.49	0.72	0.75	NS	0.81	0.55
29.11.82	47	3.84	3.53	4.86	0.78	0.79	0.94	0.74	0.49
2.12.82	50	3.01	2.64	3.94	0.75	0.76	0.93	0.84	0.56
8.12.82	56	1.47	1.24	1.72	0.78	0.81	0.89	0.72	0.44
13.12.82	61	1.99	1.71	2.00	2.13	1.62	NS	1.28	0.54
16.12.82	64	1.70	1.64	2.01	2.92	1.75	NS	2.07	0.75
20.12.82	68	1.45	1.29	1.04	2.13	1.48	1.63	1.94	0.75
23.12.82	71	1.46	1.26	1.89	1.84	1.63	1.95	1.88	1.01
28.12.82	76	1.40	1.81	2.43	2.09	1.88	2.16	5.08	3.50

Appendix 2a

Individual weights (Kg) of calves vaccinated with _O. leptospicularis_ (Group 1), _O. ostertagi_ (Group 2) or a mixture of _O. leptospicularis_ and _O. ostertagi_ (Group 3) and control calves (Group 4)

Date	Day after first Infection	Group 1				Group 2				Group 3				Group 4	
		Animal No.				Animal No.				Animal No.				Animal No.	
		41	42	43	44	45	46	48	49	50	52	53	54	38	39
13.10.82	0	93	99	71	90	85	87	68	69	82	102	89	79	71	89
19.10.82	6	83	92	71	94	80	88	65	68	85	99	91	86	67	93
26.10.82	13	83	93	76	93	79	90	69	56	85	103	94	86	74	99
1.11.82	19	86	94	76	95	71	97	72	65	90	108	89	87	75	102
8.11.82	26	82	94	73	96	68	97	71	68	90	104	79	90	79	100
15.11.82	33	89	95	73	95	*	96	64	69	85	101	80	88	82	102
22.11.82	40	86	99	74	96		97	*	69	83	98	86	84	83	104
29.11.82	47	91	99	75	98		99		70	82	95	86	86	83	102
6.12.82	54	90	104	77	100		96		70	84	95	83	87	85	103
13.12.82	61	85	100	80	98		100		76	90	103	90	97	89	91
20.12.82	68	95	108	79	112		114		76	87	102	90	98	87	98

* Animal no. 45 was necropsied on 12.11.82 and animal No. 48 was necropsied on 21.11.82.

Appendix 2b

Individual faecal egg counts (e.p.g.) of calves vaccinated with O. leptospicularis (Group 1), O. ostertagi (Group 2) or a mixture of O. leptospicularis and O. ostertagi (Group 3) and control calves (Group 4).

Date	Day after first Infection	Group 1				Group 2				Group 3				Group 4	
		Animal No.				Animal No.				Animal No.				Animal No.	
		41	42	43	44	45	46	48	49	50	52	53	54	38	39
4.10.82	-9	0	0	NS*	NS	0	NS	0	0	0	0	0	0	0	0
7.10.82	-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11.10.82	-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13.10.82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.10.82	6	0	0	0	0	0	NS	0	0	0	0	0	0	0	0
22.10.82	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26.10.82	13	0	0	0	0	NS	0	0	0	0	0	0	0	0	
1.11.82	19	450	700	450	550	500	100	350	400	750	850	500	750	0	0
4.11.82	22	450	1050	550	1050	4350	550	750	1000	1250	1300	4450	1050	50	0
8.11.82	26	1650	900	1800	1000	4400	950	1500	2200	900	1500	7300	950	0	0
15.11.82	33	250	0	450	400	*	800	1850	1800	1750	1050	2250	700	0	50
22.11.82	40	500	0	1000	750		250	*	1300	2200	700	1100	2200	0	0
29.11.82	47	200	250	800	550		300		2250	3200	1250	1000	1850	0	0

Continued overleaf

Appendix 2b (continued)

Date	Day after first Infection	Group 1				Group 2				Group 3				Group 4	
		Animal No.				Animal No.				Animal No.				Animal No.	
		41	42	43	44	45	46	48	49	50	52	53	54	38	39
2.12.82	50	0	0	0	50		0		0	100	0	0	50	0	0
6.12.82	54	0	0	0	0		0		0	0	0	0	0	0	0
9.12.82	57	0	0	0	0		0		0	0	0	0	0	0	0
13.12.82	61	0	0	0	0		0		0	0	0	0	0	0	0
20.12.82	68	0	0	0	0		0		0	0	0	0	0	0	0
23.12.82	71	0	0	0	0		0		NS	0	0	0	0	0	0
28.12.82	76	400	100	300	250		50		400	200	950	200	250	250	100

*NS - No sample taken

* Animal No. 45 was necropsied on 12.11.82 and animal No. 48 was necropsied on 21.11.82

Appendix 2c

Individual serum pepsinogen levels (i.U. Tyrosine) of calves vaccinated with O. leptospicularis (Group 1), O. ostertagi (Group 2) or a mixture of O. leptospicularis and O. ostertagi (Group 3) and control calves (Group 4)

Date	first Infection	Group 1				Group 2				Group 3				Group 4	
		Animal No.				Animal No.				Animal No.				Animal No.	
		41	42	43	44	45	46	48	49	50	52	52	54	38	39
4.10.82	-9	0.78	0.62	0.53	0.48	0.40	0.57	0.50	0.49	0.58	0.35	0.60	0.53	0.82	0.55
7.10.82	-6	0.65	0.51	0.54	0.49	0.43	0.48	0.40	0.51	0.54	0.29	0.66	0.47	0.79	0.48
11.10.82	-2	0.75	0.56	0.67	0.49	0.40	0.53	0.53	0.50	0.72	NS*	0.58	0.50	NS	0.39
13.10.82	0	0.69	0.40	0.62	0.46	0.46	0.52	0.41	0.45	0.55	0.69	0.57	0.44	0.72	0.52
15.10.82	2	0.85	0.52	0.85	0.62	0.49	0.74	0.45	0.68	0.72	0.89	0.77	0.59	0.79	0.63
19.10.82	6	0.74	0.45	0.72	1.35	0.59	0.95	0.51	0.60	0.69	0.72	0.63	0.58	0.59	0.43
22.10.82	9	0.61	0.47	0.72	1.11	0.55	0.96	0.55	0.68	0.85	0.73	0.77	0.68	0.76	0.37
26.10.82	13	0.64	0.53	0.88	0.93	0.49	1.04	0.62	0.49	0.75	0.71	0.78	0.65	0.65	0.47
28.10.82	15	0.93	0.74	1.53	1.31	0.47	1.10	0.74	0.72	1.01	0.95	0.84	0.93	0.54	0.53
1.11.82	19	1.26	0.93	2.07	1.75	0.84	2.44	2.13	2.15	1.57	1.64	1.13	2.44	0.53	0.53
4.11.82	22	1.27	1.08	1.96	1.88	2.48	3.83	4.06	4.66	2.61	2.66	1.58	NS	0.64	0.59
8.11.82	26	1.36	1.09	1.59	1.43	1.37	3.97	3.10	5.01	2.43	2.48	1.14	2.08	0.52	0.56
11.11.82	29	1.52	1.61	1.34	1.50	1.43	4.71	2.01	4.53	1.95	2.74	1.10	2.43	0.67	0.61
15.11.82	33	2.14	1.81	1.72	1.28	*	4.66	3.20	5.19	1.87	2.68	1.06	2.43	0.55	0.56

Continued overleaf

Appendix 2c (continued)

Date	Day after first Infection	Group 1				Group 2				Group 3				Group 4	
		Animal No.				Animal No.				Animal No.				Animal No.	
		41	42	43	44	45	46	48	49	50	52	53	54	38	39
18.11.82	36	1.49	1.94	1.56	1.27		4.75	2.79	4.62	1.81	1.90	1.17	2.10	0.70	0.49
22.11.82	40	1.43	2.66	1.76	1.58		5.57	*	4.01	1.64	1.78	1.34	1.46	0.78	0.55
25.11.82	43	2.18	3.65	2.58	2.62		6.23		4.8C	1.70	2.50	NS	NS	0.81	0.55
29.11.82	47	2.39	3.45	2.39	2.38		7.41		6.18	2.90	3.24	2.27	3.44	0.74	0.49
2.12.82	50	1.86	2.53	1.59	2.40		5.71		2.80	1.69	2.87	1.42	3.19	0.84	0.56
6.12.82	54	1.15	1.47	1.12	1.32		2.50		1.11	0.84	1.23	0.81	1.44	0.60	0.45
8.12.82	56	1.11	1.53	1.21	1.10		2.47		1.16	0.85	0.97	0.78	1.32	0.72	0.44
13.12.82	61	1.11	2.83	2.24	1.72		3.33		1.41	0.95	1.43	1.05	1.40	1.28	0.54
16.12.82	64	1.39	3.22	2.45	2,06		2.69		1.61	0.92	1.68	1.11	1.56	2.07	0.75
20.12.82	68	1.42	2.23	1.57	1.42		2.13		1.35	0.83	1.12	1.01	1.31	1.94	0.75
23.12.82	71	1.33	2.07	1.38	1.62		2.84		1.58	1.07	1.11	1.24	1.58	1.88	1.01
28.12.82	76	4.58	3.30	4.02	4.54		4.13		4.98	3.15	2.81	4.75	3.79	5.08	3.50

*NS - no sample taken

* Animal No. 45 necropsied on 12.11.82 and animal No. 48 was necropsied on 21.11.82.

Appendix 3a

Individual faecal egg counts (e.p.g.) of calves.

Group 1 were maintained parasite free, Group 2 were infected with 50,000 _O. ostertagi_ L_3.

Date	Days after infection	Group 1					Group 2				
		Animal No.					Animal No.				
		32	10	34	46	38	14	21	3	17	57
15.11.81	0	0	0	0	0	0	0	0	0	0	0
25.11.81	7	0	0	0	0	0	0	0	0	0	0
2.12.81	14	0	0	0	0	0	0	0	0	0	0
9.12.81	21	0	0	0	0	0	350	300	500	250	500
16.12.81	28	0	0	0	0	0	500	350	400	250	200

Appendix 3b

Individual serum pepsinogen levels (i.U. Tyrosine) of calves. Group 1 were maintained parasite free, Group 2 were infected with 50,000 _O. ostertagi_ L_3.

Date	Days after Infection	Group 1					Group 2				
		Animal No.					Animal No.				
		32	10	34	46	38	14	21	3	17	57
18.11.81	0	0.65	0.94	0.48	0.70	0.73	0.68	0.73	0.57	0.77	0.61
20.11.81	2	0.64	0.86	0.52	0.68	0.56	0.76	1.24	0.73	0.75	0.72
24.11.81	6	0.70	0.88	0.60	0.70	0.74	1.01	2.08	0.99	0.85	1.02
27.11.81	9	0.60	0.87	0.56	0.71	0.63	1.12	1.76	1.14	1.02	1.23
1.12.81	13	0.61	0.91	0.61	0.76	0.65	0.98	1.76	1.05	1.18	1.54
4.12.81	16	0.73	0.70	0.52	0.55	0.57	1.28	1.57	1.11	1.19	1.71
8.12.81	20	0.60	0.87	0.66	0.65	0.76	3.88	3.07	3.85	2.85	3.79
11.12.81	23	0.72	0.97	0.63	0.89	0.81	6.40	5.71	6.51	4.97	6.33
15.12.81	27	0.79	0.80	0.55	0.65	0.84	6.53	7.18	3.78	5.12	6.72
18.12.81	30	0.98	1.12	0.80	0.78	1.21	6.82	7.84	2.29	6.22	7.08

Appendix 3c

Individual serum gastrin levels of calves (pg/ml). Group 1 were maintained parasite free, Group 2 were infected with 50,000 _O. ostertagi_ L_3.

Date	Days after Infection	Group 1 Animal No.					Group 2 Animal No.				
		32	10	34	46	38	14	21	3	17	57
18.11.81	0	150	155	120	175	145	155	125	130	165	115
23.11.81	5	250	NS*	105	125	150	130	220	200	225	140
27.11.81	9	150	135	105	140	125	120	7	260	160	140
1.12.81	13	125	135	90	130	62	140	135	175	165	125
8.12.81	20	NS	125	130	105	115	190	150	260	410	230
15.12.81	27	140	115	NS	105	115	760	430	300	800	470
18.12.81	30	110	96	68	80	42	660	420	210	960	340

*NS - no sample taken

Appendix 4a

Individual faecal egg counts (e.p.g.) of donor calves (Group 1) and of parasite-naive (Group2) and previously infected recipients (Group 3).

	Group 1				Group 2				Group 3			
	Animal No.				Animal No.				Animal No.			
Date	39	22	58	32	50	56	55	52	57	59	42	43
23.4.82	0	0	0	0	0	0	0	0	0	0	0	0
28.4.82	0	0	0	0	0	0	0	0	0	0	0	0
3.5.82	0	0	0	0	0	0	0	0	0	0	0	0
7.5.82	0	0	0	0	0	0	0	0	0	0	0	0
10.5.82	0	0	0	0	0	0	0	0	0	0	0	0
14.5.82	0	0	0	0	0	0	0	0	0	0	0	0
17.5.82	0	0	0	0	0	0	0	0	0	0	0	0
21.5.82	1000	350	1350	800	0	0	0	0	0	50	0	0
24.5.82	2400	800	1750	1450	0	0	0	0	200	150	450	NS
28.5.82					50	450	0	0	0	0	0	0
31.5.82					100	350	100	0	100	150	150	350
1.6.82						350	0	0		250	400	350
3.6.82						550	150	0		900	150	100

Continued overleaf

Appendix 4a (continued)

	Group 1				Group 2				Group 3			
	Animal No.				Animal No.				Animal No.			
Date	39	22	58	32	50	56	55	52	57	59	42	43
7.6.82						50	100	0		1150	500	50
9.6.82							50	0			750	150
11.6.82							50	0			450	100
13.6.82								0			500	350
14.6.82											450	

Appendix 4b

Individual serum pepsinogen levels (i.U. Tyrosine) after transplantation of adult <u>O. ostertagi</u> into parasite-naive (Group 2) and previously infected recipients (Group 3).

Days After Infection	Group 2				Group 3			
	Animal No.				Animal No.			
	50	56	55	52	57	59	42	43
0							3.54	2.46
1	0.93			0.67	2.19	2.58	3.17	2.27
2	1.02	1.73	0.97	0.65	2.02	2.21	4.69	3.54
3	1.24	2.00	1.09	0.62	2.00	2.44	4.37	4.84
4	1.44	2.96	1.36	0.69	2.22	2.86	5.13	5.86
5		2.94	1.35	0.72		3.72	5.60	6.90
6		3.14	1.41	0.72		4.37	6.00	6.38
7		2.84	1.32	0.62		4.36	6.91	6.04
8		2.60	1.17	0.69		4.29	7.43	6.53
9		2.22	1.12	0.60		5.00	7.36	6.71
10		2.67	1.14	0.63		5.14	7.88	6.70
11		2.36	1.15	0.65		5.26	8.15	7.22
12		2.62	1.12	0.73			8.49	8.19
13			1.38	0.69			8.71	7.23
14			1.05	0.78			9.17	7.34
15			1.31	0.84			9.54	7.31

Appendix 5a Individual faecal egg counts (e.p.g.)

Group 1 Calves maintained parasite free until transplanted with adult <u>O. ostertagi</u>

Group 2 Calves infected with 10,000 <u>O. ostertagi</u> L_3 on alternate days for 21 days then treated with fenbendazole and transplanted with adult <u>O. ostertagi</u> 7 days later.

Group 3 Calves received the same larval infection regimen as Group 2 but were transplanted with adult <u>O. ostertagi</u> 21 days after anthelmintic treatment.

Group 4 Surgical controls

Group 5 Anthelmintic controls.

Date	Group 1				Group 2				Group 3				Group 4		Group 5	
	Animal No.				Animal No.				Animal No.				Animal No.		Animal No.	
	R11	R12	R13	R14	B62	B63	B64	B65	Y11	Y12	Y13	Y14	W19	W20	P21	P22
19.1.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.1.83	0	0	0	0	0	0	0	0	100	0	50	50	0	0	0	0
28.1.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.1.83	0	600	1350	0	NS*	NS	NS	NS	NS	NS	NS	NS	0	0	NS	NS
31.1.83	250	450	850	4260	0	0	0	0	0	0	0	0	0	0	200	50
1.2.83	NS	NS	NS	1150	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2.2.83	500	550	1350		50	100	0	50	0	0	0	0	0	0	50	200
4.2.83	500	150	850		0	0	0	300	0	0	0	0	0	0	0	0
7.2.83	650	450	2650		0	50	0	0	0	0	0	0	0	0	0	0
9.2.83		250	130		0	0	0	0	0	0	0	0	0	0	0	0

Continued overleaf

Appendix 5a (continued)

Date	Group 1				Group 2				Group 3				Group 4		Group 5	
	Animal No.				Animal No.				Animal No.				Animal No.		Animal No	
	R11	R12	R13	R14	B62	B63	B64	B65	Y11	Y12	Y13	Y14	W19	W20	P21	P22
11.2.83		500	1000		NS	100	50	150	0	0	0	0	0	0	0	0
14.2.83		400	200		0	250	100	0	0	0	0	0			0	0
16.2.84			1200		0	150		550	200	900	0	0				
18.2.83			300		NS	50		600	700	200	1150	750				
19.2.83					NS	NS		NS	500	NS	NS	NS				
21.2.83					0	150		800		300	750	650				
23.2.83					NS	100				850	850	450				
25.2.83					0	200				700	650	950				
26.2.83					NS	NS				NS	300	NS				
27.2.83					NS	150				NS		NS				
28.2.83					0					1000		1000				
2.3.83					50					850		1200				
3.3.83					0					NS		NS				
4.3.83										600		1550				
7.3.83												900				
8.3.83												750				

*NS - no sample taken

Appendix 5b

Individual serum gastrin values of Group 2 calves (naive recipients) and Group 4 calves (surgical controls) shortly before and after the transplant operation.

Date	Day	Group 2 Animal No. R11	R12	R13	R14
19.1.83	-9	195	160	150	140
24.1.83	-4	110	NS	70	NS
26.1.83	-2	190	100	130	50
28.1.83	0	160	140	140	110
29.1.83	1	150	230	NS	150
31.1.83	3	1000	500	540	330
2.2.83	5	950	520	850	390
4.2.83	7	800	90	460	
7.2.83	10	430	390	500	

Day	Group 4 Animal No. P21	P22
-6	190	84
-1	150	110
1	150	105
6	120	84

Appendix 6a

Individual faecal egg counts (e.p.g.) after transplantation of adult *O. ostertagi* into previously infected (Group 1) and parasite naive (Group 2) recipients.

Mean count for sample period	Group 1				Group 2			
	Animal No.				Animal No.			
	74	76	77	73	64	67	71	68
0 - 24 hrs	17	167	17	0	34	50	0	0
25 - 48hrs	NS	75	25	0	217	34	NS *	0
49 - 72hrs	100	NS	NS	NS	NS	NS	125	0
73 - 96hrs	NS	375	75	50	200	175	NS	NS
Day 5	NS	NS	NS	NS	200	400	NS	NS
Day 6	NS	NS	NS	NS	100	150	NS	NS
Day 7	0	NS	NS	NS	150	200	150	NS
Day 8	50	250	100	0	450	300	150	NS
Day 9	NS	200	0	50	NS	NS	NS	NS

*NS - No sample taken

Appendix 6b

Individual abomasal pepsin concentration (i.U. Tyrosine) after transplantation of adult *O. ostertagi* into previously infected (Group 1) and parasite-naive recipients (Group 2).

Hours Post Infection	Group 1				Group 2			
	Animal No.				Animal No.			
	74	76	77	73	64	67	71	68
-1	614	519	646	257	229	448	367	NS
0	634	812	932	472	171	599	232	NS
2	NS	787	885	327	44	557	NS	98
4	610	581	759	245	127	452	230	147
7	693	682	597	235	44	532	229	114
10	463	603	926	368	184	457	291	76
14	553	645	668	326	370	411	345	125
18	434	497	710	371	430	396	335	243
22	484	475	697	220	225	392	194	159
26	NS	354	672	150	283	NS	NS	244
30	391	614	672	243	510	625	253	355

Continued overleaf

Appendix 6b (continued)

Days Post Infection	Group 1				Group 2			
	Animal No.				Animal No.			
	74	76	77	73	64	67	71	68
2	392	566	736	276	188	512	202	
3	177	715	740	348	176	334	270	
4	480	365	621	292	262	333	347	
5	348	559	666	327	440	328	117	
6	451	558	517	275	369	398	231	
7		505	535	405	267	395		
8		360	538	384	43	262		

Appendix 7a The individual milk yields (kg/day) of 3 groups of adult dairy cows.

Group 1 - infected with 20,000 _O. ostertagi_ L_3 on each of 5 consecutive days.

Group 2 - infected with 100,000 _O. ostertagi_ L_3 once only.

Group 3 - control animals.

Date	Day after first Infection	Group 1 Animal No.				Group 2 Animal No.				Group 3 Animal No.			
		14	59	76	62	2	58	11	73	46	16	55	29
Day of Housing		21.8	17.8	17.0	11.8	17.6	21.8	10.8	18.6	21.2	19.4	13.2	18.8
16.10.83	-9	21.8	17.6	16.8	10.0	17.6	20.6	12.8	16.2	19.6	19.6	11.0	18.6
31.10.83	6	21.8	16.4	14.4	8.0	16.6	17.8	16.0	12.6	15.2	14.6	9.4	16.2
7.11.83	13	22.2	16.4	16.0	6.2	17.8	16.4	14.4	13.2	16.2	14.8	9.2	16.0
17.11.83	23	22.0	15.4	15.6	9.2	16.8	15.8	11.0	12.6	16.2	13.8	7.8	15.0
24.11.83	30	20.6	14.4	15.2	7.2	15.6	14.2	11.4	12.4	15.8	8.2	8.0	13.6

Appendix 7b Individual serum pepsinogen levels (i.U. Tyrosine) of 3 groups of adult dairy cows.

Group 1 - infected with 20,000 *O. ostertagi* L_3 on each of 5 consecutive days.

Group 2 - infected with 100,000 *O. ostertagi* L_3 once only

Group 3 - control animals.

Date	Day after first Infection	Group 1				Group 2				Group 3			
		Animal No.				Animal No.				Animal No.			
		14	59	76	62	2	58	11	73	46	16	55	29
25.10.83	0	1.25	1.28	1.31	1.34	0.80	1.11	1.51	1.29	1.05	0.94	0.85	1.26
28.10.83	3	1.97	1.93	1.75	1.66	1.38	1.93	1.66	1.65	0.94	0.84	0.85	1.39
31.10.83	6	2.86	2.31	2.54	1.95	1.43	1.79	1.61	1.68	0.95	0.85	0.83	1.27
2.11.83	8	2.86	2.53	2.50	1.69	1.33	1.52	1.60	1.84	1.03	0.86	0.84	1.30
4.11.83	10	2.56	2.56	2.34	1.65	1.42	1.55	1.54	1.70	0.79	0.86	0.79	1.73
8.11.83	14	1.97	2.00	1.97	1.46	1.37	1.46	1.38	1.78	0.92	0.81	0.81	1.23
11.11.83	17	1.80	1.86	1.80	1.24	1.35	1.38	1.42	1.57	0.99	0.87	0.83	1.18
14.11.83	20	1.76	2.08	1.75	1.35	1.40	1.39	1.32	1.60	1.14	0.99	0.80	1.27
16.11.83	22	1.73	2.16	1.66	1.31	1.36	1.46	1.31	1.58	1.08	0.93	0.78	1.27
18.11.83	24	1.64	2.10	1.75	1.27	1.35	1.34	1.25	1.55	1.02	0.98	0.76	1.24
21.11.83	27	1.59	1.60	1.34	1.21	1.31	1.20	1.21	1.54	1.00	0.80	0.77	1.23
7.12.83	44	1.23	1.28	1.12	1.05	0.84	1.00	1.05	1.09	0.92	0.78	0.76	0.97

Appendix 7c Individual serum gastrin levels (pg/ml) of 3 groups of adult dairy cows.

Group 1 - infected with 20,000 _O. ostertagi_ L_3 on each of 5 consecutive days.

Group 2 - infected with 100,000 _O. ostertagi_ L_3 once only

Group 3 - control animals.

Date	Day after first Infection	Group 1				Group 2				Group 3			
		Animal No.				Animal No.				Animal No.			
		14	59	76	62	2	58	11	73	46	16	55	29
25.10.83	0	520	430	330	330	380	380	310	320	500	360	120	290
28.10.83	3	500	360	210	300	450	400	500	340	390	270	110	310
31.10.83	6	490	450	300	340	320	350	480	240	280	210	90	220
2.11.83	8	620	380	235	230	250	300	480	300	170	220	150	230
8.11.83	14	370	500	400	270	300	460	520	360	430	120	130	310
16.11.83	22	330	480	270	430	310	270	400	290	250	270	140	310
21.11.83	27	300	290	170	350	290	390	400	330	260	230	60	240

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