# THE PATHOLOGY OF THE IMMUNE RESPONSE TO *HAEMONCHUS CONTORTUS* INFECTION IN SHEEP.

by

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## DECLARATION

The work described in this thesis is original and has not been submitted in any form to any other University. It was carried out by the author in the Department of Veterinary Parasitology, Veterinary School, University of Glasgow, under the supervision of Dr. J.L. Duncan.

## SUMMARY

This thesis describes the results of various experiments on the response of sheep and lambs to Haemonchus contortus infection.

Primary infections in adult sheep produced a high degree of protection against re-infection and this was associated with a marked cellular reaction in the abomasal mucosa together with a high level of local antibody production. In contrast primary infections in lambs had little or no protective effect and was associated with a weak cellular reaction in the mucosa and a poor local antibody response. There were similar qualitative changes in the type of mucin seen covering the surface mucosa after infection in both adult sheep and lambs: in worm-free animals only acid mucosubstances were detected whereas a mixture of neutral and acid mucin was observed after infection. Increased production of mucin was more obvious in the adult animals compared with the lambs.

Attempts to stimulate immunity in young lambs by using different immunising regimens with various adjuvants and immunostimulants proved unsuccessful.

There was little difference in the abomasal pathology of lambs clinically infected with small numbers of worms compared with worm-free controls. Similar infections in lambs maintained on either a high or low protein diet, however, showed that there was a marked reduction in faecal egg output and subsequent worm burdens in the group fed on a high protein diet compared with those maintained on a maintenance ration.

An investigation of the significance of passive immunity showed that prior immunisation of ewes had little apparent effect on the subsequent response of their lambs to H. contortus infection. In naturally suckled lambs, however, there was reduced worm establishment compared with that in lambs bottle-fed a dried milk diet.

The response to a challenge infection with normal H. contortus larvae in groups of previously uninfected and repeatedly vaccinated adult sheep and young lambs showed that after challenge in hyper-immune adult sheep there were increases in the size and number of lymphoid aggregates and this was associated with mild but diffuse cellular infiltrations of the mucosa. In contrast, in non-immunised adults there were fewer lymphoid aggregates but there was more obvious cellular infiltration and damage to the mucosal surface. Although hyperimmunised lambs showed a delay in the development of patent infections after challenge compared with non-immunised controls, the worm-burdens of both groups at necropsy were similar. Cellular reactions although present in both vaccinated and non-vaccinated lambs after challenge were, in contrast to the adult sheep, more marked in the vaccinated group.

In the vaccinated ewes, intra-epithelial mast cells were present in all of the animals at all stages after challenge and they were always more numerous than in the non-vaccinated animals. In the vaccinated lambs intra-epithelial mast cells were also present at all stages after challenge, although in smaller numbers than in the adult sheep, while in the non-vaccinated lambs only a few of these cells were detected three to four weeks after infection.

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# GENERAL INTRODUCTION

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Haemonchus contortus, the abomasal nematode of sheep was probably first described by Otto Fabricus in 1790 (Rudolphi, 1803). Rudolphi (1803), in describing the nematode he found in the fourth stomach of a lamb in 1801, named it Strongylus contortus. On the basis of morphological studies of this parasite, Cobb (1898) changed the name from Strongylus contortus to Haemonchus contortus.

The life cycle of H. contortus is direct (Ransom, 1906). Eggs are passed in the faeces and within 15-20 hours these hatch to produce free-living first-stage larvae ($L_1$). These larvae feed develop and grow and after a period of lethargus they moult to the second-stage ($L_2$). After a further period of activity and growth these develop to the third stage ($L_3$) but retain the second-stage sheath. This is the infective stage and its development from the egg may be reached in a minimum of 5-7 days. Infection takes place by ingestion and Silverman and Patterson (1960) showed that the rate of development of the parasitic phase is dependent upon the age and the immunological state of the host. For example they established that in susceptible lambs most $L_3$ have undergone differentiation and completed the first parasitic moult, i.e. they have become fourth-stage larvae ($L_4$) by the third day after infection. About the fifth day after infection the second parasitic moult has taken place, i.e. the larvae are now fifth-stage ($L_5$) and sexual differentiation is apparent.

In contrast in older susceptible sheep the first parasitic ecdysis was not generally completed until the fifth day and the entire cycle was delayed. In young lambs eggs were first produced before the 15th day while in older susceptible sheep, eggs were first detected in the faeces 16 to 24 days after infection (Silverman and Patterson, 1960). When mature it has been shown that a single H. contortus female may lay up to 10,000 eggs per day for several months in succession (Gordon, 1948).

Since these early studies numerous experiments have been carried out to examine the response of various groups of sheep to H. contortus infection. For example, O'Sullivan and Donald (1970) found that lactating ewes showed higher faecal egg counts and carried larger populations of H. contortus than did unmated ewes or those deprived of their lambs at birth, when these animals were grazed together on the same infected pasture during the lambing period. In 1973, the same authors reported that when lactating, pregnant and non-pregnant ewes were subjected to twice weekly infection with constant numbers of H. contortus and T. colubriformis infective larvae for a period of 8 weeks, no differences could be detected in the response of either the lactating ewes or those in late pregnancy in terms of the size, structure and fecundity of their worm burdens but that these ewes showed evidence of a diminished immunological response to infection when compared with the non-pregnant animals.

In laboratory experiments it has been shown that after primary infections with this nematode parasites may survive

for more than 133 days in sheep penned indoors (Dineen, Donald, Wagland and Offner, 1965).

However, in naturally occurring infections parasite survival appears to be more precarious in that there may be a dramatic drop in faecal egg counts due to a sudden expulsion commonly known as 'self-cure'. Self-cure was first described in 1929 by Stoll when he observed a sudden fall in faecal-egg counts of two lambs carrying H. contortus infections at pasture. Later Gordon (1948) showed that 'self-cure' often occurred shortly after a period of rain-fall and suggested that the worm expulsion was due to the presence of an 'anthelmintic factor' in freshly growing pasture. Subsequently Stewart (1953) produced 'self-cure' experimentally when he administered infective larvae to sheep rendered hypersensitive as a result of previous infection. In further studies of this phenomenon Allonby and Urquhart (1973) carried out necropsies of experimentally infected sheep which subsequently grazed infected or parasite-free pasture. The simultaneous occurrence of 'self-cure' in these animals indicated that this phenomenon was not necessarily related to fresh larval intake and the authors suggested that the new growth of pasture might be a significant aetiological factor in the field situation. A later report, however, by Dargie and Allonby (1975) confirmed Stewart's (1953) original observations i.e. that 'self-cure' can be produced by experimental re-infection of sheep: this was based on the changes in the nature and timing of anaemia in housed sheep experimentally infected and subsequently re-infected with H. contortus.

Railliet (1895) was one of the earliest workers to describe the blood-sucking activities of H. contortus. Later Fourie (1931) described in detail the anaemia which accompanies infection and attributed this to abomasal haemorrhage. In 1935, Boughton and Hardy observed that the parasites attach themselves to the stomach wall by a peculiar striking motion of the head and neck and remain attached for about 12 minutes sucking blood which then passes through the intestine of the nematode. It was later shown that about 0.05 ml of blood is removed daily by each worm (Clark, Kiesel and Goby, 1962).

Dargie and Allonby (1975) suggested that there are three stages in the development of anaemia in sheep infected with H. contortus. The first stage occurs during the initial three weeks of infection and is characterised by a progressive fall in PCV, normal serum iron levels and negative or low faecal egg counts. The second stage which may not, at least initially, involve a further reduction in PCV is accompanied by continuous abomasal haemorrhage and marked stimulation of erythropoiesis, while the third and final stage is characterised by a dramatic reduction in PCV and serum iron concentration.

Epidemiological studies on haemonchosis have been limited to certain regions of the world, e.g. Australia, and some parts of East Africa. It is surprising how little information is available from many other tropical and sub-tropical regions, where this disease is probably the most important single threat to the economic production of meat

and wool.

In Australia Gordon (1948) found that there was a marked increase in faecal egg counts of H. contortus infected sheep during a period when the rainfall was in excess of 50 mm per month and the mean maximum temperature was above 18°C and he suggested that this was due to the development of large numbers of infective larvae on pasture. He also showed that this increase was often followed by a dramatic fall, often to zero, in the faecal egg counts of the entire flock which he attributed to an anthelmintic effect of grass (vide supra).

In East Africa, the epidemiology and pathogenic significance of haemonchosis has been studied by Allonby and Urquhart (1975). They used a flock of 80 Merino ewes and their pure-bred lambs which were set-stocked on pasture where haemonchosis had been a perennial problem. The results of these studies showed that larval challenge was highly seasonal and largely related to the existence of a suitable microclimate at the soil surface. They also found that the ewes and lambs remained infected to some degree almost continually throughout the year despite the highly seasonal challenge and they were subsequently chronically anaemic and less productive than their non-infected controls.

The immune response to H. contortus infection has been the subject of intensive studies for many years. In 1950, for example, Stewart observed that the administration of infective third stage larvae stimulated an antibody response but as the infection developed the antibody response declined.

On these grounds he suggested that the third-stage larvae were highly antigenic whereas the mature worms yielded antigens of low potency. Later Neilson (1975) found that sheep parenterally hyperimmunised with metabolic products of H. contortus developed precipitating antibodies in the serum but these antibodies did not protect the sheep when they were subsequently subjected to a challenge infection. Smith (1977a) on the other hand detected antilarval antibodies in the serum and abomasal mucus of adult sheep made resistant to H. contortus by repeated infections with infective larvae. In a subsequent study in lambs using larval antigens in adjuvant (Smith 1977b) a marked serum and mucus IgG response was detected but no mucus IgA antibodies were stimulated and no protection against challenge was observed. Earlier work in Australia by Dineen, Donald, Wagland and Offner (1965) compared a single dose of 3000 infective larvae with 30 individual daily doses of 100 $L_3$. They found that retardation of development at the $L_4$ stage occurred in the serially infected animals and they concluded that an immunological response had developed which probably affected larvae administered after the tenth day.

Christie and Brambell (1967) studied the immune response of 7½ month old lambs, given daily doses of 25,000 normal larvae for 10 days and subsequently treated with thiabendazole on the 11th and 16th day after the first infection. These animals were challenged together with a group of worm-free lambs with 50,000 normal H. contortus larvae. When both groups were killed 7 days after challenge more worms had established in the control animals than in

the immunised sheep and the worms in the control group were at a later stage of development than those recovered from the immunised sheep.

For many years irradiated H. contortus larvae have been used successfully to immunise worm-free sheep over 7 months of age. The degree of protection afforded was measured by a comparison of the number of worms which established, after challenge with normal larvae, in vaccinated and control sheep (Jarrett, Jennings, McIntyre, Mulligan and Sharp, 1959, 1961; Urquhart, Jarrett, Jennings, McIntyre and Mulligan, 1966). In contrast, Urquhart, Jarrett, Jennings, McIntyre, Mulligan and Sharp (1966) and Stankiewicz and Fuksiewicz (1978) failed to protect lambs under seven months of age against challenge by single or double oral vaccination with irradiated larvae. Mansfield, Ozeral, Courter, Green and Levine (1974) attempted to vaccinate lambs under 6 months of age, using live larvae plus larval antigens. They found that after a dose of 2,000 to 8,000 $L_3$ plus antigens from 98,000 to 392,000 $L_3$, lambs showed a decrease in weight gain and PCV. A challenge inoculation with 23,000 normal $L_3$ approximately one month after the initial inoculation did not have any important additional effects. They also observed the absence of a marked increase of faecal egg counts in lambs after challenge and suggested that this was due to the presence of adult worms from the initial inoculation causing developmental arrest in the larval stages of the challenge infection. They could not determine, however, whether this was due to metabolic products of the nematodes or to an immune reaction of the host. Wilson and

Samson (1974) failed to stimulate any immunity by intraperitoneal or intravenous inoculation of exsheathed H. contortus larvae in lambs aged between 2½-5 months, although they did produce a slight degree of immunity by subcutaneous injection of exsheathed larvae. They also found that immunity was enhanced by administration of multiple doses of larvae especially in older lambs. Later Neilson (1975) failed to immunise three month old lambs against subsequent challenge with normal infective H. contortus larvae by the parenteral administration with or without adjuvant of three doses of metabolic antigens (collected and concentrated from infective third and fourth stage larvae cultured in vitro). He suggested that the antigen preparation of worm metabolic products conferred no resistance to challenge infection although, in 1965, Silverman had reported that a vaccine prepared from somatic and metabolic H. contortus $L_3 + L_4$ antigens conferred a measure of protection in 4-6 month old lambs. As noted earlier, Smith (1977b) also failed to protect lambs aged 6 months against challenge with H. contortus by vaccinating them intramuscularly with larval antigens in adjuvant; he did, however, observe a marked serum and mucus IgG antibdoy response but no increase in abomasal mucus IgA was detected. On the other hand, in 1978 Duncan, Smith and Dargie, demonstrated raised levels of abomasal mucus IgA and serum IgG antibodies in adult sheep vaccinated with irradiated larvae and subsequently immune to challenge with normal infective larvae. No increase in antibody levels and no protection was detected in lambs similarly vaccinated and challenged.

Fourie (1931) made the first detailed study of the pathology and haematology of H. contortus infection in sheep.

In his study he concluded that the anaemia observed in sheep experimentally infected with this nematode was purely haemorrhagic in character. In 1942, Andrews found on administration of infective third-stage larvae of H. contortus to lambs in single and multiple doses, that one dose of 35,000 larvae produced death whereas a larger number of larvae did not prove fatal when these were administered in small daily doses. He suggested that the multiple doses produced resistance while a single heavy dose produced a severe and sometimes fatal anaemia.

Charleston (1965) studied the pathological changes in the abomasum of lambs during infection with H. contortus. He found petechial haemorrhages, mucosal hypertrophy and an apparent deficiency in the cytoplasm of the mucus secreting cells associated with the developing larvae within or below the mucus layer. A collection of both mononuclear cells and eosinophils was observed around the deeper proprial vessels with movement of some of these cells into the mucosa.

Marked increases in the numbers of eosinophils and mast cells were detected 4 and 6 days after infection but this increase in mast cells was followed by a decline which coincided with a further rise in eosinophil numbers. He also noted an increase in the number of plasma cells 16 days after infection.

Malczewski (1970) infected lambs with 100,000 third stage H. contortus larvae, and observed a slight mucosal hypertrophy on day four: on day six there was a marked migration of eosinophils and monocytes from local capillaries

in the lower layer of lamina propria and by day 14 eosinophils were distributed throughout the mucosa.

Stewart (1953) described the histological changes in the abomasa of sheep after reinfection with H. contortus. He found oedema of the mucus membrane not seen in primary infections, a superficial aggregation of eosinophils and no increase in mucus secretion. Sommerville (1956) and Whur (1966) found that globule-leucocytes could be detected in the abomasa of sheep which had been infected with nematodes for periods in excess of 35 days although globule-leucocytes were absent from both the abomasal mucosa of worm-free sheep and from that of sheep infected with nematodes for less than 35 days. In 1973 O'Sullivan and Donald detected an increase in the numbers of mast cells, eosinophils and globule-leucocytes in the gut mucosa of non-reproductive sheep which had been subjected to twice weekly infection with constant numbers of H. contortus and T. colubriformis larvae for 8 weeks prior to slaughter.

The main object of the work described in this thesis was a study of the cellular changes occurring in the abomasa of adult sheep and lambs subjected to various regimens of infection and reinfection with H. contortus. In the interpretation of the results particular attention was paid to the significance of quantitative and qualitative changes in abomasal cell types in relation to the age of the lamb and previous exposure.

# CHAPTER 1

## A STUDY OF SOME ASPECTS OF THE LOCAL AND SYSTEMIC RESPONSES OF ADULT SHEEP AND LAMBS TO PRIMARY AND SECONDARY *HAEMONCHUS CONTORTUS* INFECTION.

## SUMMARY

In this chapter various local and systemic responses of adult sheep and lambs to primary and secondary H. contortus infection are described.

In the adult sheep parasite eggs were detected in the faeces earlier than in the lambs. High numbers of worms were recovered from both the adult and young animals after primary infection. There was, however, a marked reduction in the numbers of worms recovered from the adult sheep after the second infection in contrast with the lambs where high numbers of worms were recovered.

The red cell indices of both the adult sheep and lambs fell during the course of the experiments but this decrease was more marked in the lambs.

Histological examination of abomasal tissue from the adult sheep seven and 15 days after the primary infection showed an increase in the number of eosinophil and neutrophil leucocytes and plasma cells. Few intra-epithelial mast cells were present.

After the second infection superficial depressed lesions of the abomasum, submucosal oedema, haemorrhage and further infiltration of the mucosa by eosinophils, neutrophils, mast cells, plasma cells and lymphocytes were detected; there was also an increase in the numbers of intra-epithelial mast cells at this time. Peroxidase-conjugated antisera were used to reveal changes in the numbers of active plasma cells in the mucosa after primary and secondary infection.

Although there was an increase in the numbers of immunoglobulin-containing cells at different stages after infection IgA cells were always predominant.

In the lambs haemorrhage, partial destruction of the surface epithelium and small mucosal accumulations of lymphocytes, together with a few plasma cells, eosinophils and mast cells were seen seven and 15 days after the initial infection. No intra-epithelial mast cells could be detected at these times.

Six and 15 days after the second infection, there was obvious damage to the surface epithelium and increased accumulations of lymphocytes, plasma cells and eosinophils in the lamina propria but by day 27, the numbers of cells in the lamina propria had decreased. Intraepithelial mast cells were present 15 and 27 days after the second infection and oedema was also obvious at this time. However all of these cellular changes were less marked than in the adult sheep.

Small numbers of immunoglobulin-containing cells were present in the mucosa of worm-free lambs and there was only a slight increase in the numbers of these cells after infection with IgA-containing cells again predominating.

In both the adult sheep and the lambs, a change in the quality of the mucus covering the surface mucosa from acid in the non-infected animals to a mixture of neutral and acid mucin in the parasitised animals was detected.

Mucosal anti-H. contortus IgA antibody levels were measured by a radioimmunoassay. There was a gradual increase

in IgA-antibody levels in the mucus from the adult sheep during the course of the experiment, the highest concentrations occurring at day 27 after second infection. In contrast, the mucus IgA-antibody concentration of the lambs tended to be low throughout the experimental period.

## INTRODUCTION

Although the epidemiology, immunology and pathophysiology of Haemonchus contortus infection in sheep have been studied in some detail there are relatively few reports of the gross and microscopical changes in the abomasum during infection with this parasite. For example, Charleston (1965) observed a marked cellular infiltration of the abomasum of sheep during infection with H. contortus and he regarded this as an immune phenomenon. A similar finding was reported by Malczewski (1970) and he also suggested that the "self cure" reaction was closely related to a heavy infiltration of the abomasal mucosa by eosinophils. Another obvious change in sheep previously infected and subsequently challenged with H. contortus was oedema of the abomasal mucous membrane (Stewart, 1953). More detailed histopathological studies have been limited.

In view of the availability of improved immunopathological techniques the following work was undertaken to examine the changes in the abomasum of adult sheep and lambs during a primary and secondary infection with H. contortus.

## MATERIALS AND METHODS

Animals. A total of 22 worm free animals were used and these were divided into two groups. Group 1 consisted of 11 adult Finn-Dorset ewes and Group 2 of 11 6-8 week-old Scottish Blackface X Dorset lambs. At the start of the experiment one animal from each group was killed as an uninfected control: the remaining animals of both groups were orally infected with 350 H. contortus $L_3$/Kg bodyweight. Two animals from each group were killed seven and 15 days after the first infection. On day 15 after the primary infection the remaining animals were reinfected orally with 350 H. contortus $L_3$/Kg bodyweight and slaughtered six, 15 and 27 days later.

During the course of the experiment one lamb died reducing the number of infected lambs to ten. The design of the experiment, with the number of animals and the time of slaughter, are shown in Table 1.

Haematological and serological techniques:

Collection and storage of samples:- Blood samples were collected from the jugular vein. For haematological estimations 2 ml. of blood was withdrawn into a heparinised vacutainer and the tube shaken gently to prevent coagulation. Haematological examinations were carried out shortly after collection of the samples.

A second sample of 7 ml. of blood was withdrawn into a vacutainer and left to clot at room temperature. The serum

| NO. OF ANIMALS KILLED | | TIME OF NECROPSY | |
|---|---|---|---|
| EWES | LAMBS | DAYS AFTER PRIMARY INFECTION | DAYS AFTER SECONDARY INFECTION |
| 1 | 1 | 0 | - |
| 2 | 2 | 7 | - |
| 2 | 2 | 15 | - |
| 2 | 2 | 21 | 6 |
| 2 | 2 | 30 | 15 |
| 2 | 1 | 42 | 27 |

TABLE 1. The design of an experiment to compare the response of ewes and lambs to a primary and secondary infection with 350 H. contortus $L_3$/Kg. bodyweight.

was then harvested, transferred into plastic tubes (Metal Box Co., Portslade, Sussex, England), immediately frozen and stored at -20°C.

Packed cell volume (P.C.V.), was estimated by use of a Hawksley microhaematocrit centrifuge (Hawksley & Sons Ltd., London, England). Capillary tubes containing the blood sample were sealed at one end by heat and centrifuged for 5 minutes. The percentage PCV was determined from the scale on a Hawksley microhaematocrit reader.

Haemoglobin concentration (Hb), was estimated by the oxyhaemoglobin method (Dacie and Lewis, 1966) and its concentration was expressed as grams per 100 ml. A 1 in 200 dilution of blood was prepared in an 0.04 per cent solution of ammonium hydroxide and, after thorough mixing, the resulting solution of oxyhaemoglobin was read in a colorimeter (Evans Electro-selenium Ltd., Harlow, England) using a yellow green filter (Ilford No. 625). The colorimeter was calibrated using a cyanmethaemoglobin standard solution (cyanmethaemoglobin standard solution -C, David Keeler Ltd., London, England).

Total red blood cell counts (RBC's)

Total red blood cell counts ($x10^6$/cu.mm.) were estimated by an electronic particle counter (Coulter Model 'D', Coulter Industrial Sales Co., Elmhurst, Illinois, U.S.A.).

Parasitological techniques

H. contortus larvae were cultured from the faeces of an experimentally infected sheep. A faecal bag was used to collect the total daily output of faeces. About 300 gm. of faecal pellets were put into each of a series of 500 ml. honey jars and stored at 22°C for 14 days. The jars were then filled with water and inverted over petri dishes for 2 hrs. The fluid decanted from the faecal culture was passed through milk filter pads (Clover leaf No.9, Johnson & Johnson, England) in a Buchner funnel and clean larvae were obtained by setting up these pads containing larvae in a Baerman apparatus.

Faecal samples were collected twice weekly from the rectum and examined by a modified McMaster technique. In this technique, 3 gm. of faeces were mixed with 42 ml. of water and passed through a sieve (60 meshes per inch); 15 ml. samples of the filtrate were centrifuged in flat bottomed test tubes for 2 minutes at 2,000 r.p.m. and the supernatant poured off. The sediment of the tube was resuspended in saturated salt (NaCl) solution, the test tube inverted several times, then, using a pipette, both chambers (Volume 0.15 ml.) of a McMaster Worm Egg Counting Slide (Hawksley & Sons, London, England) were filled with the suspension. The number of eggs in both chambers was multiplied by 50 to give the numbers of eggs per gram of faeces.

Worm Counts. The total worm burdens were estimated by

counting the worms in the abomasum contents and digests of abomasal mucosa. The abomasum was opened along the lesser curvature and its contents poured into a bucket, while the mucosa was gently washed with tap water. The abomasal contents were then made up to a total volume of 2,000 ml. with tap water and after stirring thoroughly, worms in 200 ml. of the suspension were counted. The dilution was corrected for, and the total worm burden in the abomasal contents calculated.

Half of the abomasum mucosa was digested using 1% pepsin in 3% hydrochloric acid for 6 hrs. to release immature stages. The digested mucosa was then treated in the same way as the abomasal contents to estimate the number of worms present. Worms were differentiated morphologically into 3 groups namely, adults, parasites at the 4th moult (mature $L_4$) and immature $L_4$.

Collection of abomasal mucus: After gentle washing with tap water to remove any worms and adherent food debris, half of the abomasum was taken and the mucosa was scraped off with a microscope slide. One gram of the mucosal scrapings was homogenised in 5 ml. cold Tris/HCl (0.1M Tris, 0.1M NaCl, pH 8.0, with sodium azide added to 0.02%). The homogenate was then clarified by ultra-centrifugation (30,000 g) for 30 minutes; the resultant clear yellowish supernatant is subsequently referred to as abomasal mucus.

Preparation of larval antigen. *H. contortus* larval antigen was prepared as follows: Infective larvae were washed in three

changes of Ringers solution (NaCl 6 gm., KCl 0.30 gm., $CaCl_2.2H_2O$ 0.134 gm., $MgCl_2.6H_2O$ 0.20 gm./litre) then the volume reduced to 5 ml., in bottles (U.G. 300 ml. AA523) with up to 5 million H. contortus $L_3$. A mixture of air/$CO_2$ was then passed through the larvae for 5 minutes ( 1 litre air/min. 500 cc's $CO_2$/min). The larvae were then incubated for 16 hrs. at 37°C on a reciprocating agitator; 70-90% of the larvae had exsheathed by the end of this period. The larvae were then washed three times in Mapes II medium (NaCl, 3.19 gm; KCl 1.48 gm; $CaCl_2$ 0.11 gm; $MgSO_4H_2O$ 0.122 gm./litre pH 2.7 with HCl) and incubated on the agitator for 48 hrs. at 37°C. The percentage of fourth-stage larvae in the mixture by the end of the incubation period was approximately 80%. Several million of a mixture of these larvae (80% $L_4$, 20% $L_3$) were homogenised in phosphate buffered saline, pH 7.2 (PBS). The homogenate was centrifuged at low speed (3,000 RPM) for 15 minutes, then the supernatant subjected to ultracentrifugation (30,000 g.) for 45 minutes, resulting in a superficial lipid layer, an intermediate straw-coloured fluid and a pellet. This intermediate clear fluid was retained as soluble larval antigen and stored at -20°C until used.

Labelling of Rabbit Anti-Sheep IgA

Rabbit anti-sheep IgA was labelled with $^{125}I$ by a chloramine T method (Hunter and Greenwood, 1962), as described below:

1 ml. of rabbit anti-sheep IgA was added to 2 mCi $^{125}I$ ($Na^{125}I$ - Radiochemical Centre, Amersham) 0.1 ml. of

chloramine T was added at 4 mg./ml. (Dissolved 40 mg. in 10 ml. PBS), and mixed for 1 minute. 0.1 ml. Na metabisulphite 10 mg./ml. (100 mg. dissolved in 10 ml. PBS) was added to the mixture. 0.2 ml of 1% KI (10 mg./ml.) was then added. Protein bound iodine was separated from free iodine on a column of Sephadex G25 eluting with 1 ml. 1% KI then with PBS. The labelled antisera were stored at -20$^{o}$C until required and were diluted suitably in Tween 20 PBS when used.

Radioimmunoassay for the detection of antibodies to larval antigens of H. contortus

Larval antigen was diluted to 10 µg protein/ml. in 0.01M Carbonate buffer ($NaCO_3$), pH 9.6. 0.35 ml. of antigen solution was added to each plastic well in a microtiter tray (Dynatec Ltd.) and following overnight incubation at 4$^{o}$C the solution was aspirated and the well washed three times with 0.5 g/l. Tween 20 PBS. These antigen coated tubes were then stored at 4$^{o}$C until required. An equal number of untreated control wells were filled to the brim with Tween 20 PBS. After overnight incubation at 4$^{o}$C these wells were emptied, washed and again stored at 4$^{o}$C until used. For the assay, 0.35 ml. of each suitably diluted test serum (1:1000) or mucus sample (1:10) was added to each of duplicate tubes (both antigen and control tubes) and left for at least 2 hrs. at room temperature. The samples were then removed and the tubes washed three times with Tween 20 PBS. Then 0.35 ml. of $I^{125}$ rabbit anti-sheep IgA suitably diluted (1:100 - 1:500) in Tween 20 PBS was added

to each tube and incubated for 1 hr. at room temperature. The labelled antiserum was then aspirated and each tube was washed 5 times with Tween 20 PBS before being counted in a Packard automatic gamma spectrometer (Packard Instrument Co. Inc., Illinois, U.S.A.).

Each test included tubes containing serum and mucus from infected and worm-free sheep: antigen-coated and control tubes to which test serum or mucus had not been added but which had been incubated with labelled anti-globulin served as blanks. After removing the activity of these blanks, the specific activity due to antibody was obtained by subtracting the activity of the control tubes from that of the antigen-coated tubes.

Histology

The abomasum was removed immediately after slaughter: after washing with tap-water to remove any adherent coarse food debris, samples were taken from the pyloric and fundic parts of the abomasum. These were then fixed in either 10% formalin, for routine microscopic study of the histological structure and eosinophils, or Carnoy's fluid for mast cells, mucus and immunoglobulin-containing cells.

Formalin-fixed tissues were processed in a standard 24 hrs. histokinette cycle and vacuum embedded in paraffin wax. Sections cut at 6$\mu$ were examined after the following histochemical stains:

1. Haematoxylin and eosin.
2. Haematoxylin and azo-eosin.

Carnoy-fixed tissues were dehydrated and cleared in three changes each of 100% methanol and xylene at 4°C and vacuum embedded in wax. Sections cut at 6μ were examined after staining with:

1. Astra-blue/Safranin O.
2. Alcian blue/Periodic acid schiff (PAS).
3. Peroxidase labelled antisera as described below.

Horseradish peroxidase conjugated antisera to different sheep immunoglobulin classes were obtained either from Elvaibios Laboratories Limited (Horsham, West Sussex, England) or were kindly provided by Dr. W.D. Smith, Moredun Institute, Edinburgh. The sections were incubated with antiserum-horseradish peroxidase conjugate for one hour, rinsed in four changes of PBS and peroxidase activity revealed by diaminobenzidine (DAB)/Hydrogen peroxide solution (5 mg. diaminobenzidine in 10 ml. of 0.1M Tris-HCl buffer, pH 7.4 containing 2 drops of 6% $H_2O_2$) for 10 minutes which results in a deep brown colour. Suitable controls were included to check the specificity of antiserum and the presence of non-specific background staining.

Cell counts

Mast cells, plasma cells and eosinophils were counted by using a X40 objective in a microscope fitted with a graticule with grid division 10 x 10 mm. (Ernst Leitz, GMBH D-6330, Wetzlar, Germany). By systematic movement of the microscope stage, 15 or 20 graticule fields on each section were examined from each tissue sample. Thus, each cell

total given in the tables represents the mean of the average number of cells present in the 15-20 graticule fields counted from both pyloric and fundic regions from each sheep.

Statistical methods

Where applicable the student's "t" test, as described by Fisher (1934), was used to determine the level of significance of a given result.

## RESULTS

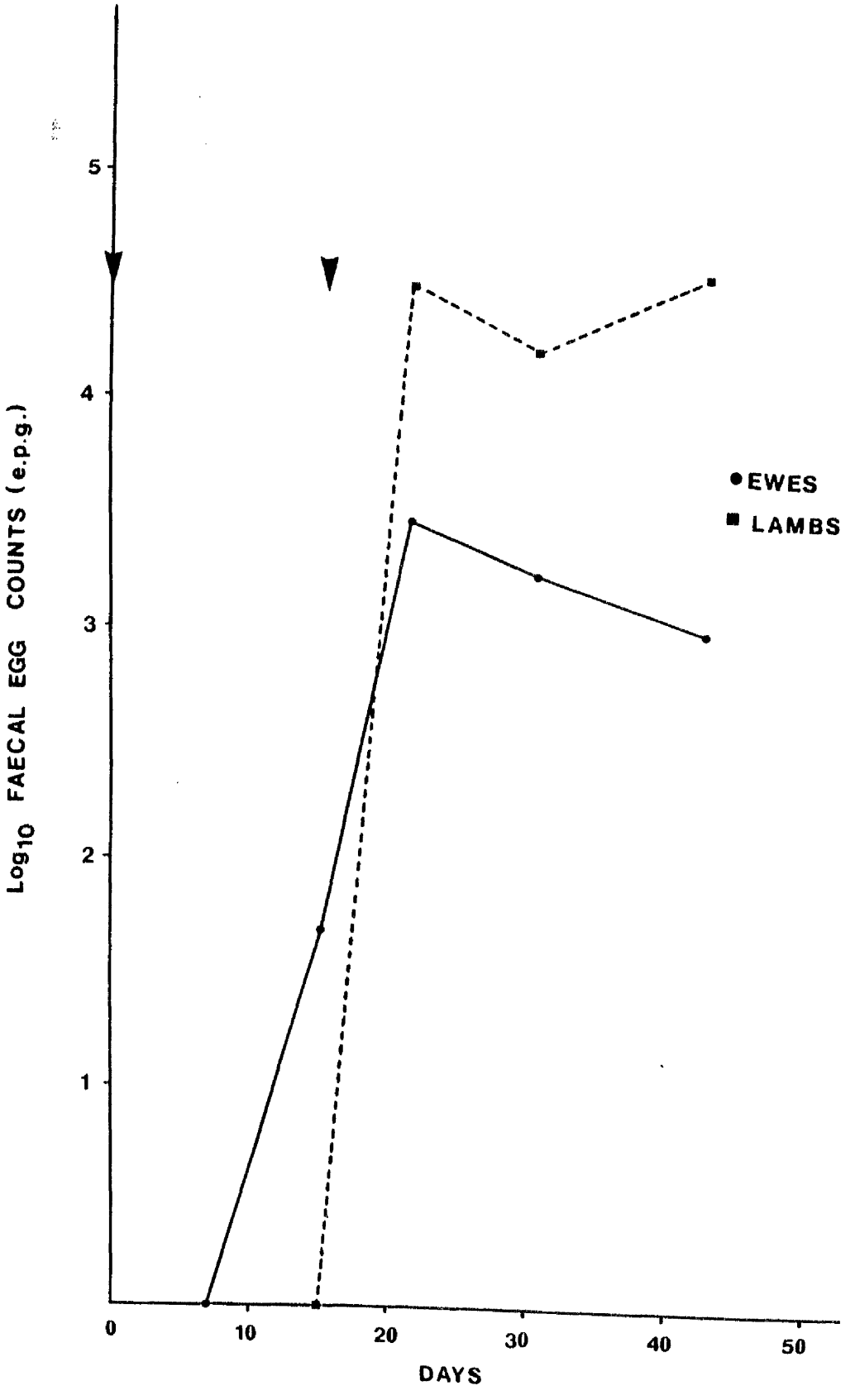
### Faecal egg counts

The mean faecal egg counts of each pair of ewes and lambs at necropsy are shown in Figure 1.

In the adult sheep, a few eggs appeared in the faeces of one animal on day 15. By day 22 (six days after the second infection) the mean faecal egg count had risen to 3050 e.p.g. By day 32 this had fallen to 1800 e.p.g. and at the end of the experiment (day 39) the mean faecal egg count was 1000 e.p.g.

Figure 1

The mean faecal egg counts of pairs of ewes and lambs after infection with 350 H. contortus $L_3$/Kg. Bodyweight on day 0 and on day 15. (▼)


Log10 FAECAL EGG COUNTS (e.p.g.)
5
4
3
2
1
0
10
20
30
40
50
DAYS
EWES
LAMBS

In the lambs no eggs were present in faeces 15 days after initial infection. By day 22 the mean count had risen to 32300 e.p.g. Although this count fell to 17400 e.p.g. by day 32 the lamb killed at the end of the experiment had a faecal egg count of 35800 e.p.g.

Worm burdens

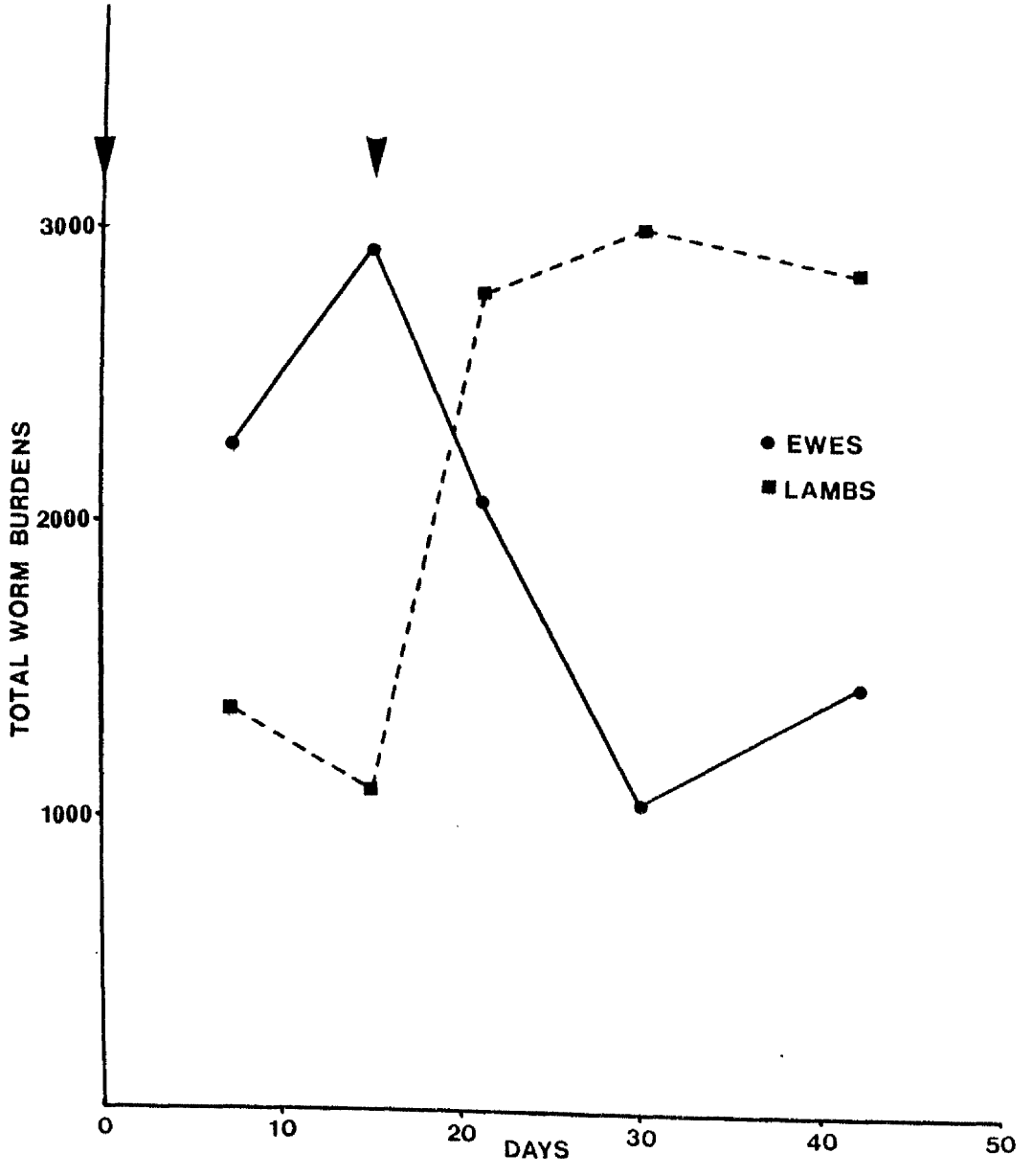
The mean numbers of worms recovered from the adult sheep and lambs are illustrated in Figure 2 and Table 2.

After the initial infection in the adult sheep, high numbers of immature and adult worms were recovered at seven and 15 days, the mean counts at this time being 2275 and 2959 respectively. The number of adult worms recovered remained high six days after the second infection but subsequently there was a substantial reduction in the mean adult worm burdens and an increase in the number of mature $L_4$.

Although the numbers of immature and adult worms recovered was lower, due to administration of infective larvae on a body weight basis, a similar pattern was observed in the lambs seven and 15 days after the initial infection i.e. 1375 and 1100 respectively. After the second infection, however, there was a marked increase in the numbers of worms recovered. For example on day six after reinfection a mean of 1825 $L_4$ and 950 adults were present. Seven days later the majority had developed to the adult stage and a high number of adult worms were still present in the lamb killed at the end of the experiment i.e. 2850 worms.

Figure 2

The mean numbers of worms recovered at necropsy from pairs of adult sheep and lambs after infection with 350 *H. contortus* $L_3$/Kg. Bodyweight on day 0 and on day 15. (▼)


TOTAL WORM BURDENS
3000
2000
1000
0
10
20
30
40
50
DAYS
EWES
LAMBS

| DAYS AFTER INFECTION | | MEAN WORM BURDENS AT NECROPSY | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | EWES | | | | LAMBS | | | |
| PRIMARY | SECONDARY | ADULT | 4M+ | $L_4$ | TOTAL | ADULT | 4M+ | $L_4$ | TOTAL |
| 7 | - | 0 | 2225 | 0 | 2225 | 0 | 1375 | 0 | 1375 |
| 15 | - | 2925 | 0 | 0 | 2925 | 1100 | 0 | 0 | 1100 |
| 21 | 6 | 2075 | 0 | 0 | 2075 | 950 | 0 | 1825 | 2775 |
| 30 | 15 | 775 | 275 | 0 | 1050 | 2875 | 125 | 0 | 3000 |
| 42 | 27 | 750 | 700 | 0 | 1450 | 2850 | 0 | 0 | 2850* |

TABLE 2. Mean number of worms recovered from pairs of ewes and lambs at different times after a primary and secondary infection with 350 H. contortus $L_3$/Kg. Bodyweight.

+4M - Larvae at fourth moult

* (one animal)

## Haematology

The mean red cell indices of the sheep and lambs seven days after the primary infection and six days after reinfection are shown in Table 3.

In general the red cell indices of both the adult sheep and lambs showed a slight decrease after the initial infection and a further decrease after reinfection. This was more obvious in the lambs.

## Histopathology

The general histological features of the abomasum of worm-free lambs and ewes, and of lambs and ewes after a primary or secondary infection with H. contortus, are described below. These are followed by details of changes in the numbers of mast cells and immunoglobulin-containing cells at different stages after infection.

### Lambs

In the worm-free lamb killed on day 0 the abomasal mucosa was normal and consisted of a columnar mucus-secreting surface epithelium which extended into the gastric pits into which opened the gastric glands (Figure 3). In the fundic region the glands were simple, branched and tubular extending to the muscularis mucosa. A thin layer of mucus originating from the mucus-neck cells and the surface

Figure 3

Section obtained from the abomasum (fundic region) of a worm-free lamb with only a few lymphocytes in the lamina propria.

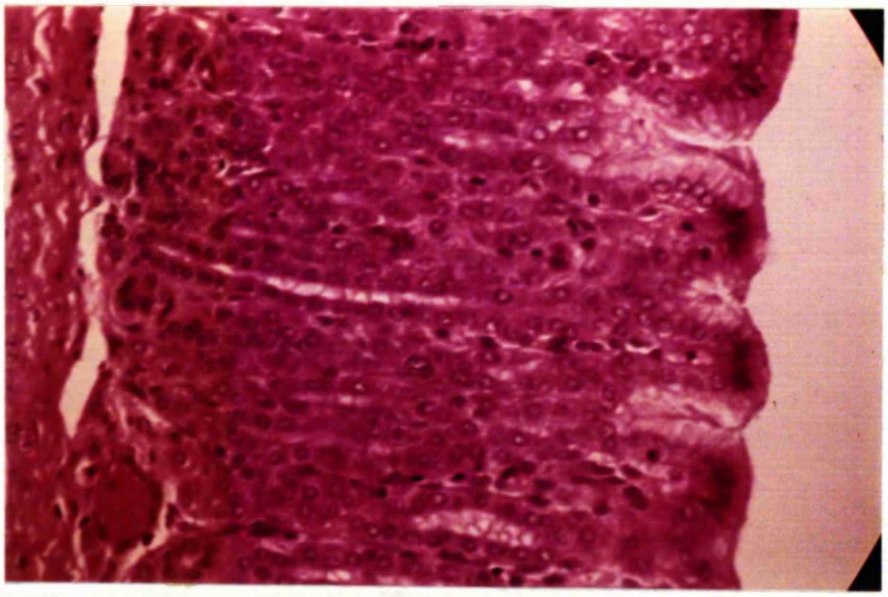
H and E. X.250

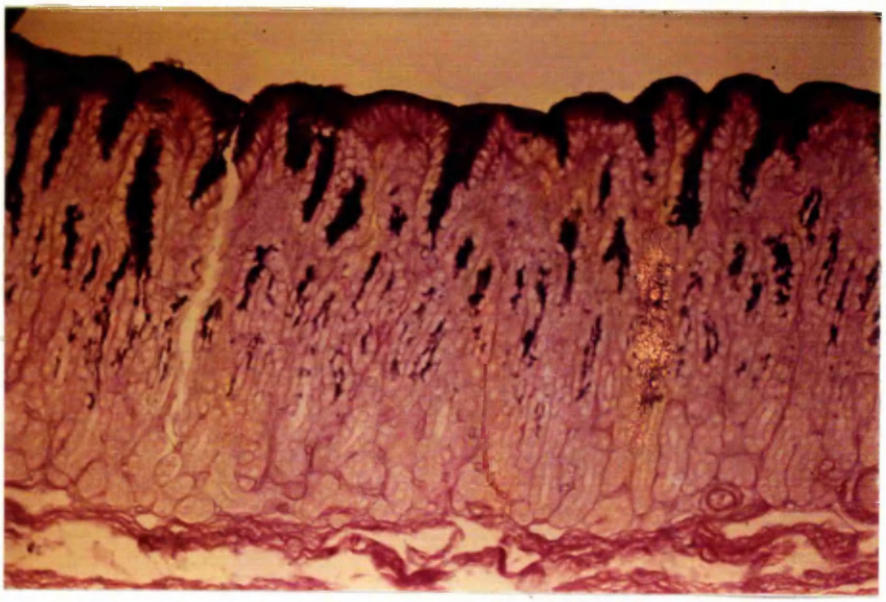
Figure 4

Section obtained from the abomasum (fundic region) of a worm-free lamb.

A thin layer of mucin is present, covering the mucosal surface.

Alcian blue/PAS. X.110





| | Day seven after primary infection | | | Day six after secondary infection | | |
|---|---|---|---|---|---|---|
| | PCV* Mean ± S.E. | RBC+ Mean ± S.E. | Hb° Mean ± S.E. | PCV* Mean ± S.E. | RBC+ Mean ± S.E. | Hb° Mean ± S.E. |
| Ewes | 30.44 ± 0.87 | 10.15 ± 0.38 | 8.94 ± 0.54 | 27.25 ± 2.10 | 7.66 ± 0.65 | 9.32 ± 0.21 |
| Lambs | 32.66 ± 0.66 | 11.51 ± 0.78 | 7.62 ± 0.35 | 23.33 ± 0.88 | 5.85 ± 0.23 | 7.43 ± 0.59 |

TABLE 3. Mean red cell indices (± standard error) of ewes and lambs at seven days after a primary infection and six days after a secondary infection with 350 H. contortus $L_3$/Kg. Bodyweight.

* PCV - Percentage

+ RBC - $X10^6$/cu. mm.

o Hb - gm/100 ml.

epithelial cells was seen covering the surface of the abomasum (Figure 4). In the pyloric area the glands were branched, coiled and relatively short compared with those in other regions of the abomasa. The lamina propria between adjacent gastric glands and between the glands and the muscularis mucosa contained only a few, scattered lymphoid cells.

Seven and 15 days after the primary infection of lambs lesions were present affecting the surface epithelium, the gastric glands and the lamina propria. Focal areas of the surface epithelium were damaged, associated with loss of the surface mucus layer. Increased mucus production by the gastric glands was suggested by apparent hyperplasia of the mucus-producing cells at the neck of the glands, which extended to occupy a greater proportion of the thickness of the mucosa (Figure 5). Small accumulations of lymphocytes, together with a few plasma cells, eosinophils and mast cells were present in the lamina propria. They occurred both at the luminal surface and around blood vessels deeper in the mucosa. Mucosal blood vessels were congested and small areas of haemorrhage were occasionally present (Figure 6).

Six and 15 days after the second infection the major lesions were damage to the surface epithelium and increased accumulations of lymphocytes, plasma cells and eosinophils in the lamina propria (Figure 7). At this stage there was an obvious decrease in the mucus content of the cells in the gastric glands (Figure 8). Oedema was

Figure 5

Section obtained from the abomasum (fundic region) of a lamb killed seven days after a primary infection with 350 H. contortus $L_3$/Kg. bodyweight.

Hyperplasia of the mucus-producing cells at the neck of the gastric glands.

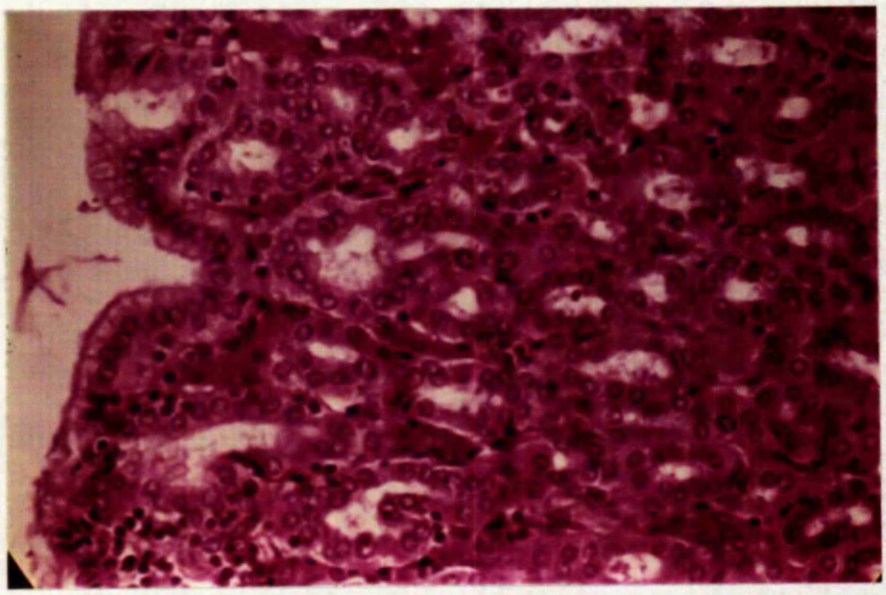
Alcian blue/PAS. X.400

Figure 6

Section obtained from the abomasum (fundic region) of a lamb killed seven days after a primary infection with 350 H. contortus $L_3$/Kg. bodyweight.

Small areas of haemorrhage are evident throughout the mucosa.

H and E. X.400



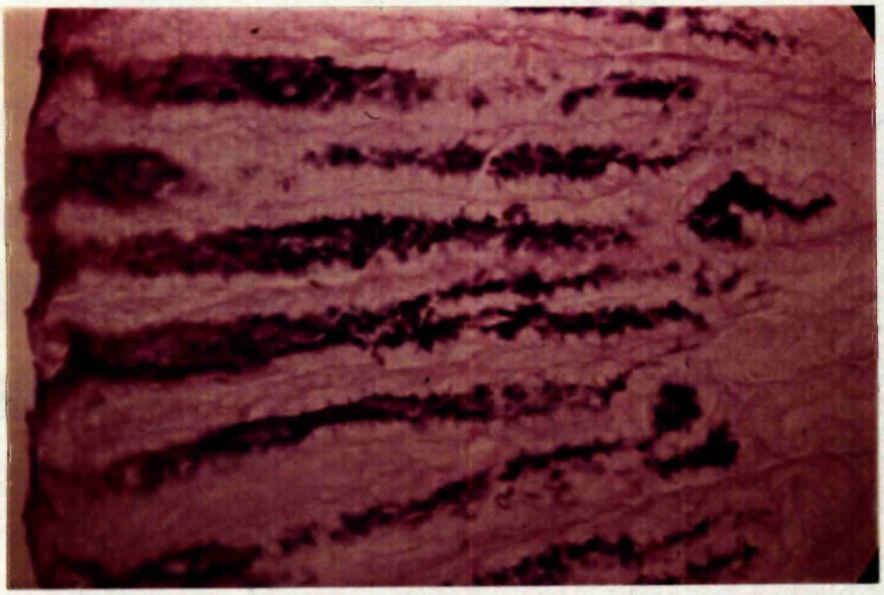


Figure 7

Section obtained from the abomasum (fundic region) of a lamb killed 15 days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

Damage to the surface epithelium is evident with obvious infiltration of lymphocytes, plasma cells and eosinophils in the lamina propria.

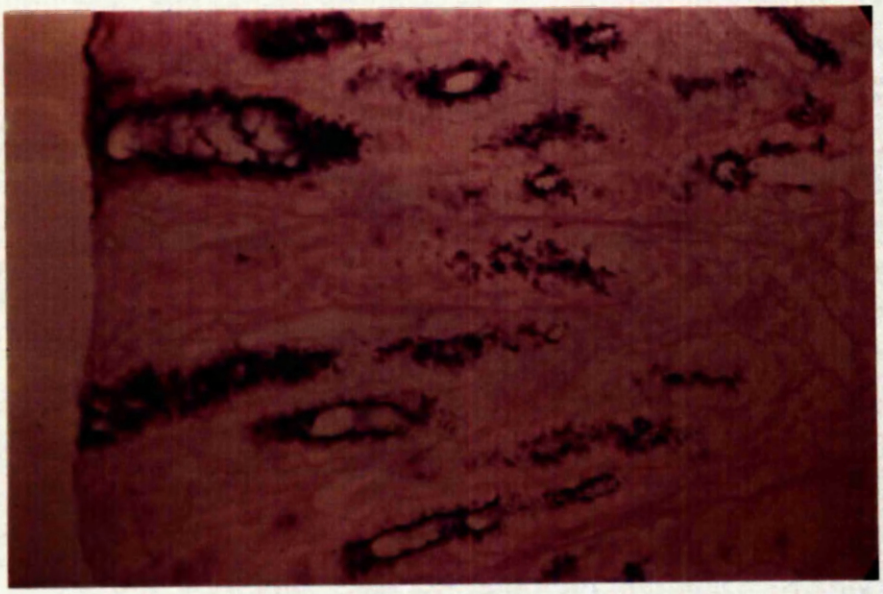
H and E. X.250

Figure 8

Section obtained from the abomasum (fundic region) of a lamb killed 15 days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

Decrease in the mucus content of the cells in the gastric glands.

Alcian blue/PAS. X.400



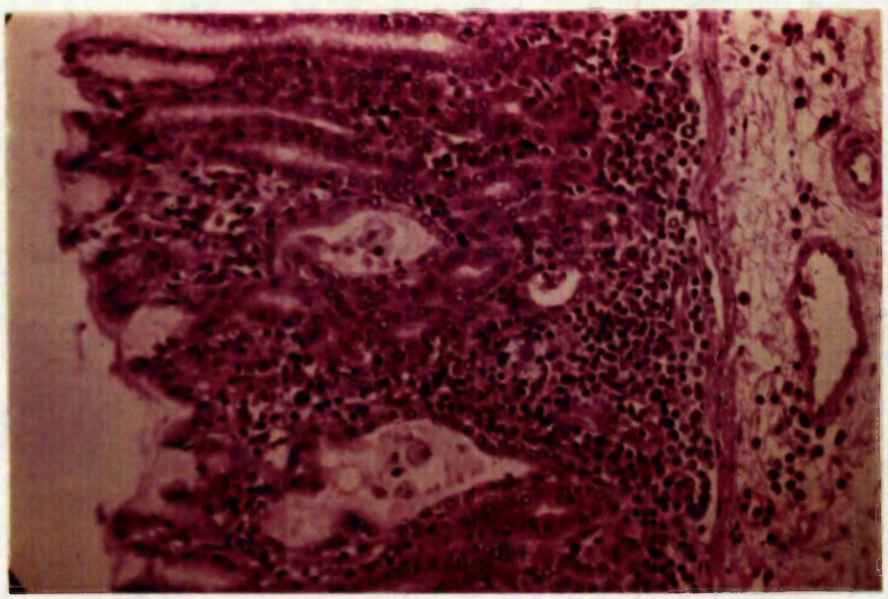
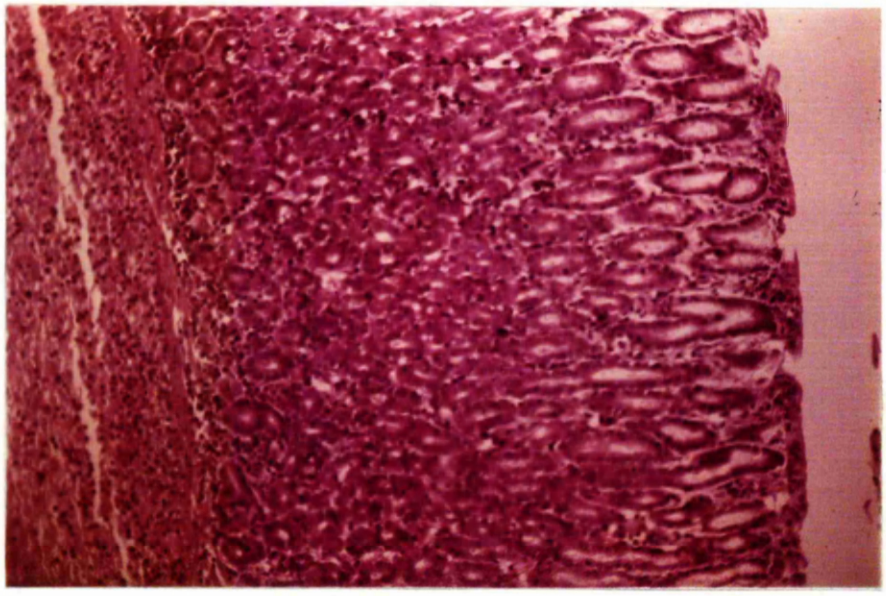


Figure 9

Section obtained from the abomasum (fundic region) of adult sheep killed seven days after a primary infection with 350 H. contortus $L_3$/Kg. bodyweight.

Heavy infiltration of the submucosa and mucosa with eosinophils, lymphocytes and plasma cells.

H and E. X.110



present in the mucosa and sub-mucosa. By 27 days mucosal and submucosal oedema was still obvious and there was a reduction in the numbers of cells in the lamina propria. There was, however, an obvious increase in mucus content of the gastric glands compared with that seen six and 15 days after reinfection.

Adult sheep

In the uninfected adult sheep the mucosa differed from that of worm-free lambs by the presence in the lamina propria of numerous mast cells together with some lymphocytes, plasma cells and eosinophils. These were distributed throughout the thickness of the mucosa. Many lymphocytes with a few mast cells and eosinophils were seen in the sub-mucosa particularly around the blood vessels.

Seven days after the primary infection of adult sheep with H. contortus the most obvious change was an increase in the number of eosinophils, neutrophils and plasma cells. The increased numbers of eosinophils and neutrophils occurred particularly in the sub-mucosa but many eosinophils, lymphocytes, plasma cells and mast cells were present in the lamina propria occurring both towards the surface and base of the mucosa, between gastric glands and around blood vessels (Figure 9). By 15 days after the primary infection similar lesions were observed but there was a further increase in the number of eosinophils in the mucosa and sub-mucosa.

In the adult sheep six days after challenge the

changes included submucosal oedema, mucosal haemorrhage and infiltration of the submucosa and mucosa by increased numbers of eosinophils, neutrophils, mast cells, plasma cells and lymphocytes. Diffuse accumulations of cells occurred particularly at the site of depressed lesions in the mucosa (Figure 10). In one animal killed six days after reinfection parasites were seen in the abomasum, both within and on the surface of the abomasal mucosa. The lesions in the immediate vicinity of these parasites were similar to those observed elsewhere in the mucosa where parasites were not detected probably due to washing of the abomasum at necropsy.

Fifteen and 27 days after reinfection of the adult sheep, depressed lesions in the mucosa were still obvious. Under these lesions there were more marked cellular accumulations than those seen at earlier stages of infection, and these extended into the submucosa. The cells present included mast cells, eosinophils, plasma cells and many lymphocytes (Figure 11). At both of these stages a marked increase in mucus production by the gastric glands was suggested by apparent hyperplasia of the muçus-producing cells.

Mast cells and Eosinophils

Mast cells occurred in the submucosa, mucosa and within the epithelium of gastric glands and changes in their combined numbers in the abomasa of the lambs and adult sheep during infection are presented in Table 4. The

Figure 10

Section obtained from the abomasum (fundic region) of adult sheep killed six days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

Accumulations of neutrophils, eosinophils and few lymphocytes at the site of a depressed lesion in the mucosal surface.

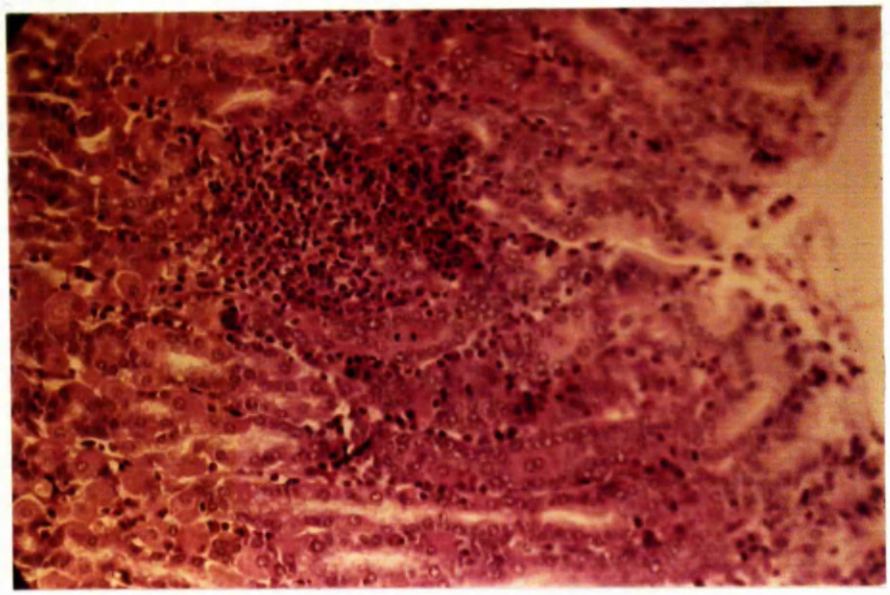
H and E. X.250

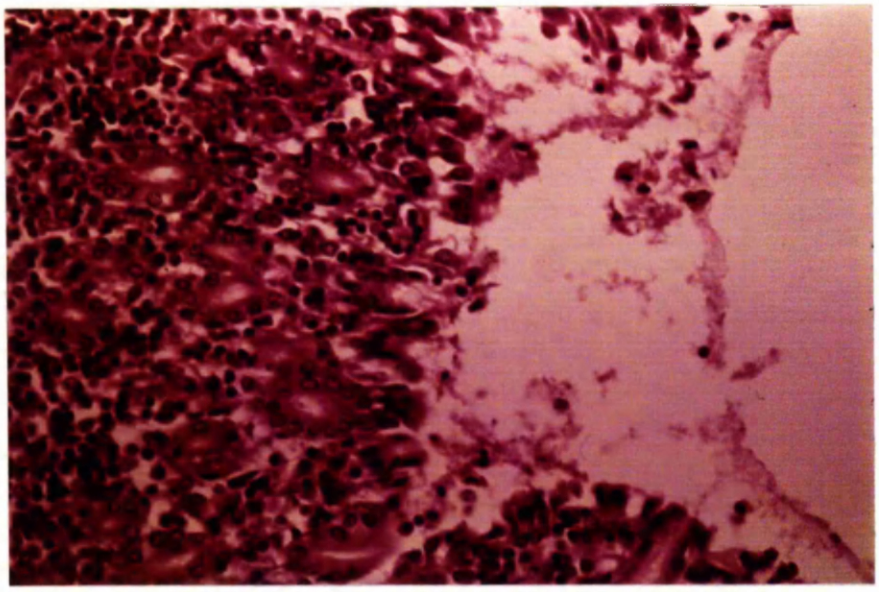
Figure 11

Section obtained from the abomasum (fundic region) of an adult sheep killed 27 days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

Accumulations of large numbers of plasma cells, lymphocytes, eosinophils and mast cells are evident beneath a depressed lesion in the surface mucosa.

H and E. X.250





| NUMBER OF ANIMALS KILLED | TIME OF NECROPSY DAYS AFTER INFECTION | | MEAN NUMBER OF CELLS IN ABOMASAL MUCOSA | | | | |
|---|---|---|---|---|---|---|---|
| | 1st | 2nd | Mast cells and Globule Leukocytes | Eosinophils | IgA-Containing cells | IgG-Containing cells | IgM-Containing cells |
| ADULTS | | | | | | | |
| 1 | Uninfected | | 15.7+ | 0.1+ | *0.5 | *0.4 | 1.2 |
| 2 | 7 | - | 9.6 | 9.4 | *17.9 | *2.4 | *2.0 |
| 2 | 15 | - | 13.5 | *13.4 | *5.9 | *1.2 | *1.8 |
| 2 | 21 | 6 | 23.2 | 18.1 | 10.9 | 1.6 | 1.6 |
| 2 | 30 | 15 | 31.3 | 16.5 | 8.1 | 1.3 | 1.7 |
| 2 | 42 | 27 | 40.0 | 31.8 | 12.9 | 8.3 | *5.9 |
| LAMBS | | | | | | | |
| 1 | Uninfected | | 1.2+ | 00+ | *0.6 | *0.2 | *0.3 |
| 2 | 7 | - | 4.7 | 0.3 | 2.7 | 0.2 | 3.7 |
| 2 | 15 | - | 4.7 | 1.9 | 1.9 | 0.1 | 2.4 |
| 2 | 21 | 6 | 00+ | 3.4 | *3.1 | 4.5 | *2.5 |
| 2 | 30 | 15 | 6.0 | 2.6 | 4.7 | 0.8 | 1.1 |
| 1 | 42 | 27 | 6.7+ | 1.6+ | *4.1 | *0.7 | *0.8 |

TABLE 4. Mean numbers of various cell types present in the abomasal mucosa of ewes and lambs after primary and secondary infection with 350 H. contortus $L_3$/Kg. Bodyweight. (calculated from the means of 20 fields from sections of both fundus and pylorus from each animal no S.E. given since there were only 1-2 animals per group).

* Mean of 40 fields from sections of both fundus and pylorus from one animal.
\+ Mean of 20 fields from sections of fundus only.

numbers of tissue eosinophils observed in sections from the abomasa of ewes and lambs at different stages after infection are also given in Table 4.

Lambs

In the worm-free lamb, small numbers of mast cells were present in the mucosa at the base of the gastric glands and in the submucosa (Figure 12).

Seven and 15 days after the primary infection mast cells were much more numerous. This increase occurred mainly at the base of the gastric glands but mast cells were also present in the lamina propria towards the luminal surface. Only a few cells were seen in the submucosal region. The number of mast cells in the abomasum of the lambs was further increased on days, 15 and 27 after reinfection but the distribution was similar to that seen after primary infection (Figure 13), with only few cells within the glandular epithelium.

There were slight increases in the numbers of eosinophils in the abomasa of lambs killed during the course of the experiment.

Adult sheep

In the uninfected adult sheep substantial numbers of mast cells were detected. The majority were distributed throughout the lamina propria but a few cells were also present in the sub-mucosa (Figure 14).

Figure 12

Section obtained from the abomasum (fundic region) of a worm-free lamb showing only a few mast cells at the base of the gastric glands.

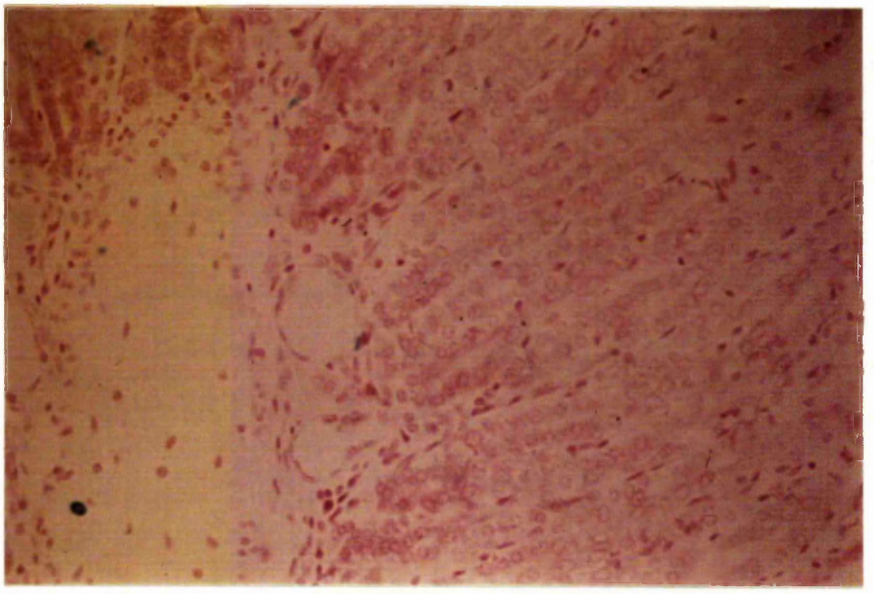
Astra blue/safranin'O. X.250

Figure 13

Section obtained from the abomasum (fundic region) of a lamb killed 27 days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

An increased number of mast cells is present in the lamina propria throughout the depth of the mucosa.

Astra blue/safranin'O . X.250



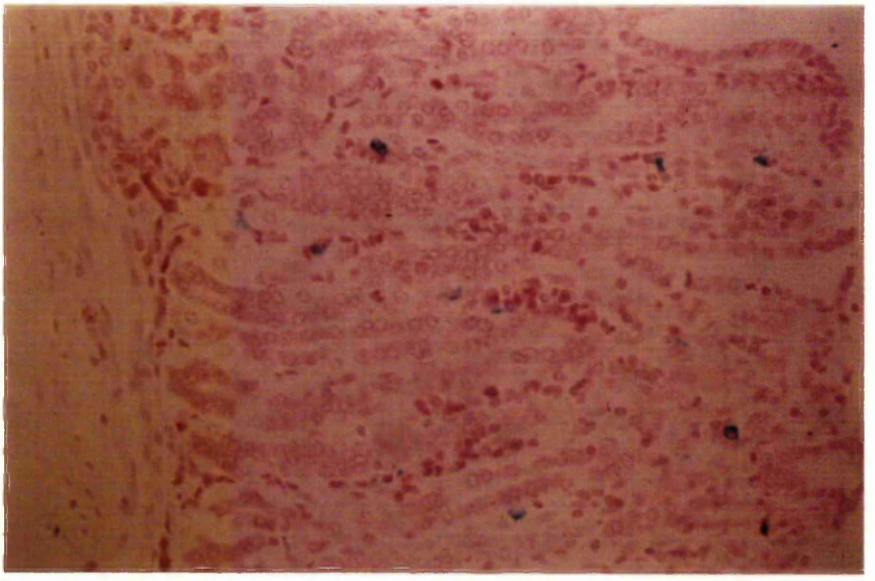


Figure 14

Section obtained from the abomasum (fundic region) of a worm-free adult sheep.

A moderate number of mast cells is seen distributed in the lamina propria throughout the depth of the mucosa.

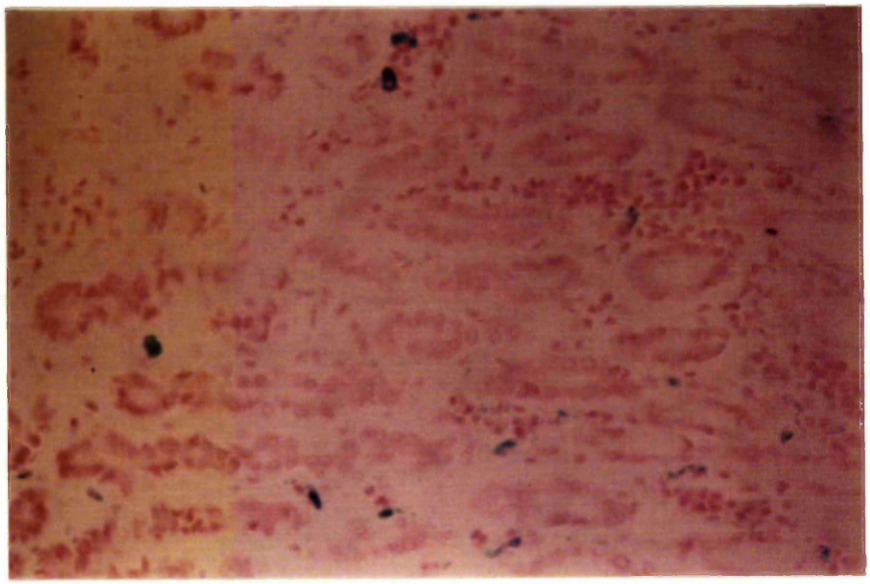
Astra blue/safranin'O. X.250

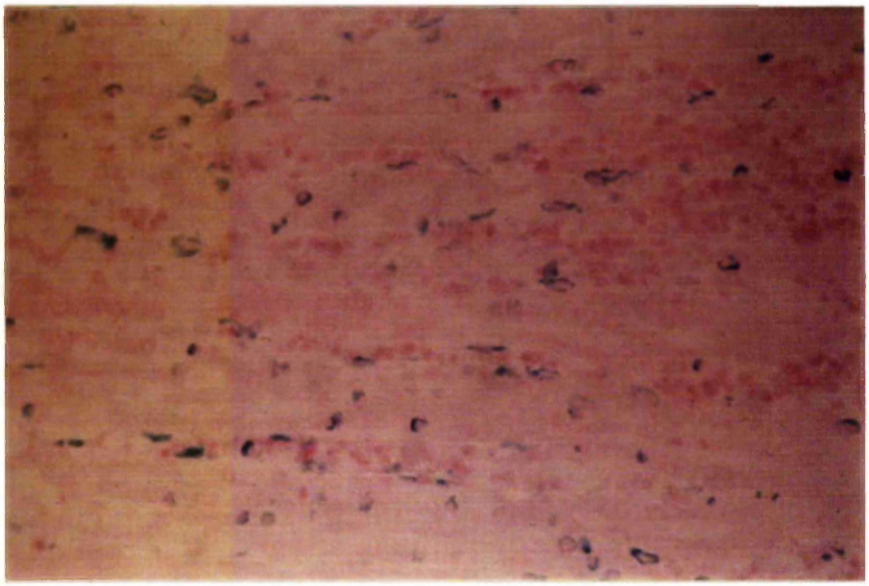
Figure 15

Section obtained from the abomasum (fundic region) of an adult sheep killed 27 days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

Large numbers of mast cells are present in the lamina propria and intraepithelially.

Astra blue/safranin'O. X.250





Seven days after infection there was a reduction in mast cell numbers but by day 15 these had returned to the level detected in the uninfected animal. A few intra-epithelial mast cells were observed at both seven and 15 days after infection. Marked increases in mast cell numbers occurred on each of days six, 15 and 27 after reinfection. At all of these stages mast cells were seen mainly in the mucosa between the gastric glands but large numbers were also present intraepithelially; small numbers of mast cells were again present in the sub-mucosa (Figure 15).

There was a progressive increase in tissue eosinophils during the course of the experiment and this was particularly marked after secondary infection.

Immunoglobulin-containing cells

Changes in the numbers of cells containing IgA, IgM or IgG in the abomasum of lambs and adult sheep during infection with H. contortus are presented in Table 4.

Lambs

In the worm-free lamb very few immunoglobulin-containing cells were present in the lamina propria. There was a slight increase in the numbers of cells containing each of the immunoglobulin classes during the course of the primary infection. Further increases in immunoglobulin-containing cell numbers occurred during the secondary infection. The numbers of cells containing different classes of immunoglobulin varied considerably between

different sections from the same animals, but IgA-containing cells always predominated.

The distribution of immunoglobulin-containing cells was the same for each class, the cells occurring mainly at the base of the gastric glands, around blood vessels and towards the surface of the mucosa (Figure 16).

Adult sheep

In the uninfected adult sheep small numbers of cells containing each of the three immunoglobulin classes were present in the lamina propria, occurring particularly at the base of the glands and around blood vessels.

Increased numbers of immunoglobulin-containing cells occurred during the course of both primary and secondary infection with H. contortus. This was particularly marked in the case of the IgA active cells. Although the numbers of these cells varied markedly in different parts of a single section and in different sections from individual animals, they occurred mainly in the mucosa at the base of the glands and around blood vessels (Figure 17). No particular increase in the number of IgA-containing cells was present in the cellular accumulations associated with the depressed mucosal lesions.

With the exception of the sheep killed twenty-seven days after reinfection the increases in IgG- and IgM-containing cells were slight and there was considerable variation in the numbers of cells present in different parts of a single section. The distribution of these cells in

Figure 16

Section obtained from the abomasum (fundic region) of a lamb killed 15 days after a second infection with 350 H. contortus $L_3$/Kg. bodyweight.

Peroxidase labelled antiserum reveals IgA-containing cells in the connective tissue throughout the depth of the mucosa.

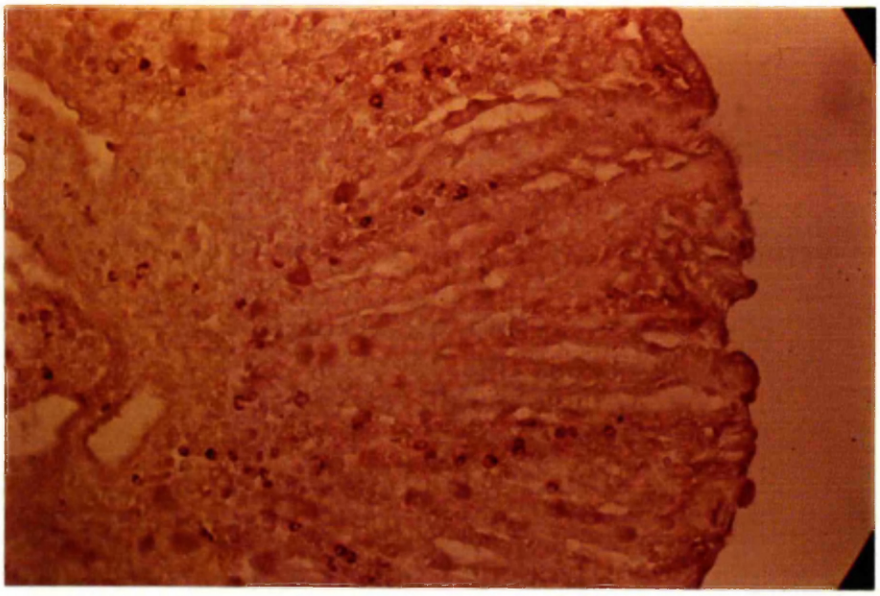
X.250

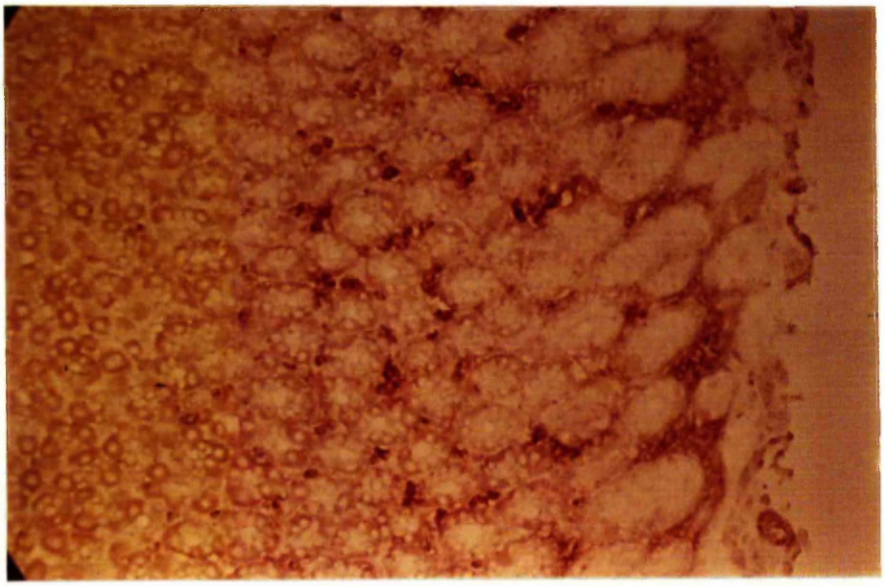
Figure 17

Section obtained from the abomasum (fundic region) of an adult sheep killed 15 days after a primary infection with 350 H. contortus $L_3$/Kg. bodyweight.

Peroxidase labelled antiserum reveals IgA-containing cells in the connective tissue in the upper half of the mucosa.

X.250





infected sheep was similar to that seen in uninfected animals.

Mucosal IgA antibody

The mean mucosal anti-H. contortus IgA antibody levels of ewes and lambs after primary and secondary infection with H. contortus were measured by a radioimmunoassay and the results are shown in Figure 18. Individual results are given in Appendix 1.

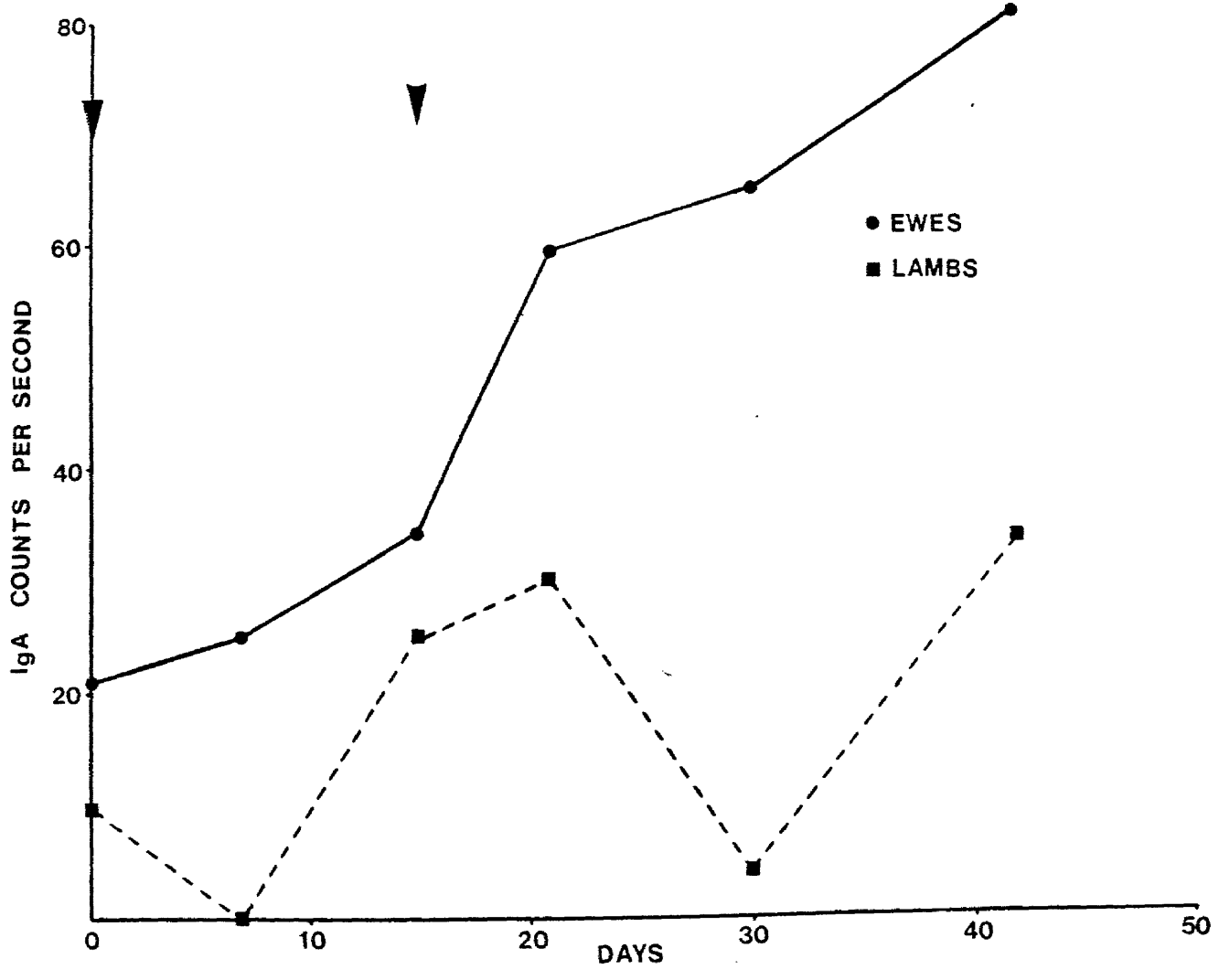
In the ewes during the initial infection with H. contortus the IgA antibody concentration increased gradually in the mucus. A marked increase in the levels of IgA antibody was detected after the second infection and the highest concentration was reached 27 days after challenge. In contrast, the mucus IgA antibody concentration of the lambs tended to be low throughout the experimental period. For example zero values were recorded seven days after the initial infection, and although these had risen at the time of challenge and six days post-challenge they had dropped to an extremely low level seven days later. A marked increase in the mucus IgA antibody level was detected in the lamb killed 27 days after the second infection but it was still less than half the mean value recorded from the two ewes killed at this time.

## DISCUSSION

In the present experiment eggs first appeared in the faeces of the adult ewes on day 15 after primary infection.

Figure 18

The mean abomasal mucus anti- *H. contortus* IgA antibody levels of pairs of adult sheep and lambs after infection with 350 *H. contortus* $L_3$/Kg Body-weight on day 0 and on day 15. (▼)


80
60
40
20
IgA COUNTS PER SECOND
EWES
LAMBS
0
10
20
30
40
50
DAYS

The highest faecal egg counts were recorded on day six after the second infection but these then declined towards the end of the experiment. A similar pattern of faecal egg output was shown by the lambs but in this case the egg counts were very much higher and were still rising at the end of the experimental period. The pattern of faecal egg output was reflected in the worm burdens of the adult sheep and lambs at various stages after infection. For example, the highest adult worm burdens were detected in the adult sheep 15 days after the initial infection. The numbers of worms recovered then started to decrease and the smallest numbers were recovered on day 15 after challenge. In contrast in the lambs the highest number of adult worms were recovered 15 days after reinfection.

An interesting feature of the worm burdens after the second infection was the proportion of immature worms in the adult sheep and lambs. For example on day six after challenge many immature $L_4$ were found in the lambs. By day 15, 28% of the total number of worms in the adult sheep were mature $L_4$ whereas the proportion in the lambs was only 4%.

At the end of the experiment only adult worms were found in the lamb killed at this time while 48% of the worm-burden of the ewes were still mature $L_4$.

Earlier work by Stewart (1953) showed that in previously infected adult sheep a sudden fall in faecal egg output occurred four days after reinfection. In a later study on the anaemia which accompanies H. contortus

infection (Dargie and Allonby, 1975) it was found that the majority of sheep, reinfected seven weeks after a primary infection, showed a sudden drop in faecal egg output approximately 7-14 days later. This was associated with a concurrent decrease in blood loss and it was considered therefore that the fall in faecal output was caused by loss or expulsion of worms.

It would appear in the present study that the adult ewes showed a typical response to primary and secondary H. contortus infection in terms of faecal egg counts and worm burdens.

Within the limitations of this experiment in which only small numbers of animals were used the adult sheep showed clear evidence that the bulk of the primary infection had been expelled by six and 15 days after challenge. Of the challenge infection only a small proportion of larvae seem to have survived and at 27 days these were still largely present as $L_4$. In comparison in the lambs there was no expulsion of the primary infection and the second infection was superimposed on this and appeared to develop normally.

It is interesting that Malczewski (1970) working with single infections of 100,000 H. contortus $L_3$ in 3½ month-old lambs, found that the worm numbers started to decrease 14 days after infection. At this time he observed a heavy infiltration of the mucosa with eosinophils which he suggested was responsible for the observed 'self cure'. This is in contrast with the findings in the present

experiment where no 'self cure' was detected in any of the lambs; in fact 15-27 days after the second infection the faecal egg counts and worm burdens of the lambs were maintained at a high level. In an earlier study where two groups of 4 and 7-month-old lambs were given either a single dose of 60,000 or daily doses of 10,000 H. contortus $L_3$ for six days then killed at two-day intervals from day four to day 20 after the first infection, Charleston (1965) found that the highest number of worms were recovered on day ten from the lambs which received a single infection. In these animals the number of worms then started to decrease gradually till the end of the experiment, whereas in the other lambs which received daily doses of 10,000 $L_3$ the number of worms recovered continued to increase throughout the course of the experiment. In a later study (Christie and Brambell, 1967) it was reported that conditions were less favourable for the early parasitic stages of H. contortus in the abomasum of sheep with recent previous experience of infection with this species than in sheep which had not been exposed previously. This conclusion was based on the results of an experiment in which two groups of 7½-month-old sheep, one of which had received repeated doses of infective larvae followed by anthelmintic treatment were challenged with 50,000 $L_3$ and killed seven days later. The mean worm burden of the immunised sheep was 5473 while that of the control animals was 15,160.

In this study only slight reductions in the PCV's and RBC counts of the adult sheep and lambs were detected one week after the initial infection. Six days after the second

infection there was no significant reduction in the PCV's and RBC counts in the adult sheep, but in the lambs there was an obvious drop in both PCV's and numbers of circulating RBC's. These results are in agreement with previous studies by Andrews (1942), Scott et al (1971) and Dargie and Allonby (1975).

Studies on the immunology of parasitic diseases have not yet reached the stage where specific dyscrasias of the immune system can be correlated with the response to a parasitic infection. Early work on immunological unresponsiveness suggested that exposure of the foetus, or the neonate to some antigens was a prerequisite for the induction of an unresponsive state but it is now known that even embryos early in gestation can respond to certain antigens, even though the response at times may be weak Fahey and Morris (1978). The immune response of the neonate to parasites is particularly important in the veterinary field since, if partial or complete non-reactivity to a particular parasite is induced by virtue of a neonatal infection, then such animals might remain susceptible throughout their lives.

It is known for example that sheep in endemic areas acquire no immunity to H. contortus. Nevertheless, experimentally, sheep may be readily vaccinated against H. contortus using either normal or irradiated larvae (Urquhart, Jarrett and Mulligan, 1962) and it is difficult to reconcile these findings with the field situation. A possible explanation depends on the fact that successful experimental immunisation can be induced only in lambs over

seven or eight months of age (Manton, Peacock, Poynter, Silverman and Terry, 1962; Urquhart et al, 1966) and this phenomenon has been attributed to immunological immaturity: it was suggested that continuous exposure to infection of lambs under natural grazing conditions had permanently depressed the immune system. Acquired unresponsiveness in adult life is not confined only to animals which have experienced antigenic stimulation in prenatal or neonatal life and under certain circumstances, frequently associated with antigenic overloading, a state of unresponsiveness or immunological paralysis may be induced in adult life (Converse, Siegel and Ballantyne, 1963; Dresser and Mitchison, 1968). A situation analogous to this, at least in its external manifestations, was described by Dineen and Wagland (1966) working with H. contortus in Merino X Border Leicester sheep which had received six fortnightly infections of 3000 larvae. Three weeks after the last infection, one group of the sheep was treated with an anthelmintic and 8 days later challenged with 3000 larvae. At autopsy six weeks later the mean worm burden per sheep was 437. The second group were not treated with an anthelmintic, the challenge infection being superimposed on the existing infection of around 6000 worms. At autopsy a mean of over 9000 worms per sheep was found, implying that the entire challenge infection had established. The authors suggested that this difference might be attributed to a possible depletion of lymphoid elements ("immunological exhaustion") caused by the prolonged and unrelieved antigenic insult in

the group which failed to exhibit resistance.

Resistance to H. contortus not attributable to previous exposure, has been recorded by other authors. Fourie (1931) wrote "it was only after many disappointments and with a great deal of difficulty that a sufficient number of typical, fatal cases of pure haemonchosis were artificially produced". Brambell, Charleston and Tothill (1964) dosed six 9-month-old worm-free sheep with 100,000 larvae and found appreciable numbers of adults in one animal only and in four of eight worm-free sheep challenged with 10,000 larvae, Urquhart, Jarrett and Mulligan (1962) found low peak egg counts and only 300, 180, 20 and 0 worms at slaughter 30 to 40 days after the challenge. In another study Bitakaramire (1966) recovered 1,040 to 6,054 worms from a challenge dose of 50,000 larvae in a group of five 10-14 month old worm-free sheep slaughtered within 16 days of challenge. Possibly the best evidence of immune inhibition is that provided by Dineen et al, (1965) who showed that a single dose of 3000 H. contortus larvae to sheep produced a population of worms of which only 2% were inhibited at the fourth larval stage. When the same dose was spread over 30 days, the percentage of inhibited forms increased to around 30%. Subsequently Dineen and Wagland (1966) showed that sheep subjected to six fortnightly doses of 3000 infective larvae and then treated with an anthelmintic were highly resistant to a subsequent challenge of 3000 larvae. Of the challenge population which did develop, 18% were inhibited larval stages, compared with 4% in the previously uninfected control group. From these

studies it is obvious that age as well as previous exposure is an important factor in the resistance of sheep to H. contortus infection shown in laboratory experiments.

It is generally accepted that the IgA system is important in mucosal defence mechanisms and it has been suggested that a slow maturation or deficiency in this system may be responsible for the unresponsiveness of young lambs to H. contortus infection (Duncan, Smith and Dargie 1978). In the present study anti-H. contortus IgA was detected in only small amounts in the abomasal mucus of lambs compared with the adult sheep. It is interesting to speculate that a more efficient IgA system in the adult animals was responsible, at least in part, for the lower faecal egg counts, lower worm burdens and increased proportion of immature parasites seen in these animals after reinfection.

Histologically the most prominent features of the worm-free lamb abomasum were the undamaged surface epithelium and the absence of a cellular reaction with only few lymphocytes moving towards a mucosal surface covered with a layer of acid mucin. This picture had changed by seven days after infection when there was mucosal haemorrhage, partial destruction of the epithelium and thick patches of neutral mucosubstances at different parts of the mucosal surface. Mucus cell hyperplasia was also detected at this stage of infection together with small aggregations of lymphocytes with a few plasma cells, eosinophils and mast cells in the mucosa. A similar picture was evident 15 days after infection but six days after reinfection there was a more marked cellular reaction. On day 15 after the second

infection oedema was the most striking feature with a slight increase in the number of eosinophils and the presence of a neutral mucin covering the surface epithelium. Oedema was still obvious 27 days after the second infection but there was less cellular reaction at this time.

In the worm-free adult sheep, small aggregations of lymphocytes with a few plasma cells were seen around the blood vessels deep in the mucosa. Few eosinophils were observed and a thin layer of mucin was seen covering the undamaged surface epithelium. Seven and 15 days after the initial infection large numbers of eosinophils were seen throughout the mucosa together with cellular aggregations which consisted of lymphocytes, plasma cells and eosinophils. Large numbers of these cells were also detected actively moving from the deeper part to the surface of the mucosa. Mucus cell hyperplasia was detected at this stage of infection and a thick layer of a mixture of neutral and acid mucosubstances was seen covering the damaged surface epithelium. Six days after the second infection there was a more extensive cellular reaction with a further increase in eosinophil numbers and haemorrhage with oedema was also evident. Larvae were seen in the mucosa, and depressed lesions on the mucosal surface were obvious. There was no change in the type of mucosubstances at this stage of infection but by 15 and 27 days after re-infection there was obvious mucosal ulceration and neutral mucosubstances were seen covering the surface epithelium. Similar histological changes were reported by Charleston (1965) in his study of two groups of 4 and 7-month old lambs given

either single or repeated doses of H. contortus $L_3$ and killed at two day intervals between four and 20 days after the first infection. He found petechial haemorrhages, mucosal hypertrophy and an apparent deficiency in the cytoplasm of the mucus secreting cells associated with developing larvae within or below the mucus layer: a collection of both mononuclear cells and eosinophils was observed around the deeper proprial vessels with movement of some of these cells into the mucosa. With infections of 100,000 H. contortus $L_3$ in lambs Malczewski (1970) observed a slight mucosal hypertrophy on day four while on day six he detected a marked migration of eosinophils and monocytes from local capillaries in the lower layer of lamina propria. By day 14 eosinophils were distributed throughout the mucosa. In an earlier study by Stewart (1953) the histological changes in the abomasum of sheep after reinfection with H. contortus were described. He found oedema of the mucus membrane, superficial aggregations of eosinophils and no increase in mucus secretion. In the present work, a thin layer of acid mucin was seen covering the mucosal surface epithelium in the worm-free adult sheep, while in the parasitised abomasum, the mucosubstances were either neutral or a mixture of acid and neutral. It is not known whether the mucins in the parasitised abomasum are chemically abnormal or whether they have accumulated to an abnormal extent.

There is a great deal of evidence that the eosinophil has antihistaminic properties (Archer, 1963) and since histamine is released by the degranulation of mast cells

(Riley, 1959) it might be expected that where a tissue eosinophilia occurs there will be an inverse relationship between the number of eosinophils and the number of mast cells present. The eosinophil is formed in the bone marrow and circulates briefly in the blood before being deposited in tissues throughout the body, the relative numbers in marrow, circulation and tissues, as estimated from studies in rodents, being in the order of 300-1-300 (Rytomma, 1960; Hudson. 1968). It has been shown that one of the major sites of localization of eosinophils is the wall of the stomach and intestine (Beeson, 1979) and increases in eosinophil numbers in the intestine of rats have been observed five, 16 and 23 days after infection with Nippostrongylus brasiliensis (Wells, 1962). Mast cell numbers in the intestine increase dramatically in rats actively immune to N. brasiliensis at the time of worm expulsion (Murray, 1972) or immediately after the worms are expelled (Kelly and Ogilvie, 1972; Wells, 1962). These cells are apparently derived from cells indistinguishable from lymphoid blast cells which undergo division and differentiation in situ (Miller, 1969). During the final rapid phase of worm expulsion, intestinal mast cells degranulate and migrate into the intestinal epithelial layer to become globule leucocytes (Miller 1969, 1971a, 1971b). Charleston (1965) found marked increases in the numbers of eosinophils and mast cells four and six days after infection of lambs with H. contortus but after this time the mast cell numbers declined coincident with a further rise in eosinophil numbers. In the present study no eosinophils and only a few mast cells were detected in the

abomasal mucosa of a worm-free lamb. Increases in the numbers of mast cells were recorded seven and 15 days after infection of the lambs while only a few eosinophils were detected at these stages. Further increases in the number of mast cells were detected 15 and 27 days after the second infection but the numbers of eosinophils tended to remain low. In 1973, O'Sullivan and Donald detected an increase in the numbers of mast cells, eosinophils and globule leucocytes in the gut mucosa of non-reproductive sheep which were subjected to twice weekly infection with constant numbers of H. contortus and T. colubriformis larvae for eight weeks prior to slaughter. Sommerville (1956) and Whur (1966) found that globule leucocytes could be detected in the abomasa of sheep which had been infected with nematodes for periods in excess of 35 days although globule leucocytes were absent from both the abomasal mucosa of worm-free sheep and from that of sheep infected with nematodes for less than 35 days. In the present study substantial numbers of mast cells were present in the abomasal mucosa of a worm-free adult sheep, but only small numbers of eosinophils were detected. Seven days after the initial infection there was a decrease in the numbers of mast cells associated with an increase in the numbers of eosinophils but the numbers of mast cells had increased to pre-infection levels by 15 days. After this time there was a further increase in the number of eosinophils and this had more than doubled by the end of the experimental period. Six days after the second infection the numbers of mast cells had markedly increased and continued to increase during the remainder of the experiment. Mast cells were seen

distributed in the connective tissue of the lamina propria of infected and worm-free animals but also within the glandular epithelium in the H. contortus infected sheep.

As stated earlier it is apparent that IgA exerts a significant protective effect on mucosal surfaces. It is known that IgA is synthesized by plasma cells in the submucosa in response to local antigenic stimulation and for this reason IgA is normally absent from newborn and germ-free animals. Curtain and Anderson (1971) could not detect any immunoglobulin-containing cells in the abomasal or intestinal mucosa of parasite-free sheep but they found large numbers of $IgG_1$ and $IgG_{1A}$-containing cells in the lamina propria of the abomasum and at the base of the villi in the small intestine of sheep infected with Ostertagia spp., Trichostrongylus spp. and Nematodirus spp. Only a few IgA-containing cells were detected in the pyloric area while the fundus was devoid of these cells.

In the present study only a few IgA-containing cells were detected in the worm-free animals but in the infected sheep variable increases in the number of IgA-containing cells occurred during the course of the experiment.

# CHAPTER 2

## A STUDY OF THE POTENTIAL BENEFIT OF VARIOUS ADJUVANTS AND IMMUNOSTIMULANTS IN THE IMMUNE RESPONSE OF YOUNG LAMBS TO *HAEMONCHUS CONTORTUS* INFECTION.

## SUMMARY

The results of the present experiment yielded information concerning the pathological changes, as well as the local immune response in the abomasum, of lambs vaccinated and challenged with H. contortus $L_3$ after treatment with various adjuvants, either alone or together with H. contortus antigens.

There were no differences in the mean faecal egg counts or in the differential worm counts of the treated and/or vaccinated lambs compared with the challenge controls. The mean PCV of the challenge control lambs had dropped markedly by the end of the experiment but the other groups of lambs showed varying reductions in PCV during the course of the experiment. The lowest PCV's recorded were in the groups which received either two doses of vaccine prior to challenge or levamisole prior to vaccination and challenge.

Various degrees of cellular reaction were observed in all of the groups compared with the worm-free control lambs, in which there was no cellular reaction. Increases in the numbers of mast cells were detected in all the lambs except the worm-free controls. No obvious changes in the numbers and distribution of tissue eosinophils were detected. There was an increase in the numbers of IgA and IgM-containing cells in all groups with the exception of the group which received a trickle infection of irradiated larvae prior to levamisole treatment and challenge where the numbers of cells

were at the same level as that seen in the worm-free control lambs. No changes were evident in the numbers of IgG-containing cells.

There were quantitative and qualitative differences in the types of mucin observed in the treated, vaccinated and challenged groups compared with the worm-free control lambs. For example, in the latter a thin layer of acid mucin was seen covering the surface mucosa whereas there was an obvious thick layer of a mixture of acid and neutral mucin on the surface mucosa in the other groups of lambs.

No obvious rise in the mucosal anti-H. contortus IgA antibodies were detected after the various treatment, vaccination and challenge regimens.

## INTRODUCTION

It has been shown that adult sheep can be immunized against the abomasal parasite H. contortus (Jarrett et al, 1959, 1961; Urquhart et al, 1966; Bitakaramire, 1966). In all of these studies two doses of irradiated larvae given orally at an interval of one month to sheep aged over six months reduced the infection resulting from subsequent challenge by 98%. However, no such protection could be induced in similarly vaccinated lambs less than six months of age (Urquhart et al, 1966; Neilson, 1975). Equivocal or negative results were reported previously in experiments where larval antigens, lymphocytes from the mesenteric lymph nodes of immune ewes or infective larvae together with larval antigens were given parenterally to lambs (Scott, Silverman, Mansfield and Levine, 1971; Silverman, Mansfield

and Scott, 1970 and Mansfield, Ozerol, Mary Courter, Cheryl Green and Levine, 1974). Neilson (1975) failed to protect lambs aged three months by injecting them with three doses of metabolic products from H. contortus larvae although earlier work by Silverman (1965) had claimed to show that a vaccine prepared from somatic and metabolic antigens derived from third and fourth stage H. contortus larvae offered a measure of protection against challenge in 4-6 month-old lambs. Christie and Brambell (1966) also produced a degree of protection in 2-3 month-old lambs by giving several large doses of H. contortus $L_3$ followed by two anthelmintic treatments prior to challenge.

More recent work by Duncan, Smith and Dargie (1978) again showed that vaccination of young lambs with irradiated H. contortus larvae did not protect them against challenge nor did it stimulate either serum IgG or mucus IgA antibodies. However, it is well known that young lambs have the ability to respond satisfactorily to bacterial and viral vaccines and humoral and cell-mediated responses have been demonstrated in foetal lambs (Sterzl and Silverstein, 1967).

In an attempt to stimulate immunity to H. contortus in 2-3 month-old Black face lambs, an experiment was undertaken using different adjuvants, either alone or together with H. contortus antigens, prior to vaccination with irradiated larvae.

# MATERIALS AND METHODS

## Animals

A total of 49 Black-face lambs were reared under worm-free conditions until 2-3 months of age. They were divided randomly into nine groups of five and one group of four animals. The immunisation regimen used for each group is described below. Twenty eight days prior to a single oral vaccination with 10,000 irradiated $L_3$ Group 1 lambs were given 5 ml. Corynebacterium parvum and 10,000 exsheathed H. contortus $L_3$ intravenously (I.V.); Group 2 animals were given 0.3 ml. Double stranded R.N.A., 5 ml. C. parvum and 10,000 exsheathed H. contortus $L_3$ I.V., Group 3 was given only 5 ml. C. parvum I.V. and Group 4 was injected I.V. with 10,000 exsheathed H. contortus $L_3$. A fifth group of lambs received two doses of 10,000 irradiated H. contortus larvae orally. Group 6 animals remained untreated until all six groups were challenged with 10,000 normal $L_3$ 28 days after the administration of irradiated $L_3$. Group 7 lambs were treated twice weekly for four weeks with levamisole subcutaneously (S.C.) at a dosage rate of 2 mg./kg. bodyweight prior to double vaccination and challenge. The lambs in Group 8 each received 500 H. contortus irradiated $L_3$ three times a week for four weeks with fortnightly doses of levamisole at 7.5 mg./kg. bodyweight S.C. while those in Group 9 only received the repeated doses of irradiated $L_3$.

One week after the last dose of irradiated $L_3$ Groups 8 and 9 were challenged with 10,000 normal H. contortus $L_3$

i.e. at the same time as Groups 1-7. The four lambs in Group 10 were kept as worm-free controls. All the animals were killed 28 days after challenge.

The experimental design is summarised in Table 5.

Preparation of larval inocula

Worm-free lambs were infected orally with 10,000 H. contortus $L_3$ using a strain regularly passaged for the past decade in the department of veterinary parasitology in Glasgow. The techniques for culturing and harvesting H. contortus $L_3$ are described in the previous chapter. Freshly harvested larvae were used either directly or after irradiation. Inocula of normal or irradiated $L_3$ were prepared by a dilution counting method.

Irradiation of $L_3$ was carried out in a $^{60}Co$-gamma-irradiation unit (Gamma chamber MK.IVB, Nuclear Engineering Limited, Reading, England). Since the output of this machine was 2.5 Kr./min. and the irradiation dose required was 60Kr., the larvae were exposed for 24 min.

Adjuvants

Double stranded RNA, kindly provided by Beecham Pharmaceuticals Research Division, Tadworth, Surrey, was given as a single dose of 0.3 ml./animal intravenously.

C. parvum was obtained from the Wellcome Research Laboratories, Beckenham, Kent and administered as a single dose of 5 ml./animal intravenously.

| Group No. | No. of Animals | Immunisation regimen prior to vaccination and/or challenge | Vaccination 10,000 Irradiated $L_3$ Day 0 | Challenge 10,000 Normal $L_3$ Day 28 | Necropsy Day 56 |
|---|---|---|---|---|---|
| 1 | 5 | I.V. C. parvum and 10,000 Exsheathed $L_3$ | + | + | + |
| 2 | 5 | I.V. Double stranded R.N.A. + C. parvum + 10,000 Exsheathed $L_3$ | + | + | + |
| 3 | 5 | I.V. C. parvum | + | + | + |
| 4 | 5 | I.V. 10,000 Exsheathed $L_3$ | + | + | + |
| 5 | 5 | Oral 10,000 Irradiated $L_3$ | + | + | + |
| 6 | 5 | NIL | - | + | + |
| 7 | 5 | Oral 2 mg./kg. levamisole 2 x weekly for 4 weeks then 10,000 Irradiated $L_3$ orally | + | + | + |
| 8 | 5 | Oral 500 Irradiated $L_3$ 3 x weekly for 4 weeks + fortnightly levamisole at 7.5 mg./kg. | - | + | + |
| 9 | 4 | Oral 500 Irradiated $L_3$ 3 x weekly | - | + | + |
| 10 | 5 | Worm-free controls | - | - | + |

TABLE 5 EXPERIMENTAL DESIGN

Details of the parasitological, haematological, immunological and histopathological techniques, are given in Chapter 1.

## RESULTS

### Faecal egg counts

The mean faecal egg counts of the challenged animals are shown in Table 6: individual counts are given in Appendix 2. No significant differences were observed in the mean faecal egg counts of the various treated and challenged groups with the exception of Group 9 in which the lambs received a trickle infection. The mean faecal egg count of this group was lower than that of any other group.

### Worm burdens

The mean worm burdens of the various groups are shown in Table 6. The individual worm burdens of all the lambs are given in Appendix 3. There was no significant difference between the mean worm burden of the challenge control animals and that of any other group.

### Packed Cell Volume

The group mean PCV's of the lambs at the start of the experiment and at necropsy are given in Table 7. Individual values recorded during the experimental period are given in Appendix 4. The mean PCV of the worm-free lambs (Group 10) was 32.8% at the beginning of the experiment and 28% on the day of slaughter. In contrast the mean PCV of the challenge

Table 6 The mean (± standard error) and the range of faecal egg counts and worm burdens at necropsy of groups of lambs given different immunising treatments prior to a single challenge infection with normal H. contortus $L_3$. The results from the various groups were not significantly different from those obtained from challenge in the control group (Group 6).

| Group No. | Treatment Regimen | Faecal egg counts after challenge | | Worm burdens at necropsy |
|---|---|---|---|---|
| | | Day 24 | Day 26 | |
| | | Mean ± S.E. (Range) | Mean ± S.E. (Range) | Mean ± S.E. (Range) |
| 1 | C. parvum/Exsheathed H. contortus $L_3$ vaccination | 8570 ± 2850.5 (1700 - 17100) | 19820 ± 3254.4 (11700 - 29600) | 2300 ± 359.9 (1350 - 3550) |
| 2 | RNA/C. parvum/Exsheathed H. contortus $L_3$ - vaccination | 7010 ± 2549.0 (950 - 15900) | 12060 ± 3113.1 (1400 - 20400) | 2420 ± 435.8 (1850 - 4150) |
| 3 | C. parvum - vaccination | 6760 ± 1166.0 (2700 - 9100) | 13380 ± 3557.9 (5000 - 26300) | 2540 ± 398.6 (1500 - 3500) |
| 4 | Exsheathed H. contortus $L_3$ - vaccination | 8190 ± 3355.7 (50 - 20400) | 16000 ± 6132.2 (0 - 37300) | 2710 ± 861.5 (150 - 4500) |
| 5 | Vaccination - vaccination | 9940 ± 6843.6 (800 - 36500) | 13480 ± 4029.0 (3900 - 25500) | 2650 ± 528.4 (1400 - 4250) |
| 6 | Challenge controls | 8410 ± 3207.0 (50 - 19700) | 16580 ± 4230.3 (7300 - 29500) | 2570 ± 395.8 (1350 - 3450) |
| 7 | Levamisole/vaccination - vaccination | 8570 ± 3433.4 (3400 - 16900) | 19660 ± 5285.7 (3600 - 30300) | 2880 ± 698.7 (850 - 5000) |
| 8 | Trickle irradiated H. contortus $L_3$/ Levamisole | 6710 ± 2816.1 (2500 - 17500) | 15080 ± 2634.7 (8200 - 20,000) | 2080 ± 346.3 (1350 - 3250) |
| +9 | Trickle irradiated H. contortus $L_3$ | 1212 ± 1195.9 (0 - 4800) | 9725 ± 621.0 (8400 - 11,100) | 2362 ± 543.7 (1400 - 3600) |

+ One animal died during the experiment.

| Group Number | Treatment Regimen | Start of the experiment Mean ± S.E. | End of the experiment Mean ± S.E. | % Reduction |
|---|---|---|---|---|
| 1 | *C. parvum*/Exsheathed *H. contortus* $L_3$ - vaccination | 29.6 ± 0.9 | 19.2 ± 1.3 | 35.1 |
| 2 | RNA/*C. parvum*/Exsheathed *H. contortus* $L_3$ - vaccination | 31.6 ± 0.8 | 22.0 ± 0.6 | 30.4 |
| 3 | *C. parvum* - vaccination | 31.0 ± 0.6 | 23.4 ± 1.4 | 24.5 |
| 4 | Exsheathed H. contortus $L_3$ - vaccination | 30.0 ± 2.7 | 22.4 ± 4.4 | 25.3 |
| 5 | Vaccination - vaccination | 31.8 ± 1.3 | *17.2 ± 0.9 | 45.9 |
| 6 | Challenge controls | 34.4 ± 1.2 | 21.5 ± 1.9 | 37.5 |
| 7 | Levamisole/vaccination - vaccination | 29.8 ± 0.9 | 17.8 ± 2.4 | 40.3 |
| 8 | Trickle irradiated *H. contortus* $L_3$/ Levamisole | 31.2 ± 1.5 | 23.6 ± 0.9 | 24.4 |
| 9 | Trickle irradiated *H. contortus* $L_3$ | 30.6 ± 0.6 | 23.3 ± 0.9 | 23.9 |
| 10 | Worm-free controls | 32.8 ± 1.6 | 28.0 ± 4.1 | 14.6 |

Table 7 The mean PCV's (± standard error) of groups of lambs prior to any immunising treatment and at necropsy 28 days after a challenge infection of normal *H. contortus* $L_3$.

* $P < 0.05$ in relation to challenge control

control lambs was 34.4% at the beginning of the experiment and 21.5% on the day of necropsy one month after challenge.

The other groups of lambs showed varying reductions in PCV during the course of the experiment but this was most marked in Group 5 which received 2 doses of vaccine prior to challenge and Group 7 which received levamisole prior to vaccination and challenge.

Histopathology

Results of eosinophils, mast cells and immunoglobulin-containing cell counts are summarised in Table 8. There was considerable variation in the numbers of different types of cells present between different sections from the same animal and between animals from the same group.

Group 10 (Worm-free controls)

No cellular reaction could be detected in any of the sections obtained from both fundus and pyloric regions of the abomasum in the worm-free control lambs. Acid mucosubstances were seen covering the surface epithelium although neutral mucosubstances were detected in the upper third of the mucosa and in the cytoplasm of the mucus cells and glandular epithelium. Few eosinophils and only small numbers of mast cells were seen mainly in the connective tissue at the base of the gastric glands but also in the subepithelial part of the mucosa. Small numbers of IgA-containing cells were detected with few IgM and IgG-containing cells. These cells were seen mainly around the blood vessels at the base of the gastric glands.

| Group No. | Treatment Regimen | Mast cells Mean ± S.E. | +P | Eosinophils Mean ± S.E. | +P | IgA-containing cells Mean ± S.E. | +P | IgM-containing cells Mean * | IgG-containing cells Mean * |
|---|---|---|---|---|---|---|---|---|---|
| 1 | C. parvum/Exsheathed H. contortus $L_3$ - vaccination | 8.29 ± 1.40 | °N.S | 1.52 ± 0.93 | N.S | 8.47 ± 2.92 | S | 14.13 | 2.33 |
| 2 | RNA/C. parvum/Exsheathed H. contortus $L_3$ - vaccination | 9.13 ± 1.92 | N.S | 2.61 ± 1.11 | N.S | 8.15 ± 2.72 | S | 4.19 | 2.33 |
| 3 | C. parvum - vaccination | 12.04 ± 4.13 | N.S | 2.32 ± 2.22 | N.S | 9.08 ± 4.41 | N.S | 4.09 | 0.33 |
| 4 | Exsheathed H. contortus $L_3$ - vaccination | 8.58 ± 1.96 | N.S | 2.48 ± 1.57 | N.S | 5.25 ± 1.83 | S | 4.63 | 0.73 |
| 5 | Vaccination - vaccination | 9.73 ± 2.36 | N.S | 1.24 ± 0.47 | N.S | 6.25 ± 4.07 | N.S | 10.56 | 6.46 |
| 6 | Challenge controls | 9.64 ± 2.50 | - | 0.26 ± 0.16 | - | 4.26 ± 1.75 | - | 4.10 | 6.53 |
| 7 | Levamisole/vaccination - vaccination | 10.21 ± 2.38 | N.S | 1.13 ± 0.59 | N.S | 20.48 ± 6.84 | S | 22.39 | 1.60 |
| 8 | Trickle irradiated H. contortus $L_3$/Levamisole | 15.36 ± 3.23 | N.S | 0.52 ± 0.49 | N.S | 0.81 ± 0.13 | N.S | 1.79 | 0.06 |
| 9 | Trickle irradiated H. contortus $L_3$ | 16.43 ± 6.22 | N.S | 0.58 ± 0.29 | N.S | 9.75 ± 3.61 | S | 2.69 | 0.13 |
| 10 | Worm-free controls | 2.96 ± 0.71 | - | 0.04 ± 0.04 | - | 0.45 ± 0.35 | - | 0.23 | 0.26 |

* Two animals were randomly chosen from each group for IgM and IgG cell counts.

Table 8 Mean numbers (± standard error) of various cell types present in the abomasal mucosa at necropsy of lambs in different groups. +'P' value using student's 't' test compared with challenge controls (Group 6)
°N.S. Not significant

GROUP 1 (C. parvum/H. contortus $L_3$ - vaccination - challenge)

A distinct reaction was detected in the abomasal fundus with large accumulations of lymphocytes, plasma cells and eosinophils; a mild cellular reaction was seen in the region of the pylorus. Lymphocytes and plasma cells were observed extending from the deeper part of the mucosa to the surface in both regions. Parasites were seen in close apposition to the surface of the mucosa covered with mucin (Figure 19). Depressions in the mucosal surface directly underneath the worms were observed. A thick irregular layer of a mixture of acid and neutral mucosubstances was seen covering the surface epithelium. Acid mucosubstances were seen in the lumen of the gastric glands and in the cytoplasm of mucus-containing cells throughout the depth of the mucosa. although the number of these cells was less than that seen in the worm-free lambs.

A few eosinophils were seen at the base of the gastric glands and an increase in the number of mast cells, distributed mainly in the connective tissue between the gastric glands was detected. There was an obvious increase in the numbers of IgA-containing plasma cells (Figure 20) and IgM-containing cells but there was no change in the number or distribution of IgG-containing cells compared with the worm-free control animals.

GROUP 2 - (RNA/C. parvum/H. contortus $L_3$ - vaccination - challenge

In these animals there were haemorrhages in the mucosa of the pyloric region (Figure 21). Only a slight cellular

Figure 19

Section from the abomasum (fundic region) of a lamb treated with C. parvum and exsheathed H. contortus $L_3$ intravenously 28 days prior to a single oral vaccination with 10,000 irradiated H. contortus $L_3$ and subsequently challenged with 10,000 normal H. contortus $L_3$ one week after vaccination: the animal was necropsied 28 days after challenge.

A parasite is seen in close apposition to the surface of the mucosa covered with mucin.

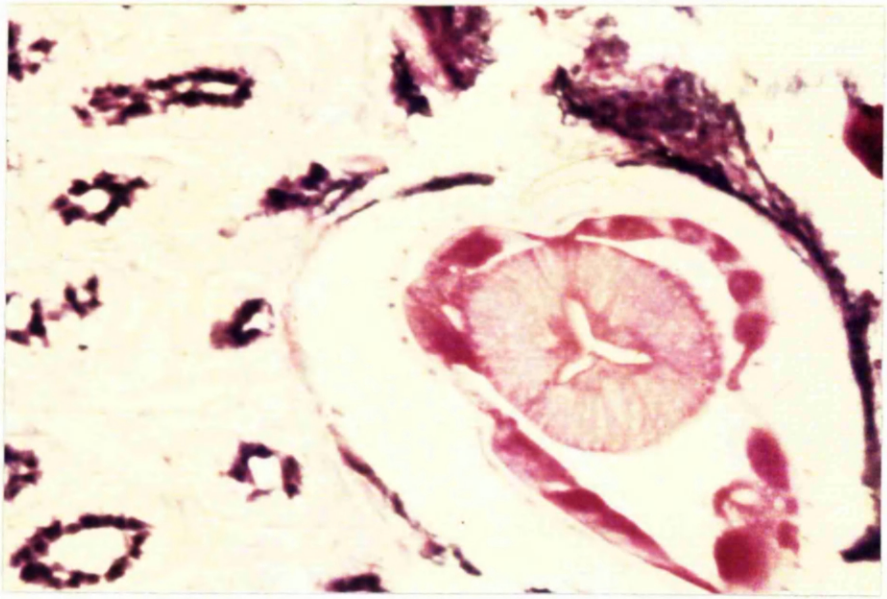
Alcian blue/PAS. X.110

Figure 20

Section from the abomasum (pyloric region) of a lamb treated with C. parvum and exsheathed H. contortus $L_3$ intravenously 28 days prior to a single oral vaccination with 10,000 irradiated H. contortus $L_3$ and subsequently challenged with 10,000 normal H. contortus $L_3$ one week after vaccination: the animal was necropsied 28 days after challenge.

Peroxidase labelled antiserum reveals IgA-containing cells in the lamina propria of the mucosa.

X.250



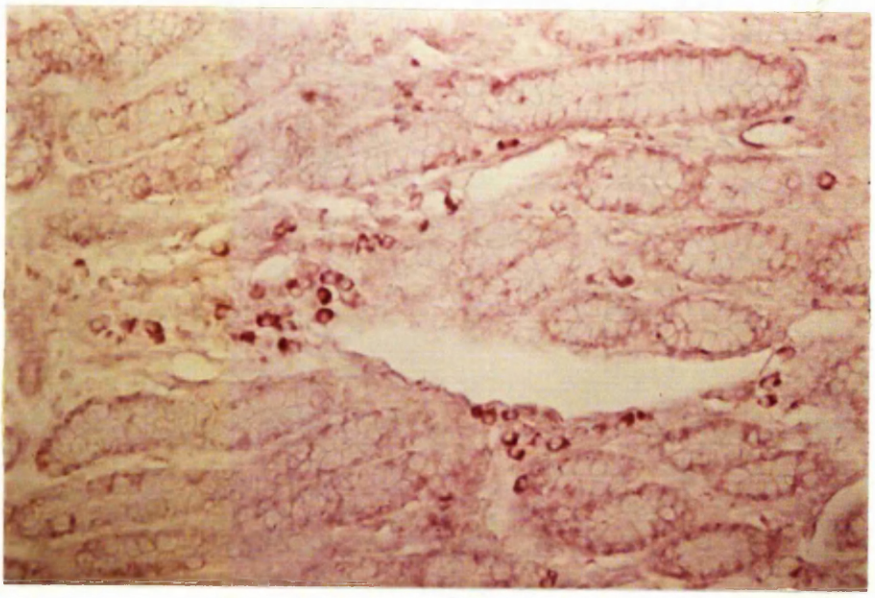
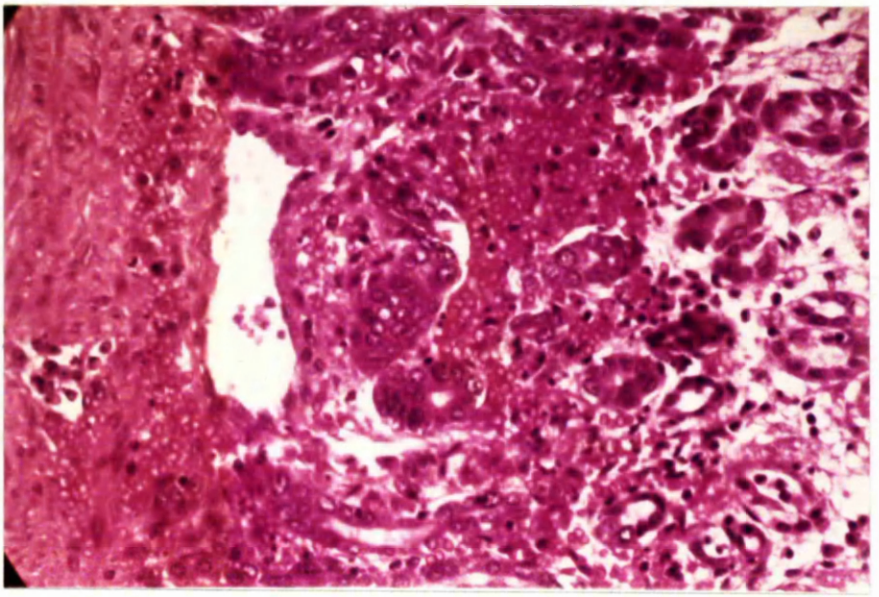


Figure 21

Section from the abomasum (pyloric region) of a lamb treated with Double stranded R.N.A., C. parvum and exsheathed H. contortus $L_3$ intravenously 28 days prior to vaccination with 10,000 irradiated H. contortus $L_3$ and subsequently challenged with 10,000 normal H. contortus $L_3$ one week after vaccination: the animal was necropsied 28 days after challenge.

Haemorrhage of the abomasal mucosa.

H and E. X.250



reaction with small aggregations of lymphocytes could be seen in the deeper part of the mucosa around the blood vessels in both fundic and pyloric regions. A mixture of acid and neutral mucin was seen covering the mucosal surface and acid mucosubstances were detected in the cytoplasm of the gastric mucus cells in the upper half of the mucosa although there was an apparent reduction in the number of these cells. Parasites were again close to the mucosal surface with associated depressed mucosal lesions filled with mucin. There was a slight increase in the number of eosinophils which were distributed throughout the mucosal connective tissue. Mast cells were also seen in large numbers in the connective tissue. Although IgA-containing cells were present in large numbers there was no apparent increase in either IgM- or IgG-containing cells.

GROUP 3 (*C. parvum* - vaccination - challenge)

A moderate cellular reaction was seen in abomasal sections from both fundic and pyloric regions in all the animals. Accumulations of lymphocytes in the deeper part of the mucosa around the blood vessels with movement of some of these cells to the surface was observed. A mixture of acid and neutral mucin was present at the surface and in the upper third of the mucosa, while only acid mucosubstances were detected in the middle third of the abomasal mucosa although there was no general increase in the numbers of mucin containing cells. In only one animal from this group was there a marked increase in the number of eosinophils. In all the animals an increase in the number of mast cells

was detected and these were distributed in the connective tissue throughout the depth of the mucosa. An increase in the numbers of both IgA and IgM-containing cells were observed, but few IgG-containing cells were detected.

GROUP 4 (*H. contortus* $L_3$ - vaccination - challenge)

A mild cellular reaction was seen in three of the five lambs. A greater cellular reaction, throughout the depth of the mucosa, was detected in sections from the remaining two animals in which parasites were seen on the surface of the mucosa surrounded by mucus containing lymphocytes and macrophages. A mixture of acid and neutral mucin was again seen covering the surface epithelium, and although acid mucosubstances were detected in the cytoplasm of the gastric gland mucus cells in the upper third of the mucosa, there was an overall decrease in the numbers of cells containing mucosubstances. There was a slight increase in the number of eosinophils whereas mast cell numbers were high and they were seen mainly in the connective tissue throughout the depth of the mucosa. There was a slight increase in the numbers of both IgA-containing cells and IgM-containing cells (Figure 22) but again little change in the number of IgG-containing cells (Figure 23).

GROUP 5 (Vaccination - vaccination - challenge)

A strong cellular reaction with accumulations of lymphocytes was seen in the pyloric region, although only a mild reaction was detected in the fundus. Larvae were seen in one section covered with mucin infiltrated with

Figure 22

Section from the abomasum (pyloric region) of a lamb treated with C. parvum I.V., 28 days prior to a single oral vaccination with 10,000 irradiated H. contortus $L_3$ and subsequently challenged with 10,000 normal H. contortus $L_3$ one week after vaccination: the animal was killed 28 days after challenge.

Peroxidase labelled antiserum reveals IgM-containing cells in the mucosal lamina propria.

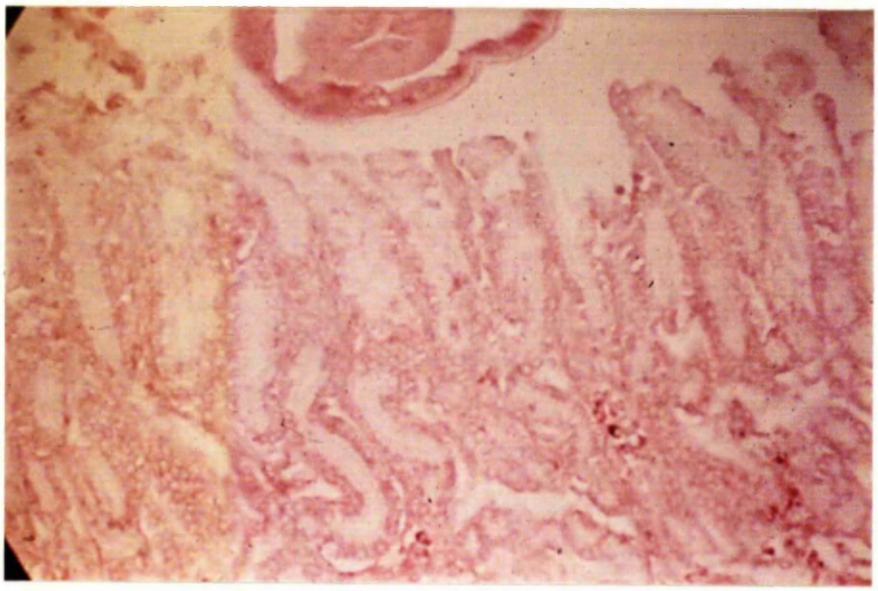
X35

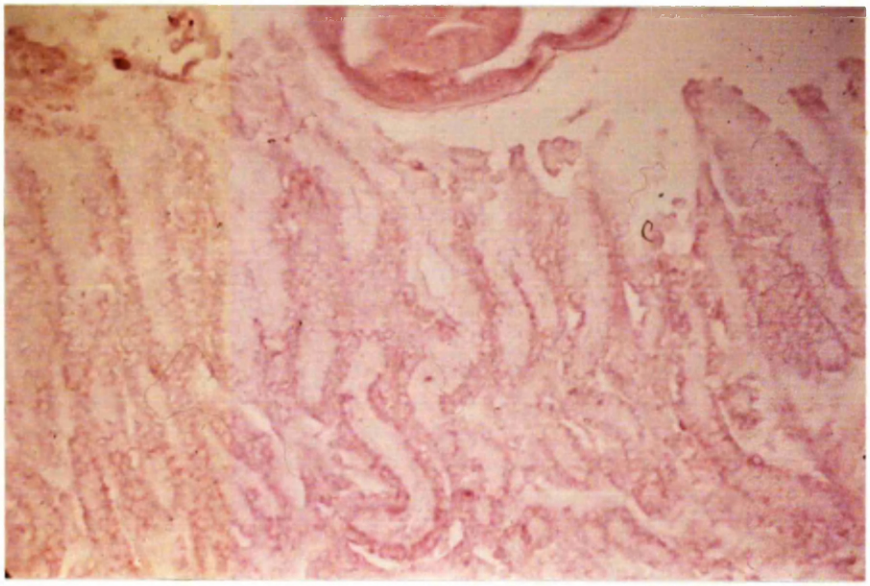
Figure 23

Section from the abomasum (pyloric region) of a lamb treated with C. parvum I.V., 28 days prior to a single oral vaccination with 10,000 irradiated H. contortus $L_3$ and subsequently challenged with 10,000 normal H. contortus $L_3$ one week after vaccination: the animal was necropsied 28 days after challenge.

No IgG-containing cells in the mucosal lamina propria could be detected using peroxidase labelled antiserum.

X.35





large numbers of lymphocytes, macrophages and few eosinophils (Figures 24,25). A thick layer of neutral and/or acid mucosubstances was seen covering the surface mucosa, and acid mucosubstances were detected in the cytoplasm of the gastric gland epithelium in the upper two thirds of the mucosa.

Although only small numbers of eosinophils and mast cells were present in the connective tissue between the gastric glands these were more numerous than in the worm-free control lambs. Slight increases in the number of IgA-containing cells were noted together with an obvious increase in the number of both IgM- and IgG-containing cells.

GROUP 6 (Challenge controls)

In most of the sections obtained from the animals in Group 6, the challenge controls, a slight cellular reaction was detected. A mixture of acid and neutral mucosubstances was seen both covering some parts of the mucosal surface, and in the cytoplasm of mucus cells and glandular epithelium in the upper third of the mucosa. In sections from one animal only acid reacting mucosubstances were detected in the upper part of the mucosa. Only a few eosinophils could be detected in this group but mast cell numbers were high. There was a slight reduction in IgA-containing cells compared with most of the other treated/vaccinated groups but they were still more numerous than in the worm-free control group. The picture was similar for IgM-containing cells, although an obvious increase in the numbers of IgG-containing cells was observed.

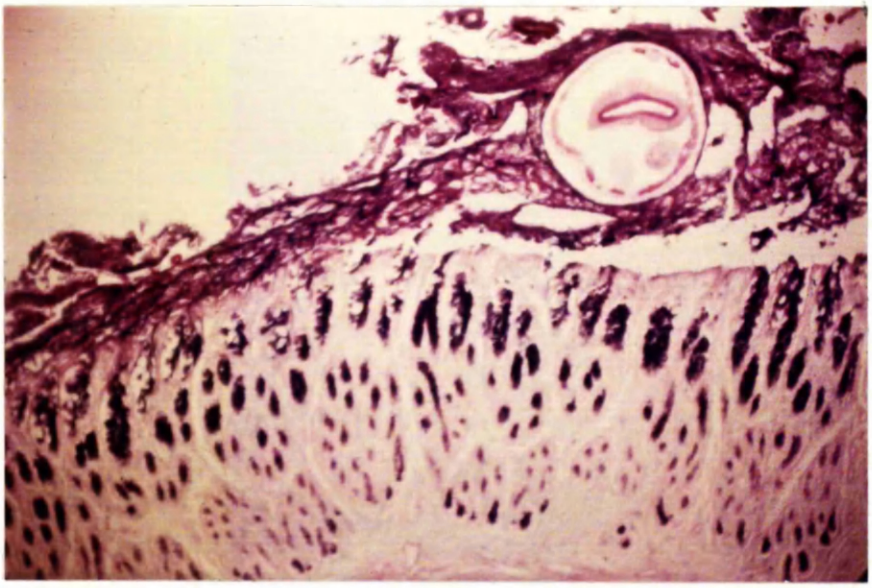
Figures 24 and 25

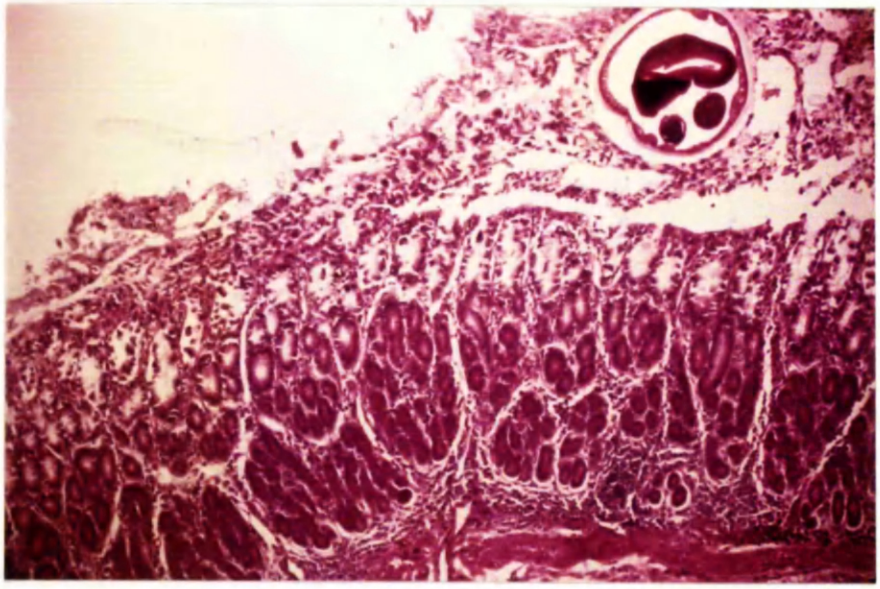
Sections from the abomasum (pyloric region) of a lamb given two doses of irradiated H. contortus $L_3$ orally 28 days prior to a further dose of 10,000 irradiated H. contortus $L_3$, and one week later challenged with 10,000 normal H. contortus $L_3$: the animal was necropsied 28 days after challenge.

A parasite is seen on the mucosal surface covered with mucin infiltrated with a large number of lymphocytes, macrophages and eosinophils.

Alcian blue/PAS. X.35 (Figure 24).

H and E. X.35 (Figure 25).





GROUP 7 (Levamisole/vaccination - vaccination - challenge)

More extensive cellular reactions were seen throughout the depth of both fundic and pyloric regions of the abomasal mucosa. Parasites were seen surrounded by muco-substances infiltrated with lymphocytes and eosinophils (Figures 26, 27). A mixture of acid and neutral mucosub-stances was detected covering the surface epithelium while neutral mucosubstances were observed in the cytoplasm of the gastric mucus cells and glandular epithelium. Acid reacting mucosubstances were seen in the deeper part of the mucosa. There was an overall increase in the number of mucus containing cells.

A small number of eosinophils were observed in sections from this group of animals but mast cell numbers and distribution were similar to that seen in the former groups. There was a marked increase in the number of IgA-containing cells and a similar increase in IgM-containing cells but no obvious change was observed in the number of IgG-containing cells compared with the worm-free lambs.

GROUP 8 (Trickle irradiated *H. contortus* $L_3$/levamisole - challenge)

A mild cellular reaction was observed in both fundic and pyloric regions of the abomasal mucosa of the animals belonging to this group. Generally acid mucosubstances were detected covering the surface epithelium, although a mixture of acid and neutral or neutral mucosubstances could be seen in sections from some animals. Acid mucosubstances were seen throughout the depth of the mucosa in the mucus cell cytoplasm and glandular epithelium. A marked increase in

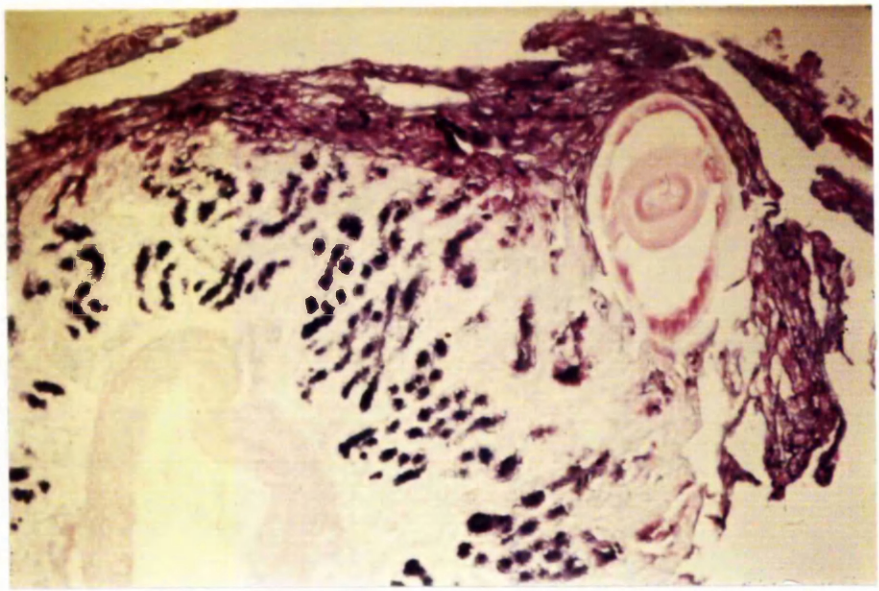
Figures 26 and 27

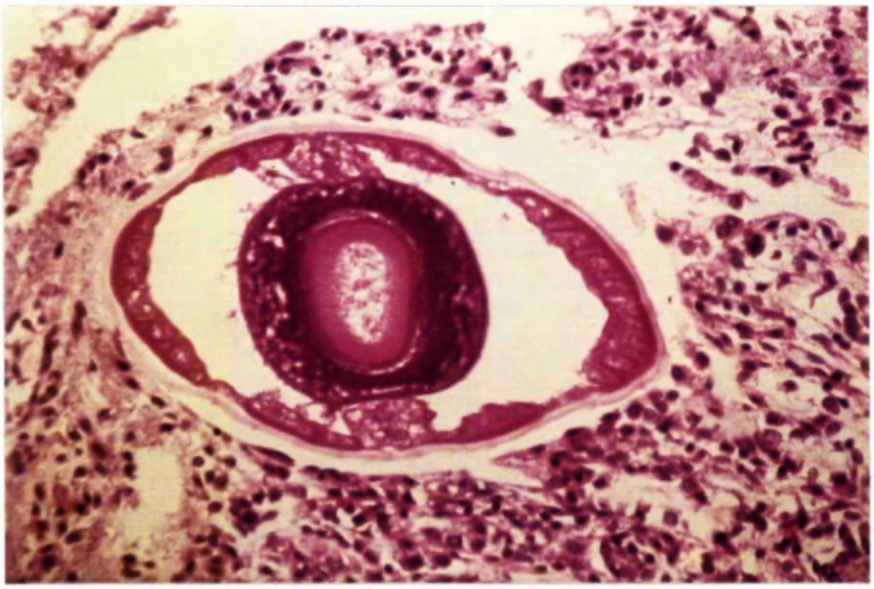
Sections from the abomasum (fundic region) of a lamb treated with twice weekly doses of levamisole subcutaneously prior to double vaccination with 10,000 irradiated H. contortus $L_3$ and one week later challenged with 10,000 normal H. contortus $L_3$. The animal was killed 28 days after challenge.

A parasite is present on the mucosal surface covered with mucin which is infiltrated with large numbers of lymphocytes and eosinophils.

Alcian blue/PAS. X.35 (Figure 26).

H and E. X.250 (Figure 27).





the number of mucosubstance-containing cells was obvious in this group of lambs.

Few eosinophils were detected but there was a marked increase in the number of mast cells. These cells were found, not only infiltrating the glandular epithelium but also in the connective tissue. No changes were observed in the number or distribution of all classes of immunoglobulin-containing cells when compared with the control group of worm-free lambs.

GROUP 9 (Trickle irradiated *H. contortus* $L_3$ - challenge)

Oedema with a slight cellular reaction was the most obvious feature in sections obtained from two of the lambs. In the remaining lambs there was no oedema but segregation of the lymphoid cells into follicular structures could be recognised throughout the depth of the mucosa (Figure 28). A mixture of acid and neutral mucosubstances was seen covering the surface epithelium and in the cytoplasm of the mucus cells and glandular epithelium in the upper part of the mucosa while acid mucosubstances were seen in the cytoplasm of the mucus cells and glandular epithelium throughout the depth of the mucosa. An increase in the number of mucosubstance-containing cells was observed in this group of animals.

Eosinophil numbers were low, although large numbers of mast cells were detected mainly in the connective tissue but also infiltrating the glandular epithelium (Figure 29).

Figure 28

Section from the abomasum (pyloric region) of a lamb which received repeated doses of 500 irradiated H. contortus $L_3$ prior to challenge with 10,000 normal $L_3$ one week later: the animal was necropsied 28 days after challenge.

Aggregations of lymphocytes into follicular structures are seen extending throughout the depth of the mucosa.

H and E. X.110

Figure 29

Section from the abomasum (fundic region) of a lamb which received repeated doses of 500 irradiated H. contortus $L_3$ prior to challenge with 10,000 H. contortus $L_3$ one week later: the animal was necropsied 28 days after challenge.

Large numbers of mast cells are present both in the mucosal connective tissue and intraepithelially.

Astra blue/Safranin O. X.250

Large numbers of IgA-containing cells were present in the mucosa but there was no increase in IgM- or IgG-containing cells.

Mucosal IgA antibody

The mucosal anti-H. contortus IgA levels of different groups of lambs at necropsy are given in Table 9.

No significant rise in IgA antibodies were detected after the various treatment, vaccination and challenge regimens.

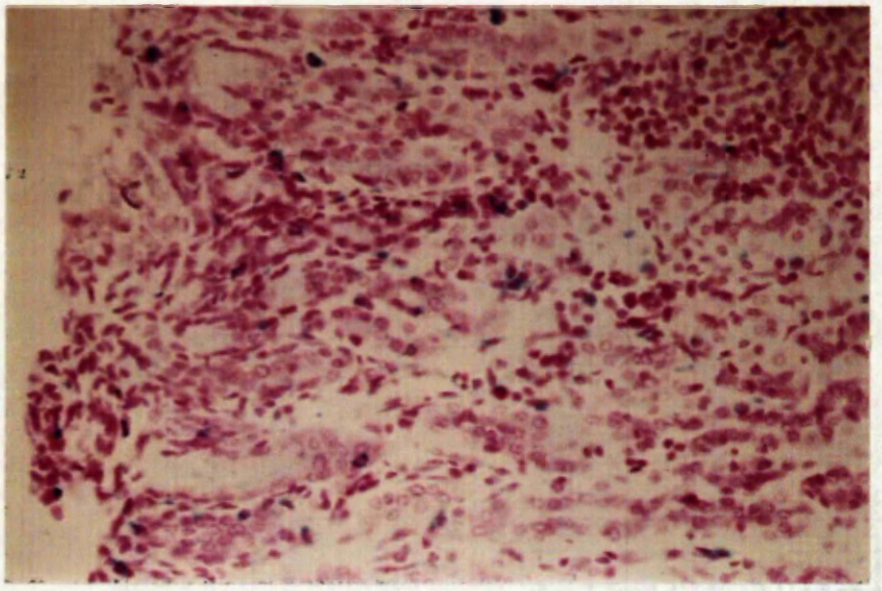
## DISCUSSION

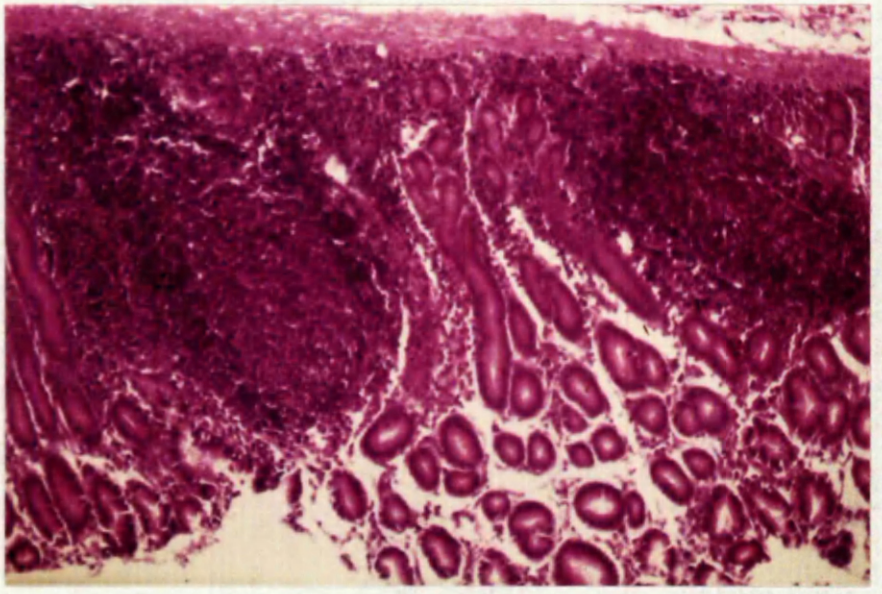
It is well recognised that immune defence mechanisms at mucosal surfaces depend largely on an IgA response Tizard (1977). It is also known that there are immuno-modulating substances which can either strengthen or weaken the immunogenic properties of antigens, or stimulate or inhibit the activity of the cells involved in the immune response e.g. in the synthesis of antibodies, anaphylaxis, delayed sensitivity, allergies, reaction to grafts. It was considered of value therefore to ascertain whether some of these compounds would correct the apparently defective immune response of lambs to H. contortus infection and in particular if the local IgA response could be augmented.

In the present work, however, no difference was seen in the faecal egg counts or in the differential worm counts of the treated and/or vaccinated lambs compared with the challenge controls. Most attempts to immunise young lambs

| Group number | No. of animals | Treatment Regimen | Individual values (counts per minute) | Mean | ± S.E. |
|---|---|---|---|---|---|
| 1 | 5 | C. parvum/Exsheathed H. contortus $L_3$ vaccination | 8, 68, 77, 114, 131 | 79.6 | 21.32 |
| 2 | 5 | RNA/C. parvum/Exsheathed H. contortus $L_3$ vaccination | 81, 90, 94, 102, 123 | 98.0 | 7.11 |
| 3 | 5 | C. parvum - vaccination | 0, 100, 117, 149, 185 | 110.2 | 31.40 |
| 4 | 5 | Exsheathed H. contortus $L_3$ - vaccination | 109, 152, 154, 157 205 | 155.4 | 15.21 |
| 5 | 5 | Vaccination - vaccination | 0, 38, 57, 98, 101 | 58.8 | 18.99 |
| 6 | 5 | Challenge controls | 45, 85, 94, 140, 142 | 101.2 | 18.22 |
| 7 | 5 | Levamisole/vaccination - vaccination | 0, 0, 37, 54, 134 | 45 0 | 24.61 |
| 8 | 5 | Trickle irradiated H. contortus $L_3$/Levamisole | 40, 60, 67, 73, 142 | 76.4 | 17.32 |
| 9 | 4 | Trickle irradiated H. contortus $L_3$ | 0, 0, 61, 74, 86 | 44.2 | 18.47 |
| 10 | 5 | Worm-free controls | 7, 66, 81, 98, 112 | 73.0 | 18.19 |

TABLE 9. Mucosal anti-H. contortus IgA levels at necropsy of groups of lambs given different immunising treatments prior to a single challenge infection with normal H. contortus $L_3$. Using the student's "t" test there were no significant differences between any of the groups compared with the challenge control lambs (Group 6) or worm-free lambs (Group 10).





against H. contortus have been similarly unsuccessful (Manton et al 1962; Urquhart et al 1966; Neilson, 1975) with the exception of Christie and Brambell (1966) who produced a degree of protection to challenge in lambs by immunising with several large doses of H. contortus $L_3$ followed by two anthelmintic treatments. Successful vaccination against H. contortus in sheep has only been achieved in animals over 7 months of age (Jarrett, Jennings, McIntyre, Mulligan and Sharp, 1961; Benitez-Usher et al 1977).

In the present experiment, a slight increase in the number of eosinophils together with a marked increase in the number of mast cells was observed in the treated and challenge control animals. The highest numbers of mast cells were seen in Group 8 which received repeated infections with H. contortus irradiated $L_3$ plus levamisole, and Group 9 which received only the trickle infection of irradiated $L_3$. On the other hand a great variation was detected in the numbers of IgA-containing cells within any single treated group and between these and the challenge control and worm-free control groups. The most remarkable increase in the numbers of IgA-containing cells was observed in the Group 7 lambs which received repeated doses of levamisole followed by a single vaccination and challenge; using the index described in Chapter 1 the mean of this group was 20.5 compared with 4.3 in the challenge controls. In contrast, only a small number of IgA-containing cells could be detected in the lambs which received a trickle infection of irradiated $L_3$ followed by levamisole treatment and this

was similar to that of the worm-free control lambs i.e. 0.80 and 0.45 respectively. No appreciable differences in IgA cell numbers could be detected between any of the other treated groups and the challenge control lambs.

Aubry, Zachowski, Simonin and Paraf (1979) reported that certain immunoadjuvants may act selectively on each of the three types of cells (i.e. B cells, T cells and macrophages) involved in the immune response both in vitro and in vivo: they also showed that some adjuvants can simultaneously activate two or three types of cells. However, some adjuvants are apparently cell specific e.g. mineral salts, and dextran sulphate for B cells, soluble concanavalin A (Con A), lymphocytosis promoting factor (LPF), and C. parvum for T cells. In previous studies on artificial immunisation against helminth infections adjuvants have occasionally been used with significant effects. For example Denham (1967) studying Trichinella spiralis in the mouse found that both Freund's complete adjuvant and B. pertussis increased protection levels significantly but that B. pertussis was more effective. Also aluminum hydroxide was found to significantly enhance protection against D. viviparus (Silverman, Poynter and Podger, 1962). On the other hand, Rothwell and Love (1974), studying T. colubriformis in guinea pigs, found that Freund's complete or incomplete adjuvant had no beneficial effect.

In the present study, by treating groups of lambs with different combinations of adjuvants and antigen prior to challenge no obvious protective response was observed.

Similar results were obtained by Urquhart et al (1966). They reported that Freund's adjuvant alone or in combination with Fasciola hepatica antigen, intraperitoneal injections of normal H. contortus $L_3$, a reduction in the number of irradiated larvae used for vaccination, and substitution of a serial daily challenge exposure for a large single challenge dose were all of no value in stimulating the immune response to H. contortus.

There are probably many reasons why young animals fail to respond to a vaccination regimen which is consistently successful in adult animals. The results described here do not eliminate the possibility that certain combinations of adjuvants, immunostimulants and different vaccination regimens would in fact be successful in immunizing young lambs; however, in the present experiment, all of the techniques of immunisation failed to produce protection in terms of reduced worm burdens. It is interesting that the cellular response particularly the eosinophils, mast cells and IgA-producing cells was generally poor when compared with that of immunized adult sheep. Also as in previous experiments the level of anti-H. contortus IgA antibody in the lambs was similar and very much less than that found in immunized adult sheep.

# CHAPTER 3

# THE PATHOLOGICAL CHANGES ASSOCIATED WITH PERSISTANT, LOW-GRADE *HAEMONCHUS CONTORTUS* INFECTIONS IN LAMBS AND THE EFFECTS OF MAINTAINING THESE ANIMALS ON DIFFERENT PLANES OF NUTRITION.

## SUMMARY

In this chapter the pathological changes associated with a persistent, low grade H. contortus infection in lambs are described. The effects of maintaining these animals on high and low planes of nutrition was also studied.

Six groups of 5-month-old Finn Dorset cross lambs were used in this experiment, four groups being maintained on a hay only diet and the remaining two groups receiving hay plus concentrates. Two of the groups on a low plane of nutrition and one of the groups which were fed additional concentrates received daily doses of 200 H. contortus $L_3$ for five consecutive days. The other groups of lambs were not infected. During the 5-6 month course of the experiment pairs of worm-free and infected lambs on the hay only diet were killed and their worm burdens and abomasal pathology recorded: at the end of the experiment the remaining infected lambs on both diets were killed.

High faecal egg counts were recorded from the hay only infected lambs until the end of the experiment. In contrast the infected lambs which received supplementary concentrates showed a marked drop in faecal egg output ten weeks after the last infection and this remained low until the time of necropsy. The faecal egg counts were reflected in the worm burdens at necropsy, a larger number of parasites being recovered from lambs on the hay only diet than from those fed hay and concentrates.

In the non-infected lambs killed during the course of the experiment small numbers of lymphocytes, mast cells, plasma cells and eosinophils were seen scattered throughout the mucosa and acid mucin was present on the mucosal surface. Occasionally well defined lymphoid tissue was seen.

In the infected lambs fed a low-plane diet and killed approximately eight weeks after the last infection, there were obvious infiltrations of the mucosa by lymphocytes, mast cells, plasma cells and eosinophils and a mixture of acid and neutral mucin was seen covering the mucosal surface. Lymphoid aggregates were larger and more numerous than in the worm-free animals. More extensive cellular reactions and depressed superficial mucosal lesions were evident in material from the infected animals fed only hay and killed later in the experiment. In all of these animals neutral mucin was present on the mucosal surface. In the lambs fed on a high plane of nutrition and killed at the end of the experiment a similar mild diffuse infiltration of the mucosa by lymphocytes, plasma cells, mast cells and eosinophils was observed. Neutral mucin was again seen covering the mucosal surface.

There was little difference in the mast cell counts of the various groups with the exception of the infected animals on a high plane of nutrition killed at the end of the experiment where there was an apparent increase. Intra-epithelial mast cells were present in two of these lambs but in all other cases mast cells were mainly confined to the mucosal connective tissue.

There were no differences in the numbers of IgA containing cells in the worm-free or infected animals killed during the experimental period. By the end of the experiment there appeared to be a slight increase in IgA-cell numbers in the infected lambs maintained on both high and low planes of nutrition.

## INTRODUCTION

In all of the major sheep rearing areas of the world clinical parasitic gastroenteritis resulting from repeated infection with various trichostrongyle nematodes is common. Probably the most important species in tropical and sub-tropical regions as well as in the warmer temperate regions of southern Europe is Haemonchus contortus. While the pathogenic effects of single infections with this parasite have been extensively studied, data on the pathology associated with repeated infections with H. contortus are very limited. Dineen, Donald, Wagland and Offner (1965), reported that the mortality rate approached 100% in lambs given one dose of 3000 H. contortus $L_3$ whereas 30 individual daily doses of 100 $L_3$ produced no deaths. They concluded that an immunological response had developed before the thirtieth day and that retardation of development, the major effect of daily intake, occurred with larvae given after the tenth day. Pradhan and Johnstone (1972 a, b) studied the immunological effects of prolonged exposure to daily and weekly doses of H. contortus infective larvae in lambs and found that the pathogenicity was greater when the lambs were infected with small numbers of larvae daily than when the same number of larvae were

given in one dose at weekly intervals. Small numbers of worms in association with a low plane of nutrition have also been shown to be pathogenic by Allonby and Dargie (1973), who gave a detailed account of the clinical signs in Merino sheep suffering from chronic haemonchosis. At necropsy of these animals they observed a generalised hyperplasia of the abomasal mucosa leading to increased thickness and opacity of abomasal folds together with an increase in abomasal pH.

There have, however, been no detailed studies on the histopathological changes in the abomasum of sheep suffering from chronic haemonchosis, and an experiment was therefore designed which would allow a study of these changes in lambs exposed to small daily doses of H. contortus infective larvae.

In addition it was planned to study the effect of high and low planes of nutrition on the pathogenesis of chronic low-grade infection.

## MATERIALS AND METHODS

Experimental design and animals: The experimental design is shown in Table 9. A total of thirty 3-month-old Finn Dorset cross lambs reared indoors under worm-free conditions were divided randomly into six groups. One group (Group 1) consisted of four uninfected controls, while three groups of six, five and five lambs (Groups 2, 3 and 4 respectively) were each infected with a total of 1000 H. contortus $L_3$ given orally in single doses of 200 $L_3$ for five consecutive days. The uninfected control group (Group 1) together with Groups 2, 3 and 5 were fed on a low plane of nutrition receiving only

hay ad lib. Groups 4 and 6 were fed a high-plane of nutrition receiving hay and a twice daily concentrate ration (at a rate of approximately 500 gm/day). The two groups of uninfected animals maintained on a low and high plane of nutrition respectively (i.e. Groups 5 and 6) were not killed. Water was available to all of the animals at all times.

The uninfected lambs of Group 1 and the six infected lambs of Group 2 fed on hay only were killed at the times shown in Table 10 i.e. two infected and two uninfected animals were killed 54 days after the infected group had received the last dose of H. contortus larvae. A further two infected lambs were killed at 107 days while the two remaining lambs from Groups 1 and 2 were killed 150 days after the last dose of larvae. All of the animals in Groups 3 and 4 were killed 164 days after receiving the last larval infection.

### Methods

Blood and faecal samples were taken and the bodyweights of the animals were recorded at weekly intervals throughout the experiment. Tissue samples for histology were taken from the abomasum as soon as possible after death. Histological, haematological and parasitological techniques were carried out as previously described.

## RESULTS

### Bodyweights

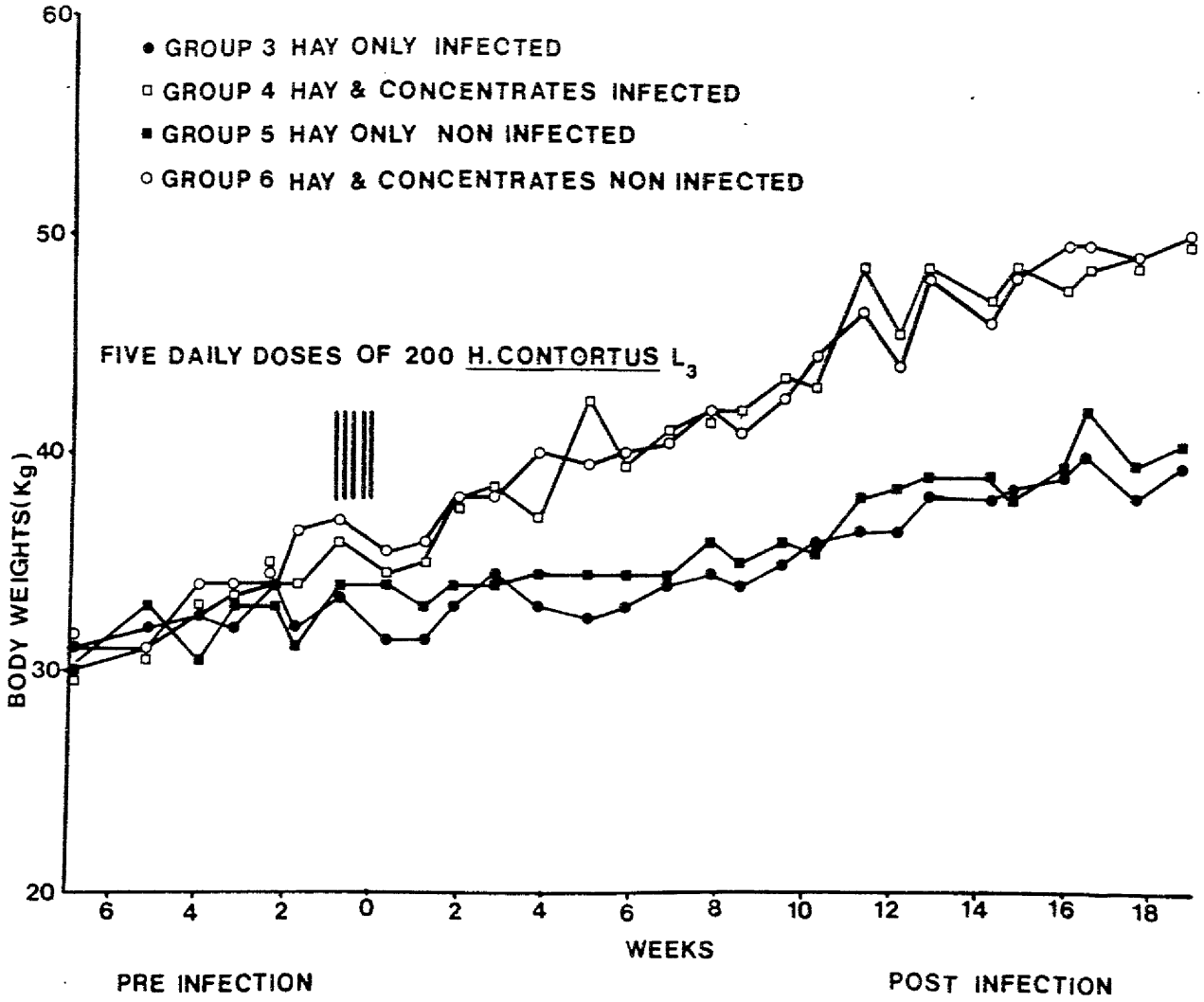
The mean bodyweights of the Group 3-6 lambs which were recorded weekly throughout the course of the experiment are shown in Figure 30.

| Group | No. of Animals | Diet | Infection | No. of animals killed and day of necropsy (Day 0 = Day of last infection) |
|---|---|---|---|---|
| 1 | 4 | Hay | Nil | 2 - 54 Days<br>2 - 150 Days |
| 2 | 6 | Hay | 5 x 200 $L_3$ | 2 - 54 Days<br>2 - 107 Days<br>2 - 150 Days |
| 3 | 5 | Hay | 5 x 200 $L_3$ | 5 - 164 Days |
| 4 | 5 | Hay and concentrates | 5 x 200 $L_3$ | 5 - 164 Days |
| 5 | 5 | Hay | Nil | Not killed |
| 6 | 5 | Hay and concentrates | Nil | Not killed |

TABLE 10 Experimental Design

Figure 30

The mean bodyweights (Kg.) of two groups of worm-free lambs and two groups of lambs infected with five doses of 200 H. contortus $L_3$ kept on a diet of hay only or hay and concentrates.


60
50
40
30
20
BODY WEIGHTS(Kg)
● GROUP 3 HAY ONLY INFECTED
□ GROUP 4 HAY & CONCENTRATES INFECTED
■ GROUP 5 HAY ONLY NON INFECTED
○ GROUP 6 HAY & CONCENTRATES NON INFECTED
FIVE DAILY DOSES OF 200 H.CONTORTUS L3
6 4 2 0 2 4 6 8 10 12 14 16 18
WEEKS
PRE INFECTION
POST INFECTION

Faecal egg counts

The mean faecal egg counts of the infected lambs in Groups 2, 3 and 4 are shown in Figure 31. Individual results are given in Appendix 5. The pattern of the mean faecal egg counts was similar in Groups 2 and 3 which were on a low level diet and the values were still fairly high at the time of necropsy (i.e. 1500 and 1540 e.p.g. respectively). In Group 4, where the animals were on a high plane of nutrition the mean faecal egg count remained high for the first ten weeks after patency but then dropped markedly and remained extremely low for the last five weeks of the experiment the mean recorded at this time being 50 e.p.g.

Worm burdens

The numbers of worms recovered from the animals at necropsy are given in Table 11.

Surprisingly the mean number of worms recovered from each pair of lambs in Group 2 which were killed on days 54, 107 and 150 after receiving the last dose of infection were exactly the same i.e. 225 worms. The lambs in Groups 3 and 4 were killed 164 days after receiving the last dose of infection, and there was a marked difference in the mean number of worms recovered in these two groups. The mean worm burden of those kept on a low-level diet was 50.8 while only a few worms (mean 3.2) were recovered from the lambs kept on a high-level diet.

Packed cell volume

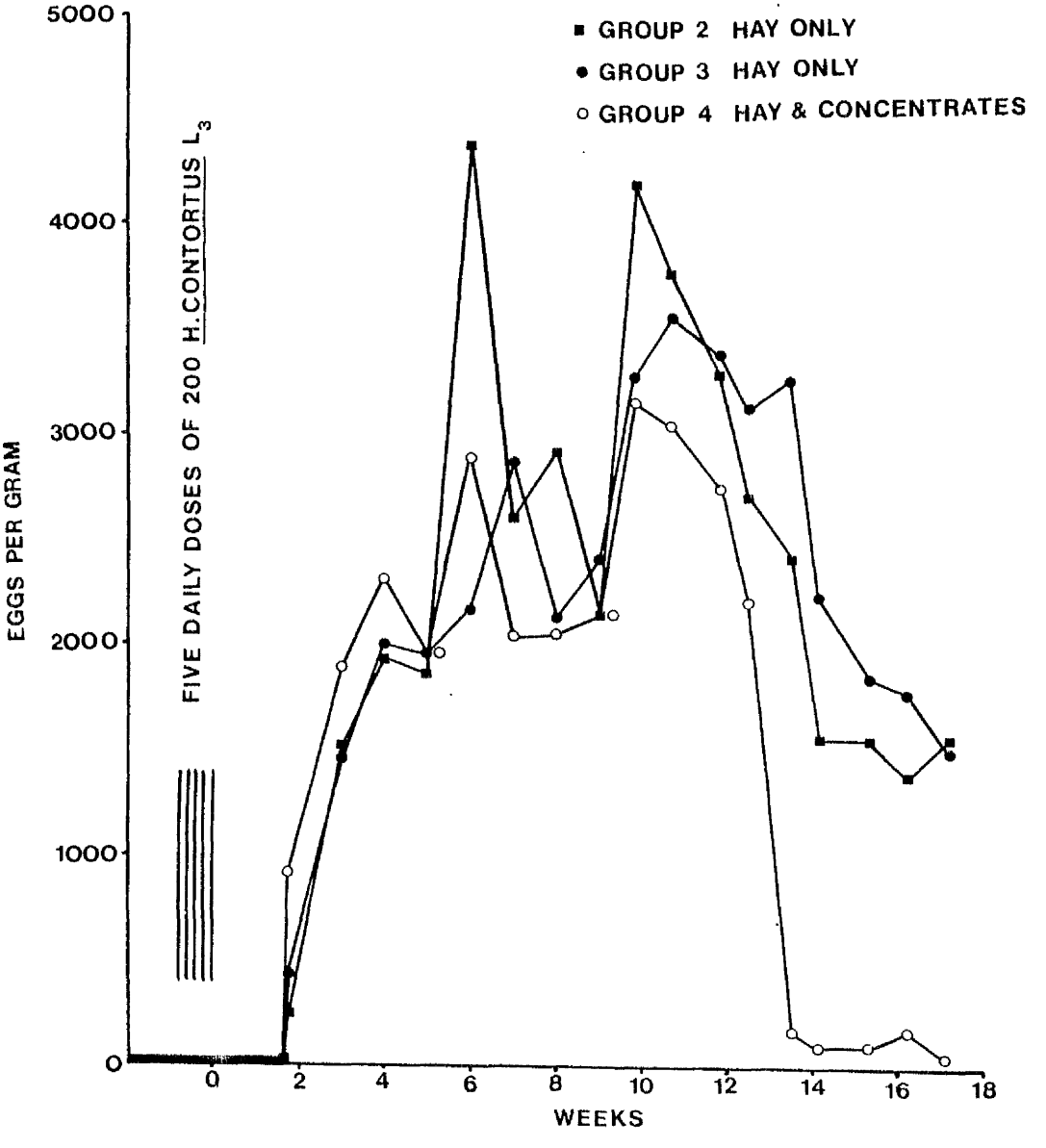
The mean PCV's of infected and non-infected lambs fed

| Group | No. of animals | Diet | No. of animals killed and day of necropsy (Day 0 = Day of last infection) | Individual worm burdens | Mean |
|---|---|---|---|---|---|
| 2 | 6 | Hay | 2 - 54 Days<br>2 - 107 Days<br>2 - 150 Days | 100, 350<br>200, 250<br>200, 250 | 225<br>225<br>225 |
| 3 | 5 | Hay | 5 - 164 Days | 0, 20, 52, 70, 112 | 50.8 |
| 4 | 5 | Hay and concentrates | 5 - 164 Days | 0, 0, 1, 1, 14 | 3.2 |

TABLE 11. The individual and mean numbers of worms recovered from the abomasa of lambs killed at different times after receiving five daily doses of 200 H. contortus $L_3$.

Figure 31

The mean faecal egg counts of groups of lambs kept on a diet of hay only or hay and concentrates and infected with five doses of 200 *H. contortus* $L_3$.


5000
4000
3000
2000
1000
0
EGGS PER GRAM
FIVE DAILY DOSES OF 200 H.CONTORTUS L3
GROUP 2 HAY ONLY
GROUP 3 HAY ONLY
GROUP 4 HAY & CONCENTRATES
0
2
4
6
8
10
12
14
16
18
WEEKS

either a high or low protein diet are illustrated in Figure 32.

Histopathology

Details of numbers of mast cells, plasma cells and eosinophils in the different groups are given in Table 12.

Group 1. Non-infected lambs. Low-plane diet.

Examination of abomasal tissue from the lambs killed on day 54 showed the presence of small numbers of lymphocytes, mast cells, plasma cells and eosinophils which were generally scattered throughout the mucosa but sometimes occurred in small accumulations. Occasionally well defined lymphoid tissue aggregates were present as previously described (Chapter 2).

Acid mucin was present on the mucosal surface while neutral mucosubstances were detected within the cytoplasm of the epithelial cells in the upper part of the gastric glands.

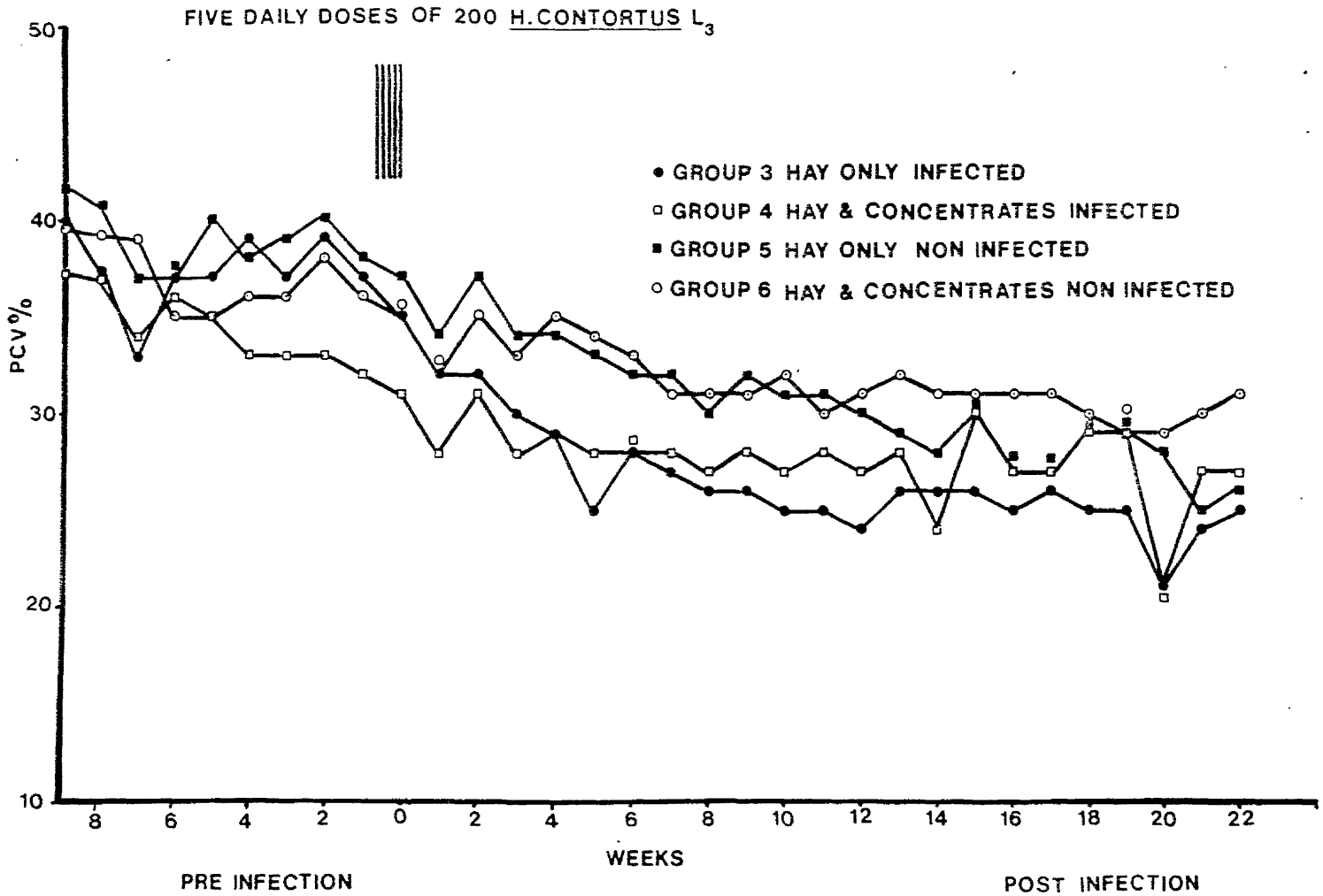
In the uninfected lambs killed on day 150 similar, mild cellular reactions were observed and acid mucin was present both on the surface epithelium and within the glandular epithelial cytoplasm of the middle and deeper part of the mucosa. Neutral mucosubstances were detected in the upper third of the mucosa.

Groups 2 and 3. Infected lambs. Low-plane diet.

In the lambs killed on day 54 there was obvious infiltration of the abomasal mucosa by lymphocytes, mast cells, plasma cells and eosinophils. Lymphoid tissue

Figure 32

The mean PCV's of two groups of worm-free lambs and two groups of lambs infected with five doses of 200 H. contortus $L_3$ kept on a diet of either hay only or hay and concentrates.


FIVE DAILY DOSES OF 200 H.CONTORTUS L3
50
40
30
20
10
PCV%
• GROUP 3 HAY ONLY INFECTED
□ GROUP 4 HAY & CONCENTRATES INFECTED
■ GROUP 5 HAY ONLY NON INFECTED
○ GROUP 6 HAY & CONCENTRATES NON INFECTED
8
6
4
2
0
2
4
6
8
10
12
14
16
18
20
22
WEEKS
PRE INFECTION
POST INFECTION

| Group | No. of animals | Diet | Infection | No. of animals killed and day of necropsy (Day 0 = Day of last infection) | Mast cells Mean ± S.E. | Eosinophils Mean ± S.E. | IgA-containing cells Mean ± S.E. |
|---|---|---|---|---|---|---|---|
| 1 | 4 | Hay | Nil | 2 - 54 Days<br>2 - 150 Days | 8.96 ± 0.04<br>8.10 ± 3.05 | 1.44 ± 1.39<br>0.32 ± 0.08 | 12.93 ± 1.11<br>25.30 ± 13.75 |
| 2 | 6 | Hay | 5 x 200 $L_3$ | 2 - 54 Days<br>2 - 107 Days<br>2 - 150 Days | 7.25 ± 1.68<br>6.31 ± 1.06<br>9.95 ± 6.65 | 0.73 ± 0.63<br>4.24 ± 2.01<br>0.75 ± 0.73 | 7.82 ± 0.46<br>13.04 ± 4.41<br>26.66 ± 1.71 |
| 3 | 5 | Hay | 5 x 200 $L_3$ | 5 - 164 Days | 8.69 ± 1.44 | 0.51 ± 0.19 | 18.46 ± 4.24 |
| 4 | 5 | Hay and concentrates | 5 x 200 $L_3$ | 5 - 164 Days | 11.27 ± 2.89 | 0.56 ± 0.32 | 21.10 ± 3.02 |

TABLE 12. Mean numbers of mast cells, eosinophils and IgA-containing cells present in the abomasal mucosa of lambs killed at different times after receiving five consecutive daily doses of 200 *H. contortus* $L_3$.

aggregates were more numerous and larger than those observed in the worm-free animals and these extended from the mucosa to the submucosa (Figure 33). Oedema was only seen in sections from one lamb and there was no obvious haemorrhage in any of the animals.

A mixture of acid and neutral mucin covered the mucosal surface and neutral mucin was seen in the glandular epithelium of the upper third of the mucosa. A mixture of acid and neutral mucin was present in the middle third of the mucosa while the deeper third of the glands contained only acid mucin (Figure 34).

One hundred and seven days after the last larval infection depressed superficial lesions were present in the abomasum. The mucosa was infiltrated by lymphocytes, mast cells, eosinophils and plasma cells and small accumulations of cells occurred in the mucosa beneath the surface epithelium; larger accumulations of lymphoid cells were also present in the mucosa and some of these extended into the submucosa.

A thick layer of neutral mucin covered the surface mucosa and neutral mucin was also detected in the cytoplasm of cells in the upper parts of the gastric glands. Acid mucin occurred in the cytoplasm of cells in the deeper parts of the gastric glands in the lambs killed 150 days after the last larval infection and marked cellular reactions and haemorrhage were observed in the mucosa; lymphocytes, mast cells, plasma cells, eosinophils and neutrophils were present throughout the mucosa. Lymphocytes occurred also as small or large aggregates in the mucosa. Acid mucin was present in

Figure 33

Section obtained from the abomasum (fundic region) of a lamb fed on a low plane of nutrition and infected orally with five consecutive daily doses of 200 H. contortus $L_3$. The animal was necropsied 54 days after the last dose of infection.

Lymphoid tissue aggregates extending from the mucosa to the sub-mucosa.

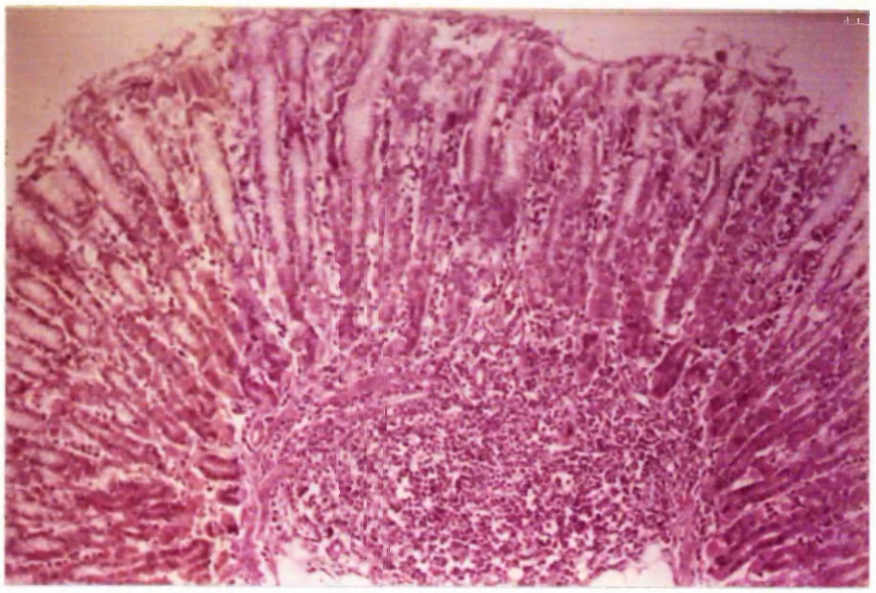
H and E. X.35

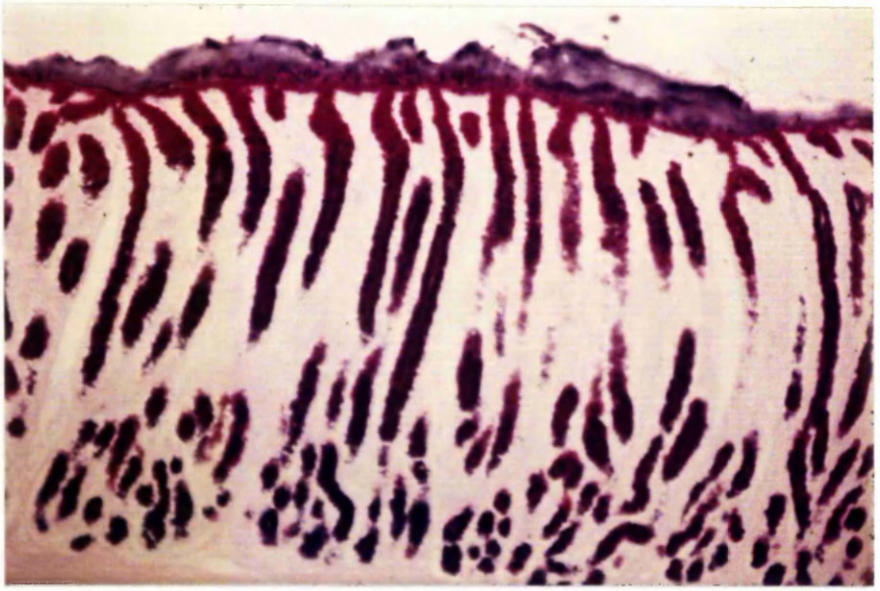
Figure 34

Section obtained from the abomasum (pyloric region) of a lamb fed on a low plane of nutrition and infected orally with five consecutive daily doses of 200 H. contortus $L_3$. The animal was necropsied 54 days after the last dose of infection.

A mixture of acid and neutral mucin is seen covering the mucosal surface and neutral mucin is present in the glandular epithelium of the upper third of the mucosa. A mixture of acid and neutral mucin is evident in the middle third of the mucosa with acid mucin in the deeper part of the mucosa.

Alcian blue/PAS. X.110





the middle and lower thirds of the epithelium of the gastric glands, while neutral mucin was present in the cells in the upper third of the glands and on the mucosal surface.

Increased numbers of mucus producing cells were seen in the gastric glands, which were sometimes dilated (Figure 35), in the five lambs of Group 3 killed 164 days after the last larval infection. Neutral mucin was seen in the upper third of the mucosa and acid mucin was present in the middle and lower thirds. Neutral mucin predominated in the surface mucus layer with, in addition, a variable amount of acid mucin. There was diffuse mucosal cellular infiltration by lymphocytes, plasma cells, mast cells and eosinophils.

Group 4. Infected lambs. High-plane diet.

In the animals in this group, killed 164 days after the last infection, histological examination revealed a generally mild, diffuse infiltration of the mucosa by lymphocytes, plasma cells, mast cells and eosinophils but occasionally more extensive cellular infiltrations were seen.

Neutral mucin was observed in the surface mucus layer and in the upper epithelial cells of the gastric glands: acid mucin was present in the middle and lower parts of the gastric glands but also occurred in small amounts in the surface mucus layer.

Mast cells

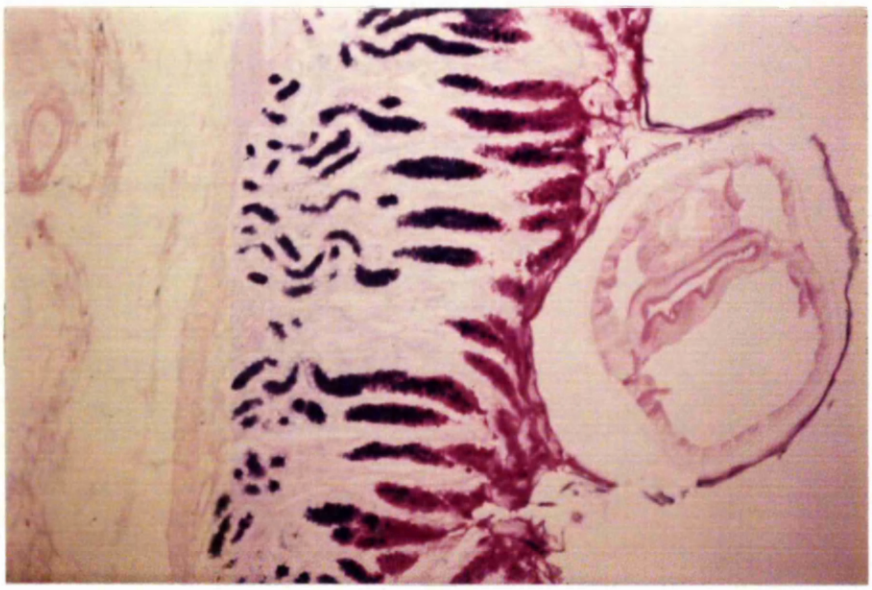
Intraepithelial mast cells (globule leucocytes) were counted as well as lamina propria connective tissue mast cells. Mean abomasal mast cells densities in the different

Figure 35

Section obtained from the abomasum (pyloric region) of a lamb fed on a low-plane of nutrition and infected orally with five consecutive daily doses of 200 *H. contortus* $L_3$: the animal was necropsied 164 days after the last dose of infection.

Large numbers of mucus producing cells are evident in the gastric glands.

Alcian blue/PAS. X.110



groups of animals are shown in Table 12.

Considerable variation occurred between different sites in any one animal and between individual animals within the same group but mast cell densities tended to be similar with the exception of one animal maintained on a low plane diet and killed 150 days after last infection: in this animal extremely large numbers of mast cells were present. In all cases mast cells were observed mainly in the mucosal connective tissue, but small numbers of intra-epithelial mast cells occurred in two of the five lambs on a high plane diet killed 164 days after the last infection. In infected lambs mast cells were present mainly towards the surface of the mucosa while in uninfected lambs they were mostly confined to the deeper parts of the mucosa. Where discreet lymphoid nodules were present in the mucosa and submucosa, mast cells occurred around but not within these.

IgA-containing cells

The numbers of IgA-containing cells in the abomasum of lambs killed at various times after daily infections with 200 $L_3$ H. contortus for five consecutive days compared with non-infected controls are given in Table 12. Despite considerable individual variation there was no apparent difference in the numbers of IgA-containing cells in sections from the first pair of lambs from Groups 1 and 2 which were killed 54 days after receiving the last dose of infection. These cells were present mainly in the lamina propria between the gastric glands throughout the depth of the mucosa but a few cells were also seen in the submucosal region. The IgA-containing cell density and distribution

was similar in the second pair of lambs from Group 2 killed on day 107. These cells were again present in the lamina propria but were confined mainly in the upper half of the mucosa (Figure 36) only a few cells being observed in the submucosa. There was no difference in the numbers of IgA-containing cells in the abomasa of the two lambs from Groups 1 and 2 killed on day 150. These cells were seen in the lamina propria mainly in the lower half of the mucosa but also around and within cellular accumulations (Figure 37).

There appeared to be a slight increase in the numbers of IgA-containing cells in the animals from Groups 3 and 4 killed 164 days after receiving the last dose of infection and these cells were distributed in the lamina propria throughout the mucosa.

Mucosal IgA antibody

The individual mucosal anti-*H. contortus* IgA antibody levels of both worm-free and infected lambs killed at various times after five daily infections with 200 *H. contortus* $L_3$ were measured by a radioimmunoassay and the results are shown in Table 13. Progressive increases in anti-*H. contortus* IgA antibody levels were seen in the Group 2 lambs which were killed in pairs on day 54, day 107 and day 150 after they received the last dose of infection, the highest values being recorded in the animals killed on day 150.

In the lambs in Groups 3 and 4 which were killed 164 days after receiving the last dose of infection there were marked individual variations and only one animal in each

Figure 36

Section obtained from the abomasum (fundic region) of a lamb fed on a low plane of nutrition and infected orally with five consecutive daily doses of 200 H. contortus $L_3$: the animal was necropsied 107 days after the last dose of infection.

Peroxidase-labelled antiserum reveals IgA-containing cells in the lamina propria in the upper half of the mucosa.

Haematoxylin was used as a counter stain.

X.250

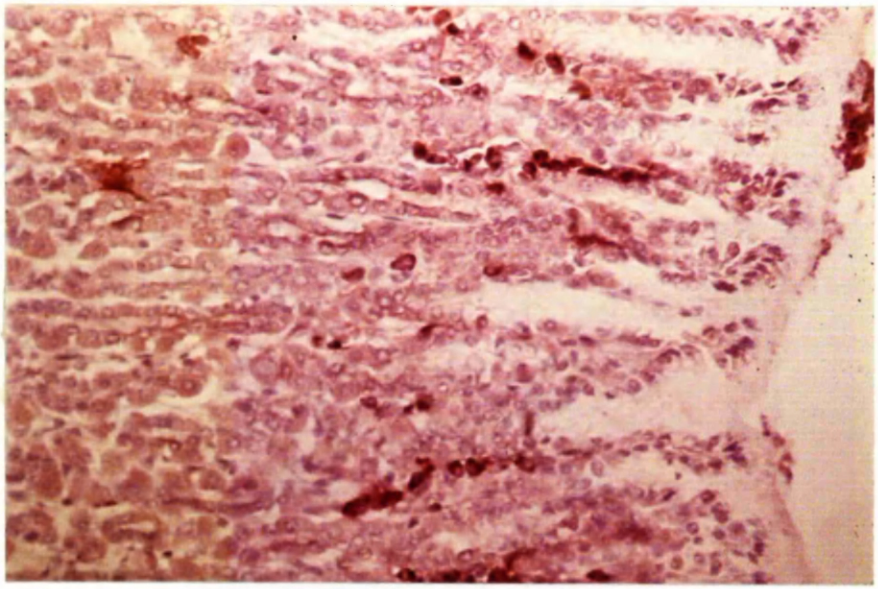
Figure 37

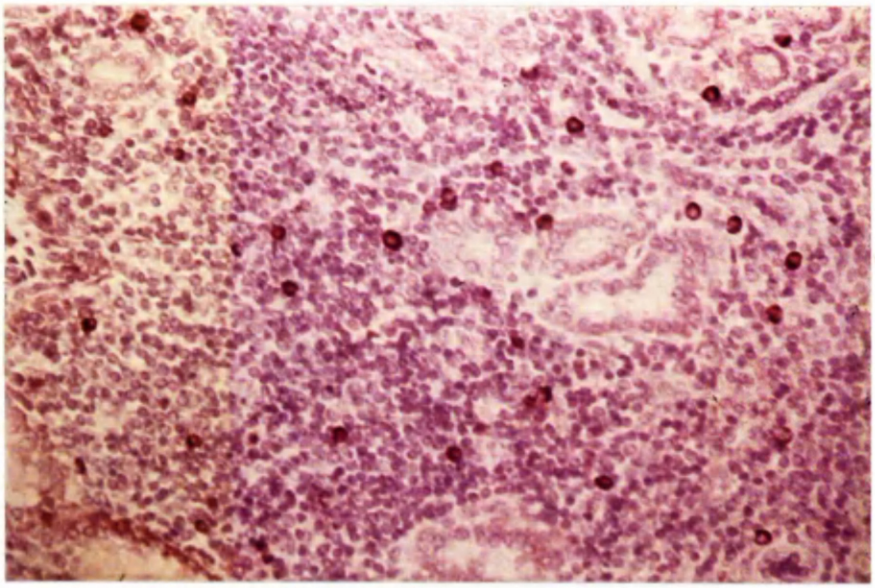
Section obtained from the abomasum (pyloric region) of a worm-free lamb.

Peroxidase-labelled antiserum reveals IgA-containing cells in the lamina propria, around and within cellular accumulations.

Haemotoxylin was used as a counter stain.

X.250





| Group | No. of animals | Diet | Infection | No. of animals killed and day of necropsy (Day 0 = Day of last infection) | Individual counts | Mean |
|---|---|---|---|---|---|---|
| 1 | 4 | Hay | Nil | 2 - 54 Days<br>2 - 150 Days | 46, 62<br>44, 51 | 54<br>47.5 |
| 2 | 6 | Hay | 5 x 200 $L_3$ | 2 - 54 Days<br>2 - 107 Days<br>2 - 150 Days | 0, 71<br>60, 97<br>106, 156 | 35.5<br>78.5<br>131 |
| 3 | 5 | Hay | 5 x 200 $L_3$ | 5 - 164 Days | 14, 26, 32, 41, 145 | 51.6 |
| 4 | 5 | Hay and concentrates | 5 x 200 $L_3$ | 5 - 164 Days | 0, 37, 38, 61, 94 | 46 |
| 5 | 5 | Hay | Nil | Not killed | - | - |
| 6 | 5 | Hay and concentrates | Nil | Not killed | - | - |

TABLE 13 The individual mucosal anti-*H. contortus* IgA antibody levels in counts per minute of both worm-free and infected lambs killed at various times after five daily infections with 200 *H. contortus* $L_3$.

group showed values markedly higher than that seen in the Group 1 worm-free controls.

## DISCUSSION

In the present work, groups of lambs fed either on hay alone or on hay and concentrates were infected with five daily doses of *H. contortus* $L_3$. After patency the mean faecal egg counts of these animals were similar but between 12 and 14 weeks after infection there was a sudden decline in the faecal egg counts but this was especially marked in the group kept on the high plane diet whereas the mean faecal egg counts of the two groups of lambs on the hay only diet remained at a fairly high level until the end of the experiment. Pradhan and Johnstone (1972a) found that with a daily regime of larval intake the number of eggs per female worm reached a peak between 50 and 60 days but this was followed by a marked suppression in egg output. Earlier work by Dineen *et al* (1965) had produced no evidence that the egg production per female worm was affected when lambs were infected with daily doses of infective larvae or were given the same number of larvae in one dose.

At necropsy of the lambs in this experiment, approximately six months after infection, smaller numbers of worms were recovered from the lambs maintained on hay and concentrates compared with those fed only hay i.e. a mean of 3.2 and 50.8 worms respectively. It is interesting that similar results have been reported by Cummins, Duncombe, Bolin, Davis and Kelly (1978) in studies on *N. brasiliensis* infection in rats

fed on a diet deficient in iron and protein compared with rats fed a normal diet. Worm expulsion in the rats on the deficient diet was delayed and they suggested that a dietary deficiency of iron and protein had directly or indirectly suppressed the immune response. In these iron and protein deficient rats, however, mesenteric lymph node cells did not appear to be depressed since they still had the capacity to cause parasite rejection when transferred to nutritionally normal recipients. Cummins et al (1978) also found that immune lymph node cells obtained from either nutrionally normal or deficient donors did not result in parasite rejection in iron and protein deficient recipients, thus suggesting that there was no permanent defect of lymphocyte function in these animals: this led to the suggestion that either some other component of the rejection mechanism was defective, or that lymphocyte function was in some way blocked in an iron and protein deficient environment.

In the present study, the infected lambs fed only hay and killed 54 and 107 days after receiving the last dose of infection showed a moderate infiltration of the abomasal mucosa by lymphocytes, mast cells, plasma cells and eosinophils while a more extensive cellular reaction of the mucosa was evident 150 and 164 days after infection.

In the lambs reared on hay and concentrates and killed 164 days after infection only a mild diffuse infiltration of the mucosa by lymphocytes, plasma cells, mast cells and eosinophils was seen but as mentioned earlier, worm expulsion had apparently occurred earlier in this group than in the animals fed only hay.

There is evidence which suggest that mucus present on the surface of the intestinal epithelium contributes to protection of that surface against penetration by antigens and microorganisms (Gibbons and Van Houte, 1975). This protection may depend on the role of the mucus coat as a physical barrier to the migration of larger molecules from the intestinal lumen to the epithelial surface as well as on the presence of specific moieties in the mucus. It has also been shown (Strombeck and Harrold, 1974) that gastric mucins interfere with the binding of toxins to receptors on enterocytes, thus preventing the stimulation of electrolytes and water secretion into the lumen. In the course of testing the uptake of immune complexes, by everted segments of rat small intestine in vitro Walker, Wu, Abel and Bloch (1976) found that complexes prepared in twofold antibody excess were absorbed in significantly smaller quantities than was antigen alone. Complexes prepared in an antibody excess appeared to stimulate the secretion of mucus and they suggested that the release of mucus might have a role in reducing the contact between immune complexes and the surface of the gut. Recently Walker, Wu and Bloch (1977) suggested an additional relationship between immune complexes and mucus, that is, the ability of the former to stimulate release of mucus by the intact small intestine of the rat, which may in turn serve to clear the surface of the gut of adherent immune complexes. An increase in the proportion of goblet cells occurs in the villous epithelium during the immune expulsion of Nippostrongylus brasiliensis from the rat intestine (Miller and Nawa, 1979): this work confirms earlier reports where increased numbers of goblet cells were observed in the

intestine of rats infected with N.brasiliensis (Wells, 1963) and in the gastrointestinal epithelium of parasitised sheep (Dobson, 1966).

In the present work there was an increase in the numbers of mucus producing cells in the gastric mucosa of infected animals and changes in their staining properties suggested that their accelerated differentiation was associated with a qualitative change in their mucin content. Similar observations have been reported by Miller and Nawa, (1979) but it is not yet known whether this is the result of an increased rate of mucin production or whether it is a function of the increased rate of differentiation of the mucus producing cells.

Over the last 20-30 years there have been repeated reports of an association between the globule leucocyte and helminth infections Sprent (1946),Kirkman (1950), and Kent (1952). For example, Sommerville (1956) showed that globule leucocytes only occurred in the abomasa of sheep after helminth infection. Later Dobson (1967) suggested that globule leucocytes could be associated with antibody transfer across the gut mucosa because they have special affinities with the mucosal surface and congregate next to mucin cells in areas of active parasite infection. He also found that the increase in the numbers of globule leucocytes was accompanied by an increase in the numbers and activity of the plasma cells which indicated increased protein synthesis and cellular turnover within the lymphoid system during infection.

Taliaferro and Sarles (1939) first described the involve-

ment of mast cells and eosinophils in the mucosal response in the intestine of rats infected with N. brasiliensis and subsequently it was shown that intestinal mast cell numbers increased at or about the time of worm expulsion (Murray, 1972; Wells 1962; Jarrett et al 1967; Miller 1969 and Miller and Jarrett, 1971). Benditt and Lagunoff (1964) also reported that such changes are common in chronic inflammatory conditions.

In the present experiment, no marked difference in mast cell densities occurred in any of the groups of animals. The most prominent feature was the change in the distribution of these cells in the mucosa. In the worm-free lambs they were located in the deeper part of the mucosa while in the parasitised animals they were mainly seen in the upper half of the mucosa. Intraepithelial mast cells were not detected in any of the worm-free or parasitised lambs kept on hay only, while they were present in two of the infected lambs fed hay and concentrates and killed at the end of the experiment. Interestingly Wells (1962) showed that in rats fed diets deficient in protein, the myeloid-amine response was altered with a reduction in eosinophil counts and increased small intestine mucosal histamine.

Plasma cells containing-IgA were detected in all of the animals used in this study and there was an apparent decrease in the numbers of these cells in the lambs killed at 54 days compared with the worm-free lambs killed at the same time. The numbers of these cells rose to the level seen in worm-free lambs 107 days after infection. Similar numbers of IgA-containing plasma cells were detected in the worm-free

and infected lambs killed at 150 days and there was little variation in the numbers of plasma cells containing-IgA in the two groups of infected lambs kept on different diets and killed 164 days after the last infection.

In rats Jenkins and Phillipson (1970) found that the administration of five infective larvae of Nippostrongylus brasiliensis daily over a period of 16 weeks produced an infection which did not provoke an obvious immune response, while a primary immune response was detected after the daily administration of 50 larvae resulting in partial worm expulsion and suppression of egg output. The resistance of these rats to reinfection, however, was not as pronounced as that seen in classical single primary and reinfection experiments.

In the present experiment progressive increases in anti-H. contortus IgA antibody levels were observed in the group of lambs which were fed a hay only diet and killed in pairs during the experiment the highest values being recorded in the animals killed on day 150. By the end of the experimental period, however, there was no marked differences between the two infected groups and only one animal in each showed values markedly higher than that seen in the worm-free controls.

# CHAPTER 4

## A STUDY OF THE PATHOLOGY ASSOCIATED WITH *H. CONTORTUS* INFECTION IN YOUNG LAMBS DERIVED FROM IMMUNISED AND NON-IMMUNISED EWES.

## SUMMARY

This chapter describes the pathological changes due to H. contortus infection in the abomasa of young lambs born to immunised and non-immunised ewes.

Eighteen Scottish Black-face cross lambs were used in this experiment. The lambs in Groups 1 and 3 were obtained from immune ewes, while those of Groups 2 and 4 were obtained from non-immune dams. Groups, 1, 2 and 3 lambs remained with and suckled their mothers while the lambs in Group 4 were separated from their mothers shortly after birth and bottle-fed.

At necropsy on day 34 there was no difference in the mean worm burdens resulting from infection with 2000 $L_3$ in the six lambs in each of Groups 1 and 2. There was, however, a marked difference in the mean worm burdens of the three lambs in each of Groups 3 and 4 which were infected with 3000 $L_3$ and killed on day 30. Relatively few worms were recovered from the lambs in Group 3 which suckled their dams compared with those in Group 4 which were bottle-fed (mean % takes of 8.3% and 57.8% respectively.

Marked cellular reactions were observed in the abomasal mucosae of lambs from Groups 1 and 3 whereas a mild cellular infiltration was detected in abomasal sections from lambs in Groups 2 and 4. Similar types of mucin were observed covering the mucosal surface in all of the lambs.

There was no significant difference in the mean numbers of mast cells in the abomasal mucosae of the suckled lambs in Groups 1, 2 and 3, although large numbers of these cells were detected in the bottle-fed lambs of Group 4.

The numbers of eosinophils observed in the abomasa of the lambs in Groups 1, 3 and 4 were similar but increased numbers of these cells were detected in sections from the Group 2 lambs.

There was no difference in the mean numbers of IgA-containing cells in the lambs from Groups 1, 2 and 4, whereas there was an obvious increase in the numbers of these cells in sections obtained from the lambs in Group 3.

## INTRODUCTION

It is well known that there is no transplacental exchange of maternal immunoglobulins in sheep, pigs and cattle (Brambell, 1970; Solomon, 1971), and little or no immunoglobulin of any class can be detected in the sera of lambs at birth (Lundjvist, 1963; Reid, 1972). For protection against neonatal disease therefore young lambs largely rely, during early life, on passive immunity derived from their dams. It is known that human milk contains IgA antibodies directed towards antigens which do not directly contact the mammary gland, for example antigens which are present in the gastrointestinal tract (Goldblum, Ahlstedt, Carlsson, Hanson,

Jodal, Lidin-Janson and Sohl-Akerlund, 1975; Holmgren, Hanson, Carlsson, Lindblad and Rahimtoola, 1976; and Stoliar, Kaniecki-Green, Pelley, Klaus and Carpenter 1976). The existence of such antibodies could be explained by the transport of IgA from the serum, for which evidence is lacking, or by the stimulation of immunocytes resident in the mammary gland by antigens absorbed from the intestine and reaching the mammary gland via the blood (Montgomery, Rosner and Cohn, 1974). It has been suggested, however, that the secretory IgA in colostrum is synthesised by plasma cells in the mammary glands which are largely derived from the gut associated lymphoid tissue (Goldblum et al, 1975) and it is known that circulating IgA appears in the serum of pigs and calves shortly after ingestion of colostrum (Porter, 1973a, 1973b).

The present work was undertaken to investigate the possibility that lambs may be passively immunised against H. contortus infection by the ingestion of colostrum and milk from hyperimmune mothers.

## MATERIALS AND METHODS

Animals A total of 18 Scottish Black-face and Black-face cross lambs were used in this experiment (Table 13). The mothers of the six lambs in Group 1 and the three lambs in Group 3 were immunised by the oral administration of weekly doses of 10,000 H. contortus $L_3$ irradiated at 60 Kr, for nine weeks prior to parturition. The mothers of the six lambs in Group 2 and the three lambs in Group 4 were not immunised.

| Group No. | No. of lambs | Prior immunisation of ewes | Method of rearing lambs indoors | Lamb larval dose normal *H. contortus* $L_3$ | Day of necropsy post infection |
|---|---|---|---|---|---|
| 1 | 6 | Immunised | Natural | 2000 | 34 |
| 2 | 6 | Non-immunised | Natural | 2000 | 34 |
| 3 | 3 | Immunised | Natural | 3000 | 30 |
| 4 | 3 | Non-immunised | Artificial (bottle-fed) | 3000 | 30 |

TABLE 13. Experimental Design

After housing and prior to immunisation all of the naturally reared Black-face ewes had received several treatments with anthelmintics to remove both nematode and liver fluke infections.

The lambs in Groups 1, 2 and 3 were allowed to remain with, and suckle, their mothers. The lambs in Group 4 were removed from their dams shortly after birth and bottle-fed until four weeks of age.

At four weeks of age the lambs were infected orally with normal H. contortus $L_3$. The lambs in Groups 1 and 2 received 2000 $L_3$ while those in Groups 3 and 4 received 3000 $L_3$.

PCV's were estimated weekly from birth. Faecal samples were examined for parasite eggs at two, three and four weeks after infection. All the lambs were killed between four and five weeks after infection. Mucus and tissue samples from the abomasum were taken directly after slaughter.

Details of the parasitological, haematological, immunological and histopathological techniques are given in Chapter 1.

## RESULTS

### Worm burdens

The numbers of worms recovered from the lambs in each group are shown in Table 14.

| Group No. | Number and background of lambs and the dose of infection | Mean No. of worms recovered | Individual worm burdens |
|---|---|---|---|
| 1 | Six lambs suckled by immunised dams and infected with 2000 $L_3$ | 17 | 0, 0, 0, 0, 0, 100 |
| 2 | Six lambs suckled by non-immunised dams and infected with 2000 $L_3$ | 17 | 0, 0, 0, 0, 50, 50 |
| 3 | Three lambs suckled by immunised dams and infected with 3000 $L_3$ | 250 | 100, 150, 500 |
| 4 | Three bottle-fed lambs infected with 3000 $L_3$ | 1733 | 900, 1300, 3000 |

TABLE 14. Worm burdens of sucking and bottle-fed lambs derived from immunised and non-immunised ewes 4-5 weeks after infection with 2000-3000 H. contortus $L_3$.

Of the animals infected with 2000 H. contortus $L_3$ only small numbers of worms were recovered i.e. 100 worms from one lamb in Group 1 and 50 worms from two lambs in Group 2 resulting in no difference between the mean worm burdens, which was 17 for both groups. There was a marked difference, however, between the mean worm burdens of the naturally suckled and bottle-fed lambs in Groups 3 and 4 which received 3000 H. contortus $L_3$. The mean worm burdens in this case were 250 and 1733 respectively.

Packed Cell Volume

The individual and group mean PCV's of the Group 1 and 2 lambs throughout the experimental period are given in Appendix 6 and illustrated in Figure 38 respectively. There was no difference in the mean PCV's between the lambs in these groups during the course of the experiment.

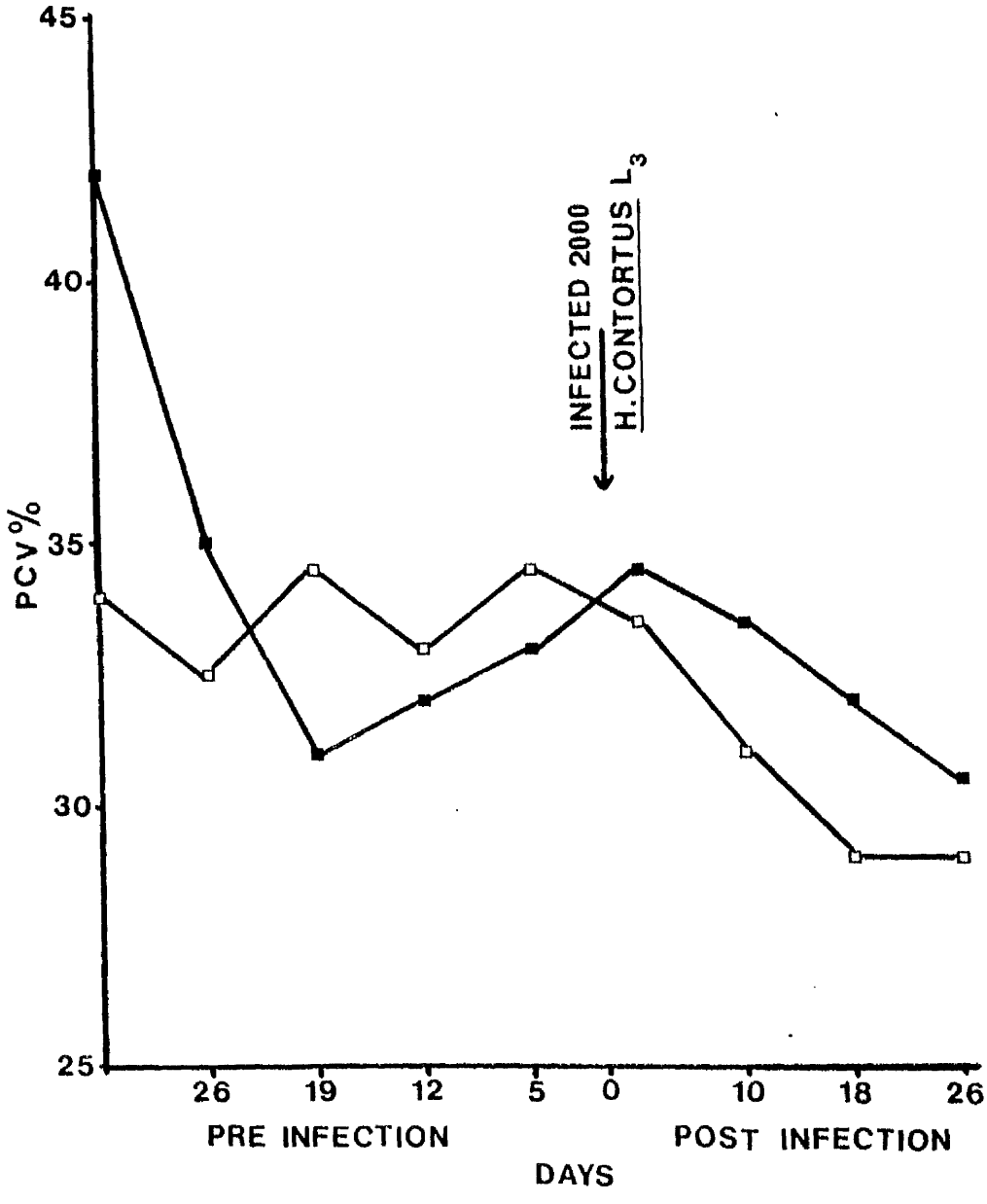
Histopathology

Differences in mast cell eosinophil and IgA-containing cell numbers in the four groups of lambs are summarised in Table 15.

No differences were noted in the histopathology of the abomasa of lambs from immunised ewes (i.e. Groups 1 and 3) which received either 2000 or 3000 H. contortus $L_3$. A marked cellular reaction with infiltration of the mucosa with lymphocytes, eosinophils and neutrophils was seen. Large aggregations of lymphocytes with eosinophils and mast cells were detected and these sometimes occupied the entire depth of the mucosa. A thick irregular layer of mainly neutral,

Figure 38

The group mean PCV's of lambs obtained from either immunised ( ■ ) or non-immunised ( □ ) ewes before and after infection with 2000 H. contortus $L_3$.


45
40
35
30
25
PCV%
INFECTED 2000
H.CONTORTUS $L_3$
26
19
12
5
0
10
18
26
PRE INFECTION
POST INFECTION
DAYS

with some acid mucosubstances was seen covering the mucosa and was also observed within the cytoplasm of the glandular epithelium in the upper third of the mucosa: acid mucosubstances were seen in the lower two thirds of the mucosa. Larvae were occasionally seen on the mucosal surface covered with mucin (Figure 39).

The histopathological appearance of the abomasa of lambs from non-immunised ewes (i.e. Groups 2 and 4) which received 2000 or 3000 H. contortus $L_3$ was similar whether or not the lambs had been naturally suckled or bottle-fed.

Shallow depressed lesions were present on the surface of the abomasum, together with a mild cellular reaction within the mucosa. In two of the lambs from Group 2 there was a more marked cellular reaction with infiltration of the mucosa with many lymphocytes, eosinophils and neutrophils. Neutrophils were also present in the lumen of the gastric glands (Figure 40).

The type and distribution of mucosubstances in the abomasa of the lambs of Groups 2 and 4 was similar to that seen in the lambs of Groups 1 and 3.

Mast cells and eosinophils

The mean numbers of abomasal mast cells of the various groups of lambs after infection with H. contortus are shown in Table 15. Considerable variation occurred between different sites and between individual animals in the different groups.

Figure 39

Section from the abomasum (fundic region) of a lamb derived from an immunised ewe and killed four weeks after infection with 2000 H. contortus $L_3$.

A parasite is present on the mucosal surface covered with mucin.

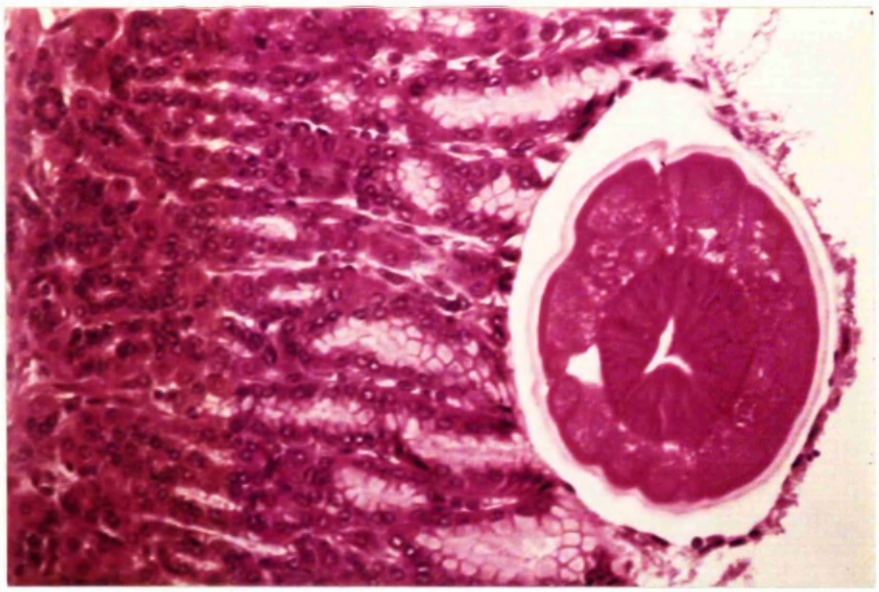
H and E. X.250

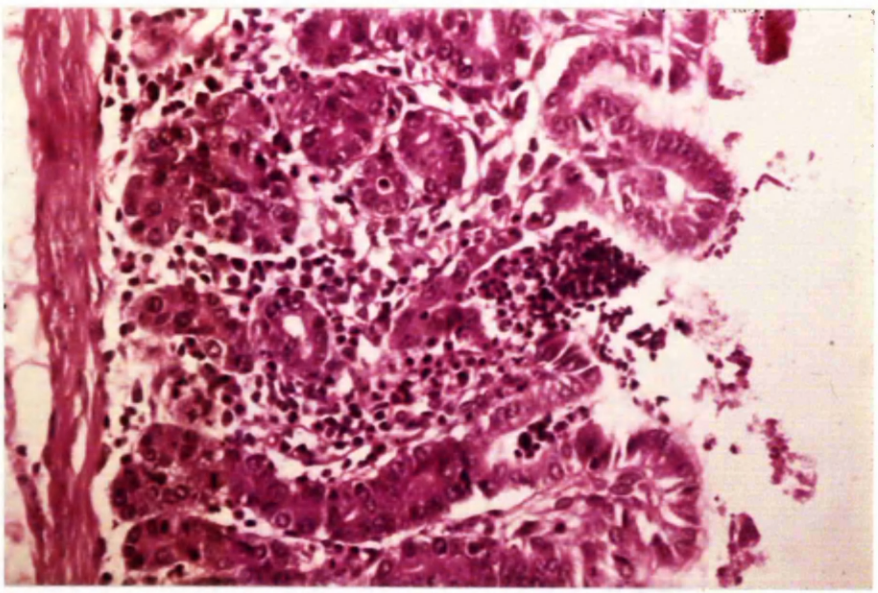
Figure 40

Section from the abomasum (pyloric region) of a lamb derived from a non-immunised ewe killed four weeks after infection with 2000 H. contortus $L_3$.

A marked cellular infiltration of the mucosa by lymphocytes, eosinophils and neutrophils is evident with many neutrophils present in the lumen of the gastric glands.

H and E. X.250





| Group No. | Number and background of lambs and the dose of infection | Mast cells Mean ± S.E. | Eosinophils Mean ± S.E. | IgA-containing cells Mean ± S.E. |
|---|---|---|---|---|
| 1 | Six lambs suckled by immunised dams and infected with 2000 $L_3$ | 5.65 ± 2.02 | 2.29 ± 1.90 | 6.37 ± 3.00 |
| 2 | Six lambs suckled by non-immunised dams and infected with 2000 $L_3$ | 4.80 ± 1.25 | 8.80 ± 5.32 | 4.42 ± 2.68 |
| 3 | Three lambs suckled by immunised dams and infected with 3000 $L_3$ | 3.38 ± 1.08 | 1.75 ± 1.25 | 13.60 ± 2.80 |
| 4 | Three bottle-fed lambs infected with 3000 $L_3$ | 8.83 ± 1.77 | 1.37 ± 0.52 | 5.35 ± 2.18 |

TABLE 15. The mean numbers (± standard error) of mast cells, eosinophils and IgA-containing cells found in the abomasal mucosa at necropsy of lambs derived from immunised and non-immunised dams after infection with 2000-3000 H. contortus $L_3$.

There was no significant difference, however, in the mean numbers of mast cells between the lambs infected with 2000 $L_3$ and these occurred in the lamina propria and within the epithelium of the gastric glands, only a few cells being seen in the submucosa.

In the lambs of Group 3 infected with 3000 larvae the number and distribution of mast cells was similar to that described above but large numbers of mast cells were present in the bottle-fed lambs of Group 4.

There was an apparent increase in the numbers of eosinophils in lambs from Group 2 compared with lambs in Group 1. A similar number of eosinophils were detected in sections from both Groups 3 and 4 although the mean numbers of these cells in the last two groups were smaller than that seen in sections from Group 2.

IgA-containing cells

Although considerable variation occurred between different sites and between individual animals in the different groups there were no differences in the mean numbers of IgA-containing cells in the lambs infected with 2000 $L_3$ in Groups 1 and 2 or in the bottle-fed lambs of Group 4 which received 3000 $L_3$. IgA-containing cells occurred in the lamina propria and around the blood vessels, a few cells being seen in the submucosa.

In the lambs from immunised ewes (Group 3) infected with 3000 larvae the distribution of IgA-containing cells was similar to that described above but large numbers of IgA-

containing cells were present.

Mucosal IgA antibody

The individual mucosal anti-H. contortus IgA antibody levels of all the lambs after infection with H. contortus, were measured by a radioimmunoassay (R.I.A.) and the results are shown in Table 16.

A low level of specific IgA antibody was detected in the mucosal samples from one lamb in Group 2 and one lamb in Group 4; the remaining samples were negative. Larger amounts of antibody were present in two samples from Group 1 and one sample from Group 3 the remaining samples being negative.

## DISCUSSION

There are few reports of successful passive immunisation against parasites. Rickard, Adolph and Arundel (1977) found that calves aged six to 11 days which were fed colostrum from cows vaccinated with Taenia saginata antigens during their last month of pregnancy were highly resistant to challenge with T. saginata eggs. They found, however, that although colostral antibody reduced the numbers of larvae which became established it did not promote destruction of those which had already undergone development. In similar studies in sheep Rickard, Boddington and McQuade (1977) reported that vaccination of pregnant ewes before lambing produced levels of maternal antibody in their lambs which were associated with a marked resistance to a challenge infection of T. ovis. In the

| Group No. | Number and background of lambs and the dose of infection | Individual mucosa IgA-levels (counts/minute) |
|---|---|---|
| 1 | Six lambs suckled by immunised dams and infected with 2000 $L_3$ | 0, 0, 0, 0, 9, 37 |
| 2 | Six lambs suckled by non-immunised dams and infected with 2000 $L_3$ | 0, 0, 0, 0, 0, 2 |
| 3 | Three lambs suckled by immunised dams and infected with 3000 $L_3$ | 0, 0, 19 |
| 4 | Three bottle-fed lambs infected with 3000 $L_3$ | 0, 0, 5 |

TABLE 16. Individual abomasal mucosa anti-H. contortus IgA-antibodies levels at necropsy of lambs derived from immunised or non-immunised dams after infection with 2000-3000 H. contortus $L_3$.

present study the worm burdens of the suckled lambs infected with 2000 H. contortus $L_3$ were extremely low with no difference in their group means suggesting no additional benefit in terms of passive protection in the lambs from the immunised ewes. In contrast, in the animals infected with 3000 $L_3$ a marked difference was observed in the group mean worm burdens, a much higher number of worms being recovered from the bottle-fed lambs. However, all of the lambs were derived from ewes which had been maintained under normal husbandry conditions for most of their lives and had therefore been previously exposed to mixed helminth infections. It appeared therefore from these limited studies that the choice of a natural or artificial milk diet for the lambs had some influence on parasite establishment and survival.

An influence of diet on parasite establishment and survival has been previously reported in experimental studies on H. placei infections in calves where fewer parasites were recovered from animals fed on milk alone than from calves fed grain and hay in addition to milk (Rohrbacher, Porter and Herlich, 1958). In addition an earlier study on Trichostrongylidosis in sheep (Whitlock, 1951) suggested that under natural conditions one of the most important factors in outbreaks of gastro-intestinal parastic disease in young animals was a deficient milk supply since untreated 'runt' lambs which were not weaned survived and suffered less severe anaemias than weaned lambs.

In rats McGhee, Michalek and Ghanta (1975) suggested that little or no colostral or milk IgA reaches the circulation of sucking rats, and they found that IgA was not

present in the serum until the animals were 20 days old. Similar results were reported by Hammerberg, Musoke, Williams and Leid, (1977) who found that serum IgA was not detectable before 17 days of age and suggested that the retention of colostral and milk IgA in the intestine might serve to enhance local defense mechanisms in newborn rats. Recently also Lloyd and Soulsby (1978) showed that normal mice may be protected against infection with Taenia taeniaeformis eggs by the administration of intestinal, colostral or serum immunoglobulins obtained from adult mice previously orally infected with this parasite. They also found that the protective capacity of these preparations was associated mainly with IgA in the case of colostrum and intestinal secretions and with serum IgG and that removal of IgA and IgG from immune colostrum and serum respectively abolished the protective effect. In the present experiment, specific IgA antibody was detected in mucosal samples from two of the lambs of Group 1 from immunised ewes, and in only one of the lambs in each of the other three groups.

At necropsy of the lambs from the vaccinated ewes, a marked cellular reaction was observed in the abomasal mucosa with large aggregations of lymphocytes, eosinophils and mast cells. In these animals a thick layer of mainly neutral with a little acid mucin was seen covering the surface epithelium. In contrast in most of the lambs from non-vaccinated ewes there was a mild cellular reaction with depressed superficial lesions in the abomasal epithelium and only a thin layer of mucin was seen covering the mucosal surface but this mucin was similar in quality to that seen in the lambs from immune mothers.

# CHAPTER 5

## A STUDY OF VARIOUS ASPECTS OF THE RESPONSE OF VACCINATED AND NON-VACCINATED EWES AND LAMBS TO A CHALLENGE INFECTION WITH NORMAL *HAEMONCHUS CONTORTUS* LARVAE.

## SUMMARY

In this chapter the response of adult sheep and lambs, previously immunised with irradiated H. contortus larvae, to a challenge infection with normal $L_3$ is compared with that of non-vaccinated controls.

On day 24 after challenge the mean faecal egg counts of the immunised and non-immunised ewes were 50 e.p.g. and 22400 e.p.g. respectively. In the non-vaccinated lambs, however, the mean faecal egg count was 8063 e.p.g. on day 19 rising to 32950 e.p.g. by day 24 whereas in the vaccinated lambs positive samples were only recorded on day 24 when the mean was 3583 e.p.g.

In the vaccinated ewes the highest numbers of worms were recovered on day three after challenge but these worms were mainly stunted adults resulting from vaccination. In the vaccinated ewes killed at the end of the experiment only small numbers of adult parasites, i.e. 50 worms, were recovered. In the non-vaccinated ewes high numbers of worms were recovered at various times during the course of the experiment and the normal development of the worms through the different larval stages was evident. In the vaccinated lambs, the numbers of worms recovered remained high throughout the course of the experiment and especially in the early stages after challenge a substantial number of these were stunted adults. In the non-vaccinated lambs, the numbers of the recovered worms also remained high through the course of the experiment but in this case natural development of the parasites through the different larval stages was evident.

There was a reduction in the PCV's of the vaccinated ewes during the course of vaccination but these had risen to prevaccination levels by the time of challenge and were subsequently maintained at this level whereas in the non-vaccinated ewes the PCV's remained within normal limits until 20 days after infection when they showed a marked decline. The PCV's of the lambs followed a similar pattern during vaccination but after challenge the PCV's of both groups fell: this decline, however, was more marked and rapid in the non-vaccinated animals.

In the non-vaccinated ewes, a mild cellular reaction was present in the abomasal mucosa three days after challenge but this increased in intensity during the course of the experiment. A mixture of neutral and acid mucin was seen as a thick, irregular layer covering the surface mucosa at most stages and dilatation of the gastric glands was evident between day 10 and 26 after infection. In the vaccinated ewes there was also a mild cellular reaction in the abomasum but larger mucosal aggregates of lymphoid tissue were present. An apparent increase in the number of mucus producing cells was detected at all stages after challenge and a thick layer of a mixture of neutral and acid mucin covered the surface mucosa. After infection of the non-vaccinated lambs a slight cellular reaction was evident only in the animals killed after day 10, but a thick layer of a mixture of acid and neutral mucin was seen covering the surface epithelium after day five. In the vaccinated lambs, a more extensive cellular reaction, with an apparent increase in the numbers of mucus producing cells was seen at all stages after

challenge and a mixture of acid and neutral mucosubstances were seen covering the mucosal surface.

There was little change in the mean numbers of mast cells in the abomasal mucosa of the non-vaccinated ewes after infection: intraepithelial mast cells were first seen on day five and the numbers of these cells subsequently increased to reach their highest levels in the animals killed on day 26 after infection. In contrast in the vaccinated ewes, IE mast cells were present in all of the animals at all stages after challenge and they were always more numerous than in the non-vaccinated animals. There was little change in the mean numbers of mast cells in the abomasal mucosae of the non-vaccinated lambs, only small numbers of IE mast cells being evident on days 24 and 26 after infection. In the vaccinated lambs, however, IE mast cells were present in all of the animals, and at all stages after challenge and the numbers of mast cells were always higher than in non-vaccinated lambs.

On histological examination of abomasal sections from the ewes IgA-containing cells were present in moderate numbers in both the vaccinated and the non-vaccinated animals: the greatest numbers of these cells were detected in the animals of both groups killed on day 10 after infection, relatively small numbers of IgA-containing cells were found in both vaccinated and non-vaccinated lambs after the challenge infection with normal H. contortus $L_3$.

There was a gradual increase in anti-H. contortus IgA-antibodies in the abomasal mucus of the vaccinated ewes over the first 10 days after challenge: in the non-

vaccinated ewes the antibody level appeared to increase until day seven but dropped by day 10 after infection. Mucosal anti-H. contortus IgA-antibodies remained at low level in both the vaccinated and non-vaccinated lambs at all stages after challenge.

## INTRODUCTION

Various experimental studies in animals have indicated that locally produced antibodies are more important than serum antibodies in the protection of mucosal surfaces against infectious agents (Pierce, 1959). It is known that after antigenic stimulation of B lymphocytes some members of the expanding clone of cells differentiate to become immunoglobulin producing cells of varying types, the plasma cell being the end-stage (Brandtzaeg, 1977). Local antibody formation takes place in the lamina propria of the mucus membrane in response to local antigen stimulation and the antibody produced is mainly IgA which is largely secreted across the mucus membrane (Tizard, 1977). Smith, Herbert and Davis (1979) reported that during the pre-patent histotropic development of Hyostrongylus rubidus in the stomach of pigs, a three-to-four-fold increase in IgA immunocyte numbers occurred but this declined to control levels about 10 days after patency. As mentioned previously there have been very few studies on the gross and microscopical changes which occur in the abomasum of sheep during infection with H. contortus.

Although Charleston (1965) and Malczewski (1970) have

described the abomasal pathology in young lambs after single infections with H. contortus, changes in the abomasum of adult sheep during infection with this parasite are more limited. Stewart (1953) described superficial aggregations of eosinophilic cells in the abomasal mucosa of sheep after reinfection with H. contortus and recently, Smith and Christie (1979) have observed numerous globule-leucocytes in the abomasal mucosa of immune sheep. More recently in this laboratory a study was made of the changes in the abomasum of adult sheep and lambs during a primary and secondary infection with H. contortus $L_3$. Basically the results of this study revealed a more extensive cellular reaction and ulcerative lesions in the abomasal mucosa of the adult sheep after reinfection compared with that seen in adult sheep after a primary infection or in young lambs exposed to both single or secondary infections.

In an attempt to gain more information on the sequence of events following H. contortus infection in immune and susceptible animals an experiment was designed in which the response to challenge in adult sheep and lambs previously immunised with irradiated larvae was compared with that of non-immunised controls.

## MATERIALS AND METHODS

The experimental design is summarised in Table 17.

A total of 28 animals, 14 Black-face ewes and 14 Black-face cross lambs were used. The ewes were naturally reared

| Group No. | No. of animals | Pre-challenge vaccination with irradiated *H. contortus* $L_3$ | Challenge dose of normal $L_3$ | Time of necropsy |
|---|---|---|---|---|
| 1 | 7 ewes | vaccinated | 100,000 | One animal from each group killed on each of days 3, 5, 7, 10 and 24 post infection. Remaining two animal from each group killed on day 26 post infection. |
| 2 | 7 ewes | - | 100,000 | |
| 3 | 7 lambs | vaccinated | 10,000 | |
| 4 | 7 lambs | - | 10,000 | |

TABLE 17. Experimental design

animals which were housed and given several anthelmintic treatments to remove existing liver fluke and roundworm infections. Seven ewes were then immunised by giving 17 weekly doses of 10,000 H. contortus $L_3$ irradiated at 60 Kr; the other seven ewes were not immunised. Seven lambs received nine similar weekly immunising infections of 10,000 irradiated larvae between 2 and 10 weeks of age; the other seven lambs were not vaccinated. At 12 weeks of age all of the lambs were challenged with 10,000 normal H. contortus $L_3$. At the same time all of the ewes were challenged with 100,000 normal H. contortus $L_3$ the challenge being given two weeks after both ewes and lambs had received their last vaccination dose.

One animal from each group was killed on each of days 3, 5, 7, 10 and 24 and the two remaining animals from each group were killed 26 days after infection.

Eight weeks prior to challenge, blood and faecal samples were taken at weekly intervals. Tissue samples from the abomasum for histological examination were taken immediately after death.

Histological, haematological and parasitological examinations were carried out according to the techniques previously described.

## RESULTS

### Faecal egg counts

The mean egg counts of all the animals after challenge

are shown in Table 18.

Ewes. In the vaccinated ewes the mean faecal egg count was consistently low, with a maximum count of 50 e.p.g. being recorded 24 days after challenge.

In the non-vaccinated ewes sampled at the same time, however, the mean faecal egg count was 22400 e.p.g.

Lambs. In the vaccinated lambs the faecal egg counts up to day 19 were negative. Positive faecal egg counts were recorded in each of the three remaining animals on day 24 the mean at this time being 3583 e.p.g.

In the non-vaccinated lambs faecal egg counts were positive on both day 19 and day 24 in each of the four animals examined at this time. The mean faecal egg counts of the non-vaccinated lambs on days 19 and 24 were 8063 e.p.g. and 32950 e.p.g. respectively.

Worm burdens

The numbers of worms recovered from individual ewes and lambs are shown in Table 19.

Ewes. In the vaccinated ewe killed three days after challenge a total of 6800 parasites were recovered from the abomasum: 94.8% of these were $L_5$ or stunted females persisting from the immunising infections. The remaining 5.2% were early $L_4$ and could have originated from the challenge infection. Development of relatively small numbers of parasites from $L_4$ and $L_5$ to adults was evident on days 10, 24 and 26 of the infection.

| Group No. | No. of animals | Pre-challenge vaccination | EPG | | | |
|---|---|---|---|---|---|---|
| | | | Days after challenge | | | |
| | | | 7 | 14 | 19 | 24 |
| 1 | 7 ewes | vaccinated | 0 | 17 | 0 | 50 |
| 2 | 7 ewes | - | 0 | 0 | 0 | 22400 |
| 3 | 7 lambs | vaccinated | 0 | 0 | 0 | 3583 |
| 4 | 7 lambs | - | 0 | 0 | 8063 | 32950 |

TABLE 18. Group mean faecal egg counts of vaccinated and non-vaccinated ewes and lambs after a challenge infection with normal H. contortus $L_3$.

| Day | Vaccinated ewes | | | | | Non-vaccinated ewes | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adult | $L_5$ | $M_4$ | $L_4$ | Total | Adult | $L_5$ | $M_4$ | $L_4$ | Total |
| 3 | *4500 | 1950 | 0 | 350 | 6800 | 0 | 0 | 0 | 3000 | 3000 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10200 | 10200 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 150 | 0 | 150 |
| 10 | 0 | 0 | 1100 | 0 | 1100 | 0 | 100 | 1300 | 0 | 1400 |
| 24 | 0 | 250 | 0 | 50 | 300 | 11300 | 600 | 0 | 0 | 11900 |
| 26 | 50 | 0 | 0 | 0 | 50 | 1600<br>3600 | 0<br>1250 | 0<br>0 | 0<br>0 | +3225 |

| Day | Vaccinated lambs | | | | | Non-vaccinated lambs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adult | $L_5$ | $M_4$ | $L_4$ | Total | Adult | $L_5$ | $M_4$ | $L_4$ | Total |
| 3 | *2400 | 1400 | 0 | 0 | 3800 | 0 | 0 | 0 | 1150 | 1150 |
| 5 | *1750 | 2950 | 0 | 500 | 5200 | 0 | 0 | 1300 | 0 | 1300 |
| 7 | *200 | 700 | 0 | 450 | 1350 | 0 | 3050 | 2800 | 0 | 5850 |
| 10 | 0 | 0 | 100 | 0 | 100 | 0 | 3800 | 0 | 0 | 3800 |
| 24 | 2450 | 0 | 0 | 0 | 2450 | 2600 | 0 | 0 | 0 | 2600 |
| 26 | 3250<br>10350 | 0<br>0 | 0<br>0 | 0<br>0 | +6800 | 6200<br>4900 | 0<br>0 | 0<br>0 | 0<br>0 | +5550 |

TABLE 19. Number of worms recovered from vaccinated and non-vaccinated ewes and lambs at different times after a challenge infection with normal H. contortus $L_3$.

*stunted females + mean of two animals

In the non-vaccinated ewes higher numbers of parasites were evident at all stages; for example, high numbers of $L_4$ were present three and five days after infection and by 24 and 26 days several thousand $L_5$ and adult parasites were recovered.

Lambs. In the vaccinated lambs substantial numbers of $L_5$ and stunted adult parasites from the immunising infections were present up to day seven after challenge. Immature larvae from the challenge infection were present on days five, seven and 10 with apparent development to the adult stage by days 24 and 26.

In the non-vaccinated lambs early $L_4$ were present by day three and these had developed to mature $L_4$ by day five. On day seven a mixture of mature $L_4$ and $L_5$ larvae were present, on day 10 only $L_5$ were recovered and only adult parasites were found by day 24. The total numbers of parasites reaching maturity, however, were similar in both vaccinated and non-vaccinated lambs.

Packed cell volume

The group mean PCV's of the ewes and lambs throughout the experimental period are illustrated in Figures 41 and 42 respectively. Individual values are given in Appendix 7.

Ewes. The mean PCV's of the non-vaccinated and vaccinated ewes were 35% and 31% respectively at the beginning of the experiment i.e. before vaccination. The mean PCV of the vaccinated ewes after receiving nine weekly doses of vaccine was reduced to 21.5%, while that of the non-vaccinated

Figure 41

The mean PCV's of repeatedly vaccinated and non-vaccinated ewes pre- and post-challenge with normal *H. contortus* $L_3$.

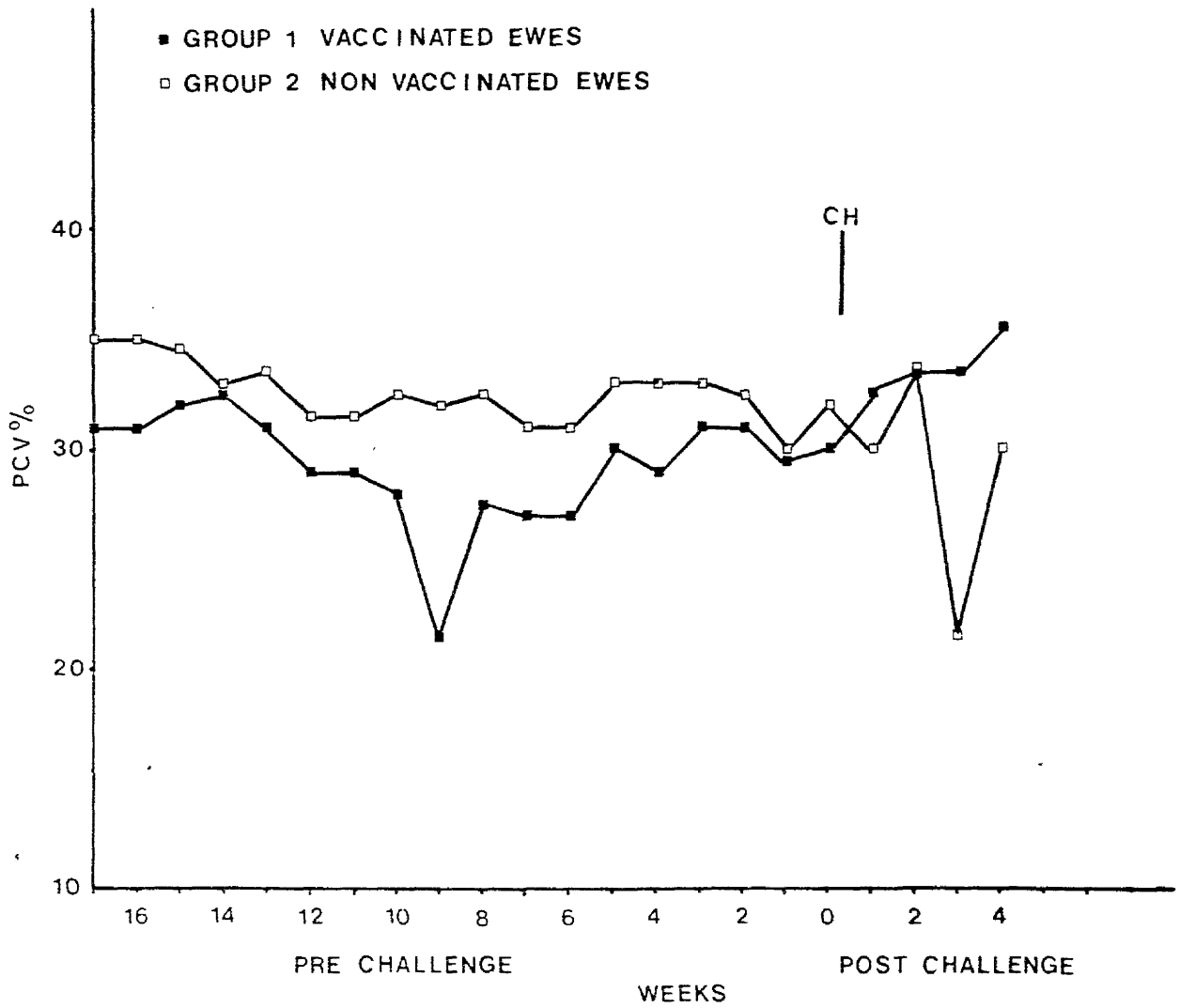
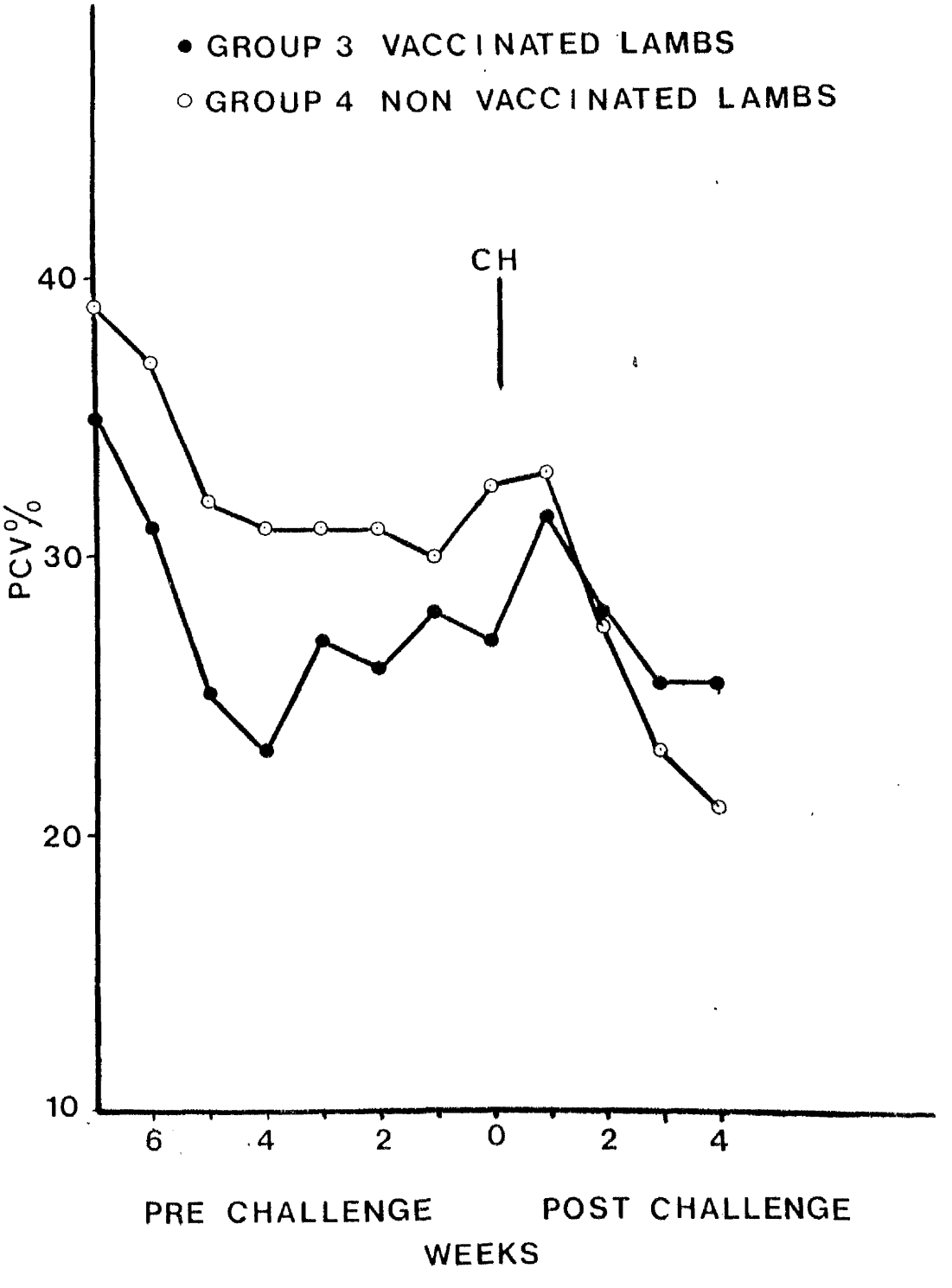

GROUP 1 VACCINATED EWES
GROUP 2 NON VACCINATED EWES
CH
40
30
20
10
PCV%
16
14
12
10
8
6
4
2
0
2
4
PRE CHALLENGE
POST CHALLENGE
WEEKS

Figure 42

The mean PCV's of repeatedly vaccinated and non-vaccinated lambs pre- and post-challenge with normal *H. contortus* $L_3$.


● GROUP 3 VACCINATED LAMBS
○ GROUP 4 NON VACCINATED LAMBS
CH
40
30
20
10
PCV%
6
4
2
0
2
4
PRE CHALLENGE
POST CHALLENGE
WEEKS

group at the same time was 32%. Over the next nine weeks the PCV's of the vaccinated ewes rose to prevaccination levels and remained around this level after challenge, whereas the mean PCV's of the non-vaccinated ewes showed a marked decrease three weeks after challenge.

Lambs. The mean PCV of the non-vaccinated and the vaccinated lambs were 39% and 35% respectively at the beginning of the experiment. In the vaccinated lambs the PCV's dropped soon after vaccination to 25% and remained at a low level until challenge. By day 5 after challenge, the mean PCV has risen to 31.5% but by the end of the experiment, this was reduced to pre-challenge levels (i.e. 25.5%). The mean PCV of the non-vaccinated lambs varied between 30-35% before challenge but started to decrease soon after the challenge infection and had fallen markedly by the end of the experiment when the mean PCV of the remaining lambs was 21%.

Histopathology

Non-vaccinated ewes. In the ewe killed three days after infection, depressed lesions on the mucosal surface with erosion of the surface epithelium were observed. A mild cellular reaction was present with infiltration of the mucosa with lymphocytes, plasma cells and eosinophils. A thin layer of a mixture of acid and neutral mucin was seen covering the surface mucosa. Neutral mucosubstances were detected within the cytoplasm of the surface epithelium and the epithelial cells of the gastric glands in the upper part of the mucosa. Acid mucosubstances were present in the middle and deeper parts of the mucosa.

Similar lesions were seen throughout the course of the infection, with an increasing mucosal cellular reaction. On day seven parasites were seen on the surface of the mucosa which was covered by a thick, irregular layer of mucus containing a mixture of a few lymphocytes and many epithelial cells (Figure 43).

Between 10 and 26 days after infection dilatation of the gastric glands was occasionally seen associated with an increase in the number of mucus producing cells.

Vaccinated ewes. In the ewe killed three days after infection, there was a mild cellular reaction with infiltration of the mucosa by mainly plasma cells, lymphocytes and few eosinophils. Larger mucosal aggregates of lymphoid tissue were also present. There was an apparent increase in the number of mucus producing cells compared with that seen in worm-free ewes (as described previously). No haemorrhage was detected but depressed superficial lesions on the mucosa were present and parasites were seen on the surface of the mucosa (Figure 44). A thick irregular layer of a mixture of acid and neutral mucin was seen covering the surface mucosa. A mixture of acid and neutral mucin was present within the cytoplasm of the surface epithelium and the mucus secreting cells of the upper gastric glands. Acid mucin was present in the middle and deeper parts of the mucosa.

A similar histopathological appearance was observed throughout the course of the infection.

Non-vaccinated lambs. In the lamb killed three days after infection, no haemorrhage and no cellular reaction was observed,

Figure 43

Section from the abomasum (pyloric region) of a non-vaccinated ewe killed seven days after an infection with normal H. contortus $L_3$.

There is obvious infiltration of the mucosa with lymphocytes, eosinophils and plasma cells. A thick irregular layer of mucus containing a mixture of few lymphocytes and many epithelial cells is present on the mucosal surface.

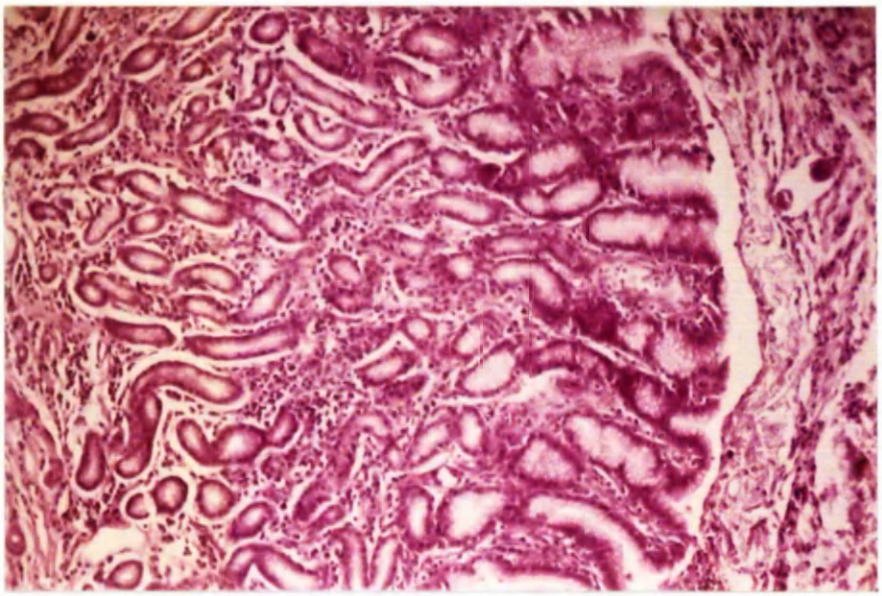
H and E. X.110

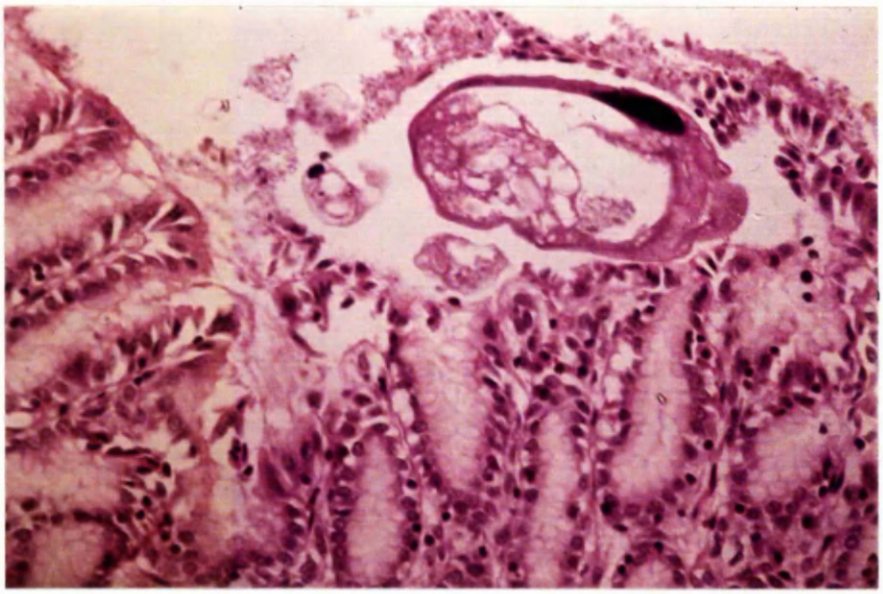
Figure 44

Section from the abomasum (pyloric region) of a repeatedly vaccinated ewe killed three days after challenge with normal H. contortus $L_3$.

A parasite is present directly over a depressed superficial lesion of the mucosa surrounded with mucin infiltrated with few lymphocytes and epithelial cells.

H and E. X.250





but there were a few depressed superficial lesions. A thin, irregular layer of acid mucin was seen covering the surface mucosa. A mixture of neutral and acid mucosubstances was present within the cytoplasm of the surface epithelium and the glandular epithelium in the upper part of the mucosa, while acid mucin was present in the middle and lower parts of the mucosa.

Five and seven days after infection the histopathological appearance was similar but there was a thick, irregular layer of a mixture of acid and neutral mucin covering the surface epithelium. Dilatation of the gastric glands was detected at day seven after infection (Figure 45).

Ten days after infection there was a slight cellular reaction in the mucosa and an apparent increase in the number of mucus secreting cells. The mucosubstances at this time appeared similar to that seen five and seven days after infection and parasites were seen on the mucosal surface covered with mucin.

A moderate cellular reaction was seen 24 and 26 days after infection. Lymphocytes, plasma cells and a few eosinophils were present around the blood vessels in the deeper part of the mucosa (Figure 46). Destruction of the superficial surface epithelium was also observed. A mixture of neutral and acid mucin was seen covering the surface epithelium, within the cytoplasm of the surface epithelium and in the epithelial cells of the gastric glands throughout the depth of the mucosa.

Figure 45

A section from the abomasum (fundic region) of a non-vaccinated lamb killed seven days after infection with normal H. contortus $L_3$. There is obvious dilatation of the gastric glands.

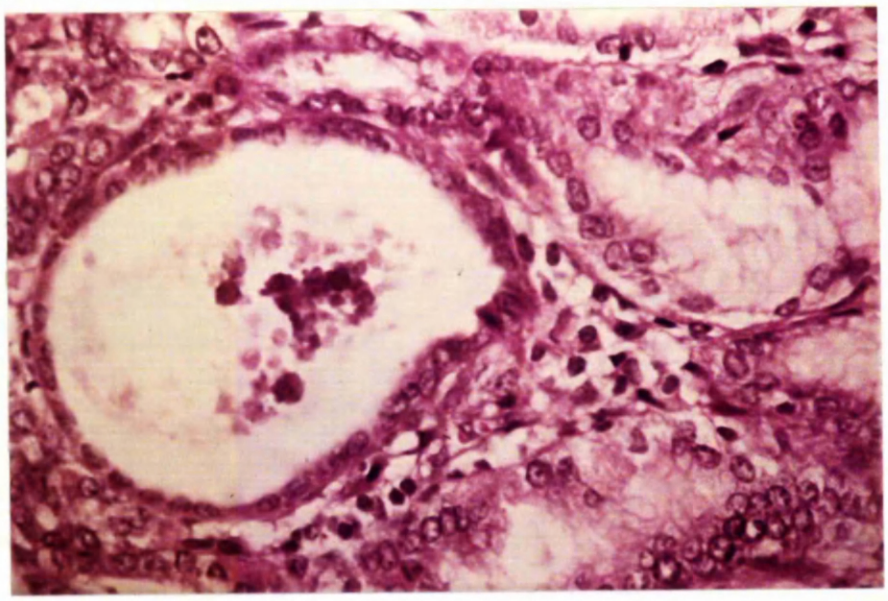
H and E. X.400

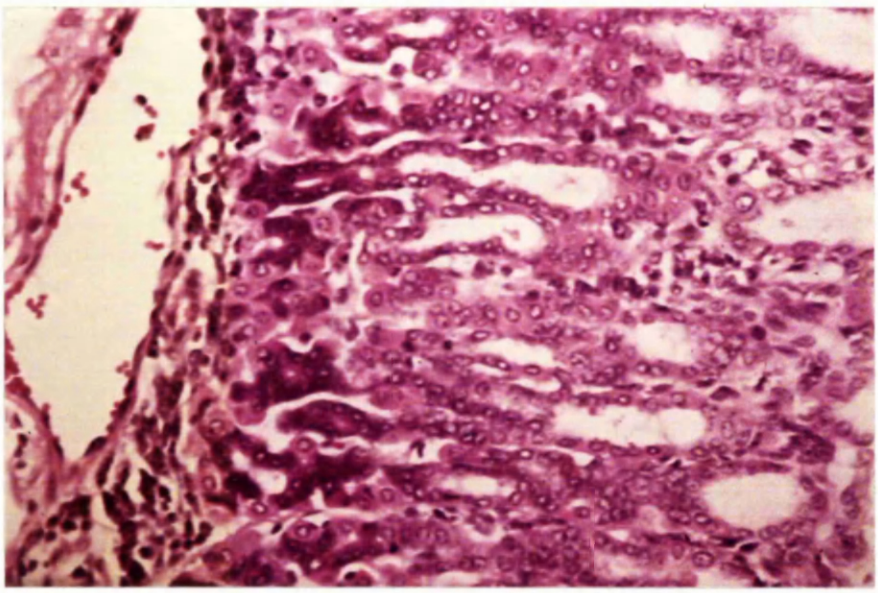
Figure 46

A section from the abomasum (fundic region) of a non-vaccinated lamb killed 24 days after infection with normal H. contortus $L_3$.

Accumulations of lymphocytes, plasma cells and eosinophils are present around a blood vessel in the deeper part of the mucosa.

H and E. X.250





abomasum and a mild cellular reaction with infiltration of the mucosa by lymphocytes, plasma cells and eosinophils. There was dilatation of some of the gastric glands and an apparent increase in the number of mucus producing cells. A thin layer of a mixture of acid and neutral mucin was seen covering the surface mucosa and similar mucin was present within the cytoplasm of the surface and glandular epithelium in the upper part of the mucosa. Within the cytoplasm of the glandular epithelium in the middle and deeper parts of the mucosa, acid mucin was present.

Five and seven days after infection there was a more obvious cellular infiltration of the mucosa mainly by lymphocytes and plasma cells with a few eosinophils. Depressed superficial lesions and haemorrhages were present. The type of mucin present at this time was similar to that seen at day three after infection.

Ten, 24 and 26 days after infection a more extensive cellular reaction was seen with diffuse and nodular accumulations of lymphocytes, plasma cells, eosinophils and neutrophils in the mucosa and occasionally in the submucosa. Mainly acid mucin, mixed with some neutral mucosubstances, was seen covering the surface epithelium but only acid mucin was seen throughout the depth of the mucosa 10 days after infection. On days 24 and 26 after infection, a mixture of acid and neutral mucin was detected throughout the depth of the mucosa; neutral mucin was dominant in the upper half while acid mucin was the main type in the lower half.

Mast cells occurred in the submucosa, mucosa and within the gastric glands and surface epithelium. Differences in their combined numbers in the abomasa of the various groups of animals are presented in Table 20.

Non-vaccinated ewes. Little change in mean numbers of mast cells occurred during the course of infection of non-vaccinated ewes. On day three, no intraepithelial mast cells (I.E.) were present. Small numbers of I.E. mast cells occurred on day five and the numbers subsequently increased to reach their highest levels on day 26 after infection.

Vaccinated ewes. Great variation in mean numbers of mast cells occurred during the course of infection in the vaccinated ewes. I.E. mast cells were present in all of the animals. At most stages they were more numerous than in non-vaccinated ewes.

Non-vaccinated lambs. Little change in the mean numbers of mast cells occurred during the course of infection in non-vaccinated lambs. At all stages they were less frequent than in the non-vaccinated ewes. No I.E. mast cells were present up to ten days after infection. Small numbers of I.E. mast cells were evident on days 24 and 26 after infection.

Vaccinated lambs. Little change in mean numbers of mast cells occurred during the course of infection of vaccinated lambs except at day ten after challenge in which large numbers of mast cells were detected. I.E. mast cells were present in all animals (Figure 47).

Figure 47

A section from the abomasum (fundic region) of a repeatedly vaccinated lamb killed 10 days after challenge with normal H. contortus $L_3$.

Large numbers of mast cells are present in the lamina propria and within the epithelial cells of the gastric glands.

Astra blue/safranin O.

X.250

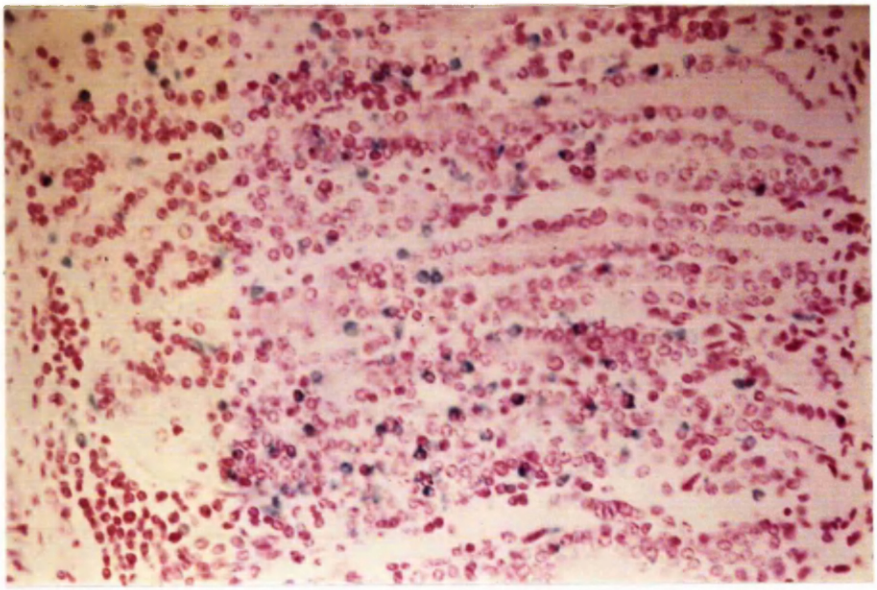
Figure 48

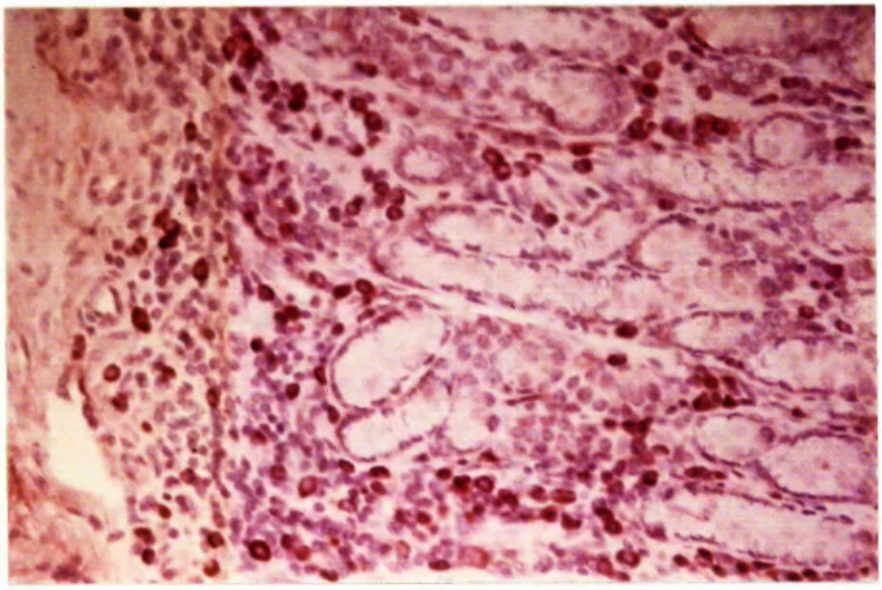
A section from the abomasum (pyloric region) of a repeatedly vaccinated ewe killed 10 days after challenge with normal H. contortus $L_3$.

Peroxidase labelled antiserum reveals IgA-containing cells in the connective tissue throughout the depth of the mucosa.

Haematoxylin was used as a counter stain.

X.250





| challenge | ewes | ewes | lambs | lambs |
|---|---|---|---|---|
| Day 3 | 75 | 48 | 26 | 28 |
| Day 5 | 181 | 175 | 13 | 0 |
| Day 7 | 282 | 436 | 18 | 35 |
| Day 10 | 463 | 291 | 72 | 48 |
| Day 24 | Not done | Not done | Not done | Not done |
| Day 26 | Not done | Not done | *11.5 | *25 |

* Mean of two animals

TABLE 21. The individual mucosal anti-H. contortus IgA-antibody levels of vaccinated and non-vaccinated ewes and lambs after a challenge infection with normal H. contortus $L_3$.

There was little difference between the numbers of tissue eosinophils between the vaccinated and the non-vaccinated ewes although fewer eosinophils were detected 24 days after challenge especially in the non-infected ewes. In the vaccinated lambs there was a progressive increase in the numbers of eosinophils until day ten after challenge then the numbers of these cells decreased but they were still more numerous than at three days after challenge. In the non-vaccinated lambs the numbers of these cells remained low throughout the course of the experiment compared with the vaccinated lambs.

## IgA-containing cells

The numbers of cells containing IgA in the abomasal mucosa of the ewes and lambs are presented in Table 20.

Ewes. IgA-containing cells were present in moderate numbers in both the vaccinated and non-vaccinated ewes. Their numbers varied somewhat in different sections or parts of a section but in both vaccinated and non-vaccinated ewes killed on day ten after infection the greatest number of IgA-containing cells were detected (Figure 48). The distribution of the IgA cells was similar in both vaccinated and non-vaccinated animals. Up to ten days after infection IgA-containing cells were present in the connective tissue throughout the depth of the mucosa. Twenty-four and 26 days after infection the majority of IgA-containing cells were present in the connective tissue of the upper half of the mucosa.

also present in all of the animals.

Lambs. There was little difference in the numbers of IgA-containing cells between vaccinated and non-vaccinated groups of lambs during the course of the experiment.

The distribution of IgA-containing cells in vaccinated and non-vaccinated lambs was similar at all stages after infection the cells being found mainly in the connective tissue throughout the mucosa.

A few submucosal IgA-containing cells were also present in all animals.

## Mucosal IgA antibody

The individual mucosal anti-H. contortus IgA-antibody levels of the vaccinated and non-vaccinated ewes and lambs killed at various times after challenge with H. contortus $L_3$ were measured by a radioimmunoassay and the results are shown in Table 21.

In the vaccinated ewes the IgA-antibody concentration increased gradually in the mucus between three and ten days. A similar increase was observed in the non-vaccinated ewes killed at three, five and seven days and although the level of IgA-antibody had fallen in the animal killed on day ten after infection it was still higher than that recorded at three and five days after infection. In contrast, the mucus IgA-antibody concentration of the lambs tended to be low in both the vaccinated and the non-vaccinated lambs throughout the experimental period.

| | Ewes | | | | Lambs | | | |
|---|---|---|---|---|---|---|---|---|
| | Vaccinated | | Non-vaccinated | | Vaccinated | | Non-vaccinated | |
| Days | Mean | ± S.E. | Mean | ± S.E. | Mean | ± S.E. | Mean | ± S.E. |
| Mast cells | | | | | | | | |
| 3 | 13.65 | 0.05 | 9.67 | 0.08 | 25.30 | 11.70 | 4.45 | 1.80 |
| 5 | 89.88 | 49.23 | 20.33 | 2.57 | 12.10 | 5.79 | 4.65 | 0.40 |
| 7 | 71.05 | - | 9.70 | - | 18.75 | 9.95 | 4.20 | 0.60 |
| 10 | 20.43 | 3.48 | 7.30 | 3.70 | 59.77 | 10.02 | 6.33 | 1.18 |
| 24 | 23.23 | 12.18 | 32.73 | 14.18 | 14.63 | 8.08 | 11.58 | 5.38 |
| 26 | 43.55 | 9.23 | 25.05 | 7.25 | 16.95 | 6.93 | 8.55 | 0.62 |
| Eosinophils | | | | | | | | |
| 3 | 3.88 | 0.13 | 11.80 | 9.70 | 1.58 | 0.33 | 0.78 | 0.73 |
| 5 | 17.78 | 9.93 | 15.65 | 2.85 | 3.18 | 0.83 | 0.33 | 0.03 |
| 7 | 13.33 | 1.13 | 16.00 | 3.95 | 15.43 | 4.68 | 3.38 | 2.08 |
| 10 | 16.60 | 3.25 | 12.10 | 2.80 | 30.80 | 5.95 | 2.38 | 1.38 |
| 24 | 6.93 | 1.68 | 0.38 | 0.08 | 5.10 | 1.80 | 1.68 | 0.88 |
| 26 | Not done | | 43.05 | 11.00 | Not done | | 4.67 | 2.28 |
| IgA-containing cells | | | | | | | | |
| 3 | +21.90 | 3.67 | 22.60 | 4.80 | 3.84 | 1.14 | 3.00 | 1.75 |
| 5 | 14.03 | 5.28 | 23.60 | 5.85 | 5.63 | 0.28 | 7.88 | 3.38 |
| 7 | 15.65 | 0.40 | *10.35 | 2.47 | 4.33 | 0.53 | 6.50 | 4.70 |
| 10 | 55.85 | 26.65 | 76.03 | 24.53 | 8.05 | 0.35 | 8.43 | 7.73 |
| 24 | 21.30 | 3.60 | 18.15 | 0.35 | 16.33 | 0.13 | 5.93 | 2.33 |
| 26 | 24.75 | 3.66 | 14.22 | 2.71 | 7.82 | 1.69 | 5.30 | 1.51 |

* Sections from the fundus region only.

\+ Sections from the pyloric region only.

TABLE 20. Mean numbers of various cell types present in the abomasal mucosa of vaccinated and non-vaccinated ewes and lambs after a challenge infection with normal *H. contortus* $L_3$.

## DISCUSSION

Twenty-four days after challenge in the present experiment, a mean faecal egg count of 50 e.p.g. was recorded in the vaccinated ewes compared with a mean of 22,400 e.p.g. in the non-vaccinated ewes. In the vaccinated lambs eggs were first present in the faeces 24 days after challenge with a mean of 3583 e.p.g. being recorded at this time. In contrast in the non-vaccinated lambs eggs first appeared in the faeces on day 19 after infection a mean of 8063 e.p.g. but by day 24 the mean faecal egg count of this group had risen to 32,950 e.p.g. In the vaccinated ewes, the largest number of worms were recovered on day three after challenge but 94.9% of these worms were $L_5$ or stunted adults which had obviously persisted from the immunizing infections. Only small numbers of parasites were subsequently seen developing through to the adult stage on days 10, 24 and 26 of the infection. In the non-vaccinated ewes relatively large numbers of worms were recovered at most stages after challenge and natural development of the parasites was evident. In the lambs, the numbers of worms recovered at the end of the experiment were similar in both, the vaccinated and the non-vaccinated groups, however, the egg output was less and eggs appeared later in the faeces of the vaccinated lambs. Although total worm burdens were similar in both groups of lambs differences in faecal egg output were probably due to a proportion of the recovered worms in the vaccinated animals being stunted adults originating from the vaccine infections and the remainder, which developed from the challenge infection, having

local cellular response of the two groups of lambs. These results are in agreement with those reported by Duncan et al (1978) who found a high reduction in the numbers of recovered worms in immune adult sheep compared with challenge control lambs whereas there was no significant difference in the mean numbers of the worms recovered from these controls and similar lambs previously given two doses of irradiated larvae. Benitez-Usher et al (1977) also failed to stimulate immunity in young lambs by repeated vaccination with irradiated larvae. There are many reports of the successful immunisation of sheep over 7 months of age with either normal or irradiated H. contortus larvae (Manton et al, 1962; Urquhart et al, 1966; Benitez-Usher et al, 1977 and Duncan et al, 1978). However, Bezubik, Stankiewicz and Byszewska-Szpocinska (1978) failed to detect any differences in either faecal egg counts or recovered worms after challenge in adult sheep and lambs immunised with repeated doses of normal larvae compared with non-immunised controls. In early studies on N. brasiliensis infection in rats, however, Taliaferro and Sarles (1939) established that in hyper-immune animals killed 15 and 16 days after receiving 3-7 large reinfections, only a few small worms were found scattered along the entire length of the small intestine and they suggested that the action of antibody on worms in the intestine was similar to its action in the skin and lungs with the exception that in the lamina propria this did not lead to nodule formation since the immobilised worms were expelled directly from the intestine.

In the present experiment, there was a decrease in the mean PCV's of the vaccinated ewes during the course of the repeated immunising infections but these had increased to pre-vaccination levels by the time of challenge. After challenge the PCV's of these animals continued to rise whereas the mean PCV's of the non-vaccinated ewes showed a marked decrease three weeks after challenge and despite some recovery by the end of the experiment they were still lower than those recorded at the time of challenge. The haematocrits of the lambs followed a similar pattern prior to challenge but subsequently the mean PCV's of both groups fell, the decrease being most marked in the non-vaccinated lambs. The fact that the PCV's of the vaccinated ewes returned to normal shortly after challenge while those of the non-vaccinated ewes showed a marked drop, reflects the immune status of the two groups of sheep whereas in terms of PCV, previous immunisation conferred little protection in the vaccinated group of lambs, indeed these results could be expected from the worm recoveries of the various groups of animals.

In rats infected with N. brasiliensis Taliaferro and Sarles (1939) reported that hyperimmunisation completely inhibited the feeding and development of the worms, that the intestine was hardly damaged either mechanically or by worm secretions, and that acute inflammation did not occur. In the vaccinated ewes in the present study, there was a mild cellular reaction with infiltration of the abomasal mucosa by mainly plasma cells, lymphocytes and eosinophils. There was, however, an increase in the numbers of mucus secreting

cells and an associated increase in the quantity detected on the mucosal surface. During infection there was also a change from acid to a mixture of acid and neutral mucin. Although there was a great variation in the numbers of mast cells occurring during the course of infection, these cells were more numerous in the vaccinated ewes and I.E. mast cells were present at all stages after challenge in these animals. Smith and Christie (1979) also found that numerous globule leucocytes were present in the abomasal mucosa of the majority of sheep immune to H. contortus. Taliaferro and Sarles (1939) showed that in hyper-immune rats infected with N. brasiliensis although the mesentery, serosa, muscular layer and submucosa showed little peri-vascular infiltration the numbers of both globular leukocytes in the epithelium of the crypts, and connective tissue basophils in the lamina propria were greatly increased. In the present work I.E. mast cells were first seen in small numbers five days after challenge in the non-vaccinated ewes. The numbers of these cells then increased gradually during the course of the infection. Taliaferro and Sarles (1939) found that in primary infections with N. brasiliensis in non-immune rats there was little evidence of inflammation of the small intestine during the first five days after infection. Between 5-8 days an inflammatory response developed which progressively increased up to 16 days after infection. Previous work (see Chapter 1) had shown that there was an increase in the numbers of eosinophils, neutrophils and plasma cells seven days after a primary infection and that a more extensive cellular reaction had developed by day 15. In the present experiment a mild

cellular reaction and infiltration of the mucosa with lymphocytes, plasma cells and eosinophils was detected in non-vaccinated ewes, three days after infection. Depressed lesions on the mucosal surface with erosion of the surface epithelium were also observed at this time and a mixture of acid and neutral mucin was seen covering the surface mucosa. Similar but more extensive cellular reactions were detected throughout the course of infection together with an increase in the quantity of the mucin on the mucosal surface.

Taliaferro and Sarles (1939) found that eosinophils appeared late in animals after a primary N. brasiliensis infection and early and in large numbers in immune animals while Wells (1962) reported an increase in the numbers of eosinophils in the intestine of rats five, 16 and 23 days after an initial infection. In this study there was no apparent difference in the numbers of tissue eosinophils in the vaccinated and non-vaccinated ewes. There was, however, a marked increase in the numbers of these cells in the mucosa of vaccinated compared with that of non-vaccinated lambs the number of these cells in the latter group remaining at a low level at all stages after challenge. The precise function of the eosinophil is still unknown but Mackenzie, Ramalho-Pinto, McLaren and Smithers (1977) have observed the adherence in vitro of rat eosinophils to Schistosomulae, adult Trichinella and N. brasiliensis larvae. It has also been found that incubation of isolated Schistosome eggs with eosinophil-rich peritoneal cells from immune mice resulted in histologic destruction and penetration of 20% of

the eggs with eosinophils (James and Colley, 1976).

IgA-containing cells were present in a moderate number in both the vaccinated and non-vaccinated ewes used in this study. In the lambs, there was a little difference in the numbers of IgA-containing cells between vaccinated and non-vaccinated groups at different stages from challenge. Beh, Husband and Lascelles (1979) found that the intraperitoneal injection of ovalbumin to 2-year-old sheep resulted in a substantial output of antibody containing cells in the intestinal lymphatic ducts and that IgA producing cells reached a peak at 8-9 weeks after the injection of the antigen. In the present study, increased numbers of IgA-containing cells were detected in both the vaccinated and the non-vaccinated ewes 10 days after infection. In pigs, by using immunofluorescent techniques to study the change in the immunoglobulin containing cells in the stomach during infection with Hyostrongylus rubidus, Smith, Herbert and Davis (1979) found that in animals infected with a single dose of 100,000 H. rubidus $L_3$ both IgM and IgA immunocytes had increased in numbers by day five and remained elevated during the histotropic phase of worm development i.e. until day 17. Although Curtain and Anderson (1971) detected increases in the numbers of $IgG_1$- and $IgG_{1A}$-containing cells in the abomasum of adult sheep naturally exposed and in the authors' opinion immune to Ostertagia, Trichostrongylus and Nematodirus spp. they did not detect any apparent increase in the numbers of IgA producing cells.

In the vaccinated ewes in the present experiment, there was a progressive increase in the levels of mucosal anti-

H. contortus IgA-antibody between day three and day ten after challenge. In contrast in the non-vaccinated ewes the specific IgA levels progressively increased until day seven after infection followed by a decrease at day ten. No samples were available from the ewes at the end of the experiment. Anti-H. contortus IgA antibodies were only detected in small amounts in both the vaccinated and the non-vaccinated lambs. These results are in agreement with previously published work. For example sheep reared worm-free until at least seven months of age, and subsequently immunised by double-vaccination with irradiated larvae have been shown to have high levels of both total IgA and H. contortus specific IgA antibodies in the abomasal mucosa whereas challenge control or uninfected animals have very low levels of both total mucosal IgA and IgA-specific antibody (Smith 1977a; Smith and Christie 1978). Duncan et al (1978) also found that young lambs vaccinated with irradiated larvae while they are still immunologically unresponsive to H. contortus and therefore susceptible to challenge, have little or no H. contortus specific IgA. Similarly Beznbik, et al (1978) found that in 7-month-old sheep immunised with normal H. contortus $L_3$ specific antibodies against larval antigens were present in the abomasal mucosa while, in 3-month-old lambs similarly immunised no local immunological response could be detected. It is interesting that in rats infected with N. brasiliensis a great increase in the specific IgA-mucosal antibody in the small intestine occurs around the point of worm expulsion (Poulain, Luffau and Pery, 1976; Sinski and Holmes, 1977).

# GENERAL DISCUSSION

## GENERAL DISCUSSION

An early report by Silverman and Patterson (1960) showed that in primary infections with *H. contortus* in adult sheep parasite eggs first appeared in the faeces between 16-24 days after infection, while in young lambs similarly infected eggs were present in the faeces before day 15. In the experiments described in this thesis eggs were detected in the faeces of adult sheep, which had been reared worm-free, 15 days after a single infection with *H. contortus* $L_3$. In contrast, similar infections of adult sheep reared under natural conditions, but subsequently housed and treated with an anthelmintic before infection failed to become patent until 19-24 days later. The delayed appearance of eggs in the faeces of the adult sheep reared under natural conditions is possibly due to the presence of a degree of resistance caused by previous exposure to a mixed nematode infection. There was no apparent difference in the pattern of egg production after infection of the worm-free lambs which were used in most of these studies with the exception of those repeatedly immunised before receiving a challenge infection: in these animals, parasite eggs appeared later and the faecal egg counts were lower than in non-immunised controls.

It is interesting that the quality of the diet had an obvious effect on the worm egg output. Whereas the number of eggs in the faeces of lambs with a low-grade infection and fed on a low protein diet remained high for 5-6 months, the faecal egg counts of lambs fed additional concentrates fell markedly 10 weeks after infection. This confirms a previous

infected with H. contortus $L_3$ and fed on a high protein diet had lower faecal egg counts than those fed on a low protein diet.

The faecal egg counts reflected the worm burdens at necropsy. Thus substantial numbers of worms were still present at necropsy of most lambs fed a low level diet, whereas only a few worms were recovered from the group of lambs fed a high level diet. This is again in agreement with the results of studies by Kates and Wilson (1955) who found lower post-mortem worm counts in infected lambs fed on a high plane of nutrition than in those fed a maintenance ration.

The present work also confirms the results of earlier re-infection experiments (Stewart, 1953; Christie and Brambell, 1967 and Dargie and Allonby, 1975) in that very few worms were recovered either from adult sheep after a second infection or from immunised adult sheep after a challenge infection. In young lambs (i.e. under 7 months of age), however, previous exposure to infection did not have any significant effect on the number of worms subsequently recovered at necropsy. These results are also in agreement with previously published reports (Manton et al, 1962; Urquhart et al, 1966; Smith, 1977a and Duncan et al, 1978).

There have been many studies on the association between H. contortus infection and anaemia in sheep and from early experimental work it was suggested that the anaemia was purely haemorrhagic in character (Fourie 1931). In the present experiments, both adult sheep and lambs infected with

H. contortus $L_3$, showed variable reductions in PCV, but this was always more obvious in the lambs. In the adult sheep repeatedly immunised by oral administration or irradiated larvae and then challenged with normal H. contortus $L_3$, the PCV's decreased during vaccination but returned to normal by the time of challenge; after challenge the PCV's remained within the normal range: in the non-immunised controls a marked post-challenge fall in PCV was observed. In the lambs, on the other hand, a similar pattern of PCV's was observed prior to challenge but both the vaccinated and the non-vaccinated lambs showed a marked drop in PCV after the chellenge infection.

In these studies, only a few lymphocytes were seen scattered in the mucosa of worm-free lambs. Small accumulations of lymphocytes together with plasma cells, eosinophils and mast cells were seen after primary H. contortus infections and increased accumulations of lymphocytes, plasma cells and eosinophils were observed after challenge. Although there were marked differences in worm burdens there was little obvious difference in the histological features in the abomasa of infected lambs fed on either a high or a low level diet. More extensive cellular reactions were, however, observed after infection of lambs obtained from and suckled by immune dams compared with those suckled by non-immune ewes or those which had been bottle-fed. However, this cellular reaction could not be clearly correlated with protection after challenge, particularly since the lambs which suckled from either immune or non-immune ewes had low worm burdens. This observation

requires further study with respect to its etiology and possible significance in the natural disease. A progressive cellular infiltration of the mucosa was also detected in lambs which received several doses of irradiated H. contortus $L_3$ prior to challenge with normal H. contortus $L_3$ but substantial numbers of worms were still present in the animals at different intervals after the challenge infection.

In the uninfected adult sheep examined in these studies numerous mast cells together with lymphocytes, plasma cells and eosinophils were seen in the mucosa. An obvious increase in the numbers of eosinophils and neutrophils was seen after primary infections with H. contortus $L_3$ and further increases in the numbers of eosinophils, neutrophils, mast cells, plasma cells and lymphocytes were detected after challenge. Only a diffuse mild cellular reaction was observed in the abomasal mucosa of immunised ewes killed at different stages after challenge with normal H. contortus $L_3$.

In worm-free lambs, only a few mast cells were detected in the mucosal lamina propria and although the numbers of these cells increased after a primary infection they were still located in the lamina propria. In general further increases in the numbers of mast cells were observed after challenge but at this time some of these cells could be seen within the glandular epithelium. Although there was no difference in the numbers of mast cells detected in lambs obtained from either immune or non-immune ewes large numbers of mast cells were present in the abomasal mucosa of bottle-fed lambs after infection. Intra-epithelial mast cells were present in the abomasal mucosa of all the vaccinated lambs

after challenge but there was no apparent variation in total mast cell numbers at different stages post-challenge except on day 10, when large numbers of these cells were detected.

In worm-free adult sheep substantial numbers of mast cells were evident distributed throughout the lamina propria. Although there was a reduction in the number of these cells seven days after a primary infection a few were seen within the glandular epithelium. Marked increases in the numbers of these cells were detected after challenge, and many of these were present intraepithelially. Intraepithelial mast cells were also seen in the abomasal mucosa of the immune ewes after challenge and the total numbers of these cells were always higher in these animals than in the non-immunised controls. Also the numbers of mast cells detected in adult sheep after infection were always higher than that in similarly infected lambs. Similar results were obtained by Gregg, Dineen, Rothwell and Kelly (1978), in their investigations of age differences in the response of lambs to Trichostrongylus colubriformis infection. They found that the numbers of both mast cells and globule leucocytes induced by infection increased with age, and suggested that only the rise in globule leucocytes was correlated with a protective immune response. Sommerville (1956) also demonstrated globule leucocytes in the intestine of sheep associated with chronic nematode infections but their position in the alimentary tract did not appear to be related to that of the parasites. Christie, Hart, Angus, Devoy and Patterson (1978) in a study of the immune response of sheep to H. contortus, found large

numbers of globule leucocytes in the gastric mucosa distributed mainly towards the lumen, whereas numerous mast cells were present at the base of the glands. Early work by Taliaferro and Sarles (1939) had demonstrated the presence of globule leucocytes in the intestine of rats after infection with Nippostrongylus muris (brasiliensis). They demonstrated a rise in 'globular leucocyte' numbers coincident with a rise in 'connective tissue basophils' in the lamina propria around the time of worm expulsion which continued for some time thereafter. Nawa and Miller (1979) studied the intestinal mast cell response after N. brasiliensis infection in normal rats and those adoptively immunised with thoracic duct lymphocytes (T.D.L.) obtained from rats infected with this parasite 10 days previously. They observed an increase in the number of mast cells in infected recipients of T.D.L. and found that T.D.L. cells lacking surface immunoglobulin could also induce an intestinal mast cell response. Later MacDonald, Murray and Ferguson, (1980) studied mast cell kinetics during infection with N. brasiliensis at various sites in the small intestine of rats and in heterotopically transplanted isografts of foetal small intestine placed under the kidney capsule. They found that infection produced an increase in the number of mast cells not only in the proximal jejunum but also in the distal ileum and in the kidney isografts of small intestine, while globule leucocyte infiltration of the gut epithelium was confined to the proximal small intestine and did not occur in either the distal ileum or in isografts.

In the present experiments, IgA-containing cells were detected in the abomasal mucosa of both worm-free and

parasitised animals but very few IgA-containing cells were present in the abomasal lamina propria of worm-free lambs. Generally only slight increases in the number of these cells were observed after primary or challenge infections of lambs and although a marked increase in IgA-cell numbers was detected in a group of lambs which received repeated doses of levamisole prior to single vaccination with irradiated larvae and challenge, this was not associated with any protection.

Small numbers of IgA-containing cells were detected in the abomasal mucosa of worm-free adult sheep but increased numbers of these cells were observed after primary or repeated infections with H. contortus $L_3$. In worm-free animals the cells were distributed at the base of the gastric glands while they were seen mainly in the upper third of the mucosa in infected animals.

An increase in the numbers of IgA-containing cells has been observed in the gastric mucosa of pigs 5-17 days after infection with Hyostrongylus rubidus $L_3$ (Smith et al, 1979). On the other hand Curtain and Anderson (1971) were not able to detect any apparent increase in the numbers of IgA-producing cells in the abomasal mucosa of adult sheep naturally exposed and in the authors' opinion immune to various gastro-intestinal nematodes.

Similar changes in type of mucus covering the surface mucosa of all the infected adult sheep and lambs was observed. Whereas acid mucin was seen on the mucosal surface in worm-free adult sheep and lambs this changed to a mixture of

neutral and acid mucosubstances after infection. Increases in the quantity of mucin together with increases in the numbers of mucus producing cells were associated with the changes in mucin type observed after infection and this was always more obvious in the adult sheep. It is interesting that in experiments on N. brasiliensis infection in rats Miller and Nawa (1979) demonstrated that immune expulsion was associated with a concomitant rise in goblet cells.

Mucosal anti-H. contortus IgA-antibodies were only detected in small quantities in worm-free lambs after primary and secondary infection or after challenge of vaccinated lambs. Gradual increases in the concentration of these antibodies were detected in the abomasal mucosa of adult sheep after a primary infection and further increases were detected on re-infection. In both vaccinated and non-vaccinated ewes there were again increases in the level of anti-H. contortus IgA-antibodies at different stages after challenge, but this appeared to be higher and more sustained in the immunised animals. Smith (1977a) examined the abomasal mucus and serum at slaughter from sheep hyperinfected and therefore immune to H. contortus and found the presence of anti-H. contortus IgA- and IgG-antibodies. Subsequently Duncan et al (1978) found that young lambs vaccinated with irradiated H. contortus larvae while they were still immunologically unresponsive had little or no H. contortus specific IgA whereas adult sheep similarly immunised were protected against challenge and had high levels of IgA antibodies. Beznbik et al (1978) also found that in 7-month-old sheep immunised with normal H. contortus $L_3$, specific antibodies against larval antigens

lambs similarly immunised no local immunological response could be detected.

In summary the work carried out in this thesis demonstrated that primary infections with normal H. contortus $L_3$ in adult worm-free sheep produced a high degree of protection against re-infection. This was associated with a high level of specific antibodies at the site of infection together with a strong cellular reaction which consisted of lymphocytes, plasma cells and mast cells, including I.E. mast cells. In contrast primary infections in worm-free young lambs had no effect on the establishment of a second infection and was associated with both a weak cellular reaction and a poor local antibody response.

Attempts to stimulate immunity in young lambs by using various immunising regimens with different adjuvants and immunostimulants together with parasite antigens, were unsuccessful despite the fact that in some circumstances immunisation appeared to stimulate certain immunoglobulin-cell and mast cell responses.

In a study of long standing H. contortus infection in lambs with small numbers of worms, there was little obvious difference in the pathological changes in the abomasum between infected animals and worm-free controls: however, similar infections of groups of animals maintained either on a high or low protein diet showed that approximately three months after infection in the group of lambs fed concentrates in addition to hay, there was a marked reduction in faecal egg output and only a few worms were recovered at necropsy

two to three months later. In the group fed only a maintenance ration faecal egg output was sustained at a fairly high level and more worms were recovered at necropsy.

An investigation of the significance of passive immunity showed that prior immunisation of ewes had little apparent effect on the subsequent response of their lambs to H. contortus infection. In naturally suckled lambs, however, there was reduced worm establishment compared with that in lambs bottle-fed a dried milk diet. The significance of this observation requires investigation.

After challenge in hyperimmune adult sheep, there were increases in the size and number of lymphoid aggregates and this was associated with mild but diffuse cellular infiltrations of the mucosa. In contrast in non-immunised adults there were fewer lymphoid aggregates but there was more obvious cellular infiltration and damage to the mucosal surface. Although hyper-immunised lambs showed a delay in the development of patent infections after challenge compared with non-immunised controls, the worm burdens of both groups at necropsy were similar. Cellular reactions, although present in both vaccinated and non-vaccinated lambs after challenge were, in contrast to the adult sheep, more marked in the vaccinated group. Also in the non-vaccinated lambs only a few I.E. mast cells were detected 24 days after infection whereas they were present in small numbers at various times after challenge in the vaccinated animals.

From these studies it would appear that the unresponsiveness

of young lambs to H. contortus infection is associated with both a poor cellular and antibody response in the abomasum.

# APPENDICES

| TIME OF NECROPSY | | ADULT SHEEP | | LAMBS | |
|---|---|---|---|---|---|
| Days after first infection | Days after second infection | Animal number | Counts per second | Animal number | Counts per second |
| 0 | - | R100 | 21 | G70 | 10 |
| 7 | - | R87 | 3 | G43 | 0 |
| | | R91 | 47 | G69 | 0 |
| 15 | - | R85 | 39 | G35 | 14 |
| | | R86 | 28 | G50 | 40 |
| 21 | 6 | R88 | 62 | G48 | 40 |
| | | R89 | 55 | G52 | 20 |
| 30 | 15 | R90 | 10 | G56 | 7 |
| | | R96 | 117 | G63 | 1 |
| 42 | 27 | R98 | 75 | G67 | 33 |
| | | R99 | 85 | | |

APPENDIX 1. Mucosal IgA values of sheep and lambs at different times after primary and secondary infection with 350 H. contortus $L_3$/Kg. Bodyweight.

| Group Number | Treatment Regimen | No. of animal | | Days after challenge 24 | 26 |
|---|---|---|---|---|---|
| 1 | C. parvum/H. contortus $L_3$ - vaccination | W | 62 | 17100 | 13800 |
| | | | 63 | 11800 | 20500 |
| | | | 64 | 9300 | 23500 |
| | | | 65 | 2950 | 11700 |
| | | | 66 | 17000 | 29600 |
| 2 | RNA/C. parvum/ H. contortus $L_3$ - vaccination | O | 9 | 7300 | 14000 |
| | | | 11 | 15900 | 15000 |
| | | | 15 | 7600 | 10800 |
| | | | 16 | 3300 | 20400 |
| | | | 17 | 950 | 12700 |
| 3 | C. parvum - vaccination | Y | 1 | 5700 | 26300 |
| | | | 2 | 9100 | 9400 |
| | | | 3 | 8500 | 13000 |
| | | | 4 | 2700 | 13200 |
| | | | 5 | 7800 | 5000 |
| 4 | H. contortus $L_3$ - vaccination | BK | 104 | 5700 | 18100 |
| | | | 106 | 50 | 10000 |
| | | | 109 | 8500 | 14600 |
| | | | 110 | 20400 | 37300 |
| | | | 107 | 6300 | - |
| 5 | Vaccination - vaccination | R | 60 | 36500 | 25500 |
| | | | 72 | 9700 | 8100 |
| | | | 81 | 1550 | 9700 |
| | | | 83 | 800 | 20200 |
| | | | 84 | 1150 | 3900 |
| 6 | Challenge controls | FR | 92 | 9000 | 7300 |
| | | | 93 | 7600 | 9400 |
| | | | 94 | 5700 | 23200 |
| | | | 96 | 19700 | 13500 |
| | | | 91 | 50 | 29500 |
| 7 | Levamisole/vaccination - vaccination | P | 1 | 7300 | 27500 |
| | | | 2 | 3400 | 10600 |
| | | | 3 | 10700 | 3600 |
| | | | 4 | 4550 | 26300 |
| | | | 5 | 16900 | 30300 |
| 8 | Trickle irradiated H. contortus $L_3$/ Levamisole | G | 9 | 2500 | 8200 |
| | | | 10 | 17500 | 19000 |
| | | | 11 | 3700 | 19100 |
| | | | 12 | 2800 | 9100 |
| | | | 14 | 7050 | 20000 |
| 9 | Trickle irradiated H. contortus $L_3$ | BL | 90 | - | 9000 |
| | | | 96 | - | 10400 |
| | | | 97 | 4800 | 11100 |
| | | | 98 | 50 | 8460 |
| | | | 100 | - | - |

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| Group number | Treatment Regimen | No. of animal | Days after challenge 24 | 26 |
|---|---|---|---|---|
| 10 | Worm-free controls | EL 865 | - | - |
| | | 869 | - | - |
| | | 870 | - | - |
| | | 872 | - | - |
| | | 871 | - | - |

APPENDIX 2. Individual faecal egg counts (e.p.g.) of lambs given different immunising treatments prior to a single challenge infection with normal H. contortus $L_3$.

| Group number | Treatment regimen | No. of animal | No. of ♂ worms | No. of ♀ worms | Total |
|---|---|---|---|---|---|
| 1 | C. parvum/H. contortus $L_3$ - vaccination | W 62 | 1150 | 950 | 2100 |
| | | 63 | 1400 | 2150 | 3550 |
| | | 64 | 1650 | 800 | 2450 |
| | | 65 | 1250 | 800 | 2050 |
| | | 66 | 850 | 500 | 1350 |
| 2 | RNA/ C. parvum/H. contortus $L_3$ - vaccination | O 9 | 2100 | 2050 | 4150 |
| | | 11 | 900 | 950 | 1850 |
| | | 15 | 800 | 1100 | 1900 |
| | | 16 | 1200 | 850 | 2050 |
| | | 17 | 1200 | 950 | 2150 |
| 3 | C. parvum - vaccination | Y 1 | 1400 | 1400 | 2850 |
| | | 2 | 1850 | 1650 | 3500 |
| | | 3 | 1050 | 650 | 1700 |
| | | 4 | 1650 | 1500 | 3150 |
| | | 5 | 650 | 850 | 1500 |
| 4 | H. contortus $L_3$ - vaccination | BK 104 | 1450 | 1750 | 3200 |
| | | 106 | 950 | 350 | 1300 |
| | | 109 | 2100 | 2300 | 4400 |
| | | 110 | 2500 | 2000 | 4500 |
| 5 | Vaccination - vaccination | R 60 | 850 | 550 | 1400 |
| | | 72 | 2850 | 1400 | 4250 |
| | | 81 | 1100 | 850 | 1950 |
| | | 83 | 2000 | 1500 | 3500 |
| | | 84 | 1550 | 600 | 2150 |
| 6 | Challenge controls | FR 92 | 850 | 1300 | 2150 |
| | | 93 | 800 | 550 | 1350 |
| | | 94 | 1150 | 1350 | 2500 |
| | | 96 | 1850 | 1550 | 3400 |
| 7 | Levamisole/vaccination - vaccination | P 1 | 1700 | 1400 | 3100 |
| | | 2 | 1900 | 1550 | 3450 |
| | | 3 | 550 | 300 | 850 |
| | | 4 | 1050 | 950 | 2000 |
| | | 5 | 2750 | 2250 | 5000 |
| 8 | Trickle irradiated H. contortus $L_3$/Levamisole | G 9 | 950 | 850 | 1800 |
| | | 10 | 600 | 950 | 1550 |
| | | 11 | 1050 | 300 | 1350 |
| | | 12 | 1150 | 1300 | 2450 |
| | | 14 | 1800 | 1450 | 3250 |
| 9 | Trickle irradiated H. contortus $L_3$ | BL 90 | 800 | 700 | 1500 |
| | | 96 | 1500 | 1450 | 2950 |
| | | 97 | 1650 | 1950 | 3600 |
| | | 98 | 700 | 700 | 1400 |
| | | 100 | Died | - | - |

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| Group number | Treatment regimen | No. of animals | No. of worms | No. of worms | Total |
|---|---|---|---|---|---|
| 10 | Worm-free controls | EL 868 | - | - | - |
| | | 869 | - | - | - |
| | | 870 | - | - | - |
| | | 872 | - | - | - |
| | | 871 | - | - | - |

APPENDIX 3. The individual worm burdens at necropsy of lambs given different immunising treatments prior to a single challenge infection with normal H. contortus $L_3$.

| Group No. | Treatment Regimen | Number | WEEKS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Prior to challenge | | | | | | | | | Post challenge | | | | |
| | | | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 0 | 1 | 2 | 3 | 4 |
| 1 | C. parvum/H. contortus $L_3$ - vaccination | W 62 | 33 | 33 | 27 | 30 | 31 | 27 | 30 | 30 | 28 | 24 | 26 | 28 | 21 | 20 |
| | | 63 | 30 | 31 | 27 | 30 | 31 | 27 | 26 | 29 | 26 | 23 | 25 | 27 | 19 | 24 |
| | | 64 | 28 | 33 | 29 | 28 | 29 | 26 | 25 | 26 | 25 | 24 | 24 | 26 | 18 | 18 |
| | | 65 | 28 | 28 | 25 | 27 | 28 | 26 | 26 | 27 | 25 | 23 | 25 | 25 | 30 | 17 |
| | | 66 | 29 | 29 | 26 | 25 | 25 | 27 | 25 | 28 | 25 | 24 | 24 | 25 | 16 | 17 |
| 2 | RNA/C. parvum/H. contortus $L_3$ - vaccination | O 9 | 29 | 30 | 30 | 32 | 31 | 30 | 29 | 30 | 26 | 26 | 27 | 28 | 19 | - |
| | | 11 | 33 | 32 | 27 | 27 | 28 | 27 | 27 | 28 | 28 | 26 | 26 | 26 | 20 | 21 |
| | | 14 | 33 | 35 | 29 | 29 | 31 | 26 | 29 | 18 | 26 | 25 | 28 | 29 | 22 | - |
| | | 15 | 31 | 32 | 26 | 27 | 27 | 24 | 27 | 29 | 25 | 27 | 27 | 28 | 24 | 22 |
| | | 16 | 32 | 32 | 29 | 31 | 20 | 28 | 29 | 30 | 27 | 25 | 26 | 28 | 22 | 23 |
| 3 | C. parvum - vaccination | Y 1 | 32 | 35 | 30 | 31 | 28 | 29 | 23 | 27 | 24 | 22 | 25 | 24 | 17 | 18 |
| | | 2 | 32 | 29 | 28 | 28 | 29 | 29 | 28 | 31 | 25 | 23 | 28 | 26 | 21 | 26 |
| | | 3 | 29 | 30 | 27 | 26 | 28 | 25 | 27 | 28 | 24 | 24 | 25 | 25 | 23 | 23 |
| | | 4 | 30 | 31 | 28 | 27 | 30 | 27 | 30 | 31 | 27 | 27 | 27 | 28 | 23 | 25 |
| | | 5 | 32 | 32 | 30 | 30 | 28 | 27 | 28 | 28 | 27 | 24 | 25 | 27 | 21 | 25 |
| 4 | H. contortus $L_3$ - vaccination | BK 104 | 27 | 32 | 17 | 24 | 26 | 26 | 27 | 27 | 25 | 25 | 26 | 26 | 18 | 22 |
| | | 106 | 22 | 19 | 26 | 19 | 18 | 18 | 19 | 22 | 19 | 18 | 19 | 21 | 14 | 14 |
| | | 109 | 31 | 29 | 26 | 27 | 28 | 27 | 25 | 27 | 23 | 23 | 24 | 23 | 21 | 21 |
| | | 110 | 32 | 25 | 26 | 27 | 27 | 29 | 28 | 28 | 27 | 25 | 27 | 27 | 19 | 16 |
| | | 107 | 38 | 39 | 31 | 34 | 34 | 32 | 32 | 32 | 28 | 29 | 30 | 31 | 31 | 39 |
| 5 | Vaccination - vaccination | R 72 | 30 | 29 | 24 | 25 | 16 | 17 | 19 | 20 | 25 | - | 19 | 18 | 14 | - |
| | | 81 | 31 | 31 | 27 | 28 | 19 | 22 | 20 | 25 | 24 | 23 | 25 | 26 | 17 | - |
| | | 83 | 31 | 32 | 30 | 29 | 16 | 18 | 21 | 23 | 23 | 22 | 22 | 23 | 18 | - |
| | | 84 | 30 | 29 | 27 | 26 | 17 | 22 | 22 | 22 | 25 | 23 | 23 | - | 19 | - |
| | | 60 | 37 | 34 | 32 | 32 | 18 | 18 | 22 | 18 | 20 | 20 | 22 | 25 | 18 | - |

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| Group No. | Treatment Regimen | Number | WEEKS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Prior to challenge | | | | | | | | | | Post challenge | | | |
| | | | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 0 | 1 | 2 | 3 | 4 |
| 6 | Challenge controls | FR 92 | 31 | 33 | 28 | 31 | 30 | 31 | 30 | 29 | 31 | 28 | 32 | 31 | 25 | 27 |
| | | 93 | 33 | 34 | 31 | 31 | 33 | 33 | 32 | 33 | 32 | 33 | 32 | 32 | 24 | 21 |
| | | 94 | 34 | 34 | 30 | 34 | 28 | 29 | 28 | 28 | 27 | 28 | - | 28 | 18 | 18 |
| | | 95 | 37 | 35 | 31 | 30 | 32 | 33 | 32 | 33 | 35 | 31 | 33 | 32 | 18 | 20 |
| | | 91 | 37 | 36 | 30 | 31 | 29 | 30 | 31 | 29 | 30 | 29 | 31 | 31 | 19 | - |
| 7 | Levamisole/vaccination - vaccination | P 1 | 28 | 30 | 28 | 29 | 18 | 20 | 25 | 27 | 24 | 26 | 25 | 27 | 20 | 17 |
| | | 2 | 33 | 34 | 27 | 29 | 17 | 20 | 22 | 26 | 24 | 23 | 24 | 26 | 17 | 16 |
| | | 3 | 30 | 32 | 32 | 30 | 26 | 23 | 26 | 32 | 31 | 30 | 30 | 32 | 24 | 27 |
| | | 4 | 29 | 29 | 24 | 24 | 16 | 19 | 22 | 28 | 22 | 22 | 24 | 23 | 15 | 15 |
| | | 5 | 29 | 30 | 26 | 27 | 19 | 23 | 26 | 30 | 27 | 27 | 25 | 25 | 19 | 14 |
| 8 | Trickle irradiated H. contortus $L_3$/Levamisole | G 9 | 33 | 34 | 29 | 26 | 33 | 22 | 29 | 30 | 31 | 29 | 30 | 29 | 23 | 25 |
| | | 10 | 27 | 28 | 25 | 28 | 30 | 28 | 24 | 26 | 30 | 28 | 28 | 27 | 18 | 21 |
| | | 11 | 34 | 33 | 30 | 32 | 32 | 29 | 30 | 31 | 33 | 30 | 28 | 21 | 20 | 22 |
| | | 12 | 34 | 32 | 32 | 33 | 32 | 29 | 27 | 31 | 30 | 29 | 31 | 27 | 20 | 26 |
| | | 14 | 28 | 32 | 33 | 30 | 34 | 32 | 27 | 28 | 28 | 29 | 30 | 29 | 20 | 24 |
| 9 | Trickle irradiated H. contortus $L_3$ | BL 90 | 30 | 32 | 29 | 29 | 29 | 29 | 27 | 26 | 26 | 25 | 27 | 28 | 21 | - |
| | | 96 | 30 | 32 | 26 | 26 | 29 | 30 | 27 | 26 | 30 | 29 | 31 | 31 | 23 | - |
| | | 97 | 30 | 35 | 29 | 29 | 32 | 31 | 27 | 29 | 30 | 31 | 31 | 29 | 25 | - |
| | | 98 | 33 | 36 | 27 | 28 | 26 | 25 | 21 | 29 | 9 | - | 33 | 29 | 24 | - |
| | | 100 | 30 | 36 | 30 | 27 | 25 | 19 | 15 | 12 | Died | - | - | - | - | - |

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| Group No. | Treatment Regimen | Number | WEEKS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Prior to challenge | | | | | | | | | Post challenge | | | | |
| | | | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 0 | 1 | 2 | 3 | 4 |
| 10 | Worm-free controls | EL 868 | 35 | 34 | 32 | 33 | 33 | 35 | 32 | 35 | 30 | 29 | - | 33 | 33 | 21 |
| | | 869 | 36 | 36 | 32 | 37 | 30 | 37 | 33 | 31 | 31 | 32 | 33 | 31 | 32 | 34 |
| | | 870 | 27 | 29 | 26 | 27 | 29 | 30 | 27 | 27 | 29 | 29 | 29 | 28 | 30 | 36 |
| | | 872 | 33 | 34 | 30 | 30 | 31 | 32 | 30 | 34 | 33 | 34 | 34 | 35 | 34 | - |
| | | 871 | 33 | 34 | 31 | 28 | 30 | 31 | 32 | 27 | 20 | 27 | 30 | 29 | 12 | 21 |

APPENDIX 4. The individual PCV's (%) of lambs given different immunising treatments prior to a single challenge infection with normal H. contortus $L_3$.

| Weeks after Infection | Group 2 HAY | | | | | | Group 3 HAY | | | | | Group 4 HAY AND CONCENTRATES | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of animal | | | | | | No. of animal | | | | | No. of animal | | | | |
| | G2 | G18 | G5 | G6 | G3 | G7 | Y7 | Y8 | Y9 | Y10 | Y11 | P14 | P15 | P16 | P17 | P18 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 100 | 100 | 150 | 100 | 550 | 1750 | 450 | 200 | 0 | 300 | 300 | 1650 | 550 | 1250 | 800 | 300 |
| 4 | 950 | 1300 | 550 | 1050 | 2450 | 2600 | 1300 | 1400 | 400 | 2900 | 1600 | 2650 | 1700 | 700 | 1650 | 2700 |
| 5 | 1900 | 3500 | 900 | 1450 | 2400 | 1850 | 3200 | 900 | 700 | 2450 | 2450 | 2950 | 2250 | 550 | 2250 | 3600 |
| 6 | 1850 | 1800 | 1700 | 3300 | 950 | 2300 | 3250 | 250 | 300 | 1200 | 4300 | 1700 | 50 | 1900 | 950 | 5200 |
| 7 | 2050 | 3100 | 2800 | 2250 | 2050 | 850 | 5600 | 5000 | 750 | 3050 | 7500 | 5250 | 2100 | 200 | 4500 | 2400 |
| 8 | 2650 | 3100 | 3150 | 3000 | 3350 | 2200 | 1750 | 1100 | 1050 | 3650 | 5450 | 2950 | 1000 | 100 | 3050 | 3050 |
| 9 | - | - | 600 | 1350 | 2400 | 3900 | 4150 | 3300 | 1400 | 2750 | 3000 | 2850 | 1950 | 150 | 2750 | 2550 |
| 10 | - | - | 2450 | 2250 | 1950 | 2950 | 3050 | 1550 | 900 | 2550 | 2650 | 4850 | 1950 | 50 | 2200 | 1600 |
| 11 | - | - | 3250 | 3150 | 2300 | 4350 | 4900 | 4850 | 4550 | 2900 | 3800 | 4400 | 2650 | 550 | 5550 | 2600 |
| 12 | - | - | 2850 | 3450 | 4350 | 3550 | 4600 | 2450 | 3600 | 3350 | 4800 | 4750 | 2500 | 350 | 4700 | 2900 |
| 13 | - | - | 4250 | 2800 | 4300 | 2200 | 4200 | 2500 | 4500 | 2300 | 2900 | 3500 | 2500 | 500 | 4900 | 2300 |
| 14 | - | - | 3400 | 2200 | 4350 | 2300 | 3500 | 1600 | 2800 | 2700 | 2900 | 3000 | 2600 | 400 | 5000 | 0 |
| 15 | - | - | 5200 | 2950 | 1300 | 3600 | 2500 | 2300 | 1350 | 2050 | 3850 | 400 | 450 | 0 | 0 | 0 |
| 16 | - | - | 3000 | 2250 | 2050 | 1650 | 1050 | 1500 | 1300 | 1500 | 2450 | 100 | 300 | 0 | *N.S | 0 |
| 17 | - | - | - | - | 2050 | 1650 | 1050 | 1500 | 1300 | 1500 | 2450 | 100 | 300 | 0 | N.S | 0 |
| 18 | - | - | - | - | 2000 | 1550 | 2450 | 750 | 1250 | 1150 | 1300 | 500 | 300 | 50 | 0 | 0 |
| 19 | - | - | - | - | 1700 | 1300 | 2500 | 850 | 950 | 1150 | 2300 | 50 | 200 | 0 | 0 | 0 |
| 20 | - | - | - | - | 4500 | 1750 | 1300 | 700 | 700 | 1100 | 1500 | 550 | 150 | 0 | 0 | 0 |
| 21 | - | - | - | - | 750 | 50 | 1100 | 200 | 200 | 200 | 1150 | 0 | 0 | 0 | 0 | 0 |
| 22 | - | - | - | - | - | - | 50 | 200 | 550 | 0 | 0 | 0 | 0 | 0 | 150 | 0 |

APPENDIX 5. Individual faecal egg counts (e.p.g.) of lambs infected with five consecutive daily doses of 200 H. contortus L

* N.S. = No sample.

| Lambs from vaccinated ewes | PCV% | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Days pre-infection | | | | | | Days post-infection | | | |
| | 34 | 26 | 19 | 12 | 5 | 0 | 2 | 10 | 18 | 26 |
| LY 90 | 31 | 29 | 35.5 | 34 | 33 | | 33.5 | 33 | 28 | 30 |
| 91 | 35 | 32 | 31.5 | 32 | 32 | | 31 | 30 | 25.5 | 28.5 |
| 92 | 36 | 35 | 34 | 34.5 | 36.5 | | 35 | 32 | 26 | 27.5 |
| 96 | 32.5 | 34 | 34.5 | 33 | 36.5 | | 35 | 32.5 | 31 | 28 |
| 97 | 37 | 36.5 | 37.5 | 35.5 | 34 | | 35 | 25 | 33.5 | 32.5 |
| 98 | 32 | 29 | 34 | 31 | 34 | | 32 | 35 | 30.5 | 29 |
| Mean | 34 | 32.5 | 34.5 | 33 | 34.5 | | 33.5 | 31 | 29 | 29 |
| Lambs from non-vaccinated ewes | | | | | | | | | | |
| LP 85 | - | 33 | 27 | 30.5 | 37 | | 43 | 38.5 | 31.5 | 32 |
| 86 | 57 | 38 | 31 | 31.5 | 33 | | 32.5 | - | 31 | 33.5 |
| 87 | 38 | - | 34.5 | 33.5 | 31.5 | | 30 | 30 | 30.5 | 27 |
| 88 | 40 | 37 | 29.5 | 31 | 29.5 | | 30 | 30 | - | 25 |
| 90 | 33 | 31 | 32 | 34 | 34 | | 37.5 | 35 | 36 | 34 |
| 97 | - | - | - | - | - | | - | - | - | - |
| Mean | 42 | 35 | 31 | 32 | 33 | | 34.5 | 33.5 | 32 | 30.5 |

APPENDIX 6. Individual PCV's of groups of lambs derived from vaccinated and non-vaccinated ewes pre- and post-infection with 2000 *H. contortus* $L_3$.

| Group No. | Animals | Group Mean PCV | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Weeks pre-challenge | | | | | | | | Weeks post-challenge | | | |
| | | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 1 | 2 | 3 | 4 |
| 1 | Vaccinated ewes | *27 | 27 | 30 | 29 | 31 | 31 | 29.5 | 30 | 32.5 | 33.5 | 33.5 | 35.5 |
| 2 | Non-vaccinated ewes | 31 | 31 | 33 | 33 | 33 | 32.5 | 30 | 32 | 30 | 33.5 | 21.5 | 30 |
| 3 | Vaccinated lambs | 35 | 31 | 25 | 23 | 27 | 26 | 28 | 27 | 31.5 | 28 | 25.5 | 25.5 |
| 4 | Non-vaccinated lambs | 39 | 37 | 32 | 31 | 31 | 31 | 30 | 32.5 | 33 | 27.5 | 23 | 21 |

* Received first dose of vaccine 10 weeks earlier than the lambs.

APPENDIX 7. Mean PCV's of groups of vaccinated and non-vaccinated ewes and lambs pre- and post-challenge with normal H. contortus $L_3$.

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