Changes in the zymogenic cell populations of the abomasa of sheep infected with *Haemonchus contortus*

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SUMMARY

The effects of dietary urea supplementation and of a 10-week trickle infection regime, simulating chronic exposure to *Haemonchus contortus*, on the zymogenic population of the abomasa of Hampshire Down lambs was examined. At necropsy a variety of parameters including plasma pepsinogen concentrations, the wet weights of abomasal fundic mucosal pieces and the amounts of pepsinogen contained in them, were assessed. Tissue pepsinogen concentration was measured as the total, acid-stable proteolytic activity present in mucosal homogenates, as well as immunohistochemically. The immunohistochemical findings were quantified using computer-aided image analysis. Elevation of plasma pepsinogen concentrations in infected animals was of borderline significance ($P = 0{\cdot}06$). The fundic mucosae of infected animals were heavier ($P < 0{\cdot}02$) than those of control animals, but there was no overall change in the pepsinogen content of tissues. Immunohistochemistry revealed that infected animals had increased numbers of zymogenic cells, due to mucous cell hyperplasia and the adaptation of cells to produce both mucins and pepsinogen. The pepsinogen content of chief cells, the major source of pepsinogen in uninfected animals, was reduced in infected lambs. Image analysis confirmed that at a mid-point of the mucosa of infected animals there was increased pepsinogen-specific immunoreactivity that corresponded with areas of mucosal hyperplasia. Mucous cell hyperplasia might therefore allow the maintenance of pepsinogen secretion in infected animals even if chief cell output is reduced.

Key words: *Haemonchus contortus*, sheep, pepsinogen, immunohistochemistry, peptic activity, mucous cell hyperplasia.

INTRODUCTION

Haemonchosis is associated with hyperplasia of the abomasal epithelium and biochemical changes which include elevated abomasal pH and increased concentrations of plasma pepsinogen and plasma gastrin (Mapes & Coop, 1970; Hunter & MacKenzie, 1982; Nicholls *et al*. 1988; Simpson *et al*. 1997). Similar changes occur in association with infection with *Ostertagia* spp. parasites (Armour, Jarrett & Jennings, 1966; Jennings *et al*. 1966; Ritchie *et al*. 1966; Anderson, Hansky & Titchen, 1981; Fox *et al*. 1987).

In infected animals, pepsinogen was originally thought to leak into the bloodstream through immature cell junctions that exist between hyperplastic epithelial cells (Murray, 1969), but elevated plasma concentrations of the zymogen may also be due to the direct stimulation of host secretion by the parasites themselves (McKellar *et al*. 1986, 1987; McKellar, Mostofa & Eckersall, 1990; Lawton *et al*. 1996; Simpson *et al*. 1997) or by gastrin (Fox *et al*. 1989).

Hyperplasia in ostertagiosis is largely confined to the vicinity of parasitized glands and appears macroscopically as nodules. Hyperplasia within nodules affects the full mucosal thickness, with the mature secretory cells of the gastric glands, the pepsinogen-producing chief cells and acid-secreting parietal cells, being replaced by a population of immature cells phenotypically closer in appearance to mucous cells (Murray, Jennings & Armour, 1970). In contrast, *Haemonchus* larvae emerge from the gastric glands at the 4th larval stage, 2–3 days after gland penetration and are thereafter ambulatory over the abomasal surface (Charleston, 1965; Nicholls, Lee & Sharpe, 1985). In haemonchosis, hyperplasia is therefore generalized and is thought to occur only in the more superficial layers of the epithelium, so that parietal and chief cell numbers are not necessarily affected (Charleston, 1965; Hunter & MacKenzie, 1982).

Cybulski & Andren (1990) localized pepsinogen to chief cells in the gastric glands of the bovine fundus, but also demonstrated the presence of pepsinogen in surface mucous cells of the gastric pits and mucous

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neck cells. Changes in the numbers of any group of cells could therefore affect the quantity of pepsinogen contained within the abomasum and consequently affect rates of secretion. If hyperplasia resulted in an increase in the total number of cells secreting pepsinogen then enhanced secretion of zymogen could be an important component of the mechanism of elevation of plasma pepsinogen operating in haemonchosis.

In the present study, the production of pepsinogen in ovine abomasa in the presence and absence of infection with *Haemonchus contortus* was investigated biochemically and immunohistochemically. Chronic haemonchosis was simulated by trickle infections of Hampshire Down lambs over a 10-week period. Lambs were fed either a basal, maintenance diet or one supplemented by urea. Various parasitological, haematological and blood biochemistry parameters were monitored during the trickle infection period and these and the effects of diet on the resilience and resistance of animals to infection are discussed fully elsewhere (Wallace *et al*. 1998).

MATERIALS AND METHODS

Experimental design

Twenty-four 5-month-old Hampshire Down lambs, that had been reared in helminth-free conditions, were allocated into 4 groups by stratified random sampling (Wallace *et al*. 1998). Half the lambs were fed a basal diet (BD) and the other half (SD) were fed a ration supplemented with urea at 22 g/kg dry matter. Feeding was begun initially at the rate of 1·4 kg fresh matter/day and was increased at fortnightly intervals to maintain the same rate of feeding/kg liveweight. Lambs of the BDC and SDC groups ($n = 4$) remained as uninfected controls, whereas animals in the BDI and SDI groups ($n = 8$), each received a single oral dose of 100 *H. contortus* 3rd-stage larvae/kg bodyweight, followed by a trickle infection regime of 200 L_3, three times a week, for the next 10 weeks. Infective larvae were obtained from the Moredun Research Institute (Edinburgh, Scotland). All lambs were killed at the end of the 10-week period.

Procedure at necropsy

Animals were killed by exsanguination following electrical stunning. Blood was collected as the sheep were bled out, into heparinized containers which were later centrifuged at 2000 ***g*** in a Beckman GS-6R centrifuge for 10 min. Plasma was then separated and stored at −20 °C. Blood was also collected into glass tubes containing EDTA for haematological examination. Faeces were collected from each animal to allow the determination of faecal egg counts (Gordon & Whitlock, 1939). Abomasa were removed and were later opened along the greater curvature and their contents were collected. This was generally accomplished within 30 min of necropsy. One half of the abomasal mucosa was digested in pepsin-hydrochloric acid for 6 h at 42 °C to allow the estimation of tissue-dwelling stages. Worm burdens were determined using standard parasitological techniques (Armour *et al*. 1966).

Samples of fundic mucosal tissue were taken from the other abomasal half, using a biopsy punch (9·5 mm diameter). Mucosal pieces were therefore of identical areas. Ten tissues were taken from the fundic mucosal folds of each animal in a random pattern, 5 were immediately immersed in Brunnel's fixative (Laboratory Supplies and Instruments Limited, Antrim, Northern Ireland), the other 5 were added to plastic tubes on ice (0–4 °C) and were later weighed and homogenized to allow estimation of tissue pepsinogen concentrations. In addition to immunohistochemical stains, tissue sections were also stained with haematoxylin and eosin (H & E) and periodic acid–Schiff (PAS).

Total plasma protein and plasma albumin concentrations were measured by continuous flow analysis (Standard Technicon Auto Analyser II). Packed cell volumes were measured by micro-haematocrit centrifugation.

Plasma pepsinogen concentrations were measured using a previously validated method (Scott, Stear & McKellar, 1995). The method was based on the digestion of glycine-buffered, ovine serum albumin, following the conversion of pepsinogen to active pepsin at pH < 2. Peptic activity breaks the substrate into small peptide fragments that are measurable colorimetrically using copper complexes. Plasma pepsinogen concentrations were expressed in international units (1 i.u. = 1 μmol tyrosine equivalents released/min/l of plasma).

Biochemical estimation of tissue pepsinogen concentration

Tissue pepsinogen concentrations were estimated by measuring the total, acid-stable proteolytic activity of tissue homogenates using a modification of the method employed by Basson, Adrian & Modlin (1990). Weighed tissue pieces were thoroughly homogenized in 10 ml of a solution containing 0·1 % Triton X-100 (BDH Chemicals, Poole, UK) in 0·01 M HCl (pH 2·0). Pepsinogen concentration was then determined in the supernatant produced following centrifugation (2000 ***g*** for 30 min) using the same method as that for plasma pepsinogen estimation.

Pepsinogen immunohistochemistry

Immunolocalization of pepsinogen was determined on 2 μm thick paraffin sections of abomasal mucosae.

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Endogenous peroxidase was blocked with 0·5% hydrogen peroxide in methanol for 30 min, and sections were then treated with 0·1% trypsin solution (Sigma Chemical Co., Poole, UK) at 37 °C for a further 30 min. Pepsinogen was visualized using a commercial antiserum for rabbits immunized with highly purified bovine pepsinogen type I (Chris Hansen's Laboratories Limited, Reading, UK) and a commercial, Avidin Biotinylated-enzyme Complex kit (Vector Laboratories Limited, Peterborough, UK). The primary antiserum, used at 1:10000 dilution, was incubated on the sections overnight at 4 °C, after which the secondary, biotinylated and peroxidase-complexed antibody was incubated on the sections for a further 30 min. Bound peroxidase was then visualized using 0·1% diaminobenzidine tetrahydrochloride (DAB; BDH Chemicals Limited, Poole, UK). Two types of negative staining control were performed, the first by omitting the primary antiserum and the second by replacing the primary antiserum with normal, non-immune rabbit serum (Vector Laboratories Ltd). Sections were prepared with or without haematoxylin counterstain and non-counterstained sections were used in computer-aided image analysis to quantify the amount and distribution of pepsinogen-specific immunoreactivity.

Image analysis

Image analysis was performed using the NIH Image (version 1.52) software package (National Institutes of Health, Research Services Branch, USA) and a Sony CCD video camera module (model XC-77CE) linked to an Olympus BH2 light microscope. The system utilized captured monochrome images of each mucosal section, using a constant illumination setting and a constant magnification. Pepsinogen-specific immunoreactivity was then measured in a standard plot measuring 400×550 screen pixels. The plot was placed on the mucosal image with the glands in longitudinal section so that the base of the plot was adjacent to the base of the glands. The long side of the plot was oriented parallel to the longitudinal axis of the glands and was sufficient to encompass the total mucosal depth of all animals, from base to luminal surface. The width of the plot allowed approximately 20 adjacent glands to be scanned. The computer was then able to generate a plot profile that was, in essence, the average densities for each of 550 pixel columns. Staining patterns were consistent from one part of a tissue section to another so that a single scan was performed for each of the 5 sections from any one animal and therefore the overall results represent measurements of approximately 100 glands per animal.

The 550 separate data points from each scan were condensed to 55 by taking the mean of every 10 consecutive values. The background pixel density, i.e. the pixel density of non-stained areas, was subtracted, then an overall mean profile was obtained from the 5 scans for each animal. From this data an 'area under the curve' (AUC) for each animal was calculated by the summation of all data points and this represented the total of pepsinogen-specific immunoreactivity.

Statistical analyses

Faecal egg counts at necropsy, for infected animals on the basal and supplemented diets, were compared using a 2-sample *t*-test. Total worm burdens were similarly compared with a 2-sample *t*-test. Packed cell volumes, and the concentrations of total plasma protein, plasma albumin and plasma pepsinogen for individual animals were compared by analyses of variance, using general linear model procedures. Tissue wet weights, tissue pepsinogen concentrations (both i.u./area and i.u./g data) and AUCs were similarly compared. Because tissue pepsinogen concentration, measured as peptic units/mucosal area, and AUC values for the quantity of immunostaining, were essentially measures of the same thing, the 2 parameters were compared using a Pearson product moment correlation. The image analysis data for all animals were compared with a multivariate, mixed model analysis of variance. This analysis compared staining intensities amongst the 4 groups, at each position of the mucosa, from the deepest (position 1) to the most superficial (position 42). Position 42 was the highest position for any animal (i.e. the most superficial) at which a positive value for pixel density was recorded.

RESULTS

Parasitological and blood biochemistry data

Total worm burdens, the numbers of the different parasite stages and faecal egg counts, for each group are given in Table 1. Despite a lower mean faecal egg count in supplemented animals versus animals on the basal ration, the difference was not significant by *t*-test ($P = 0{\cdot}20$) and total worm burdens in the 2 groups of infected animals were not significantly different ($P = 0{\cdot}90$). Values for packed cell volumes, total plasma protein, plasma albumin, and plasma pepsinogen concentrations for all 4 groups are shown in Table 2. Packed cell volumes were significantly lowered by infection ($P < 0{\cdot}001$), but were not affected by diet ($P = 0{\cdot}17$). Similarly, total plasma proteins were reduced by infection ($P < 0{\cdot}02$) and were unaffected by diet ($P = 0{\cdot}14$). Albumin levels were affected by both diet ($P < 0{\cdot}01$) and infection ($P < 0{\cdot}001$) with lowest values recorded in infected animals fed the basal ration. The effect of infection on plasma pepsinogen concentration was only of borderline significance ($P = 0{\cdot}06$), the effects of diet

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Table 1. Mean (±S.E.) larval and adult worm burdens, and faecal egg counts of animals of the 4 groups of lambs

(BDC, control animals on the basal diet; BDI, infected animals, basal diet; SDC, control animals, supplemented diet; SDI, infected animals, supplemented diet.)

Group	*n*	Worms L_4	L_5	Male	Female	Total	FEC (epg)
BDC	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BDI	8	44 (20)	256 (73)	1156 (160)	806 (110)	2262 (293)	9688 (2185)
SDC	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	288 (169)
SDI	8	112 (92)	112 (28)	1144 (113)	944 (108)	2312 (248)	6469 (1007)

Table 2. Mean (±S.E.) values for packed cell volume (PCV), total plasma protein, plasma albumin and plasma pepsinogen concentrations of animals of the 4 groups of lambs

(BDC, control animals on the basal diet; BDI, infected animals, basal diet; SDC, control animals, supplemented diet; SDI, infected animals, supplemented diet.)

Group	*n*	PCV (%)	Total protein (g/l)	Albumin (g/l)	Pepsinogen (i.u.)
BDC	4	33 (1·14)	65 (1·60)	23 (0·85)	1·84 (0·81)
BDI	8	24 (1·06)	55 (2·46)	18 (0·61)	4·08 (0·40)
SDC	4	33 (1·20)	68 (1·63)	25 (1·25)	2·14 (0·40)
SDI	8	27 (0·90)	58 (1·36)	21 (0·74)	3·04 (0·92)

Table 3. Mean (±S.E.) wet weights and tissue pepsinogen concentrations (expressed as i.u./standard area and as i.u./g wet weight of tissue) of animals of the 4 groups of lambs

(BDC, control animals on the basal diet; BDI, infected animals, basal diet; SDC, control animals, supplemented diet; SDI, infected animals, supplemented diet.)

Group	*n*	Wet weight (g)	Tissue pepsinogen concentration (i.u./area)	(i.u./g)
BDC	4	0·0907 (0·0055)	4179 (2345)	42732 (23056)
BDI	8	0·1000 (0·0032)	5207 (517)	51719 (4402)
SDC	4	0·0784 (0·0011)	6733 (1362)	86748 (18039)
SDI	8	0·0958 (0·0054)	3289 (851)	31977 (7231)

and of the interactive term, between diet and infection, were not significant ($P = 0·65$ and 0·40 respectively).

Tissue pepsinogen concentrations

Mean group values for tissue wet weights and tissue pepsinogen concentrations are given in Table 3. Tissues from infected animals were significantly heavier ($P < 0·02$) and this change was unaffected by diet ($P > 0·05$). Variation in tissue pepsinogen concentration among individual sheep was considerable; values ranged from a mean (±S.E.) tissue pepsinogen of 172 (100) i.u./area of mucosa for one animal (SDI group) to 10800 (744) i.u./area for another (SDC). Higher overall mean tissue pepsinogen concentrations (measured as i.u./area and as i.u./g) were recorded in the uninfected sheep receiving the supplemented diet (SDC), but were lower in the infected animals on the same diet (SDI). In contrast, the infected animals on the basal diet (BDI) had slightly more pepsinogen than the control animals (BDC). From the analyses of variance for tissue pepsinogen, the term for the interaction between diet and infection was either of borderline significance ($P = 0·07$, for i.u./area data) or was

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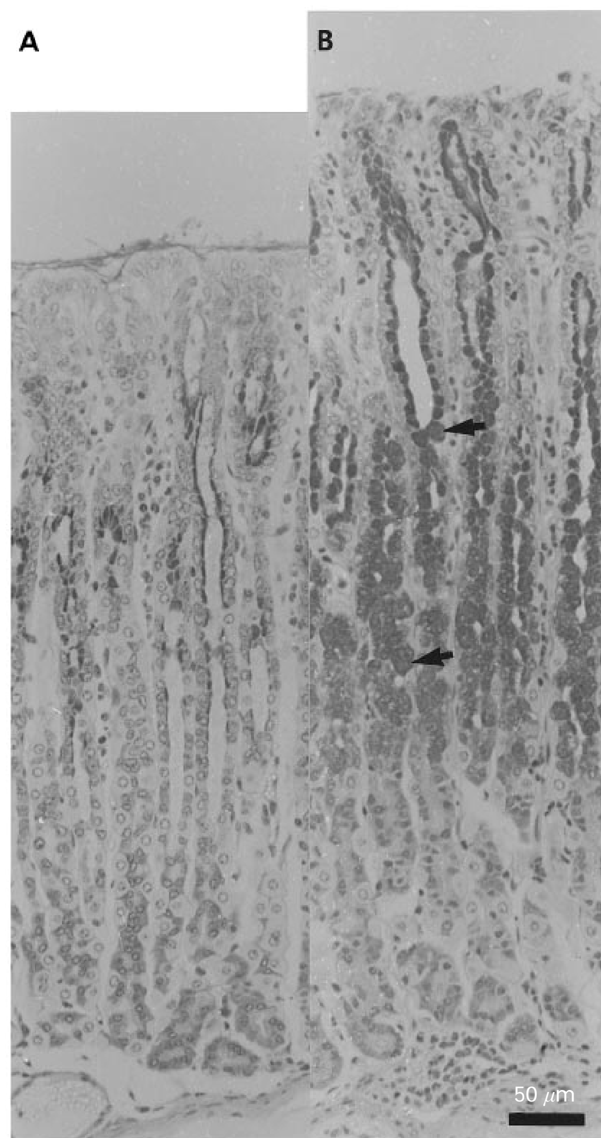

Fig. 1. Composite micrograph showing the distribution of mucins within the mucosae of an uninfected control animal (A) and an animal infected with *Haemonchus contortus* (B). The arrows indicate the hyperplastic and hypertrophied mucous cells in the infected animal. (H & E and PAS.)

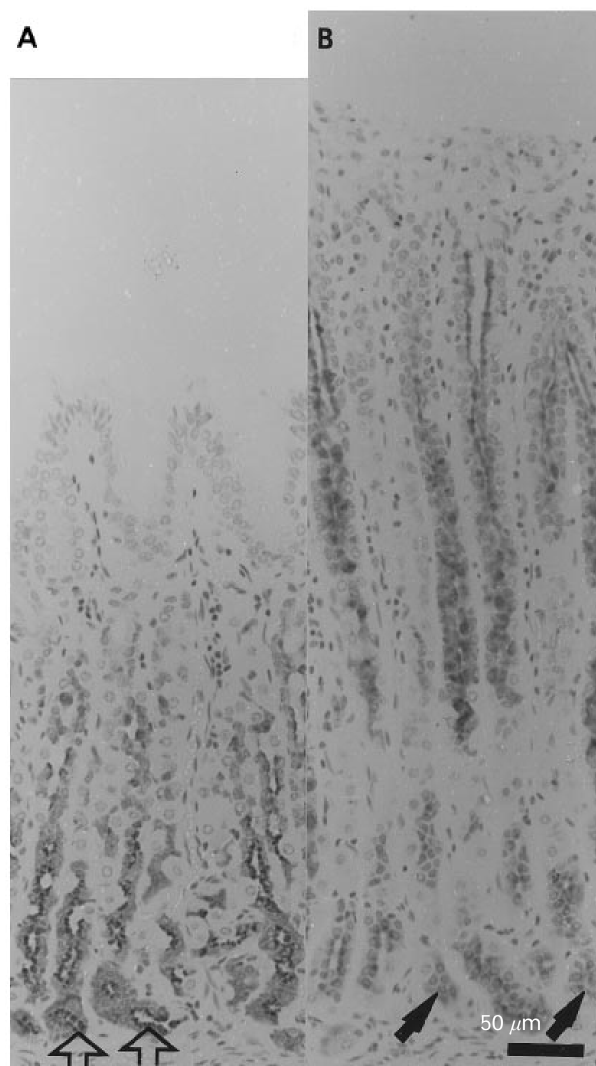

Fig. 2. Composite micrograph showing the distribution of pepsinogen within the mucosae of an uninfected control animal (A) and an animal infected with *Haemonchus contortus* (B). Note the paler staining of chief cells (solid arrows) at the deep gland base in (B) compared with the stronger apical immunoreactivity (open arrows) of normal chief cells in (A). (Haematoxylin counterstain.)

significant ($P < 0{\cdot}05$, for i.u./g data), and the effects of both diet and infection on their own were not significant.

Pathology

The mucosae of infected animals appeared thicker at necropsy, but no other gross pathological changes were detected within the abomasa or other viscera of any of the animals. The fundic mucosae of all control animals, examined in haematoxylin and eosin (H & E) and periodic acid–Schiff (PAS)-stained sections, appeared normal. The pits were short and lined by tall surface mucous cells, containing moderate amounts of neutral mucus, as indicated by the presence of PAS-positive material (Fig. 1 A) and the epithelium was continuous across the mucosal surface. Pits were short, seldom greater than $\frac{1}{5}$ to $\frac{1}{4}$ of the total depth of the mucosa and each pit was usually associated with 2 or 3 glands.

Histopathological changes in infected animals were similar in both dietary groups. There was minimal disruption and loss of epithelial cells at the mucosal surface. Increased mucosal depth was predominantly due to hyperplasia of mucous cells, with moderate hypertrophy, occurring mid-way through the mucosa and in PAS-stained sections there was increased staining for mucins (Fig. 1 B). Pits were narrower, appeared more numerous, and were tightly packed in the superficial mucosa of infected animal abomasa. The whole pit/gland unit had become straight and tubular and the isthmus

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was no longer as distinct. Parietal cells, although not quantified in the present study, appeared fewer in number in mid-mucosal levels, but were still numerous in deeper gland levels.

Immunohistochemistry and image analysis

In uninfected animals, the chief cell was the major site of pepsinogen-specific immunoreactivity and was characterized by large, dense granules packed within the apical cytoplasm (Fig. 2A). Smaller amounts of immunostaining were detected within narrow populations of mucous neck cells. Infected animals featured an expanded population of cells staining for pepsinogen at the mid-mucosal level (Fig. 2B) and in some sections pepsinogen was present in epithelial cells right to the mucosal surface. Chief cell granule content in some infected animals appeared lower than normal. In H&E-stained sections affected chief cells had paler basal cytoplasm than normal, with an even paler perinuclear area, and had reduced eosinophilia of the apical cytoplasm due to a reduction in the number of granules (Fig. 3).

The 2 animals with the lowest tissue pepsinogen concentrations measured biochemically, animals 15 (SDI) and 19 (BDC), with values of 172 (100) and 312 (37) i.u./area respectively, both had negligible pepsinogen immunostaining at any level of their mucosae.

Quantitative differences in the amount of pepsinogen-specific immunoreactivity and its distribution were investigated using image analysis. The plot profiles generated for individual animals and the overall mean profiles of control and infected animals are shown in Figs 4A–D and 5. The profiles for the majority of uninfected control animals were similar in shape with the greatest staining intensities recorded in the deepest parts of the mucosa, corresponding to the chief cells. Towards the middle of the mucosa, lower staining intensities corresponded to mucous neck cells. The plot profiles of many infected animals had a broader outline when compared to controls, with more immunoreactivity present in the more superficial levels. In the analysis of staining intensities in relation to mucosal position, the following results were obtained; at position 1 (mucosal base) only the interaction between diet and infection was significant ($P < 0{\cdot}05$), but this was of borderline significance by position 2 ($0{\cdot}05 < P < 0{\cdot}10$) and not significant by position 3 ($P > 0{\cdot}10$). The effect of infection became of borderline significance by position 16, and was fully significant ($P < 0{\cdot}05$) by position 21 and remained so for many of the positions thereafter (Fig. 5).

The results (in arbitrary units) for AUC data for each of the 4 groups were as follows: BDC group, 2·49 (0·72); BDI group, 4·71 (0·98); SDC group, 4·21 (0·64); SDI group, 4·52 (0·69). The correlation of individual AUC and total tissue pepsinogen (i.u./area) was positive, but poor ($r = 0{\cdot}20$). Values for individual AUCs for all animals varied widely (from 0·55 to 9·64) and the lowest values were again found in animals 15 and 19, 0·55 and 0·65 respectively. Analysis of variance showed that AUC data were not significantly affected by either diet or infection ($P > 0{\cdot}05$).

DISCUSSION

In the present study the mucosae of infected animals were significantly heavier than those of controls and this was due primarily to mucous cell hyperplasia. The phenotype of the hyperplastic mucous cells was similar to mucous neck cells since they contained pepsinogen as well as mucus. Hyperplasia was associated with a likely increase in the total number of cells secreting pepsinogen, however, the total mucosal content of pepsinogen did not increase in infected animals and, based on the immunohistochemical evidence, a decline in the granule content of chief cells may have been responsible.

A small number of trichostrongyle eggs were counted in the faeces of 2 animals of the SDC group, yet no worms were detected within the abomasa of either animal at necropsy. It is likely that this anomaly was the result of a contamination of faecal samples rather than an indication that parasite-naïve animals had been inadvertently infected.

The paucity of both immunoreactivity and peptic activity in tissues from 2 of the animals examined, is a good indication that the anti-bovine pepsinogen antiserum was recognizing only ovine pepsinogen. Interestingly, both animals with low tissue pepsinogen also had very low plasma pepsinogen concentrations. It is also probable that the lack of pepsinogen-specific immunoreactivity in the normal ovine surface mucous cells means that this cell type does not ordinarily produce the enzyme, and this is the only significant departure of the present work from the study of Cybulski & Andren (1990) who demonstrated pepsinogen-specific immunoreactivity in chief cells, mucous neck cells and surface mucous cells of the bovine fundus.

The decline of chief cell granule content seen in some infected animals might indicate hypersecretion. Prolonged, strong stimulation of pepsinogen production and secretion could result in the direct release of pepsinogen without granule formation (Hirschowitz, 1984; Hersey, 1987). Other features of the pepsinogen-depleted chief cells, such as the paler basal cytoplasm, suggest rather that pepsinogen production by these cells was in fact reduced, but this requires confirmation by, for example, an examination of the cellular contents of pepsinogen-specific mRNA. Similar histological features of depleted chief cells have been observed in the chief

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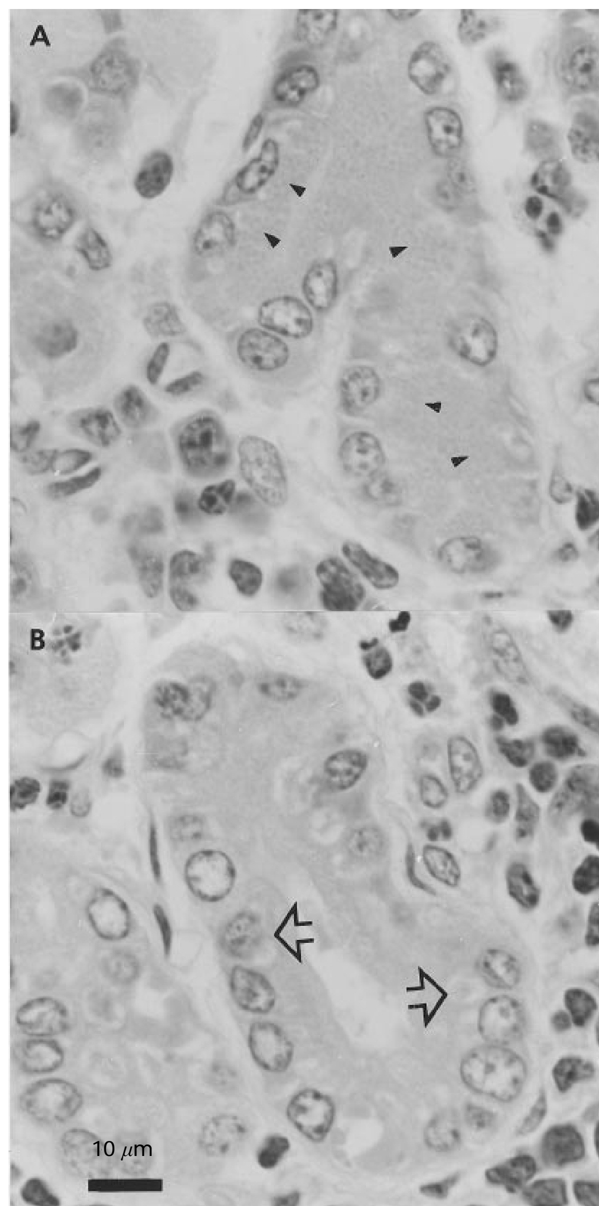

Fig. 3. The appearance of cells at the deep gland base of an infected animal, showing chief cells with abundant eosinophilic granules (arrowheads) in their apical cytoplasm (A) and abnormal cells (B) with granule depletion and pale perinuclear cytoplasm (arrows). (H & E.)

cells of transgenic mice over-expressing the mucosal growth regulator transforming growth factor-alpha (Bockman, Sharp & Merlino, 1995), where increased growth factor levels block the terminal differentiation of chief cells resulting in hyperplasia of cells with a mucous cell phenotype. In the present study, mucins were not demonstrable in cells in the deeper portions of the glands of any animal, but were nevertheless detected in more cells at lower gland levels than in control animals, suggesting that altered cells at the deep gland base may eventually express mucus following more prolonged exposure to the parasites or a heavier worm burden.

Individuals varied markedly in the amounts of pepsinogen present in mucosae, measured both immunohistochemically and biochemically and this variation could translate into real differences in actual rates of secretion. McLeay *et al.* (1973) demonstrated that the outputs of both pepsinogen and acid from separated pouches of ovine fundic mucosa were continuous over time, and other work has shown that continuous secretion of pepsinogen in sheep was reflected by adjacent cells showing all phases of secretory activity, from granule repletion to depletion (Hill, 1965) so that, taken overall, the mucosal content of pepsinogen should be relatively constant with time and any differences in total tissue pepsinogen should relate to the capacity of individual mucosae to secrete.

The present study has shown that, in considering the potential for pepsinogen secretion in haemonchosis or any parasitic gastritis, all zymogenic cells should be examined and not just chief cells. Stringfellow & Madden (1979) monitored a decline in chief cell granule number in the mucosae of calves infected with *O. ostertagi*, but plasma pepsinogen concentrations remained elevated in these animals. They argued that production of pepsinogen had ceased and that the continued high plasma levels were due to a long plasma half-life for pepsinogen, but they may have overlooked the potential for the continued secretion of pepsinogen by other zymogenic cell types.

Pepsinogen concentrations in the present study, in either plasma or in abomasal tissues, were not significantly elevated by infection. A second study in Hampshire Down lambs has confirmed many of the results of this study, including the marked degree of inter-animal variation in tissue pepsinogen content and the existence of sheep that appear to produce little pepsinogen if any (unpublished observations), and in this second study, plasma pepsinogen concentrations were significantly elevated in infected animals.

The consequences for the digestive function of animals of the lack of pepsinogen in abomasal secretions are unknown, however, pepsin has been considered more important in the digestion of proteins of animal origin such as collagen than in the breakdown of plant matter, and more important in animals where mastication is limited or absent (Hersey, 1987). Interestingly, the chief cells of the 2 ‘pepsinogen deficient’ animals (15 and 19) bore many typical eosinophilic granules, the possible content of which is unknown. Any animal that does not produce pepsinogen will be unlikely to develop an elevated plasma pepsinogen concentration following exposure to parasites. These findings may help explain, at least in part, the occasional inability of animals to react to similar worm burdens with corresponding elevations of zymogen in plasma. Other workers have had findings of sheep reacting to

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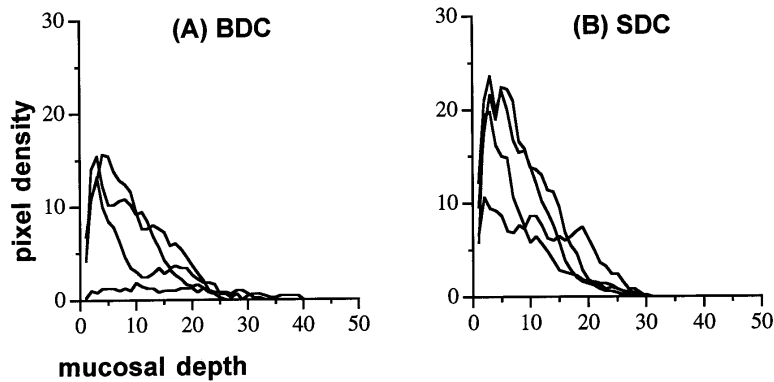

Fig. 4. Individual profiles for animals of the BDC (A), SDC (B), BDI (C) and SDI (D) groups showing pepsinogen-specific staining intensity plotted against mucosal depth (arbitrary units). BDC, control animals on the basal diet; BDI, infected animals, basal diet; SDC, control animals, supplemented diet; SDI, infected animals, supplemented diet.

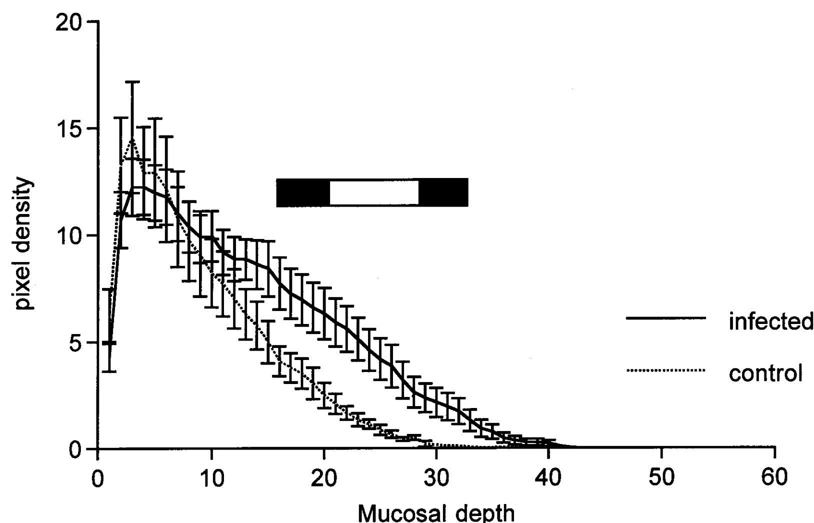

Fig. 5. The overall mean (±S.E.) immunoreactivity profiles for both uninfected, control animals and animals infected with *Haemonchus contortus*, plotted against mucosal depth (arbitrary units). The bars denote regions where the differences between control and infected animals were either of borderline significance ($0{\cdot}05 < P < 0{\cdot}10$ – white) or were significant ($P < 0{\cdot}05$ – black).

trichostrongyle infection with low plasma pepsinogen responses (Coop, Sykes & Angus, 1977; Lawton *et al*. 1996; Simpson *et al*. 1997) suggesting that this phenomenon is widespread and may be an additional, important consideration when assay of plasma pepsinogen concentration is used to establish the status of an individual in terms of abomasal infection with helminth parasites.

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