Effects of *Ostertagia ostertagi* on gastrin gene expression and gastrin-related responses in the calf

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1. Infection with the bovine abomasal nematode *Ostertagia ostertagi* results in a loss of acid-secreting parietal cells and an increase in gastric pH. The effects of an experimental infection on gastrin mRNA expression, blood and tissue gastrin concentrations, the different molecular forms of gastrin in each, and pyloric mucosal chromogranin A-derived peptides were investigated in the calf.

2. An increase in blood gastrin concentrations in the infected group reached a peak by day 28 postinfection (635 pg ml^{-1}; $P < 0.01$). Gel chromatography analysis of blood samples revealed that the hypergastrinaemia comprised largely gastrin-34 (G-34) in parasitized calves while gastrin-17 (G-17) predominated in control animals.

3. An 11-fold increase in gastrin mRNA expression was recorded in the parasitized animals which was accompanied by a 23·8% reduction in pyloric mucosal gastrin content and an apparent drop of 24·7% in the number of gastrin-producing G cells detected. There was no major change in the relative abundance of G-17 and G-34 in the pyloric mucosa of infected calves. No significant differences in the concentration of pyloric mucosal chromogranin A-derived peptides were recorded between infected and control groups.

4. These data suggest that the hypergastrinaemia seen in parasitized calves results largely from an increase in gastrin synthesis and that depletion of previously stored peptide makes virtually no contribution to elevated blood gastrin concentrations.

Infection with the abomasal nematode, *Ostertagia ostertagi*, in the calf results in a loss of acid-producing parietal cells which leads to a rise in gastric pH (Murray, Jennings & Armour, 1970). This is accompanied by a marked hypergastrinaemia (Fox, Gerrelli, Pitt, Jacobs, Hart & Simmonds, 1987) and a significant reduction in both pyloric mucosal gastrin concentrations and in gastrin-producing G cell numbers (Fox, Carroll, Hughes, Uche, Jacobs & Vaillant, 1993). This suggests that the release of previously stored gastrin, and not an increase in G cell population size, is at least partly responsible for the increase in circulating peptide concentrations. The same authors have also demonstrated, using radioimmunoassay techniques, that while there is a rise in blood total gastrin, levels of circulating heptadecapeptide (G-17), which predominate in the blood of worm-free animals (Reynolds, Simpson, Carr & McLeay, 1991), remain largely unaltered. The present study was therefore designed to investigate the effect of *Ostertagia ostertagi* on: (i) pyloric mucosal gastrin mRNA as an indirect indicator of peptide synthesis; (ii) the relative abundance of the different molecular forms of gastrin in blood and pyloric mucosa using gel chromatography techniques; and (iii) concentrations of chromogranin A-derived peptides, including the putative autocrine regulator, pancreastatin, whose biosynthetic pathways are closely linked to that of gastrin in pyloric G cells (Watkinson *et al.* 1991; Cetin, Bargsten & Grube, 1992).

METHODS

Experimental design

Eight, 2½-month-old Friesian heifer calves, reared under conditions designed to minimize the risk of parasitic infection, were kept in individual pens and maintained on good quality hay *ad libitum*. All were treated with fenbendazole (Panacur™; Hoechst, Milton Keynes, Bucks, UK) by oral administration at the manufacturer's recommended dose 7 days before the start of the study. The calves

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were then ranked according to weight (mean ± S.E.M., 123·0 ± 4·1 kg) and allocated to either control or infected groups, the latter receiving 100000 *Ostertagia ostertagi* infective larvae *per os* (by mouth) on day 0. The animals were weighed at weekly intervals during the study and also on day 28 when all eight were killed.

Blood collection and analysis

Jugular blood samples were collected from each calf between 10.00 h and 11.00 h thrice weekly throughout the study. Plasma, collected in lithium heparin containing 100 kallikrein-inactivator Units of proteinase inhibitor (Trasylol™; Bayer) per millilitre of blood, and serum were divided into aliquots and stored at −20 °C prior to analysis. Plasma samples were assayed for total gastrin using the technique described below while pepsinogen determinations were made using the Moredun modification of the method described by Mylrea & Hotson (1969) using bovine albumin as substrate in a glycine–hydrochloric acid buffer (5·85 g NaCl + 7·5 g glycine in 1 l distilled water adjusted to pH 2·0 by the addition of 5 M HCl). Pepsinogen concentrations were expressed as milliunits (mU) of tyrosine, where 1 unit equals 1 μM tyrosine released per litre of plasma per minute at 37 °C.

Post-mortem procedures and processing of tissue samples

The calves were weighed and then killed by intravenous injection of pentabarbitone sodium (Euthasate™; Willow Francis Veterinary, Crawley, West Sussex, UK) at 133 mg (kg body weight)$^{-1}$. The abomasum was removed intact from each animal, opened along its greater curvature and gastric fluid collected for subsequent pH estimation using a Kent-EIL meter (model number 7020; Fisher Scientific UK, Loughborough, Leics, UK). The gastric mucosa was rinsed thoroughly under cold running water and adhering worms collected on a wire-mesh screen with an aperture of 0·15 mm. The mucosal surface was then blotted dry and fundic and pyloric regions divided.

Fifteen replicate samples of pyloric mucosa (mean ± S.E.M., 1·36 ± 0·12 g) were collected from each animal. Five samples were 'snap frozen' individually in liquid nitrogen, placed in separate preweighed, prefrozen, sterile universal bottles, weighed and then stored at −70 °C prior to RNA extraction. Five further samples were prepared for the extraction of gastrin and chromogranin A-derived peptides using a technique similar to that described by Gregory & Tracy (1964). Briefly, the samples were placed in preweighed glass tubes to which 5 ml of hot deionized and distilled water were added. Each tube was then maintained at 100 °C in a heating block for 30 min. After cooling and centrifugation at 2750 *g* for 30 min, the supernatant was removed and stored at −20 °C prior to gastrin assay. The last five mucosal samples were collected in Bouin's fluid for immunohistochemistry, details of which are given below. The remaining fundic and pyloric mucosae were then stripped from the muscularis layer and weighed prior to the recovery of tissue-dwelling worms by acid–pepsin digestion. Mucosal weights are expressed per unit of body weight (g (kg body weight)$^{-1}$).

Gastrin mRNA recovery and quantification

Total RNA was extracted using a commercially available kit (Promega), which is based on the method of Chomczynski & Sacchi (1987), and samples quantified by absorbence at 260 nm. Aliquots of 10 or 15 μg of each sample were denatured using deionized glyoxal and agarose gel electrophoresis was performed as described by Sambrook, Fritsch & Maniatis (1989). RNA was then immobilized onto a GeneScreen Plus membrane (Du Pont) by Northern blotting and hybridization performed under stringent conditions using an homologous bovine gastrin DNA probe (kindly donated by Dr T. Lund, University College and Middlesex School of Medicine, UK), as described in the manufacturer's protocol. In brief, the probe was prepared by eluting a 407 bp *Hind*III fragment, which harbours the open reading frames responsible for bovine G-17 and G-34 gene expression (Lund, Olsen & Rehfeld, 1989), from a low-melting agarose gel. The probe was labelled to a high specific activity with α-[^{32}P]dCTP (deoxycytidine triphosphate) using the Multiprime DNA labelling kit (Amersham). The blotted filters were incubated for 4 h at 60 °C in pre-hybridization buffer (1% SDS, 0·1 M sodium chloride, 10% dextran sulphate) and then for 16–20 h at 60 °C in the same buffer containing denatured salmon sperm DNA (100 μg ml^{-1}) and the denatured radioactive probe. The filters were then washed twice in 2 × salium sodium citrate (SSC; 1 × SSC is 0·15 M NaCl plus 0·015 M sodium citrate; at room temperature (19–23 °C) for 5 min), twice in 2 × SSC and 1% SDS (at 60 °C for 30 min) and twice in 0·1% SSC (at room temperature for 30 min) and visualized by autoradiography using intensifying screens at −70 °C. After 72 h exposure, the hybridization signal was quantified using a laser densitometer (Chromoscan 3; Joyce-Loebel Ltd, Tyne and Wear, UK; absorbence measured in standard optical density (OD) units and the results expressed per unit weight of mucosa). The signal was washed from the membranes with boiling 0·01% SDS and 0·01 × SSC, and successful removal of the probe confirmed by 72 h exposure to X-ray film. The membranes were then rehybridized with a radiolabelled β-actin probe (supplied by Dr R. Dimaline, The Physiology Laboratory, University of Liverpool; Dockray, Hamer, Evans, Varro & Dimaline, 1991) as described above.

Radioimmunoassay

The total gastrin immunoreactivity was determined using a commercially available human radioimmunoassay kit (Becton-Dickinson UK Ltd, Oxford; kit code 255025) that had been previously validated for use in cattle using standard procedures outlined by Bolton (1982). The kit antiserum exhibited the following cross-reactivities with the various molecular forms of gastrin: G-17-I (100%), G-17-II (81%), G-34-I (40%) and G-13-I (57%). Cross-reactivity was determined using bovine plasma, from which endogenous gastrin had been removed by charcoal stripping (Gerrelli, 1989), spiked with different forms of human gastrin (Sigma) which, in the case of G-17, differs from bovine gastrin by only two amino acid substitutions in the middle of the linear peptide chain (Nilsson, 1980). A further four substitutions are present in bovine G-34 (Lund *et al.* 1989). Cross-reactivity was calculated as the ratio of gastrin standard to related peptide, expressed as a percentage, that gave a 50% displacement of tracer binding.

The immunoreactivity of chromogranin A-derived peptides in extracts of pyloric mucosa was quantified using techniques described by Watkinson *et al.* (1991). Briefly, these employed the use of two antisera; L331, which reacts with the C-terminus of pancreastatin itself, and L300, which is directed towards a sequence of chromogranin A (306–313) immediately C-terminal to pancreastatin. Chromogranin A immunoreactivity was measured using L300 on tissue extracts previously digested with trypsin and carboxypeptidase B. The latter exposes the C-terminal sequence to which L300 is directed and results in a marked increase in immunoreactivity, in comparison with non-digested material, which approximates to total intact chromogranin A. The immunoreactivity of material from both this and a previous study (Fox *et al.* 1993), in which calves were exposed to the same level, pattern and strain of *Ostertagia ostertagi* and killed on day 28, are presented here.

Chromatography

Pooled plasma (2 ml) or diluted tissue extract (0·4 ml) samples from each group were fractionated by gel filtration on Sephadex G50 Superfine columns (1·6 cm × 100 cm) equilibrated and eluted with 0·06 M phosphate buffer (7·2 g NaH_2PO_4 per litre distilled water adjusted to pH 7·4 by the addition of 8·3 g $Na_2HPO_4.H_2O$ per litre distilled water) at a flow rate of 3·2 ml h^{-1}. Fractions of 0·8 ml were collected at 4 °C. The columns were calibrated using the non-sulphated forms of human G14, G-17 and G-34 (Sigma), while void and total volumes were established using Dextran Blue (Sigma) and $Na^{125}I$ (Amersham International), respectively.

Immunohistochemistry

Samples of pyloric mucosa fixed in Bouin's fluid (Raymond A. Lamb, London) were embedded in wax and 7 μm-thick sections taken perpendicular to the muscularis mucosae and mounted on glass slides coated with chrom–alum gelatin (0·5% w/v gelatin, 0·09% w/v chromic potassium sulphate, 0·1% w/v sodium azide; Merck). Gastrin immunoreactivity was demonstrated using the peroxidase–antiperoxidase (PAP) technique described previously by Fox *et al.* (1993). Briefly, sections were dewaxed, immersed in phosphate-buffered saline (PBS) and then incubated for 22 h at 4 °C with rabbit antigastrin antiserum, L39, diluted to 1 : 300 in PBS containing 2·5% w/v bovine serum albumin and 0·25% w/v sodium azide. Sections were then incubated for 1 h each at room temperature with goat antirabbit IgG (ICN Flow) then rabbit PAP (Sigma), both diluted to 1 : 50 in PBS. The slides were rinsed in PBS after each incubation. Sites of antibody binding were demonstrated by immersing the sections in glucose oxidase–diaminobenzidine–nickel substrate for 6 min (Shu, Ju & Fan, 1988) before counterstaining with Eosin. Antiserum L39, raised against human and porcine heptadecapeptide gastrin (G-17), was used to identify immunoreactive G cells. Normal rabbit serum (1 : 50) and L39, preincubated with natural porcine G-17 (provided by the late Professor R. A. Gregory and Dr H. J. Tracy (retired); both from The Physiology Laboratory, University of Liverpool), at a concentration of 10 nmol G-17 per millilitre of L39 (1 : 300), were used as specificity controls. The number of cells is expressed per area of mucosa overlying 0·5 mm of muscularis mucosae. Immunoreactive cells were counted only where the plane of section passed vertically through the mucosa and a minimum of ten counts was obtained per animal.

Parasitological procedures

Faecal samples were collected at regular intervals and screened for the presence of nematode eggs using the modified McMaster method. Post-mortem worm counts were performed using techniques similar to those described in the Ministry of Agriculture, Fisheries and Food reference book (No. 418, Manual of Veterinary Parasitological Laboratory Techniques, 1986) for the recovery of nematodes from the abomasal lumen and mucosa. Briefly, this involved opening the abomasum along its greater curvature, collecting the contents and washing the mucosa to facilitate recovery of adult stages and digestion of the gastric mucosa in an acid–pepsin mixture overnight at 37 °C to recover tissue-dwelling, larval stages.

Statistical analysis

Data are expressed as means ± S.E.M. Statistical comparisons were made using Student's t test for unpaired values, with a level of significance at $P < 0·05$.

RESULTS

Clinical observations

The majority of infected calves passed soft faeces at some time during the patent phase of the infection. There was no significant difference in live-weight between groups at the end of the study.

Post-mortem data

Terminal abomasal pH was significantly elevated in the infected calves by day 28 (6·6 ± 0·3 and 2·9 ± 0·4 in infected and control animals, respectively; $P < 0·01$; Fig. 1). Wet weight of the fundic mucosa was 95·6% heavier (2·7 ± 0·3 and 1·4 ± 0·1 g (kg body weight)$^{-1}$ in infected and control animals, respectively; $P < 0·01$) while that of the pyloric mucosa was 31·4% heavier (0·67 ± 0·03 and 0·51 ± 0·03 g (kg body weight)$^{-1}$ in infected and control calves, respectively; $P < 0·05$) in infected animals by the end of the study (Fig. 1).

Parasitology data

Ostertagia infections became patent on day 18 with faecal egg output reaching a peak on day 23 (160 ± 47 eggs per gram). No strongyle eggs were recovered from any of the control animals during the study.

Nematodes were recovered from the abomasum of each of the infected calves. The number of worms found in the infected group on day 28 was 24055 ± 2106 of which 83·5 ± 2·7% were found in the lumen. No roundworms were found in the abomasum of any of the control animals.

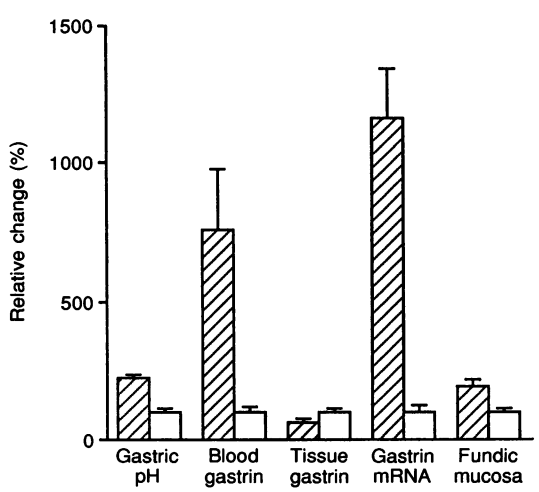

Figure 1. Gastrin gene expression and gastrin-related responses to *Ostertagia* infection in the calf

Relative changes in gastric pH, blood gastrin, tissue gastrin, tissue gastrin mRNA and fundic mucosal weight in parasitized (▨) and control animals (□) on experiment day 28. Results have been normalized to control calf values in each case. Vertical bars represent + S.E.M.

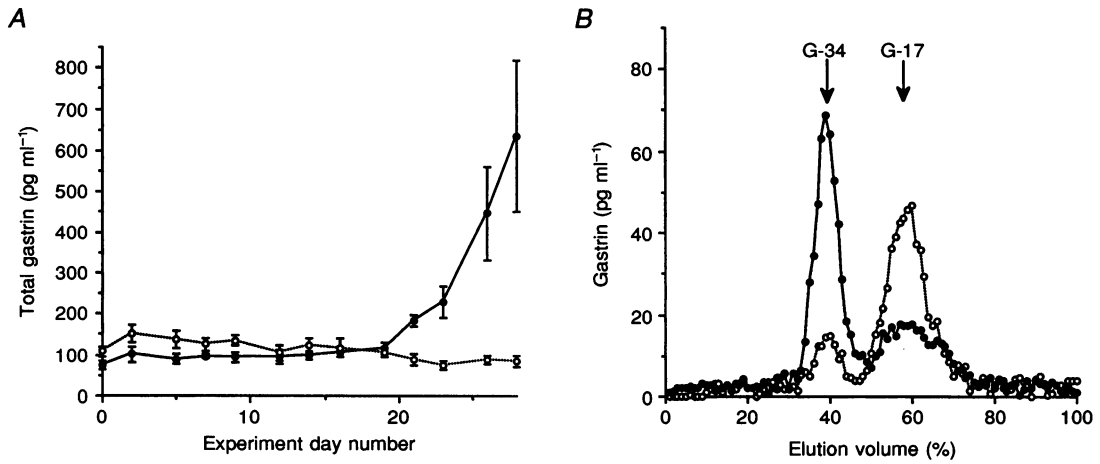

Figure 2. Total blood gastrin and molecular forms of gastrin in the blood of *Ostertagia*-infected and control calves

A, graph of total blood gastrin illustrates infected (continuous line) and control (dotted line) calf data; vertical bars represent ±S.E.M. *B*, separation of two molecular forms of gastrin in plasma from infected (continuous line) and control (dotted line) calves on experiment day 28 using Sephadex G50 Superfine columns. The elution positions of human gastrin-34 (G-34) and gastrin-17 (G-17) are indicated by arrows at the top of the figure. Elution volume is expressed relative to the void volume indicated by Dextran Blue (0%) and $Na^{125}I$ (100%).

Blood data

Pepsinogen. Serum pepsinogen levels were significantly elevated in the infected calves from day 14 ($P < 0{\cdot}01$) reaching a peak on day 28 (3175 ± 360 mU tyrosine). This represented a 14·3-fold increase over the mean control group value.

Total gastrin. Total gastrin concentrations were significantly elevated in the infected group from day 21 ($P < 0{\cdot}01$) reaching a peak on day 28 (635 ± 183 pg ml^{-1}; Fig. 2*A*). This represented a 7·6-fold increase over the corresponding control group value (83·6 ± 13·8 pg ml^{-1}; Fig. 1). Furthermore, a highly significant correlation was established between blood total gastrin levels and terminal abomasal pH in infected and control calves ($y = 157{\cdot}8x - 387{\cdot}2$, correlation coefficient $r = 0{\cdot}86$; $n = 8$, $P < 0{\cdot}01$).

Gastrin gel chromatography. Gel filtration of pooled blood from infected calves (day 28) revealed two peaks eluting in positions corresponding to G-34 and G-17 (Fig. 2*B*). Comparison of the area under these two peaks, using the Solitaire Image-Analysis System (Seescan plc, Cambridge, UK), indicated that G-34 and G-17 accounted for 63·1 and 36·9% of gastrin immunoreactivity, respectively. When cross-reactivity of the kit antiserum with these two peptides was taken into account the relative abundances of G-34 and G-17 were 81·0 and 19·0%, respectively. However, comparison of the area under these two peaks in samples taken from control animals on day 28 indicated that G-34 and G-17 accounted for 16·4 and 83·6% of gastrin immunoreactivity, respectively. When cross-reactivity of the kit antiserum was taken into account the relative abundances of G-34 and G-17 were 32·9 and 67·1%, respectively.

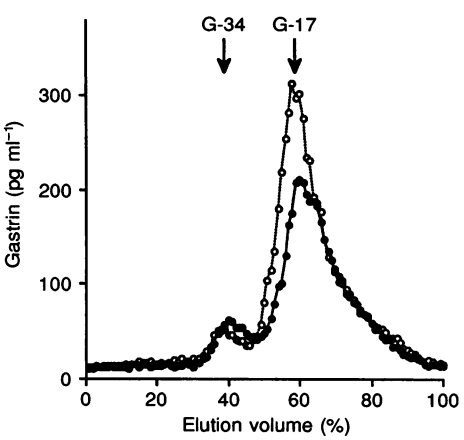

Figure 3. Molecular forms of gastrin in pyloric mucosal extracts from *Ostertagia*-infected and control calves

Separation of gastrins in pyloric mucosal extracts from infected (continuous line) and control (dotted line) calves on experiment day 28 using Sephadex G50 Superfine columns. The elution positions of G-34 and G-17 are indicated by arrows at the top of the figure. Elution volume is expressed relative to the void volume indicated by Dextran Blue (0%) and $Na^{125}I$ (100%).

Table 1. Effect of *Ostertagia ostertagi* on the number of immunoreactive G cells in the pyloric mucosa, as demonstrated by antigastrin antiserum L39 (1 : 300), in calves killed 28 days postinfection

	G cell numbers*	Number of areas examined
Control calves (4)	29·5 ± 0·7	228
Infected calves (4)	22·2 ± 0·8	156

Numbers are expressed as means ± S.E.M. per area of mucosa overlying a 0·5 mm length of muscularis mucosae in 7 μm-thick sections. Values in parentheses represent the number of calves; * significant difference between means, $P < 0·001$.

Table 2. Effect of *Ostertagia ostertagi* on chromogranin A-derived peptide immunoreactivity of the pyloric mucosa in calves killed 28 days postinfection

	Pancreastatin (pmol g^{-1})	Total chromogranin A (pmol g^{-1})
Control calves	43·7 ± 12·2 (5)	246·0 ± 33·0 (7)
Infected calves	37·5 ± 7·7 (7)	203·0 ± 56·8 (6)

Data are taken from this and a previous study (Fox *et al.* 1993) and are expressed as means ± S.E.M. Values in parentheses represent the number of calves.

Tissue data

Total gastrin. Pyloric mucosal gastrin concentrations were 40·4% lower in the infected calves at the end of the study (1302 ± 369 and 2186 ± 312 ng g^{-1} in infected and control animals, respectively; Fig. 1). This drop in concentration was partly offset by the higher pyloric mucosal weight resulting in the total gastrin content being only 23·8% lower in this group (1199 ± 316 and 1573 ± 260 ng, respectively).

Gastrin gel chromatography. Gel filtration of pooled pyloric mucosal extracts from infected and control calves (day 28) revealed two peaks eluting in positions corresponding to G-34 and G-17 (Fig. 3). Comparison of the area under these two peaks indicated that G-34 and G-17 accounted for 11·7 and 88·3%, and 6·8 and 93·2% of gastrin immunoreactivity in control and infected calves, respectively. When cross-reactivity of the kit antiserum was taken into account the relative abundances of G-34 and G-17 were 24·9 and 75·1%, and 15·4 and 84·6% in infected and control animals, respectively.

By taking account of the difference in tissue gastrin content between the two groups, it is possible to estimate the relative contribution made by the depleted pyloric mucosal reserves of peptide to the hypergastrinaemia seen in the parasitized calves. However, such data must first be transformed into G-17-equivalent values to take account of the difference in half-life between G-17 and G-34 (up to 8 times; Nilsson, 1980). If, to begin with, the difference in half-life is assumed to be 4-fold, the total pyloric mucosal gastrin content in the infected calves would be (1199 ng × 75·1%) = 900 ng G-17 plus (1199 ng × 24·9% × 4) = 1194 ng G-17-equivalent value of G-34 fraction, i.e. a total of 2094 ng. The corresponding total pyloric mucosal gastrin content in the control calves would be (1573 × 84·6%) + (1573 × 15·4% × 4) = 2300 ng, i.e. a difference in total tissue gastrin content of 206 ng (G-17 equivalent) between the two groups.

On the same basis, total blood gastrin content in the infected animals on day 28 would be (635 × 19%) + 635 × 81% × 4) = 2178 pg ml^{-1} and (83·6 × 67·1%) + (83·6 × 32·9% × 4) = 166 pg ml^{-1} in the control group, i.e. a difference in total blood gastrin content of 2012 pg ml^{-1} (G-17 equivalent) between the two groups. Assuming that the difference in tissue gastrin content (206 ng) was released into the blood (plasma volume of parasitized calves taken as 8·1% of live-weight (Halliday, Mulligan & Dalton, 1968), i.e. 11·4 l, the resulting increase (18·1 pg ml^{-1}) would represent only 0·9% of the total increase in blood gastrin (2012 pg ml^{-1}) seen in the infected group. A similar calculation, based on the assumption that the difference in half-life between G-17 and G-34 was 8-fold, revealed no difference in total pyloric mucosal gastrin content between the infected and control calves (3289 ng and 3269 ng G-17 equivalent, respectively). These calculations suggest that the release of previously stored gastrin into the blood of parasitized calves makes little if any contribution to the observed hypergastrinaemia in this group. Such calculations estimate the maximum contribution that previously stored gastrin is likely to make to the hypergastrinaemia. The actual contribution will be less than that shown since depletion of tissue gastrin is likely to take place over several days and not just on day 28 (assumed for the purposes of this calculation).

Gastrin mRNA. The relative abundance of gastrin mRNA in the pyloric mucosa of infected calves increased 11·4-fold over corresponding control values by day 28 ($P < 0·01$). This difference did not, however, reflect non-specific changes in RNA or differences in the quantity of RNA applied to the membranes. When expressed relative to a non-regulated mRNA species (β-actin), the ratio of gastrin to β-actin mRNA was 0·71 ± 0·11 in infected calves and 0·061 ± 0·016 in control animals, an 11·6-fold difference ($P < 0·01$; Fig. 1). Similar results were obtained when the same experiment was repeated using the β-actin probe for the first hybridization followed by washing and subsequent hybridization with the gastrin probe (results not shown). This ensured that the washing procedure, which removed the first signal, did not remove significant amounts of RNA prior to rehybridization.

G cell numbers. Staining with antiserum L39 demonstrated cells in the gastric epithelium at all levels of the pyloric mucosa in animals from both infected and control groups. However, no staining was obtained when L39 was replaced by normal rabbit serum or by L39 preabsorbed with gastrin. The number of immunoreactive G cells detected in pyloric mucosal samples from infected animals was, however, 24·7% lower than in corresponding samples from control calves ($P < 0·001$; Table 1). Furthermore, the intensity of immunoreactive staining was reduced in cells from parasitized calves in comparison with that in cells from control animals.

Chromogranin A-derived peptides. There was no significant difference in the pyloric mucosal concentration of either pancreastatin or chromogranin A between infected and control groups (Table 2).

DISCUSSION

Abomasal infection with *Ostertagia ostertagi* in the calf is accompanied by a loss of acid-secreting parietal cells as the parasite burden matures (Murray *et al.* 1970). In the present study, this resulted in a significant rise in gastric pH which, in turn, stimulated secretion of the gut peptide, gastrin, from G cells in the pyloric antrum leading to a marked hypergastrinaemia (Fig. 2*A*) (Reynolds, Stiffe & Titchen, 1978). The highly significant correlation established between terminal abomasal pH and blood total gastrin levels in the parasitized calves lends further support to the view that the main stimulus for the hypergastrinaemia was the concurrent rise in abomasal pH.

Parasite infection was also accompanied by a marked increase in wet weight of the fundic mucosa in the infected group (95·6% increase over corresponding control values by day 28). Similar increases in fundic mucosal weight have also been reported by Anderson, Reynolds & Titchen (1988) in lambs exposed to *Ostertagia circumcincta* and Fox *et al.* (1993) in calves infected with *Ostertagia ostertagi*. Both groups of authors suggested that the fundic mucosal hyperplasia associated with *Ostertagia* may at least be partly attributable to the concurrent rise in blood gastrin levels. This would stimulate gastric acid secretion (Reynolds *et al.* 1978) and growth of the acid-secreting, parietal cell region of the abomasal mucosa (Johnson, 1981), both effects attempting to restore gastric pH to normal.

Gel chromatography analysis of blood samples from parasitized calves revealed that most of the gastrin response to *Ostertagia* consisted of G-34 (Fig. 2*B*) which was surprising bearing in mind that G-17 normally predominates in the blood of worm-free animals (Reynolds *et al.* 1991). This does, however, support the results of previous work cited, though not described, by Anderson *et al.* (1988) in *Ostertagia*-infected sheep and the results of preliminary studies by Fox *et al.* (1993) in parasitized calves. The increase in G-34 could have been due to either: (i) preferential release of newly synthesized gastrin prior to completion of post-translational processing. Incomplete processing might have been caused by an actual impairment in processing or the rate of progastrin synthesis and subsequent secretion exceeding the ability of the G cells to process the peptide (Dimaline, 1988); (ii) the longer half-life of G-34. In man, the half-life of G-34 is up to 8 times longer than that of G-17, depending upon the method of determination used (Nilsson, 1980), and may be further extended by sulphation (Pauwels, Dockray & Walker, 1987; Dimaline, 1988); and/or (iii) an increase in contribution made by gastrin of duodenal origin. In sheep, G-34 accounts for approximately 50% of the total gastrin recovered from the last quarter of the duodenum although the total gastrin content of the latter organ is relatively small (Reynolds *et al.* 1991). It was apparent, however, that most of the gastrin produced by the pyloric G cells was passed directly into circulation with very little, if any, being stored because of the absence of any major change in the relative abundance of G-17 and G-34 recovered from pyloric mucosal extracts from parasitized and control calves (Fig. 3).

Mechanisms that might have contributed to the hypergastrinaemia seen in the *Ostertagia*-infected calves include either: (i) an increase in peptide synthesis; (ii) direct and/or indirect stimulation of gastrin secretion by the autocrine peptide, pancreastatin, in the pyloric mucosa; (iii) a depletion of previously stored reserves of gastrin; and/or (iv) a reduction in the rate of gastrin turnover.

The 11-fold rise in abundance of gastrin mRNA in the pyloric mucosa of infected calves strongly supports the hypothesis that an increase in peptide synthesis made a significant contribution to the hypergastrinaemia. While mRNA quantitation involving the hybridization techniques described here does not allow a distinction to be made between changes in mRNA content due to alterations in rate of transcription initiation, RNA turnover/degradation or transport and those due to changes in synthesis (Pearson, 1993), the magnitude of the increase in expression reported here suggests that an increase in synthesis was likely to be the main cause in the parasitized calves.

Pancreastatin has been shown to inhibit acid secretion by parietal cells (Lewis, Zdon, Adrian & Modlin, 1988), although the absence of a significant response in the present study (Table 2) suggests that the peptide was not directly involved in initiating or sustaining the drop in acid output here. The tendency towards a decline in pyloric mucosal concentrations would, however, be consistent with a secondary homeostatic response to the change in gastric pH, i.e. pancreastatin levels were lowered in order to remove any inhibitory effect of the peptide on acid secretion. A similar reduction in tissue chromogranin A concentrations was also observed. This again is not surprising since a close inverse relationship has been established between gastrin and

chromogranin A in individual G cells by Cetin *et al.* (1992). They demonstrated that upregulation of gastrin synthesis, shown in the present study by increased gastrin gene expression (and not increased storage as in their study), was coupled with downregulation of chromogranin A and vice versa. The authors postulated that this relationship was related to the putative function of chromogranin A in the G cell, i.e. packaging and sorting of gastrin, control of post-translational processing of progastrin or autocrine control of synthesis and secretion.

Surprisingly perhaps, the depletion of previously stored reserves of gastrin in the pyloric mucosa appeared to make only a minor contribution (0·9% or less) to the hypergastrinaemia. It was, however, interesting to note that the reduction in pyloric mucosal gastrin content of infected calves (23·8%) was very similar to the apparent drop in G cell numbers (24·7%; Table 1). Since the intensity of staining was generally reduced in G cells from the parasitized group, it is probable that the apparent drop in G cell numbers was due to the gastrin stores in a proportion of the cells falling below the limit of detection of the technique, rather than an absolute drop in numbers.

A reduction in the rate of gastrin turnover or clearance would also contribute to elevated blood gastrin concentrations in *Ostertagia*-infected animals although this was not investigated in the present study. Such changes could be identified by monitoring the rate of disappearance of exogenous gastrin following infusion, similar to work conducted in rats by Dockray *et al.* (1991).

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