*Leading article**Campylobacter pyloridis* – A new factor in peptic ulcer disease?

Reports of a new bacterium – *Campylobacter pyloridis* – which may represent a previously unrecognised genus in most patients with chronic gastritis and peptic ulceration raises important questions about the relationship between the two conditions and the role of this organism. Human gastric spiral organisms are being rediscovered, having been originally noted earlier.^{1–3} In 1975 Steer and Colin-Jones⁴ reported the presence of gram negative bacteria on the surface epithelium of 80% of gastric ulcer patients. Warren⁵ and Marshall⁶ in 1983 described numerous S-shaped spiral bacteria on the antral epithelium of patients with chronic gastritis and successfully cultured the slow growing microaerophilic organism which has now achieved official recognition as *Campylobacter pyloridis*.⁷ Steer⁸ in a scanning electron microscopy study of gastroduodenal mucosa and Rollason *et al*⁹ in a retrospective study of gastric biopsies reported the presence of these bacteria on gastric type epithelium. Numerous other reports have now confirmed the presence of gastric spiral organisms in gastritis.^{10–19}

All reported studies agree that *C pyloridis* is strongly associated with non-autoimmune chronic gastritis. Some workers have also confirmed Warren's original observation that the organisms are particularly associated with active chronic gastritis.^{18–20} The organisms are occasionally found on histologically normal body type gastric mucosa, but only in association with chronic gastritis in the antrum.²¹ Areas of intestinal metaplasia are not colonised.^{8–15–16–18} *C pyloridis* is uncommon in reflux gastritis (Dixon M, personal communication) and has been reported in only three of 14 patients with type A autoimmune gastritis,²² which is considered to have a different pathogenesis to type B, non-immune gastritis.²³

In the duodenum *C pyloridis* is found only in the presence of active duodenal inflammation²⁴ in areas of gastric metaplasia.^{8–15–16} The association between *C pyloridis* and duodenal ulceration has been confirmed by several workers.^{8–10–12–16–18–20} In one study, sixty three of 70 patients with duodenal ulcers and 27/40 gastric ulcer patients, had positive antral cultures for *C pyloridis*.²⁵ Interestingly, two of seven bacteria-negative duodenal ulcer patients and nine of 13 negative gastric ulcer patients were taking non-steroidal anti-inflammatory drugs and did not have antral gastritis.

C pyloridis can be detected histologically on the mucosal surface of most biopsies showing active chronic gastritis. In our experience at least two biopsy sites (antrum and body) are necessary to avoid sampling error. The bacteria are faintly visible on conventional haematoxylin and eosin sections, but the Warthin-Starry silver stain shows the organisms more

reliably.⁵ As this method is expensive, time consuming and sometimes unpredictable, a number of other techniques have been explored. Phase contrast microscopy²⁶ and fluorescent labelling with acridine-orange²⁷ have been recently recommended, but these methods rely on specialised microscopes which may not be readily available in all laboratories. The technique we now use is a modified Giemsa (without differentiation) which is quick, simple and as reliable as the Warthin-Starry stain. All of these methods are non-specific and will label any other bacteria present in the stomach. The characteristic curved morphology of *C. pyloridis* and its close association with the enterocyte surface, however, allows presumptive histological diagnosis in almost all patients.

A polyclonal antibody raised in rabbits labels *C. pyloridis* in tissue sections.²⁸ There was no cross reaction with *C. jejuni* and attachment of antibody to the cell membrane and flagellae of *C. pyloridis* has been shown by immunoelectronmicroscopy. We have developed a rabbit antibody from a different preparation of antigen, but have found it to cross react with other campylobacter species. Interestingly, a common campylobacter antibody which has been used for fluorescent identification of campylobacter in stools, reacts with *C. pyloridis*.¹⁷

Scanning electron microscopy studies have shown *C. pyloridis* to have a smooth surface with four to six polar sheathed flagellae, each with a terminal bulb, not found in other campylobacters.²⁹ The bacteria are seen predominantly in the 'gutters' between epithelial cells, where they adhere to microvilli.^{8, 16} The plasma membranes of involved mucus secreting epithelial cells have been shown to be intact but indented, with the number of microvilli considerably depleted.³⁰ At sites of close contact with the bacterium shallow cup shaped surface elevations form in the epithelial cells (unreported observations), which are morphologically similar to the adherence pedestals of enteropathic *E. coli*.³¹ The number of bacteria correlates with the presence of polymorphonuclear leucocytes²⁰ and by transmission electron microscopy the organisms have been seen in phagocytic vacuoles, suggesting that they can attract and activate polymorphs.³²

C. pyloridis was first identified using campylobacter isolation techniques.³³ A recent study has evaluated different methods for isolating *C. pyloridis* from gastric biopsies and details the most satisfactory method.³⁴ Although now officially termed *Campylobacter pyloridis*, considerable doubt remains as to its true taxonomic status.³⁴ *C. pyloridis* are micro-aerophilic, gram negative, oxidase and catalase-positive bacteria.³⁰ Unlike most campylobacters they are not nitrate reducing, will not hydrolyse hippurate and are resistant to nalidixic acid.¹¹ One of the remarkable properties of the organism is its great ability to split urea,¹¹ a feature not seen in other campylobacters affecting man. So marked is this phenomenon that the urease activity of a homogenised biopsy can be used for specifically diagnosing colonisation.³⁵

C. pyloridis differs from other campylobacters in various biochemical characteristics. Polyacrylamide gel electrophoresis with 15 different *C. pyloridis* strains has shown similar protein bands.¹² These, however, differ from those of campylobacter reference strains. The major cellular fatty acids are also different to those of other campylobacters.²⁹ Unlike all previously known campylobacter species, *C. pyloridis* lacks methylated

menaquinone-6 and it shows phosphatase activity in the phenolphthalein phosphate test.³⁶ The DNA base pair ratio is, however, in the campylobacter range (guanine+cytosine 36%).³⁰ Although it is generally convenient to include *C pyloridis* as a campylobacter for classification purposes at present, studies of cistron similarities will confirm whether or not it represents a new bacterial genus.³⁶

Whilst culturing gastric biopsies for *C pyloridis*, a curved rod has also been identified in a small number of patients and called gastric campylobacter-like organism type 2 (GCLO-2).³⁷ The biotyping³⁷ and cellular fatty acid profile³⁶ resembles that of *C jejuni*, indicating that it is a true campylobacter and not related to *C pyloridis*.

If *C pyloridis* is indeed a pathogenic organism, a local and perhaps a systemic antibody response might be expected. Serum complement fixation, haemagglutination and bacterial agglutination tests have all been reported and bacteria positive patients found to have raised titres.^{14 38} In order to examine the humoral response in more detail ELISA techniques have been used. Serum IgG titres in peptic ulcer patients are raised compared with children and laboratory staff.³⁹ The IgA response is of more relevance to local gut immunity and in this issue an ELISA study using whole organisms as antigen is reported. Serum IgA and IgG titres to *C pyloridis* are significantly raised in the bacteria-positive patients. Interestingly, no differences were found in serum IgM titres which is consistent with longstanding *C pyloridis* colonisation. The results also suggest a degree of antigenic variation in *C pyloridis* strains and some antigenic similarity between *C pyloridis* and other campylobacter species. Choice of antigen, both the strains used and preparation, are important. Our preliminary results comparing single strain, whole and sonicated *C pyloridis* as antigens for an IgG assay, suggest that a sonicate gives good differentiation between positive and negative patients. Absorption studies with *C jejuni* demonstrate the IgG assay to be specific, but some cross reaction occurs in the IgA assay.

In the study reported in this issue p. 642 local gastric juice antibodies were also demonstrated in a proportion of the bacteria-positive patients. Further evidence of local antibody production comes from immunohistochemical and biopsy culture studies. Using gastric biopsy cultures, we have found *C pyloridis* specific IgG and IgA in bacteria-positive patients only. With an immunoperoxidase technique IgG, IgA and IgM coating of organisms in tissue sections has also been shown.⁴⁰ Organisms deep within the gastric pits appear uncoated, suggesting that they may be protected in this site from host antibody.

Evidence supporting a pathogenetic role for *C pyloridis* in man comes from an Australian study in which a volunteer ingested *C pyloridis* and from the sporadic reports of apparently infective gastritis. Ingestion of live *C pyloridis* after a dose of cimetidine in a subject known to have normal gastric histology, resulted in a variety of non-specific symptoms.⁴¹ Histology carried out 10 days after ingestion, showed the presence of spiral organisms, diminished intracellular mucus and polymorph infiltration of the mucosa. At electron microscopy the microvilli were depleted. A repeat biopsy four days later showed no organisms or polymorphs present. The mucus content of the epithelial cells had improved, but was not normal and the ultrastructural changes remained.

Instances of gastritis have been reported in volunteers undergoing gastric secretion studies using the same sets of equipment which were not sterilised between subjects.^{42 43} It has been suggested that these might have been caused by *C pyloridis*⁴⁴ and retrospective analysis of the available biopsy material has shown *C pyloridis* colonisation, implicating this as the infective agent.⁴¹

In vitro studies have shown *C pyloridis* to be sensitive to a number of antibiotics including tetracycline, erythromycin, penicillin, cephalothin, and metronidazole. Resistance has been shown to vancomycin, sulphamethoxazole, and trimethoprim.^{25 45} Sensitivity testing using ulcer healing agents has shown *C pyloridis* to be susceptible to bismuth, but relatively insensitive to cimetidine, carbenoxolone, and sucralfate.⁴⁵ These sensitivities are of considerable interest in view of a report suggesting that metronidazole heals ulcers⁴⁶ and studies suggesting decreased relapse rates in patients with duodenal ulcer treated with bismuth compounds.⁴⁷ Preliminary observations of therapy with bismuth eradicating *C pyloridis* in most patients tested, are now appearing.⁴⁸⁻⁵⁰ Similar results were also seen in a small number of patients treated with amoxycillin, but not erythromycin.⁴⁸⁻⁵⁰ Where eradication of *C pyloridis* has been achieved, there was histological improvement with decreased polymorphonuclear and mononuclear cellular infiltrates. Follow up of these patients has, however, shown a significant relapse rate.^{48 49} Restricted endonuclease analysis of the bacterial DNA before treatment and after relapse shows that the same strain is involved, suggesting relapse rather than reinfection.⁴⁹ A combination of bismuth and an antibiotic has been used and appears to result in a lower relapse rate one month after treatment.⁵¹ Studies where histamine H2 antagonists, sucralfate and carbenoxolone have been investigated show no alteration in *C pyloridis* colonisation nor improvement in gastritis.^{48 49}

Many questions remain unanswered concerning the role of *C pyloridis* in the pathogenesis of human disease. The evidence accumulating from morphological, serological and therapeutic data strongly implicates *C pyloridis* in its unique ecological niche in the stomach, in the pathogenesis of active chronic gastritis. Whether this condition is a cause of symptomatic dyspepsia is a matter of dispute. Langebenberg¹¹ for instance found six of 25 healthy medical students to have chronic gastritis and *C pyloridis*. Dyspeptic symptoms have recently been linked with the activity of gastroduodenitis, as judged by the presence of mucosal neutrophils,^{52 53} a feature not taken into account in previous population studies. Most patients with peptic ulcer disease have chronic gastritis. Therefore, does the sequence of events after *C pyloridis* infection, lead in some patients to peptic ulceration?

Marshall *et al*⁴¹ have described an entity they called 'acute pyloric campylobacter gastritis' with achlorhydria. They suggested that in a proportion of patients the infecting organism is not cleared by the normal immune processes and persists to produce a chronic phase. Gastric acid secretion eventually returns, giving the potential for ulceration of the chronically inflamed gastroduodenal mucosa. The mucus blanket covering the gastroduodenal epithelium maintains a neutral pH at the epithelial cell surface and is considered to be important in mucosal protection.⁵⁴ Disturbance of enterocyte mucus production is a characteristic feature of mucosa colonised by *C pyloridis* and may predispose to peptic ulceration

by exposing gastric type epithelium to damaging luminal factors. A chronic, indolent infection by this highly adapted organism, may yet emerge as the common pathogenic denominator in the spectrum of gastroduodenitis and peptic ulceration.

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