ORIGINAL ARTICLES

Attempt to fulfil Koch's postulates for pyloric campylobacter

(see also page 431)

Barry J. Marshall, John A. Armstrong, David B. McGechie and Ross J. Glancy

ABSTRACT: A volunteer with histologically normal gastric mucosa received pyloric campylobacter by mouth. A mild illness developed, which lasted 14 days. Histologically proven gastritis was present on the tenth day after the ingestion of bacteria, but this had largely resolved by the fourteenth day. The syndrome of acute pyloric campylobacter gastritis is described. It is proposed that this disorder may progress to a chronic infection which predisposes to peptic ulceration.

(Med J Aust 1985; 142: 436-439)

The association between the newly described bacterium, pyloric campylobacter (PC), and gastritis has now been confirmed in several countries,[1,2] and is further substantiated by the study of Marshall et al. which also appears in this issue of the Journal.[3] Nevertheless, the possibility remains that these bacteria are not the primary cause of gastritis, but are merely opportunistic commensals of previously abnormal mucosa.

We therefore attempted to fulfil Koch's postulates[4] for pyloric campylobacter. The requirements for the fulfilment of Koch's first two postulates may be obtained from the recent literature.

First postulate. "The organism, germ, should always be found microscopically in the bodies of animals having the disease and in that disease only; it should occur in such numbers, and be distributed in such a manner as to explain the lesions of the disease." The disease in this case is an inflammatory condition of gastric mucus-secreting epithelium at present variously termed "type B gastritis", "antral gastritis", "active chronic gastritis", "superficial gastritis", and "acute gastroduodenitis".[5,6] In this disorder, PC is almost always present, adherent to the gastric mucosa. The bacteria do not attach to cells of intestinal type, so they are not found in the duodenal cap, except on islands of gastric epithelium.[7] Such "metaplastic" gastric epithelium can be found on the borders of duodenal ulcers, and in the duodenal cap of patients with duodenitis.[8-10]

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Second postulate. "The germ should be obtained from the diseased animal and grown outside the body." PC was first isolated in 1982 from a patient with gastritis.[11] Since January 1983 PC has been cultured from over 150 patients in our hospital. In almost all patients, an infiltration of polymorphonuclear cells has been found in the specimens of antral mucosa on the initial or a subsequent biopsy.

The experiment described in this paper was undertaken in order to fulfil Koch's third and fourth postulates; that is, to demonstrate that PC could colonize histologically normal mucosa and induce gastritis.

Methods and results

The histological and microbiological techniques used in this experiment were identical with those described in the following paper.[3] Electron microscopic specimens were fixed in buffered glutaraldehyde, embedded in epoxy resin for thin sectioning, and stained with uranyl acetate and lead citrate.

Third postulate. "The inoculation of these germs, in pure cultures, freed by successive transplantations from the smallest particle of matter taken from the original animal, should produce the same disease in a susceptible animal." The subject was one of the writers (B.J.M.), a 32-year-old man, a light smoker and social drinker, who had no known gastrointestinal disease or family history of peptic ulceration. Biopsy specimens of the mucosa of the gastric body, antrum and duodenal cap were taken at endoscopic examination. No inflammation or ultrastructural abnormality was detected in any of these specimens (Figure 1, A, B). PC could not be seen in Gram-stained smears of gastric mucus, nor could the organism be cultured from the biopsy specimens, despite the successful isolation of PC from two patients with duodenal ulcers and one patient with gastritis in the same gastroscopy session. The test isolate was taken from the latter patient, a 66-year-old man with non-ulcer dyspepsia. Before the human experiment was conducted, a portion of this isolate was inoculated intraperitoneally into two rats which suffered no ill effects after the inoculation. The isolate was sensitive to ampicillin, erythromycin, tinidazole, doxycycline and rifampicin. It was freeze-dried, then revived for the experiment.

One month later, when electron microscopic results were available and any lesion which resulted from the initial biopsies could be presumed to have healed, the subject fasted overnight and received premedication with cimetidine (600 mg) at 8 a.m. to produce temporary achlorhydria. At 11 a.m., the subject swallowed the growth from a flourishing three-day culture of the isolate (about 10^9 colony-forming units) which was suspended in 10 mL of alkaline peptone water (pH 8.0). He took no food for two more hours, after which he ate normal food. No medication nor alcohol was taken during the subsequent fortnight.

Increased abdominal peristalsis (audible gurgling at night) was noted by the subject during the first 24 hours after ingestion of the bacterial suspension; he had no other symptoms for one week. On the seventh day after ingestion, he had a feeling of fullness in the epigastrium after his evening meal and felt hungry on waking early in the morning. On the eighth day, he woke early and vomited a little mucus at 6 a.m. No pH recording was taken, but the subject was surprised that the vomitus did not have an acidic taste. He had no fever, but had a headache on four occasions during the

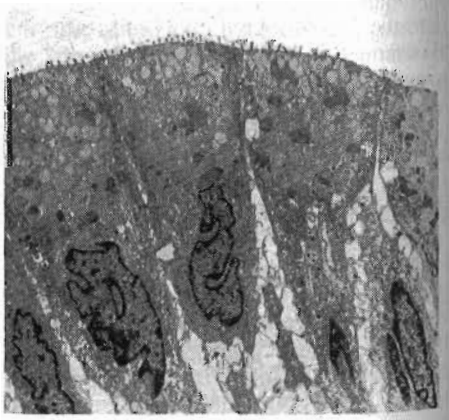

Figure 1: Baseline histology findings. Antrum. **A.** *Haematoxylin–eosin-stained plastic-embedded section. Polymorphs are absent and 50% of the depth of the epithelial cells is mucus (original magnification × 250).* **B.** *Electron micrograph from the same block. The cells have a flat surface with short microvilli and the abundant mucin granules characteristic of antral epithelial cells (original magnification × 4800).*

FIGURE 2: Ten days after ingestion of PC. **A.** *Haematoxylin–eosin-stained section shows PMN infiltration of the lamina propria and glands (original magnification × 250).* **B.** *PAS-stained section shows that the epithelial cells contain very little pink-staining intracellular mucus (original magnification × 250).* **C.** *Numerous PC organisms demonstrated by Warthin–Starry's silver staining (original magnification × 900).* **D.** *Electron micrograph of the reprocessed paraffin material demonstrates PC organisms adhering to an epithelial cell with altered smooth-surface membrane (original magnification × 16 500).*

second week. His faeces softened slightly, but did not progress to diarrhoea. The subject was irritable, and appeared ill during the second week of the experiment; several colleagues observed that he developed "putrid" breath.

Gastroscopy was repeated on the tenth day solely for the purpose of seeing if infection had been established. Unlike the first procedure, the subject tolerated it poorly with much heaving and regurgitation of mucus, which made it necessary to obtain biopsy specimens quickly and terminate the procedure. The gastric mucosa appeared normal, but the endoscopist stated that it was "soft", that is, the biopsy tissue could be pulled away very easily, compared to the initial biopsies. The subject did not feel a "tug" when the biopsy specimens were taken, as he did at the initial and subsequent endoscopies. Two samples were taken from the antrum, one for histological examination and one for culture.

The biopsy specimen obtained for histological examination was small and fragmenting, but many polymorphonuclear neutrophil leucocytes (PMNs) were present in the lamina propria and on the surface of the mucosa (Figure 2A). Epithelial cells were abnormal, and intracellular mucus was almost absent as evidenced by the periodic acid–Schiff (PAS) staining (Figure 2B). Spiral bacilli could be seen adhering to the surface and glandular epithelium, as well as among PMNs in the mucus (Figure 2C). As separately fixed tissue was not available, electron microscopy was performed later on the reprocessed paraffin block. Ultrastructural preservation was less than ideal, but sectioned PC organisms were clearly adherent to the luminal surface of the antral epithelial cells, the smooth bulging surfaces of which were almost devoid of microvilli (Figure 2D).

The subject continued to feel hungry on wakening, early in the morning, but no new symptoms developed and no more vomiting occurred. Gastroscopy was repeated on the fourteenth day, when biopsy specimens for light and electron microscopy were taken. It was expected that continuing gastritis and PC infection would be present, but no organisms could be seen in any of the specimens. At this time, the histological changes had diminished, PMNs were once more absent, and only a minimal accumulation of mononuclear cells had remained (Figure 3A). In PAS sections, the mucus content of the epithelial cells had increased but was still less than normal (Figure 3B). The ultrastructural changes usually associated with PC colonization[12] were, however, still present (Figure 3C).

On the fourteenth day, the subject began therapy with tinidazole (500 mg twice a day), which he continued for one week. His symptoms resolved completely within 24 hours of the start of therapy.

Antibodies to PC, as measured by a passive haemagglutination test, did not develop.[13]

Fourth postulate. "The germs should be found in the diseased areas so produced in the animal." Gram-stained specimen of the biopsy taken on the tenth day showed the presence of spiral bacteria, and these were cultured after 48 hours. The organism was identified as pyloric campylobacter by the methods previously described. Antibiotic sensitivities were the same as in the inoculating strain. The isolate has been freeze-dried, and is available from the writers on request.

Discussion

We have shown that ingested PC organisms are able to colonize the completely normal gastric mucosa. This colonization is associated with acute inflammatory changes; PMN infiltration and exudation from the mucosa; mucus depletion; and reversible epithelial cell damage. These changes may now be referred to as being characteristic of "acute pyloric campylobacter gastritis". In our subject, the host defences eradicated the

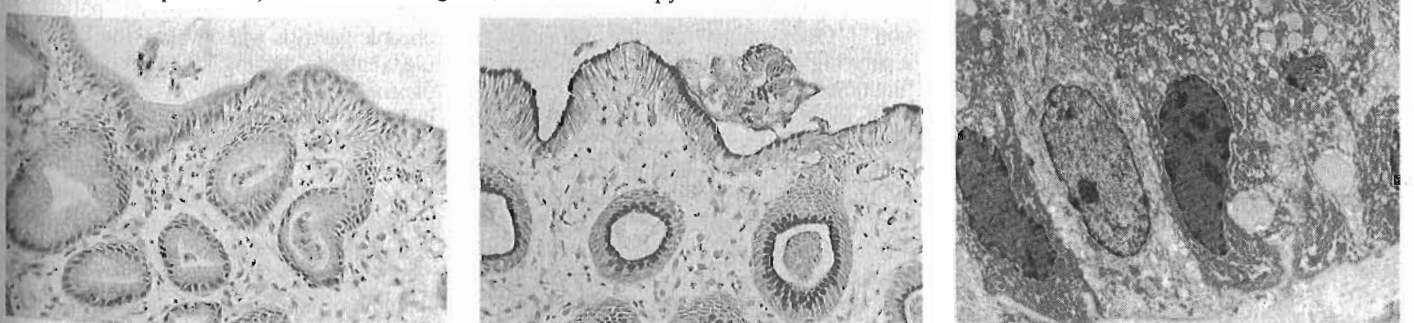

FIGURE 3: Fourteen days after ingestion of PC. **A.** *Haematoxylin–eosin-stained paraffin-embedded section. PMNs are rare, epithelial cells contain mucus, but not yet in normal quantities (original magnification × 250).* **B.** *Mucus content (pink-staining) of epithelial cells is now about 50% of normal (PAS stain; original magnification × 250).* **C.** *Electron micrographs show that many epithelial cells are still abnormal. Microvilli are lacking, the cell surfaces are smooth and bulging, and mucin granules are still depleted (compare with Figure 1B) (original magnification × 4800).*

organism, but, in individuals who are unable to clear a PC infection, chronic gastritis may develop. It is likely that, when the organism persists, acute inflammatory changes (represented by a PMN infiltrate) give way to a chronic inflammatory infiltration of lymphocytes and plasma cells.

Acute pyloric campylobacter gastritis and the subsequent development of chronic gastritis may be the syndrome described by Osler in his *Principles and Practice of Medicine*.[14] Osler described the symptoms of acute and chronic gastritis with achlorhydria as if the disorders were commonplace, and progressed from one to the other. As they are not paralleled by any description in modern textbooks, he is best quoted verbatim:

> *ACUTE GASTRITIS.* In mild cases the symptoms are those of slight indigestion — an uncomfortable feeling in the abdomen, headache, depression, nausea, eructations, and vomiting, which usually gives relief. The tongue is heavily coated and the saliva is increased. In children there are intestinal symptoms — diarrhoea and colicky pains and often slight fever. The duration is rarely more than 24 hours. In the severer forms the attack may set in with a chill and febrile reaction, in which the temperature rises to 102° or 103°F [38.9°C or 39.4°C]. The tongue is furred, the breath heavy, and vomiting is frequent. The ejected substances, at first mixed with food, subsequently contain much mucus and bile-stained fluids. There may be constipation, but very often there is diarrhoea. The urine presents the usual febrile characteristics, and there is a heavy deposit of urates. The abdomen may be somewhat distended and slightly tender in the epigastric region. Herpes may appear on the lips. The attack may last from one to three days, and occasionally longer. The examination of the vomitus shows, as a rule, absence of hydrochloric acid, the presence of lactic and fatty acids, and marked increase in the mucus ... Recovery is usually complete though repeated attacks may lead to subacute or chronic gastritis.
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> *CHRONIC GASTRITIS.* The affection persists for an indefinite period, and, as is the case with most chronic diseases, changes from time to time. Many of the symptoms are due to functional disturbance. The disease itself probably does not cause many symptoms. The appetite is variable, sometimes greatly impaired, at others very good. Among early symptoms are feelings of distress or oppression after eating, which may become aggravated and amount to actual pain. When the stomach is empty there may also be a painful feeling. The pain differs in different cases, and may be trifling or of extreme severity ... There is pain on pressure over the stomach, usually diffuse and not severe. The tongue is coated, and the patient complains of a bad taste in the mouth. The tip and margin of the tongue are often very red. Associated with this catarrhal stomatitis there may be an increase in the salivary and pharyngeal secretions. Nausea is an early symptom and is particularly apt to occur in the morning hours ... Eructation of gas, which may continue for some hours after taking food, is a very prominent feature in cases of so-called flatulent dyspepsia, and there may be marked distension of the intestines. With the gas, bitter fluids may be brought up. Vomiting, which is not very frequent, occurs either immediately after eating or an hour or two later. In the chronic catarrh of old topers a bout of morning vomiting is common, in which a slimy mucus is brought up. The vomitus consists of food in various stages of digestion and slimy mucus, and the chemical examination shows the presence of abnormal acids, such as butyric, or even acetic, in addition to lactic acid, while the hydrochloric acid, if present, is much reduced in quantity. The digestion may be delayed, but usually there is not much disturbance of motility ... Of other symptoms headache is common, and the patient feels constantly out of sorts, indisposed to exertion, and low spirited. In aggravated cases melancholia may occur.

Under the heading of "acute gastritis", Osler described a brief gastrointestinal disturbance of adults and children, characterized by achlorhydria. Achlorhydria is due to either gastric atrophy (very rare in children) or severe superficial gastritis.[15] The latter cause is, therefore, more likely in the syndrome described by Osler, the inflammation causing either parietal cell failure or a back-diffusion of hydrogen ions into the mucosa. The fatty acids which he described would have been the result of bacterial metabolism within the stomach. Acetic acid could have been produced by large numbers of PC bacteria, and butyric acid would have originated from anaerobic bacteria which had colonized the stomachs of Osler's patients with achlorhydria. (Gas-liquid chromatography of gastric juice obtained from 10 patients at gastroscopy, showed a pH of less than 2.0, and neither acetic acid nor butyric acid was present. In one patient, in whom achlorhydria had been induced with high doses of cimetidine, the gastric juice had a pH of 5.4 and contained small amounts of acetic and butyric acids [personal observations].)

Today, the disorder described by Osler would be labelled "acute viral gastroenteritis" and, as it is no longer fashionable to test the pH of vomitus in such cases, the achlorhydria would go undetected. The "chronic gastritis" described by Osler includes many of the symptoms observed in patients with "nervous dyspepsia" or "non-ulcer dyspepsia", particularly that of "eructation".[3] Osler points out the difficulty of separating these patients from those with functional disorders.

Of the more recent descriptions of this disorder in the literature, the most detailed is Ramsey et al.'s "epidemic gastritis with hypochlorhydria".[16] They described an epidemic which occurred in healthy individuals who were undergoing multiple gastric intubations for acid secretion studies. Of their 37 subjects, hypochlorhydria developed in 17 and, in one-half of the cases, this was preceded by symptoms similar to those described by Osler. Where antral mucosa was obtained, PMN infiltration was noted; the histological findings were identical with those in our case. The disorder was more common in their younger volunteers, and was associated with an elevated serum level of pepsinogen. The hypochlorhydria lasted about three months on average, but in three subjects some achlorhydria still persisted after one year.

At the time the epidemic occurred, it was presumed that an infectious agent, possibly a virus, was responsible. However, PC organisms have now been demonstrated by retrospective examination of the biopsy specimens taken from the subjects of Ramsey et al. (W.L. Peterson, personal communication). One similar case reported from Holland by Wiersinga and Tytgat[17] has also been found to be caused by PC (G.N. Tytgat, personal communication).

Pyloric campylobacter gastritis: a hypothesis

Although the disease described by Osler has been forgotten, it is probable that all patients with antral gastritis pass through such an acute stage. By piecing together the previous reports and combining the data with those obtained in our own experiment, it may be possible to reconstruct the natural history of antral gastritis.

The acute form of the disease should be most common in previously unexposed individuals, that is, children or young adults. It will be seen in families with a history of peptic ulceration or chronic dyspepsia. After ingestion of the infective agent (PC bacteria), the patient will have no symptoms for about one week. Subsequently, an acute gastrointestinal disturbance, characterized by epigastric discomfort, nausea and vomiting, will develop in 50% of cases, the remainder being asymptomatic.[16] In the acute stage, the vomitus will contain acetic and fatty acids with a reduced amount of hydrochloric acid. Halitosis may be a feature.

If the organism is not cleared by natural immune processes, a mild gastrointestinal disturbance may persist (?Osler's chronic gastritis), but the patient will have achlorhydria, and so will not suffer from acidity to any degree. The achlorhydric phase will last three to 12 months during which time the histological pattern of chronic gastritis will develop, the PMNs being replaced largely by lymphocytes and plasma cells. As immunity to the infection increases, the inflammation in the body of the stomach will regress and acid secretion will return. The most severely affected mucosa will then be in the antrum and pyloric canal where bacterial growth will be less inhibited by acid secretion. In this final, "chronic" stage of the infection, chronic inflammation of the gastroduodenal mucosa will persist, but acid secretion will return to baseline levels. The patient will then have the potential for the development of peptic ulceration.

By obtaining serial microbiological and histological data from patients with the acute syndrome, it should now be possible to confirm that pyloric campylobacter is, indeed, the common factor linking acute achlorhydric gastritis, chronic gastritis, and peptic ulceration.

Acknowledgements

We wish to thank Dr J.R. Warren for advice on the historical aspects; Dr I.G. Hislop for performing the gastroscopies; Mr N. Nokes for the cultures; and S.H. Wee (EM) and J.E. Holdsworth for the histological preparations.

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(Received October 30, 1984; accepted January 9, 1985)

Pyloric campylobacter infection and gastroduodenal disease

(see also page 431)

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ABSTRACT In 1982, a new spiral Gram-negative bacterium which was similar to those of the genus *Campylobacter* was isolated from the gastric mucosa of 11 patients with gastritis. From then on, the organism was isolated in a further 114 of 267 patients who underwent antral biopsy in Fremantle Hospital between January 1983 and September 1984. During 1984, the bacterium was cultured from 88% of patients in whom it was detected histologically, and was not cultured from any patient with histologically normal gastric mucosa. The new bacterium, pyloric campylobacter, grew in three days on brain–heart infusion blood-agar at 37°C in an atmosphere with added CO_2. All isolates tested were sensitive to penicillin, erythromycin, tetracycline, cephalosporins, gentamicin and bismuth citrate; 80% of isolates were sensitive to metronidazole or tinidazole. It is suggested that pyloric campylobacter infection is a major factor in the causation of dyspeptic disease and peptic ulceration. Antibacterial regimens directed against the bacterium may provide a permanent cure for these chronic disorders.

(Med J Aust 1985; 142: 439-444)

After Warren observed that campylobacter-like organisms inhabited the gastric epithelium of patients with gastritis,[1] a study which was conducted between April and June 1982 in the Royal Perth Hospital led to the isolation of a new, Gram-negative spiral bacterium, pyloric campylobacter (PC). The bacterium was present in 57 of 100 patients who underwent gastroscopy, including all 13 patients with a duodenal ulcer and 14 of 18 patients with a gastric ulcer.[2-4] The new organism was very uncommon in patients with histologically normal mucosa, but was found in 38 of the 40 patients who were found to have polymorph infiltration of the mucosa representative of active disease. It appeared to inhabit the deeper (alkaline) layers of the antral mucus and, thus, was protected from the luminal gastric acid.

Because of the investigatory nature of the original work, the new bacterium was isolated only from 20% of the biopsy specimens in which it was evident on histological examination. The taxonomic status of the new bacterium was uncertain, and it could not be isolated reliably. This research was continued in Fremantle hospital, and, in January 1983, isolation methods suitable for routine use were developed and the original observations of Warren[1] and Marshall[2] were confirmed.

The purpose of this paper is to communicate our preliminary data, so that others may test the validity of our observations. We found that pyloric campylobacter can be easily isolated in a standard clinical microbiology laboratory. The organism is associated with biopsy-proven gastritis and is rarely found in gastric biopsy specimens which show no evidence of inflammation. We postulate that pyloric campylobacter is a common and important gastrointestinal pathogen.

Patients and methods

The patients were seen by B.J.M. at a joint endoscopy session with a gastroenterologist during 1983, and at a dyspepsia research clinic established by B.J.M. in 1984. In 1983, biopsies were performed only in patients with ulcers. When microbiological techniques became sufficiently advanced, biopsy specimens were taken also from patients with normal findings at gastroscopy, so that histologically normal samples of gastric mucosa could be obtained. These specimens were cultured to see if pyloric campylobacter were present as a commensal of normal tissue, perhaps in numbers too small to be visible in histological sections.

In 1984, most of the patients who underwent biopsy came from the dyspepsia research clinic, and were referred specifically to have the diagnosis of pyloric campylobacter infection excluded after other investigations had failed to provide a diagnosis. Patients with known duodenal ulcer disease were also referred for investigation after reports of the new bacterium appeared in the local press. The 1984 group of patients represented a consecutive series in which data were complete, and these data were analysed separately.

At the time of writing, antral biopsy specimens for histological examination had been obtained from 350 patients; in 267 of these the material was also sent for culture. Microbiological data were entered onto a computer database. A brief recording of the endoscopy findings was made at the time of the examination. Gastric ulcers were defined as those which were visible from within the stomach and which did not have their major portion within the pyloric canal. Duodenal ulcers, therefore, included nearly all pyloric canal ulcers. The findings in all other patients, except those with carcinoma, were recorded as "dyspepsia, no ulcer". No records were kept of patients in whom a biopsy was not performed.

Analysis of data

The data were analysed to find the answers to the following three questions: What proportion of patients with peptic ulceration had pyloric campylobacter infection?; In patients without pyloric campylobacter infection, what other factors could be indentified which might predispose to ulceration?; How efficient were the microbiological techniques in use?

Names of patients with ulcers were obtained from the endoscopy record book. The microbiology record was then examined and pa-

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