The *Campylobacter pylori* Story

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The *Campylobacter pylori* story began before the turn of the century, with early works describing 'spirochaetes' in the gastric mucosa of animals. Culture of the organism in 1982 enabled investigators to make sense of the many previous works concerning the microbiology, biochemistry, and histology of the gastric mucosa. Whereas some physicians remain skeptical of *C. pylori*'s pathogenic role, those who have studied the new organism believe it is a major gastrointestinal pathogen and see the possibility of curative therapy for what is now called 'acid peptic disease'.

Key words: Campylobacter pylori; gastritis; non-ulcer dyspepsia; peptic ulcer; urease

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The spiral bacterial that colonize the stomach of mammals have been studied since before the turn of the century. Bizzozero (1) and Salomon (2) reported that spiral bacteria were present in the stomach of cats and dogs, but less commonly in kittens. These and other early investigators knew that the spiral organisms inhabited the glands and oxyntic cells of acid-secreting fundus and therefore must have been acid-tolerant (Fig. 1).

In 1938 Doenges (3) extended these studies to report similar spiral bacteria in postmortem human stomachs, and in 1940 Freedberg & Barron (4) noted their presence in resected gastric tissue from patients with peptic ulcer disease or cancer. Ten years later, Palmer (5) denied the existence of the spiral bacteria, and they were subsequently forgotten.

In the 1960s and 1970s physicians and microbiologists overestimated their ability to culture fastidious microorganisms and assumed that negative bacterial cultures from gastric samples meant that the stomach was sterile. They ignored the previous human and animal literature and failed to include light microscopy in their study of the gastric mucosa. Thus the large numbers of *C. pylori* present in many samples of gastric tissue were not noticed or were discounted as unimportant commensal flora.

An example of the above-mentioned error was the lack of attention paid to the work of Steer and Colin-Jones in 1974–5. They found bacteria associated with diffuse gastritis in gastric ulcer patients and observed that neither the bacteria nor the inflammation was affected when the ulcers were healed with carbenoxolone (6, 7). The importance of the finding went unappreciated when the organisms were incorrectly identified as *Pseudomonas spp.*

In parallel with early studies of the spiral gastric bacteria was interest in gastric mucosal urease. Murray Luck (8) studied the urease enzyme of gastric mucosa in 1924. Luck was unaware of the gastric spiral bacteria and he concluded that urease arose from the gastric mucosal cells. The purpose of gastric urease was unclear, but large amounts of ammonia were observed in the vomitus of uremic persons (9), leading to the thesis that gastric urease acted as a 'safety valve', enabling the excretion of excess nitrogen, as ammonia, in the vomitus.

Fitzgerald & Murphy (10) studied gastric urease from human stomachs resected for peptic ulcer disease. They believed that urease protected the gastric mucosa from acid by buffering hydrogen ions with ammonia. They even treated ulcers with urea, apparently rendering their patients

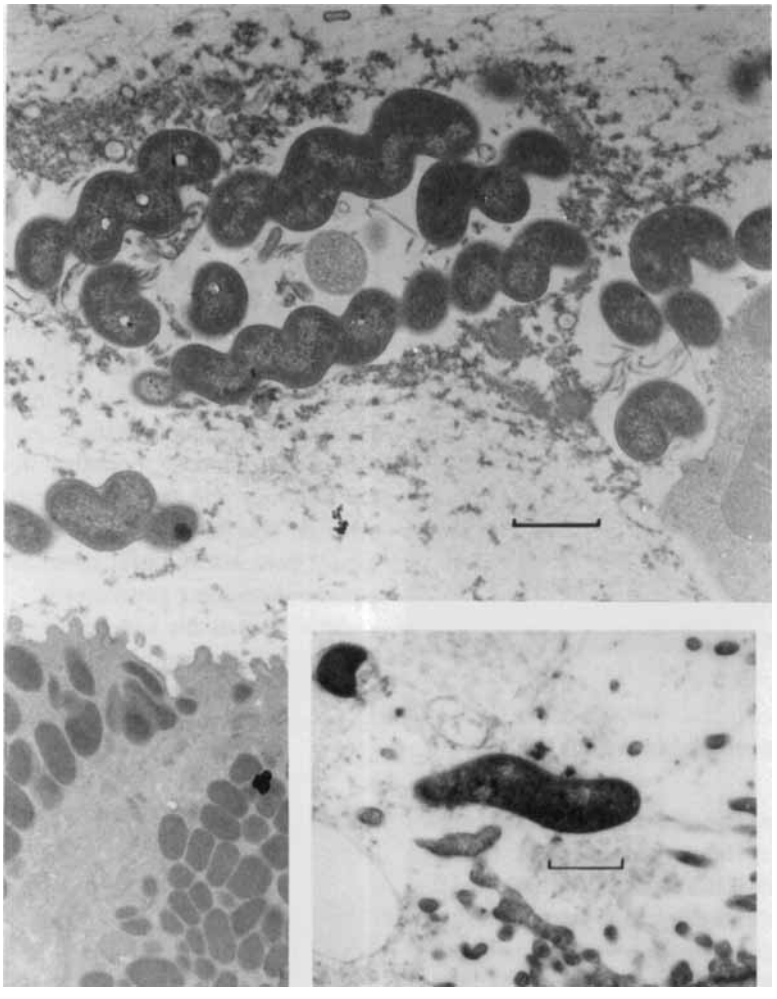


Fig. 1. Electron micrograph showing gastric spirilla in a parietal cell gland from a cat stomach. Note that these bacteria have multiple tight spirals and are easily distinguished from *C. pylori* (inset shows *C. pylori* for comparison). Bars = 1 μm.

achlorhydric subsequent to the neutralization of gastric acid with ammonia in the following equation:

$$\begin{array}{l} NH_2 \\ | \\ C{=}O + 2H_2O + H^+ \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^- \\ | \\ NH_2 \end{array}$$

Liebre & LeFevre (11) noted the disappearance of gastric urease after tetracycline administration, thus indicating that the enzyme was bacterial in origin. In 1968 Delluva et al. (12) proved that the urease was bacterial when they observed that gastric urease was never present in germ-free animals.

My own experience with *C. pylori* began in 1981. During a gastroenterology elective term I met Robin Warren, a pathologist, who had taken an interest in spiral bacteria he had seen after silver staining a gastric biopsy specimen in 1979 (Fig. 2 and 3). In 1981 I had no first-hand knowledge of patients with gastritis because it was regarded somewhat as a 'non-diagnosis' and a finding of little practical relevance to patient management.

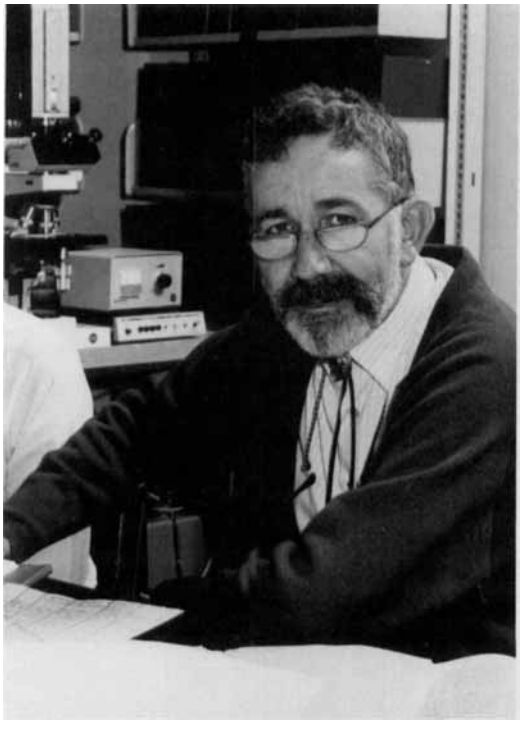


Fig. 2. Robin Warren, taken in 1985.

Warren gave me the names of 25 patients in whose gastric biopsy specimens he had seen bacteria. I studied the case records but could find no clinical syndrome linked to bacteria. Most of the patients had dyspepsia but had widely different diagnoses, ranging from duodenal ulcer to normal endoscopy and 'functional dyspepsia'.

One patient was an elderly Russian man who carried a diagnosis of 'abdominal angina' and was losing weight because of postprandial epigastric pain. His endoscopy was normal, and with the patient's permission, I treated him with oral tetracycline suspension for 2 weeks, and Warren observed disappearance of the bacteria and healing of antral gastritis. The patient swore he was improved by this therapy, but because of a language problem we were uncertain as to the symptoms that had responded.

We then commenced a study of 100 consecutive patients undergoing elective endoscopy in an attempt to find the origin of the bacteria, identify any associated syndrome, and define the association with gastritis. By that time we had enlisted the help of microbiologists, who were becoming adept at demonstrating the bacteria in Gram stains of mucus but could not culture the bacterium.

In the study protocol, all patients were asked about smoking, alcohol, animal contact, and the state of their oral hygiene. We referred to the bacteria as '*Campylobacter*-like organisms' or CLO. We suspected that the bacteria were commensals of the mouth or perhaps acquired from eating chicken (a known source of *Campylobacter jejuni*), or colonized the stomach of patients whose gastric acidity was impaired from taking H_2-receptor antagonist therapy, antacids, or milk.

Two antral biopsy specimens were taken, one for histology and one for microbiology. There were no side effects from the study, and since then I have taken antral specimens from every patient undergoing gastroscopy.

After 30 patients had been studied, the bacterium was first cultured, partly due to luck because, although the plates were being incubated under conditions suitable for *Campylobacter*, they

Fig. 3. The author, taken in 1988.

were being read at 3 days and discarded if no growth occurred. Luckily, over the Easter break, plates were left in the incubator for 5 days, by which time numerous colonies of *C. pylori* were evident (13). We now know that *C. pylori* takes 3–6 days to form visible colonies.

After that time the bacterium was isolated from 11 patients in the study. *Campylobacter* organisms were only just gaining popularity as an enteric pathogen, and our laboratory had little experience with culture of these microaerophilic organisms. Today all microbiology laboratories routinely culture for *Campylobacter* and can easily set up methods for isolating *C. pylori*.

Ultrastructural examination of our biopsy specimens and cultures showed that the bacterium was at least a new species, and probably a new genus, because it had multiple sheathed flagella, unlike the usual *Campylobacter* organisms, which had simple, single unsheathed flagella.

Although our clinical studies were generally unrewarding, we did note an association between burping and the presence of the organism and proposed that some patients with 'flatulent dyspepsia' might have *C. pylori*.

The histologic studies were remarkable. Warren observed that nearly all patients with the new bacterium had gastritis, whereas patients without the bacterium had normal mucosa. The association was so strong that most statistical programs could not calculate the p value accurately, and returned a value <0.0000.

All 13 patients with duodenal ulcer had the bacterium and gastritis. Eighty per cent of the patients with gastric ulcer and about half of the patients without an ulcer also had the bacterium (Fig. 4). Thus this new *Campylobacter* was associated with gastritis and, as a consequence, with peptic ulcer disease (14).

An extensive literature search revealed numerous references to the spiral bacteria. Starting with the work of Ito (15) and following his references, we uncovered reports by Palmer, Freedberg, and Doenges, and later the first paper of Steer & Colin-Jones (6), who have since published a series on the work. As a result of reading these papers and the extensive early original literature on histologic gastritis, I concluded that the new bacterium was probably the cause of peptic ulcer disease, non-ulcer dyspepsia, and possibly also gastric cancer. Further work on a larger series of patients (16) and self-infection with the bacterium (17) strengthened this belief and defined the acute *C. pylori* syndrome, 'hypochlorhydric gastritis'.

The original study has been repeated in many

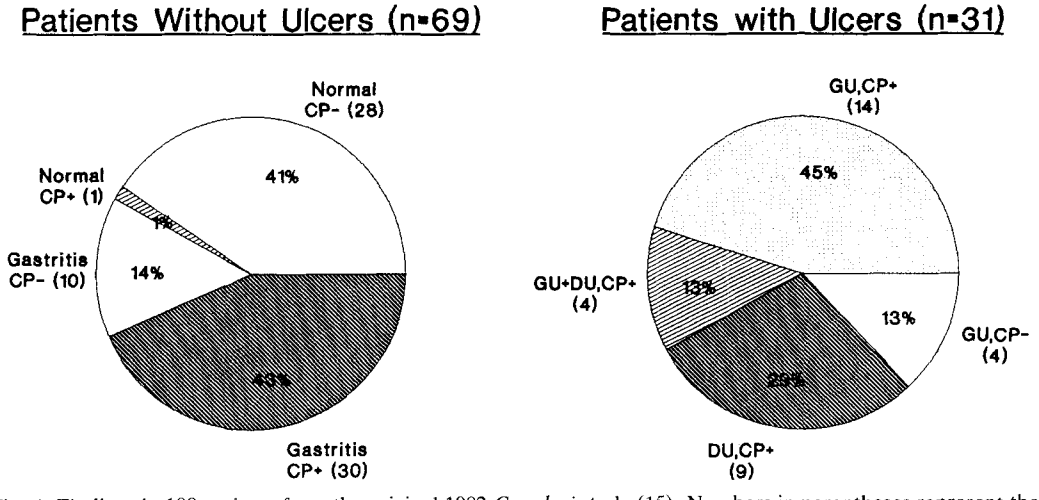
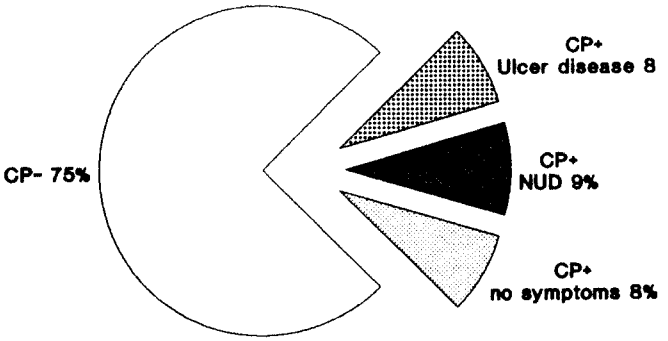
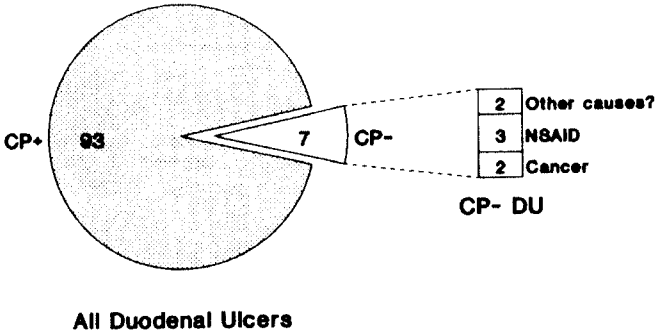


Fig. 4. Findings in 100 patients from the original 1982 *C. pylori* study (15). Numbers in parentheses represent the number of patients.

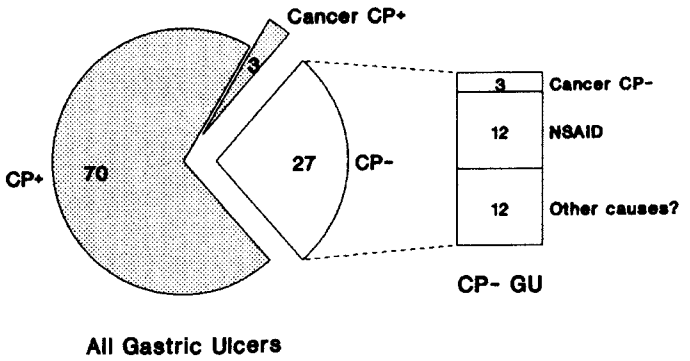
a *C.Pylori* epidemiology-All Adults



b *C.Pylori* and Duodenal Ulcer



c *C.Pylori* and Gastric Ulcer



d *C.Pylori* and Non-Ulcer Dyspepsia (NUD)

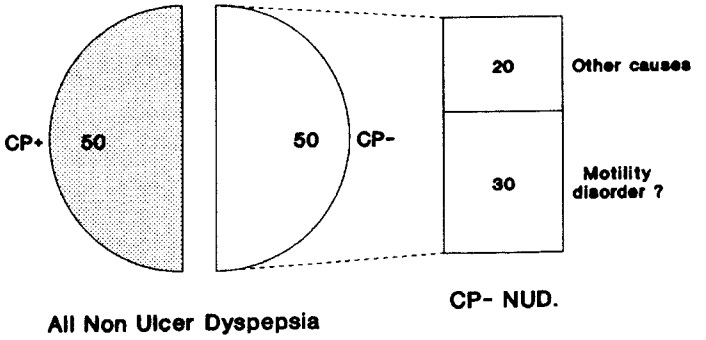


Fig. 5a. Epidemiology of *C. pylori*. Note that *C. pylori* may be commoner in Third-World countries. 5b. *C. pylori* in duodenal ulcer. 5c. *C. pylori* in gastric ulcer. 5d. *C. pylori* in non-ulcer dyspepsia. Note that the percentage CP+ varies: USA, 40%; Australia, 55%; Italy, 80%.

countries now, and these associations have been consistently reported (18) (Fig. 5a–d). There are some 150 independent works on *C. pylori*, but the role of the new bacterium is still controversial.

After *C. pylori* was isolated and its massive urease production noted by Langenberg et al. (19), the origin of gastric mucosal urease became evident. We now know that the bacterium is uniquely adapted to life in the stomach because of its ability to break down urea and generate alkaline ammonia. Unlike other *Campylobacter* organisms, and weak urease producers, *C. pylori* can survive for at least 30 min at a pH less than 2.0 (20). Thus it can colonize the acid-secreting stomach and does not rely on absence or a buffered gastric acid barrier for its infectivity.

The urease of *C. pylori* now enables us to study its epidemiology conveniently and monitor the progress of patients undergoing antibacterial therapy. McNulty & Wise (21) used Christensen's medium, containing urea and phenol red, to test for urease production in bacterial colonies. They then placed gastric mucosal specimens in Christensen's medium (21), and showed that a color change was present, as predicted by Delluva's earlier studies, only when the specimens were infected by *C. pylori*. With enhanced versions of this test such as the CLOtest® the sensitivity and specificity rival both histology and microbiology (22). Urease (urea amidohydrolase) is one of the most rapidly acting enzymes known, so the test does not rely on amplification with a secondary enzyme to detect *C. pylori* in tissue. The biopsy urease test should therefore remain one of the most rapid means of diagnosing *C. pylori* in patients undergoing endoscopy.

Urease is also the basis for a sensitive and specific non-invasive test for *C. pylori*. In the ^{14}C urea breath test the fasting patient drinks a small amount of water to which has been added up to 10 μCi of ^{14}C urea. If gastric urease is present, the urea is rapidly broken down in the stomach. Breath samples after 15 min contain the isotope as $^{14}CO_2$, which can be detected in a scintillation (beta) counter. The radiation exposure from this test is trivial (less than half that of a chest roentgenogram), and the test can be performed on up to four patients at a time in a 1-h period. The sensitivity and specificity of the test are both 90–100%. Variations include the use of a non-radioactive isotope (23) and giving the isotope in a meal (24). Both these increase the cost of the test.

Urease tests are an effective means to assess response to therapy because they detect the presence of *C. pylori* organisms. Serologic tests assess antibodies, which often remain in high titer after the bacterium is eradicated. For this reason the enzyme-linked immunosorbent assay (25), the commonest serologic test for *C. pylori*, is useful to screen for the infection but inevitably gives false-positive results in the months after eradication of the bacterium.

The epidemiology of *C. pylori* is being studied in many countries but is not yet understood. The prevalence varies with regard to sex, age, social class, race, geographic location, and disease state. *C. pylori* is present in 90% of patients with duodenal ulcer and 70–80% of patients with gastric ulcer, although it may be commoner in gastric ulcers unassociated with non-steroidal anti-inflammatory drugs or carcinoma (26). The bacterium can be found in about 25% of all adults but is much commoner after 50 years of age (40–50%) than below the age of 30 (10–25%).

We have difficulty understanding *C. pylori* epidemiology because the mode of transmission of the infection is not known. In the acute infection abdominal pain, nausea, and vomiting occur, and gastric secretions could be a means of transmission. Like duodenal ulcer disease, *C. pylori* gastritis may affect several members of the same family, implying infection from a common source or person-to-person spread (27). It is not known whether *C. pylori* can be spread by the fecal/oral route, but live organisms have been observed in the human rectum (28), so they probably are excreted in the stool at times.

There is some evidence that the epidemiology of *C. pylori* is changing. At the turn of the century

gastritis with hypochlorhydria was described by Osler as if it were commonplace. Today, in the United States and elsewhere, acute infections with *C. pylori* are not reported, except when they occur in research laboratories (29). Our own experience in Virginia is that the disease is uncommon in children and young adults (B. J. Marshall, J. Sutphen. Unpublished observations). In contrast, *C. pylori* is common at all ages in Peru (30), but children from lower socioeconomic groups are more likely to have the infection (R. Leon-Barua. Unpublished observations). This suggests that *C. pylori* is very similar to other chronic gastrointestinal infections, being acquired early in childhood, especially in areas of overcrowding and poor hygiene.

The increased prevalence of *C. pylori* in older persons in the United States may mean that when those persons were children, they had a greater chance of acquiring the disease. Today's young adults, however, are rarely exposed and are less likely to acquire *C. pylori* during their life (31).

In the United States the apparent decline in duodenal ulcer since 1972 (32) may be due to less *C. pylori* in young adults since that time—that is, persons born after 1950. In addition, the advent of powerful, convenient, safe antibiotics and their widespread use in childhood since the 1970s may have decreased the number of children in whom the infection persisted into adulthood.

The data with regard to *C. pylori* and ulcer disease will increase exponentially now that accurate non-invasive methods of *C. pylori* diagnosis have been described. Almost certainly, the discovery of *C. pylori* will affect the management of duodenal ulcer, gastric ulcer, and non-ulcer dyspepsia. If *C. pylori* is a major etiologic factor, we can expect cheap antibacterial agents gradually to replace the antisecretory drugs and surgery as therapy for these afflictions.

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Discussion

Sewing:

The microbiologists have told me that there exist other urease-positive microorganisms that appear to be more innocent than *Campylobacter pylori.* If you just use the CLO test for diagnosis, how many false-positive results do you get?

Marshall:

The CLO test is buffered, so it needs a considerable amount of gastric urease to generate a positive result. We have rarely seen a false-positive result. *Proteus,* which is the other urease producer we all know about, has 100 times less urease than *C. pylori.* If a urease test turns positive within the first 3 h, it is definitely *C. pylori.* I always withdraw H_2-receptor antagonists 12 h before endoscopy because if a patient has acid in his stomach, the only way he can have urease is if he has *C. pylori. C. pylori* is protected from the acid. It lives under the mucus. In reference to the last speaker, the presence of urease in gastric mucosa means that the pH at the bottom of the gastric mucus layer is greater than a 5.0. *C. pylori* urease is denatured in acid, so that if the pH at the bottom of the mucus layer was less than 5, we would not expect to see a positive urease test.

Ward:

May I ask whether the urease that produces the positive reaction is produced by the organisms in vitro, or is it urease that you happened to collect on your biopsy specimen before you removed it?

Marshall:

It is preformed urease. Most of the tests have a bacteriostat in them; thus you can incubate the tests for 24 h without contaminating commensal flora affecting the result. It is interesting that CLO tests, which are an indeterminate color (faintly orange), usually declare themselves by 24 h, turning red (positive) or more yellow (negative). Acid is released by the specimen as it decomposes, but this acid cannot overcome the ammonia generated by a urease-producing specimen. The optimum pH for urease is about 7, so if CLO test pH starts at pH 6, then once it reaches pH 7, any urease that is present is even more active, so it rapidly turns red. Only about 5% of them that end up an orange color.

Allen:

Please comment on the resistance of *Campylobacter pylori* to acid pH. If urease is inactivated below pH 5, how can it be protective at these lower pH values?

Marshall:

C. pylori is killed in acid when urea is absent. When urea is present, the organism can raise its environmental pH if the starting pH is >3.5. At lower pH's *C. pylori* still survives, apparently generating enough intracellular ammonia to maintain a satisfactory microenvironment within itself. Other bacteria, even *Proteus* spp., are killed in this situation.

Hunt:

We found survival of the organisms in the presence of urea down to a pH of 2.6, but replication of the organism does not occur below about pH 4.5.

Marshall:

Very interesting findings.

Tygat:

Does acid-suppressive therapy have any effect on the intensity of CP colonization?

Marshall:

No; patients who have pernicious anemia, for example, do not have increased prevalence rates of *C. pylori* infection.

Hunt:

What do you think the natural reservoir of this organism is for man?

Marshall:

At the moment, I think it is family members.

Hunt:

Do you have evidence for that? Are there any epidemiologic studies yet available?

Marshall:

The epidemiology is hard to study, but in my clinic more than half the wives of DU patients have *C. pylori,* and most of those are symptomatic.

Bianchi Porro:

Do you think the acute or chronic administration of non-steroidal anti-inflammatory drugs might favour the colonization of *C. pylori*?

Marshall:

There is no evidence to suggest that. I note that the major selling point for any new non-steroidal anti-inflammatory drug is that it does not cause gastric irritation. Either NSAID leads to gastric irritation or, alternatively, people who have arthritis may also be predisposed to gastric problems for some reason. Whether that could be *C. pylori* I do not know, but, again, there are no data yet on non-steroidal drugs and *C. pylori.*