Infection with *Campylobacter pylori*

T. ROKKAS, MD, *Research Fellow**
G. E. SLADEN, DM, FRCP, *Consultant Gastroenterologist,*

*Gastroenterology Unit of United Medical and Dental Schools of Guy's and St Thomas' Hospitals, London**

History

In 1982, campylobacter-like organisms, currently called *Campylobacter pylori (C. pylori)*, were first cultured in Perth, Australia, by Warren and Marshall [1]. This bacterium had been the subject of much attention prior to this date. It had been seen in 1938 by Doenges [2] in post-mortem stomachs and by Freedberg and Barron [3] in gastrectomy specimens. It was also the source of gastric urease noted in gastrectomy specimens in 1950 by Fitzgerald and Murphy [4]. The bacterium was forgotten for approximately 25 years and its significance was not recognised until Steer and Colin-Jones [5] reported its association with gastritis in 1975.

Microbiology

This gastric bacterium is Gram-negative and grows best by culturing gastric biopsy specimens on blood agar plates for 3–5 days at 37°C in a humid microaerophilic atmosphere containing 5–12% CO_2 and 5–15% O_2. Typical *C. pylori* colonies are flat or concave with surrounding haemolysis. *C. pylori* is identified as a curved or coccobacillary rod (Fig. 1) which is oxidase-, catalase- and urease-positive [6], the latter distinguishing it from other campylobacters such as *Campylobacter jejuni* [7]. It also differs in that flagellar stains show 4–5 sheathed flagella, in contrast to other campylobacters which have single unsheathed flagella [8]. Furthermore, the flagella of *C. pylori* have bulbs on the ends similar to those seen in *Vibrio* species. Other differences have also been noted between *C. pylori* and the other campylobacter species: the cellular fatty acid content differs considerably [9] and *C. pylori* lacks the respiratory quinone (methylated menaquinone-6) found in all other members of the genus [10]; finally, protein composition studies by sodium dodecyl sulphate polyacrylamide gel electrophoresis have shown different patterns for *C. pylori* and other campylobacters [11–13].

Diagnosis of *C. pylori* infection

In addition to culture of biopsy material, *C. pylori* can also be detected in fixed sections using different staining processes, ie routine haematoxylin and eosin or silver stain such as the Warthin-Starry method [14] (Fig. 2). Other techniques such as phase contrast microscopy, fluorescent labelling with acridine-orange and a modified Giemsa stain, also give reliable results [15]. Gram staining of cytology specimens, obtained by brushing the gastric mucosa, is simple and may be more effective at showing *C. pylori* than silver stains of biopsy specimens [16]. Recently, monoclonal antibodies specific for *C. pylori* have been produced for rapid immunofluorescent detection of these bacteria in tissues [17].

The ability to produce urease in large amounts and to split urea [18], a feature not seen in other campylobacters affecting man [7,19], is currently being used to diagnose infection with *C. pylori*. There is an excellent correlation between isolation of *C. pylori* and the presence of a

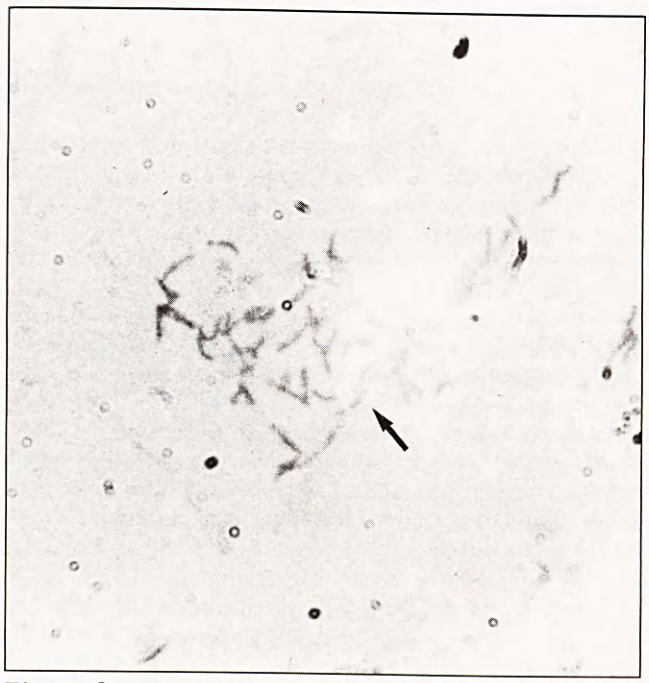

Fig. 1. *Gram stain preparation for a colony of bacteria isolated from a gastric biopsy specimen, where the characteristic spiral appearance of* C. pylori *is shown (arrow).*

*Address for correspondence: Gastroenterology Unit, UMDS of Guy's and St. Thomas' Hospitals, London SE1 9RT.

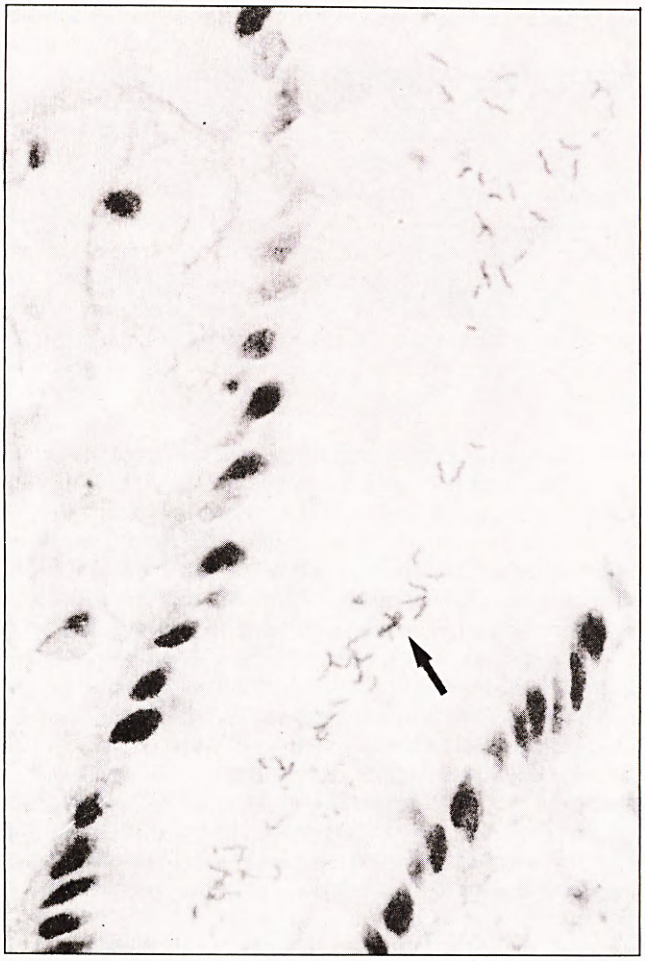

Fig. 2. *A gastric biopsy specimen from a patient with non-ulcer dyspepsia, stained by the Warthin-Starry technique, showing colonisation with* C. pylori *(arrow).*

positive urease action when gastric biopsy specimens are placed in Christenson's medium [10]. A commercially available test (CLO-test, Delta West Ltd., Canning Vale, W. Australia) detects preformed urease in gastric biopsy specimens within 20 minutes to three hours [21]. Alternatively, the biopsy specimens may be crushed and immediately placed in a urea broth; most are positive within an hour [20]. High urease activity is also the basis of a newly developed breath test which is non-invasive, accurate and rapid [22]. In patients with *C. pylori* infection ^{13}C-urea is split by urease in the stomach to produce ammonia and $^{13}CO_2$ which appears in the breath within 20 minutes and accumulates for more than 100 minutes [24]. Similarly, *C. pylori* infected patients have low urea and high ammonia levels in the gastric juice compared with non-infected controls [25].

Immune response to *C. pylori* infection

Several studies have dealt with the systemic and local immune response to *C. pylori,* using a variety of antigens and antibody techniques such as serum complement fixation, haemagglutination, bacterial agglutination tests and enzyme linked immunosorbent assay (ELISA) [26–32]. Although the results of the studies do not fully agree, the major conclusions can be summarised as follows [7]: patients with gastritis, peptic ulcer disease or non-ulcer dyspepsia have elevated *C. pylori*-specific serum antibody levels; specific serum IgA and IgG appear to correlate with infection; local secretory IgA antibodies may be found in gastric secretions; severity of gastritis and level of antibody response do not correlate; in patients with gastritis and in control populations the percentage of subjects with antibodies rises with age and specific antibodies persist for years.

Epidemiology

Graham *et al.* [24] investigated the prevalence of *C. pylori* infection by using the ^{13}C-urea breath test. In these studies, *C. pylori* infection was significantly more common in patients with peptic ulcer than in asymptomatic controls (88.7% vs 20.6% respectively, $p < 0.005$) and *C. pylori* infection was present in 97 per cent of duodenal ulcer patients. In asymptomatic individuals the prevalence of *C. pylori* infection showed an age-related increase that paralleled the age-related increase of gastritis [33]. In detail, the frequency of *C. pylori* infection in asymptomatic individuals was less than 5 per cent for the 25–44 year age group, 20 per cent for the 45–54 year age group, 50 per cent for the 55–64 year age group and 75 per cent for those aged 65–84 years. A similar age-associated increase was not present in patients with peptic ulcer disease.

Pathogenic role of *C. pylori*

C. pylori is closely associated with active chronic gastritis, in which polymorphs, plasma cells and lymphocytes infiltrate the mucosa [6,7,34,35]. There is now good evidence that *C. pylori* can cause gastritis and that it is not merely a commensal on a previously damaged mucosa. The most convincing evidence derives from studies in which normal volunteers have swallowed *C. pylori* with the subsequent development of antral gastritis and colonisation of the antral mucosa by the organism [36,37]. In the literature there are two reports of epidemic hypochlorhydric gastritis in subjects undergoing intubation for gastric secretion studies [38,39]. Evidence to date suggests that *C. pylori* could have been transmitted by inadequately sterilised intubation equipment [7,10].

Acute infection results in an episode of acute gastritis with reduced acid secretion. If *C. pylori* is not eradicated in the acute stage, chronic active gastritis develops and acid secretion returns [10].

The chronic gastritis associated with *C. pylori* infection resembles type B or antral gastritis [7,10] (Fig. 3). *C. pylori* is not associated with type A or autoimmune gastritis which affects only the body mucosa and is associated with pernicious anaemia [40], nor is it associated with gastritis secondary to duodeno-gastric reflux [41]. The organism only colonises the mucus overlying gastric epithelial cells and it is not found in areas of

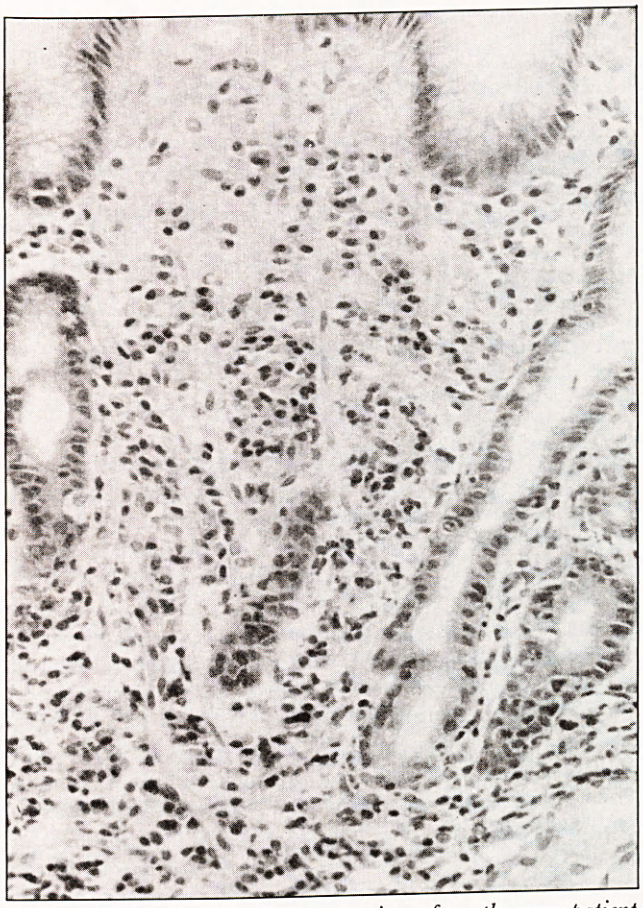

Fig. 3. *H and E stain of biopsy specimen from the same patient as Fig. 2, showing severe gastritis.*

intestinal metaplasia [7,42]. In the duodenum, it colonises areas of gastric metaplasia and is associated with duodenitis [26,43].

C. pylori infection and active chronic gastritis are present in about 90 per cent of duodenal ulcer patients and in about 70 per cent of patients with benign gastric ulcers, which are not associated with NSAIDs consumption [34,44]. This strong association between *C. pylori* and peptic ulcer disease has been the focus of much attention. There is increasing evidence favouring a cytopathogenic role for *C. pylori* with mucus secreting cells as the preferential target [6]. Thus, it is believed that *C. pylori* may play a role in the pathogenesis of peptic ulcer, most probably by weakening the gastric mucosal barrier and exposing the mucosa to the damaging effects of HCl and pepsin [45]. Apart from peptic ulcer, *C. pylori* is also present in association with active chronic gastritis in approximately 50 per cent of the population under the age of 50 years complaining of non-ulcer dyspepsia (NUD), whereas in the general population about 10–25 per cent of adults of comparable age are affected by the bacterium, a difference which is statistically significant [34,35]. There is thus substantial evidence to suggest that *C. pylori* plays a pathogenic role in non-ulcer dyspepsia [35]. A strong association between *C. pylori* and chronic active gastritis has also been reported in association with ulcers and NUD in children, strengthening the possibility that it plays a pathogenic role [45–49].

Treatment of *C. pylori* infection

The isolation of *C. pylori* was followed by *in vitro* antibiotic sensitivity testing. The bacterium appears to be sensitive to many antibiotics (penicillin, amoxycillin, erythromycin, tetracycline, gentamicin and metronidazole) and resistant to others (vancomycin, trimethoprim, sulphamethoxazole and nalidixic acid) [16,20]. Several drugs used in peptic ulcer disease were also tested *in vitro* against *C. pylori*. These tests showed that the most effective drugs were bismuth salts, with minimal inhibitory concentrations in the range of 4–32 mg/l [20,50]. In a placebo-controlled trial erythromycin proved to be ineffective [51], but bismuth salts have proved effective *in vivo*. In double-blind trials, colloidal bismuth subcitrate (De-Nol) was compared with cimetidine in patients with duodenal ulcer and *C. pylori* infection and was shown to be superior in eradicating *C. pylori* from the stomach, improving the associated gastritis and significantly reducing the number of ulcer relapses over a long period (1–2 years) [52,53]. These studies confirmed former observations that bismuth salts are equal to H2 receptor antagonists in healing peptic ulcers and superior in reducing the number of relapses over a long period [54–57]. On the other hand, 4–8 weeks treatment with bismuth salts proved to be superior to placebo in non-ulcer dyspepsia, in that it significantly cleared *C. pylori* from the stomach, improved or restored the associated gastritis [51] and improved dyspeptic symptoms [58,59]. The addition of an antibiotic, such as tinidazole or amoxycillin for the first 10 days of treatment has been reported to be more effective in reducing the risk of recolonisation [52].

Conclusions

The isolation of *C. pylori* has opened a new exciting chapter in gastric microbiology which may have great clinical significance and may change medical thinking about the aetiology and treatment of chronic gastritis and peptic ulcer.

Up to 90 per cent of patients with duodenal ulcer, 70 per cent with gastric ulcer and 50 per cent with NUD have *C. pylori* infection. In these patients there is a striking association between the presence of *C. pylori* and chronic active antral gastritis.

Clearance of *C. pylori* with bismuth salts results in resolution of gastritis, with subsequent ulcer healing and a lower incidence of relapse.

References

1. Warren, J. R. and Marshall, B. J. (1983) *Lancet,* **i,** 1273.
2. Doenges, J. L. (1939) *Archives of Pathology,* **27,** 469.
3. Freedburg, A. S. and Barron, L. E. (1940) *American Journal of Digestive Diseases,* **7,** 443.
4. Fitzgerald, O. and Murphy, P. (1950) *Irish Journal of Medical Science,* **292,** 97.
5. Steer, H. W. and Colin-Jones, D. G. (1975) *Gut,* **16,** 590.

6. Goodwin, C. S., Armstrong, J. A. and Marshall, B. J. (1986) *Journal of Clinical Pathology,* **39,** 353.
7. Blaser, M. J. (1987) *Gastroenterology,* **93,** 371.
8. Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W. and Jewell, L. D. (1987) *American Journal of Clinical Pathology,* **87,** 49.
9. Goodwin, G. S., McCulloch, R. K., Armstrong, J. A. and Wee, S. H. (1985) *Journal of Medical Microbiology,* **19,** 257.
10. Marshall, B. J. (1986) *Journal of Infectious Diseases,* **152,** 650.
11. Pearson, A. D., Ireland, A., Bamforth, J. *et al.* (1984) *Lancet,* **i,** 1349.
12. Perez-Perez, G. I. and Blaser, M. J. (1987) *Infection Immunology,* **55,** 1256.
13. Buck, G. G., Gourley, W. K. *et al.* (1986) *Journal of Infectious Diseases,* **153,** 664.
14. Warren, J. R. (1983) *Lancet,* **i,** 1273.
15. Rathbone, B. I., Wyatt, I. I., Worsley, B. W. *et al.* (1985) *Lancet,* **i,** 1217.
16. Gilman, R. J., Leon-Barua, R., Koch, J. *et al.* (1986) *Digestive Diseases and Sciences,* **31,** 1089.
17. Engstrand, L., Pahlson, C., Gustavsson, S. and Schwan, A. (1986). *Lancet,* **ii,** 1403.
18. Langenberg, M. L., Tytgat, G. N. J., Schipper, M. E. I. and Rietra, P. I. G. M. (1984) *Lancet,* **i,** 1348.
19. Hazell, S. L. and Lee, A. (1986) *Lancet* **ii,** 626.
20. McNulty, C. A. M. and Wise, R. (1985) *Lancet* **i,** 1443.
21. Marshall, B. J., Warren, J. R., Francis, G. J. *et al.* (1987) *American Journal of Gastroenterology,* **82,** 200.
22. Graham, D. Y., Klein, P. D., Evans, D. G. *et al.* (1986) *Gastroenterology,* **90,** 1435.
23. Bell, G. D., Weil, J., Harrison, G. *et al.* (1987) *Lancet,* **i,** 1367.
24. Graham, D. Y., Klein, P. D., Evans, D. G. *et al.* (1987) *Lancet,* **i,** 1174.
25. Marshall, B. J. and Langton, S. R. (1986) *Lancet,* **i,** 965.
26. Jones, D. M., Lessels, A. M. and Eldridge J. (1984) *Journal of Clinical Pathology,* **37,** 1002.
27. Von Wulffen, H., Heeseman, T., Butzow, G. H., Loning, T. and Laufs, R (1986) *Journal of Clinical Microbiology,* **24,** 716.
28. Rathbone, B. J., Wyatt, J. I., Worsley, B. N. *et al.* (1986) *Gut,* **27,** 642.
29. Morris A., Nicholson, G., Lloyd, G., Haines, D. and Taylor, D (1986) *New Zealand Medical Journal,* **99,** 657.
30. Kaldor, J., Tee, W., McCarthy, P., Watson, J. and Dwyer, B. (1985) *Lancet,* **i,** 921.
31. Eldridge, J., Lessels, A. M. and Jones, D. M. (1984) *Lancet,* **i,** 1237.
32. Marshall, B. J., McGechie, D. B., Francis, G. I. and Utley, P. J. (1984) *Lancet,* **ii,** 281.
33. Siurala, M., Isokoski, J. M., Varis, K. and Kekki, M. (1968) *Scandinavian Journal of Gastroenterology,* **3,** 211.
34. Marshall, B. J. and Warren, J. R. (1984) *Lancet,* **i,** 1311.
35. Rokkas, T., Pursey, C., Simmons, N. A., Filipe, M. I. and Sladen, G. E. (1987) *American Journal of Gastroenterology,* **82,** 1149.
36. Marshall, B. J., McGechie, D. B., Rogers, P. A. and Glancy, R. J. (1985) *Medical Journal of Australia,* **142,** 439.
37. Morris, A. and Nicholson, G. (1987). *American Journal of Gastroenterology,* **82,** 192.
38. Gledhill, T., Leicester, R. T., Addis, B. *et al.* (1985) *British Medical Journal,* **290,** 1393.
39. Ramsey, E. T., Carey, K. V., Peterson, W. L. *et al.* (1979) *Gastroenterology,* **76,** 1449.
40. O'Connor, J. H., Axon, A. T. R. and Dixon, M. C. (1984) *Lancet,* **ii,** 1091.
41. O'Connor, J. H., Wyatt, J. I., Dixon, M. C. and Axon, A. T. R. (1986) *Journal of Clinical Pathology,* **39,** 403.
42. Thomas, J. M (1984) *Lancet,* **ii,** 1217.
43. Steer, H. W. (1985) *Journal of Pathology,* **146,** 355.
44. Lusak, B. K., Kouroupos, E., Sherman, A. and Lustbader, I. (1987) *American Journal of Gastroenterology,* **82,** 938.
45. Marshall, B. J. (1987) *Hospital Practice,* **22,** 87.
46. Czinn, S. J., Dahms, B. B., Jacobs, G. H., Kaplan, B. and Rothstein, F. C. (1986) *Journal of Pediatrics,* **109,** 80.
47. Hill, R., Pearman, J., Worthy, P. *et al.* (1986) *Lancet,* **i,** 387.
48. Cadranel, S., Goosens, H. and DeBoeck, M. (1986) *Lancet,* **i,** 735.
49. Drumm, B., Sherman, P., Cutz, E. and Karmali, M. (1987) *New England Journal of Medicine,* **316,** 1557.
50. Skoglund, M. L., Watters, K. and Taubee, J. (1987) *American Journal of Gastroenterology,* **82,** 933.
51. McNulty, C. A. M., Gearty, J. C. and Crump, B. (1986) *British Medical Journal,* **293,** 645.
52. Marshall, B. J., Goodwin, C. S., Warren, J. R. *et al.* (1987) *Gastroenterology,* **92,** 1518.
53. Coughlan, G., Gilligan, D., Humphries, H. *et al.* (1987) *Gastroenterology,* **92,** 1355.
54. Martin, D. F., Hollanders, D., May, S. J. *et al.* (1984) *Lancet,* **i,** 7.
55. Bianchi-Porro, G., Lazzaroni, M., Petrillo, M. and DeNicola, C. (1984) *Lancet,* **ii,** 698.
56. Hamilton, I., O'Connor, H. J., Wood, P. C., Bradbury, I. and Axon, A. T. R. (1986) *Gut,* **27,** 106.
57. Lee, F. I., Samloff, I. M. and Hardman, M. (1985) *Lancet,* **i,** 1299.
58. Rokkas, T., Pursey, C., Simmons, N. A., Filipe, M. I. and Sladen, G. E. (1987) *Gastroenterology,* **92,** 1599.
59. Borody, T., Hennesy, W., Dascalopoulos, G., Carrick, J. and Hazell, S. (1987) *Gastroenterology,* **92,** 1488.