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Campylobacter pyloridis and Gastritis: Association with Intercellular Spaces and Adaptation to an Environment of Mucus as Important Factors in Colonization of the Gastric Epithelium

Stuart L. Hazell, Adrian Lee, Lynette Brady, and William Hennessy

From the School of Microbiology, University of New South Wales, Kensington, Sydney; and the Microbiology Department and Gastroenterology Unit, St. Vincent's Hospital, Darlinghurst, New South Wales, Australia

Stomach biopsy specimens from >40 individuals with *Campylobacter pyloridis*-associated gastritis were examined by light and electron microscopy. The bacteria were consistently seen in two locations: within the gastric mucus and associated with intercellular junctions of gastric epithelial cells. *C. pyloridis* is suggested to be one of a broad group of spiral bacteria that are adapted to the peculiar niche provided by intestinal mucus. The spiral morphology and the form of motility of these organisms give them a selective advantage in a viscous environment. This point has been demonstrated in vitro by measurement of the velocity of clinical isolates in solutions of methyl cellulose of varying viscosity. The localization of *C. pyloridis* close to intercellular junctions is proposed to be due to the presence of preferred metabolites or growth factors, e.g., urea and hemin. All isolates show an extremely high urease activity and require hemin for growth.

Over the past 100 years there have been sporadic reports of bacteria associated with the human gastric mucosa [1]. Little interest was generated in these organisms until 1983, when Warren [2] reported the presence of curved bacilli on the gastric epithelium of a series of patients with active chronic gastritis, and Marshall [3] described the isolation of the organism, which was given the name *Campylobacter pyloridis* [4]. A series of publications have established a strong association between these organisms and the presence of gastritis, and a pathogenic role has been suggested [3, 5–7]. Because of our previous interest in spiral bacteria that colonize intestinal mucus, we undertook an investigation of human gastric biopsy specimens with a view to delineating more clearly the mechanism of association of these organisms with the gastric epithelium.

Materials and Methods

Microscopy. During routine gastroscopic investigations antral biopsy specimens were obtained. A small portion of each specimen was cut away and retained for culture, and the remainder was fixed in Karnovsky's mixture containing 0.05% (wt/vol) ruthenium red.

After fixation for 4 hr, the tissues were washed in 0.1 *M* cacodylate buffer (pH 7.2), dehydrated in a graded series of alcohol, and embedded in acrylic resin (LR white; London Resin Co. Ltd., Basingstoke, Hampshire, England) without further processing. Sections were cut for both light and electron microscopy.

Culture. The portion of tissue retained was cultured on 5% lysed horse blood agar selective for *C. pyloridis* (supplement SR69; Oxoid Ltd., Basingstoke). The plates were incubated at 37 C in a microaerophilic environment of high humidity for four to five days. Isolates were considered to be *C. pyloridis* if they grew microaerophilically, had characteristic colonial morphology, were catalase and urease positive, and showed consistent cell morphology and multiple sheathed flagella by transmission electron microscopy. NCTC strain 11639 was used as the reference culture.

Physiological properties. Urease activity was tested by inoculation of a slope of urea agar (Oxoid) with a dense suspension of bacteria from a lysed horse blood agar plate. The slope was then held at room temperature (~22 C) for 48 hr if required.

Hemin requirement was determined by inoculation of a nutrient agar plate (15 ml of blood agar base 2; Oxoid) that had been prepared and allowed

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Please address requests for reprints to Dr. Stuart L. Hazell, School of Microbiology, P.O. Box 1, Kensington, Sydney, New South Wales, Australia 2033.

to stand overnight with a small amount of growth from a two-day culture of the *C. pyloridis* isolates. The inoculum was spread over the surface of the plate, and an X-factor (hemin) disk (Difco Laboratories, Detroit) was placed at the center of each plate. Plates were incubated for five days at 37 C in a microaerophilic environment.

Motility in a viscous environment. Motility in viscous environments was determined by a modification of the methods of Schneider and Doetsch [8]. Solutions of methyl cellulose (concentration range, 0.1%–1.25%) were prepared in 1% peptone and sterilized by autoclaving. Slopes of lysed horse blood agar were prepared and inoculated with *C. pyloridis* or *Escherichia coli*. These slopes were then overlaid with 5 ml of one of the sterile methyl cellulose solutions. These slopes were then incubated at 37 C in a microaerophilic environment for three days, in the case of *C. pyloridis*, and overnight in air in the case of *E. coli*.

After incubation, one drop of culture overlay and one drop of corresponding sterile methyl cellulose solution were placed on a clean glass slide, and a coverslip was added. The slide was viewed under dark-field illumination with use of a video camera mounted on the microscope. The movements of bacteria at the interface of the drops of methyl cellulose were recorded on tape. The velocity of at least 30 "fast" bacteria was determined on replay for all the solutions used. The measurements were made with use of a path trace on plastic overlaying the television monitor, a planimeter, and a stop watch. The 10 fastest recordings were averaged and plotted against the relevant solution viscosities.

Viscosities were determined with use of glass flow viscometers. Both velocities and viscosities were measured at 21 C.

Results

Distribution of C. pyloridis in gastric mucosa. Mucus-associated bacteria. Bacteria were seen in two different areas of the tissue biopsy specimens. The first was in the layer of mucus that covered the mucosa (figure 1). The spiral morphology of these organisms could be seen, and organisms were characteristically arranged in parallel.

Cell-associated bacteria. The second area of localization was adjacent to the gastric epithelial cell surface. On close examination of many biopsy specimens, it became clear that the organisms were congregating around a specific site, i.e., the intercellular junctions (figures 2 and 3). When the orientation of the section was appropriate, it was possible to quantitate this association. Twenty-two fields in thin sections from six biopsy specimens were counted. Only electron micrographs that showed clear tight junctions were considered true cross-sections and were used for counting. More than 80% of the bacteria were seen to lie within 2 μm of a junction; only rarely were organisms seen to congregate around the center of the epithelial surface. There was little evidence of cell invasion; however, at certain of the junctions, bacteria could be seen that had penetrated part of the way down the junction between the cells (figure 2). The consistency of this distribution convinced us this observation was not a fixation artifact.

Motility of C. pyloridis in a viscous environ-

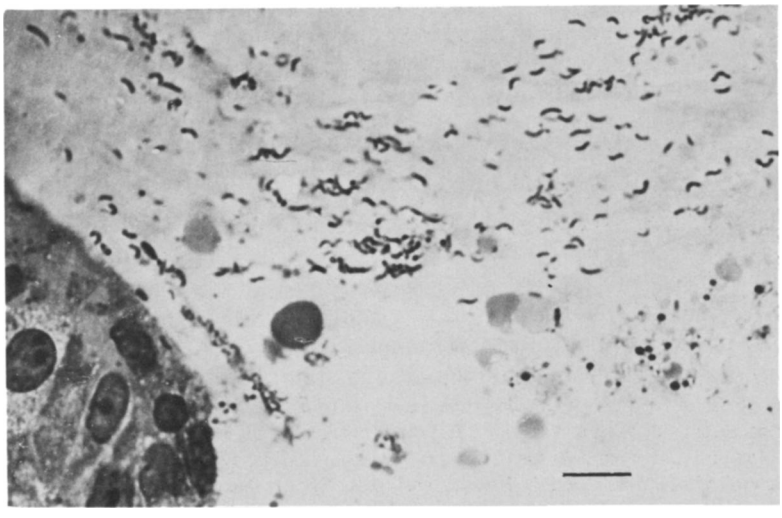

Figure 1. Section showing mucus-associated bacteria overlying the gastric epithelia (methylene blue stain). Bar = 6 μm.

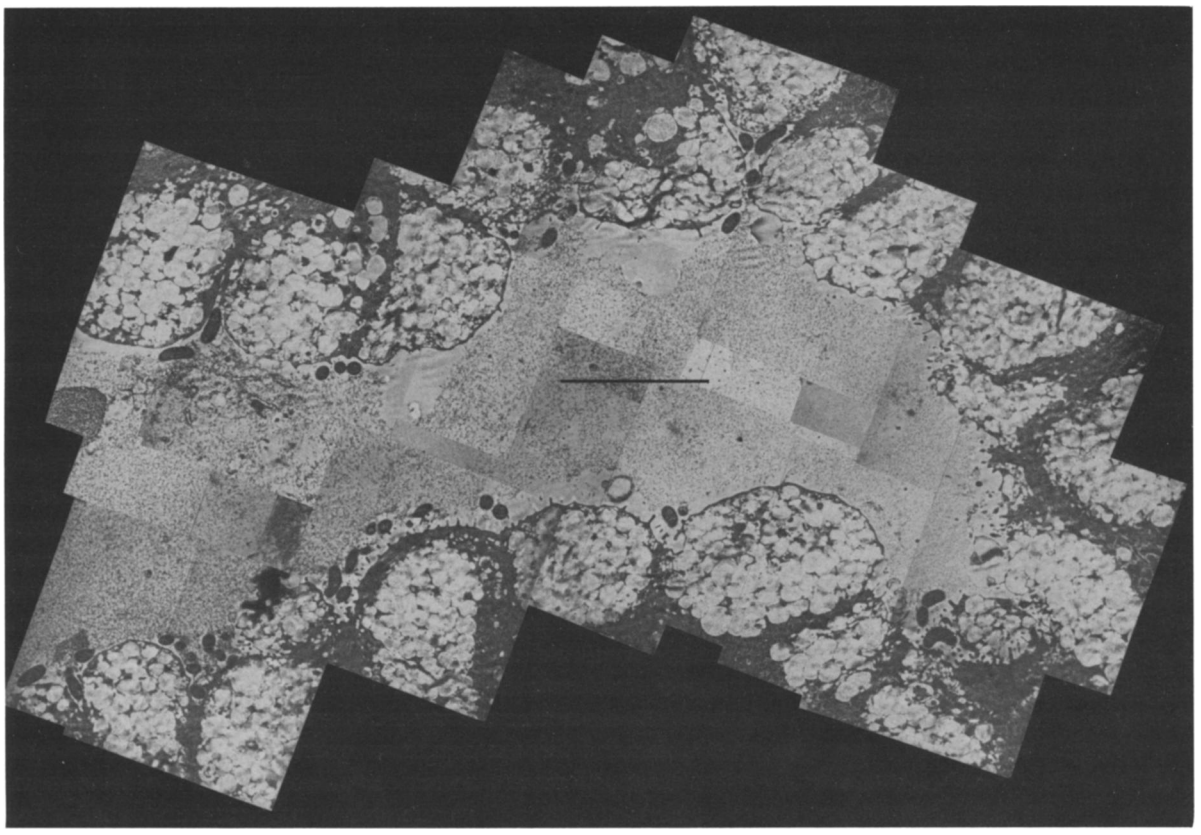

Figure 2. Montage of electron micrographs shows the association of bacteria with the intercellular spaces. Bar = 5 μm.

ment. The spiral campylobacters moved more freely in viscous solutions than did *E. coli* organisms, typical motile rod-shaped intestinal inhabitants (figure 4). Even at 200 centipoise, the spiral organisms could still move, albeit sluggishly, whereas the *E. coli* bacteria were immobilized at 20 centipoise.

Metabolic activity of C. pyloridis. Urease activity. The results of urease tests for 11 isolates are shown in table 1. All strains showed extremely high enzyme activity, i.e., provided a large inoculum was placed on the urea slopes, the reaction could be seen within 20 min. This reaction is so consistent that this test is now used in this laboratory as the presumptive test for *C. pyloridis*, together with characteristic colonial morphology on selective agar.

Hemin requirement. Also shown in table 1 are the hemin requirements of 11 isolates. All cultures required hemin; however, the growth stimulation provided by the concentration present in the commercially obtained disks varied considerably.

Discussion

In the original descriptions of spiral bacteria associated with the gastric mucosa, the organisms were described as underlying the blanket of mucus [3]. Close examination of biopsy specimens reveals that the bacteria can be consistently seen in two locations: (*1*) within the gastric mucus and (*2*) in close proximity to epithelial cells. These bacteria were considered to be *C. pyloridis* because the morphology was the same as that previously reported. This proposal was confirmed in a few tissues by Immuno-gold electron microscopy (authors' unpublished observation). Spiral bacteria appear to have a special affinity for gastrointestinal mucus. Indeed, the mucus-filled crypts of the small and large intestines of most animal species studied are normally colonized with large numbers of spiral organisms [9]. Mucus in the gastric pits of the stomach of normal cats and dogs is colonized with characteristic spiral bacteria [10].

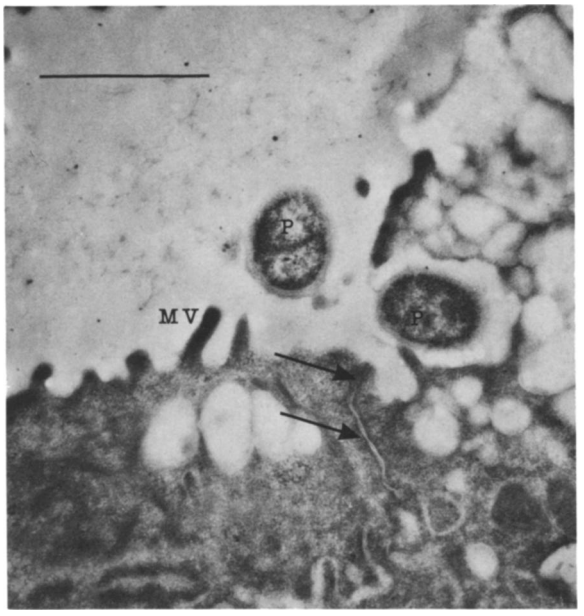

Figure 3. Electron micrograph shows the localization of *C. pyloridis* (P) close to the intercellular junction (arrows) of mucus-secreting gastric epithelial cells. Bacteria can be seen in among the microvilli (MV). Bar = 1 μm.

Based on comparison with our studies in rodents [11, 12], other reports on spiral bacteria in the literature, and the above observations on human stomach biopsy specimens, it would appear that *C. pyloridis* is one of an interesting group of bacteria that are especially adapted to the ecological niche provided by the intestinal mucus. Previously it has been proposed that the special motility of spiral bacteria enables them to move easily in the viscous environment provided by the mucus [13]. Observation

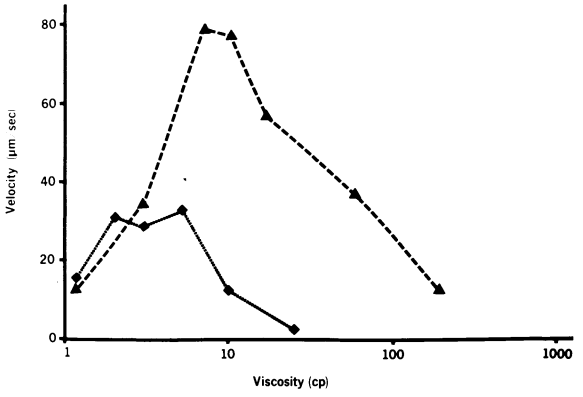

Figure 4. Effect of increasing viscosity on the velocity of *C. pyloridis* (- -▲- -) as compared with *E. coli* (· · ♦ · ·).

Table 1. Urease production and hemin requirement of 11 strains of *C. pyloridis*.

No. of strains	Urease production	Hemin (annular radius of growth, in mm)
3	Positive	1–2
6	Positive	2–5
2*	Positive	5–20
Medium		2–5

* Includes NCTC strain 11639, supplied by Dr. B. Marshall (Gastroenterology Unit, Royal Perth Hospital, Perth, Western Australia, Australia).

of the motility of cultures of *C. pyloridis* is consistent with this hypothesis. The bacteria are extremely flexible, and when put in various solutions of methyl cellulose in growth medium, they can be seen to corkscrew their way rapidly through highly viscous concentrations that severely impede the movement of more conventional rod-shaped organisms. The parallel orientation of the organisms seen in figure 1 is also similar to that seen with other colonizers of mucus, e.g., intestinal spiral bacteria in scrapings of mucus from rat bowel observed under phase contrast microscopy are seen to track in parallel streams. Direct observation by phase contrast microscopy of material from patients infected with *C. pyloridis* shows that this characteristic arrangement of the bacteria is due to an alignment with the parallel strands of mucus.

Predilection for the viscous environment of intestinal mucus alone cannot explain why these bacteria colonize the gastric epithelium. The organisms can be found deep in the gastric pits and therefore have to travel deep into the mucus, presumably under some attractive stimulus. This proposal suggests that nutrients from the stomach lumen are not the major energy source for these bacteria. The organisms are also acid sensitive (authors' unpublished observation), an unlikely property if they were adapted to the nutrients available in the host's foodstuff, which would be in highest concentration in the acidic outer periphery of the layer of mucus. In this regard the clear concentration of the organisms around intercellular junctions, as reported above, is likely to be significant. Although this point is not discussed in a recent article by Steer [1], an excellent scanning micrograph in his article shows the localization of spiral bacteria around the margins of gastric epithelial cells.

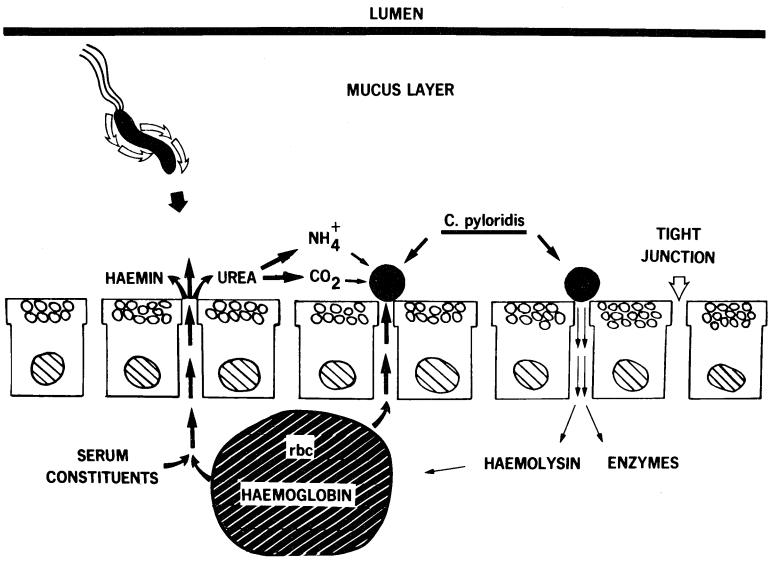

Figure 5. Diagrammatic representation (not to scale) of possible events in colonization of the gastric mucosa by *C. pyloridis*. rbc = red blood cell.

Potential chemotaxins or growth substances, e.g., urea, have been shown to transverse the gastric epithelium via the paracellular route [14]. The highest concentrations of these substances would be close to the cell junction. Wallace et al. [15] have shown that many bacteria in the rumen wall hydrolyze urea diffusing across the mucosa. Savage [16] has suggested that urea may be of value to organisms colonizing the mucosal surfaces of the gastrointestinal tract. It is significant that urea constitutes up to 50% of the nonprotein nitrogen in human serum. Levels of enzymatic activity are a reflection of the normal environmental milieu of a bacterium. The high level of urease activity in all freshly obtained isolates tested would suggest that urea is readily accessible at the cell surface and therefore is a preferred metabolite. The presence of gastritis may also alter gastric permeability to further increase extracellular leakage.

Another possible growth factor available at this site was hemin, which we found stimulated the growth of *C. pyloridis* on basal medium. Because the hemin in blood is usually bound within the red blood cell, it is unlikely that there would be significant leakage through cell junctions in normal tissue. However, *C. pyloridis* is a hemolytic bacterium; thus it is possible hemolysin could contribute to the accessibility of hemin.

Campylobacter pyloridis is now firmly established as a major etiologic agent of human gastritis. Understanding of the means whereby this bacterium associates with tissue is important to an appreciation of the pathogenesis of this disease. Based on the observations reported above, our current hypothesis is that this organism is one of a group especially adapted to an environment of mucus. When *C. pyloridis* comes in contact with the mucus, the peculiar motility of this bacterium allows it to move freely in this viscous environment. Under chemotactic stimuli the organism moves into the gastric pits and toward the intercellular junctions from which potential nutrients and growth factors are diffusing. The organisms have the enzymatic capacity to utilize these factors, e.g., urease. Cells either multiply or accumulate at this site. Inflammatory products are released, inducing gastritis and thus causing the release of further growth factors. This possible sequence of events is diagrammatically represented in figure 5. Given that the natural habitat of these bacteria is likely to be intestinal mucus, the origin of the original inoculum could be endogenous, i.e., from further down the bowel via the fecal-oral route, or even from another animal species. As yet unknown predisposing factors may be required to cause changes in the gastric mucosa to change the intestinal milieu to allow initial colonization.

References

1. Steer HW. Surface morphology of the gastroduodenal mucosa in duodenal ulceration. Gut 1984;25:1203–10
2. Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis [letter]. Lancet 1983;1:1273

3. Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis [letter]. Lancet 1983;**1**:1273–5
4. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. International Journal of Systematic Bacteriology 1985; **35**:223–5
5. McNulty CAM, Datson DM. Spiral bacteria of the gastric antrum [letter]. Lancet 1984;**1**:1068–9
6. Steer H. Mucosa-related bacteria in the stomach [letter]. Lancet 1984;**2**:528
7. Eldridge J, Lessells AM, Jones DM. Antibody to spiral organisms on gastric mucosa [letter]. Lancet 1984;**1**:1237
8. Schneider WR, Doetsch RN. Effect of viscosity on bacterial motility. J Bacteriol 1974;**117**:696–701
9. Lee A. Neglected niches: the microbial ecology of the gastrointestinal tract. In: Marshall KC, ed. Advances in microbial ecology. 1985;**8**:115–62
10. Weber AF, Schmittdiel EF. Electron microscopic and bacteriologic studies of spirilla isolated from the fundic stomachs of cats and dogs. Am J Vet Res 1962;**23**:422–7
11. Lee A, Phillips M. Isolation and cultivation of spirochaetes and other spiral-shaped bacteria associated with the cecal mucosa of rats and mice. Appl Environ Microbiol 1978;**35**:610–3
12. Lee A, O'Rourke J, Phillips M, Barrington P. In: Pearson AD, Skirrow MB, Rowe B, Davies JR, Jones DM, eds. Campylobacter II: proceedings of the second international workshop on campylobacter infections. London: Public Health Laboratory Service, 1983:112–114
13. Greenberg EP, Canale-Parola E. Relationship between cell coiling and motility of spirochetes in viscous environments. J Bacteriol 1977;**131**:960–9
14. Powell DW. Barrier function of epithelia. Am J Physiol 1981;**241**:G275–88
15. Wallace RJ, Cheng K-J, Dinsdale D, Ørskov ER. An independent microbial flora of the epithelium and its role in the ecomicrobiology of the rumen. Nature 1979;**279**:424–6
16. Savage DC. Mechanisms by which indigenous microorganisms colonize gastrointestinal epithelial surfaces. Prog Food Nutr Sci 1983;**7**:65–74