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Enzyme-Linked Immunosorbent Assay for *Campylobacter pyloridis:* Correlation with Presence of *C. pyloridis* in the Gastric Mucosa

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Antibody to *Campylobacter pyloridis* was measured by ELISA in the sera of 160 patients from whom gastric biopsy specimens were also obtained. The antigen was an acid-glycine extract of *C. pyloridis*, and titers ranged from 80 to 22,000 ELISA units (EU). Of 117 patients in whom *C. pyloridis* was detected microbiologically or histologically, 87 (74%) had a titer ≥300 EU, and only one had a titer <150 EU. Of 43 patients in whom *C. pyloridis* was not detected, only two (5%) had a titer >300 EU. Thus, for a titer of 300 EU the ELISA test had a specificity of 97% and a sensitivity of 81%. At 150 EU the specificity was 78%, and the sensitivity was 99%. Histological diagnosis of active chronic gastritis was associated with a high median ELISA titer (485 E), chronic gastritis with a much lower titer (150 EU), and normal histology with a titer of 110 EU. Discriminating use of this serological test could be of assistance to detect *C. pyloridis* in the gastric mucosa.

The name *Campylobacter pyloridis* has now been validated [1] for the campylobacter-like spiral bacteria first cultured at Royal Perth Hospital in 1982 from specimens of the gastric antral mucosa obtained by endoscopic biopsy [2, 3]. This new organism may be the etiologic agent in gastritis-associated dyspeptic disease and most cases of duodenal ulcer [4], and possible pathogenic mechanisms have been delineated [5]. Thus in patients with nonulcer dyspepsia, when the presence of *C. pyloridis* is indicated by detecting specific antibody, an attempt at curative antibacterial therapy may be justified. A discriminatory serological test could replace the difficult and expensive procedures such as upper gastrointestinal endoscopy and biopsy, which are presently required to demonstrate the presence of *C. pyloridis* in the stomach. However, as Svedheim [6] has stated, "the antigen is crucial in diagnostic serology." For *Campylobacter jejuni*, an acid-glycine extract in an ELISA is the most satisfactory preparation [6–8], but for *C. pyloridis* the antigens for serological tests in published reports were sonicated whole bacteria [9–11] or bacteria killed with formalin [12, 13]. Some healthy volunteers had histological gastritis and *C. pyloridis* in their stomachs [14]. In this study, serological results were obtained with an acid-glycine extract of *C. pyloridis* in an ELISA assay, and from every patient, a gastric biopsy specimen was obtained and examined microbiologically and histologically.

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Materials and Methods

Bacterial strain and antigen preparation. The antigen was prepared from a pool of 11 isolates of *C. pyloridis* obtained from the gastric mucosa and included strains 11637 and 11638 from the National Collection of Type Cultures (London). The preparation of the antigen and the ELISA technique were similar to those described for *C. jejuni* by Blaser and Duncan [8]. Isolates were grown on heated blood agar with IsoVitaleX® 1% (BBL Microbiology, Cockeysville, Md) for three days at 37 C in a *Campylobacter* gas mixture [15]. Bacterial cells were harvested in sterile distilled water, washed twice in sterile distilled water, and suspended in 0.2 *M* glycine-hydrochloride buffer (pH 2.2) at a concentration of 0.1 g (wet weight) of cells to 2.5 ml of buffer. Suspensions were stirred at 25 C for 15 min and centrifuged at 11,000 *g* for 15 min at 4 C. The supernatant was retained, and the pH was neutralized with sodium hydroxide. The supernatant was dialyzed against sterile distilled water for 24 hr at 4 C. The protein was filtered by using the Amicon Diafiltration system membrane type UM10 (Amicon, Dan-

vers, Mass), and protein concentrations were determined by the Lowry technique [16]. Preparations were stored at −20 C until use. Before coating the ELISA trays, we diluted the concentrated antigen preparation in 0.5 *M* carbonate buffer (pH 9.6) to give a final concentration of 2.5 μg of protein/ml. Polyvinyl chloride, "high activity" microtiter, flat-bottomed plates (Flow Laboratories, McLean, Va) were coated with the diluted antigen preparation by adding 0.2 ml to each well. The plates were covered and incubated for 24 hr at 4 C. Each well was then aspirated dry and refilled with 0.3 ml of PBS containing thimersol-Tween® 20 plus gelatin (1 mg/ml). The plates were kept at 4 C until use.

Proteins in the antigen. Protein profiles of the acid-glycine preparation were examined by discontinuous SDS-PAGE, as described by Laemmli [17]. After centrifugation at 6,000 *g* for 15 min, the supernatant was heated at 100 C for 5 min with a disintegration buffer that gave a final concentration of 50 nmol Tris hydrochloride (pH 6.8), 5% beta-mercaptoethanol (vol/vol), 2% SDS (wt/vol), 10% glycerol (vol/vol), and 0.01% bromophenol blue. The proteins were separated on a SDS-PAGE gel system [17] that consisted of a 3% stacking gel and a 6%–18% gradient gel. Electrophoresis was performed at 500 V for 4 hr with cooling, and the gels were stained with coomassie blue. The molecular weights of the peptides resolved were calculated on the basis of a calibration curve of marker proteins.

Patients, biopsy specimens, and sera. The patients were consecutive referrals to the Gastroenterology Unit at Royal Perth Hospital from September 1984 to August 1985, from whom endoscopic biopsy specimens were obtained. Patients who had been treated with antibiotics or antacid were excluded from the study. One hundred sixty patients were entered in the trial, including 101 men and 59 women with an age range of 20–85 years. The mean age for the men was 53 years, and mean age for the women was 48 years. Endoscopic biopsy specimens were obtained and cultured for *C. pyloridis* on a selective medium in a microaerophilic environment, as previously described [15]. One specimen was taken for microbiological culture and one specimen for histological diagnosis and detection of spiral bacteria. Serum specimens were stored at −20 C, but 66 specimens were studied unfrozen for the presence of IgM antibodies to *C. pyloridis* and were then studied again after being frozen at −20 C.

ELISA. For the ELISA, optimal dilutions of all reagents were determined by checkerboard titration. Antigen-labeled plates were removed from 4 C and brought to room temperature (~23C) before use. Wells were washed three times with PBS containing thimersol-Tween 20. Each test serum was diluted 1:100 in serum diluent (PBS containing thimersol-Tween 20 with 5 mg of bovine gammaglobulin/ml and 1mg of gelatin/ml). A 100-μl sample of each test serum dilution was added in triplicate to the microtiter plate and incubated for 90 min at 25 C in an incubator to maintain a stable temperature. Wells were aspirated and washed three times with 0.3 ml of PBS containing thimersol-Tween 20. Peroxidase-labeled goat antibody to human IgA, IgG, and IgM (heavy- and light-chain specific) was used at a dilution of 1:16,000, and specific conjugates were used as follows: IgG, 1:50,000; IgM, 1:4,000. All conjugates were obtained from Kirkegaard-Perry Labs, Maryland. Conjugates were appropriately diluted in PBS containing thimersol with 20μg of bovine serum albumin/ml and 1.0 mg of gelatin/ml, and 100 μl was added to each well and was placed in an incubator for 90 min at 25 C. Wells were washed three times with PBS containing thimersol-Tween 20, and then twice with PBS containing only thimersol. A 100 μl sample of substrate consisting of 2.2′-azino-di-(3-ethyl-benzthiazoline) sulfonate with 0.005% hydrogen peroxide was added to each well and incubated at 25 C for 15 min. The reaction was stopped with 50 μl of 0.001% wt/vol sodium azide in 0.1 *M* citric acid. Plates were read within 2 hr on a Titertek Uniscan® (Flow Laboratories, Sydney, Australia) at 405 nm. This machine allowed a single blank well to be used to make a baseline measurement.

Each plate contained dilutions of three control sera that had to meet stringent requirements for the plate to be accepted. One control was the calibration serum consisting of a pool of 11 positive sera with high titers of antibody. This was diluted from 1:50 in doubling dilutions to 1:12,800, which allowed the construction of a standard curve as described under analysis of data below. The second control was a pool of 14 negative sera that was put on each plate in duplicate at a dilution of 1:100, and the reading of these wells had to be <0.1 absorbance units for the plate to be accepted. The third control was a "weakly positive" serum that was diluted at 1:400 and assayed in three wells. The results were analyzed as described below. Before the procedure was accepted, the weakly positive serum was tested in 18 plates to ensure reproducibility.

Inhibition assay. To asess the specificity of our test, we incubated a pool of sera with high positive titers in four aliquots at 37 C for 30 min—one with 10μg of the *C. pyloridis* extract and the three others with an acid-glycine extract of *C. jejuni*, *Campylobacter fetus*, or *Escherichia coli*. After incubation, 200-μl samples of the serum were doubly diluted from 1:10 to 1:320 and placed in triplicate wells; the ELISA was performed as described above.

Calculation of ELISA. The absorbance readings obtained for each of the dilutions of the calibration serum were used to construct a standard curve in the following manner. The highest dilution of the serum, 12,800, was arbitrarily designated as being equal to one ELISA unit (EU), and with the dilution factor of 100, this gave a result of 100 EU. The results were plotted as absorbance versus dilution of serum (figure 1). The 12 results usually covered a range of absorbances from 0.1 to 1.2 and were entered onto a programmed calculator. The plate was accepted if the correlation coefficient of this standard curve was >0.980. For each test serum done in triplicate the results were calculated from the standard curve by linear regression analysis and expressed in EU [18]. The mean of the weak positive control serum was 359 EU, with a standard error of 85.5 EU. For the sera of patients without *C. pyloridis*, the upper limit of two standard deviations from the mean was 300 EU.

Results

Inhibition assay. The effect of different acid-glycine extracts on the titer of *C. pyloridis* antibody in a highly positive serum is shown in figure 2. First, it can be seen that the *C. pyloridis* acid-glycine extract adsorbed out antibody in the positive control serum. Second, at 1:20 and 1:40 there was a greater than fourfold difference in absorbance between *C. pyloridis* and the other three extracts. These results also indicate, however, a degree of cross-reactivity between antibodies to *C. pyloridis* and extracts from the other bacterial species tested.

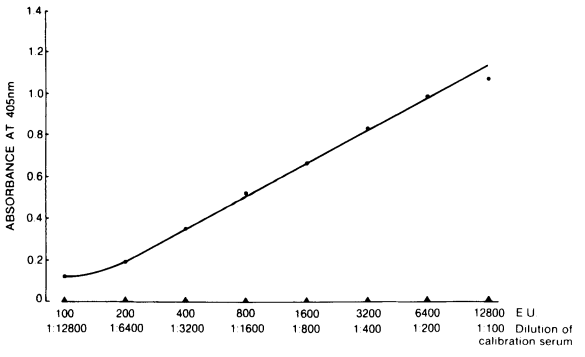

Figure 1. Standard curve of calibration serum used in the ELISA test.

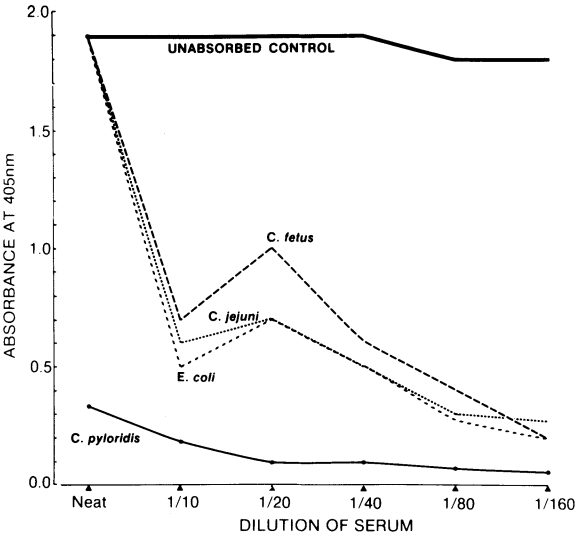

Figure 2. The effect of adsorption by acid-glycine extracts of four different bacteria on optical densities of serum that contained antibodies to *Campylobacter pyloridis*.

Protein bands in the ELISA antigen. The SDS-PAGE profile of the acid-glycine extact of *C. pyoridis* is shown in figure 3. A major triplet of protein bands was observed at 57 kDa, 62 kDa, and 64 kDa, with lesser bands at 24.5 kDa, 28 kDa, 33 kDa, and 84 kDa.

Detection of C. pyloridis by culture and histology, and histological diagnosis. For the 160 patients in the study, *C. pyloridis* was detected by culture or histology in 117 (73%) of the patients. In one patient, histology failed to reveal the organism, but *C. pyloridis* was cultured from the biopsy specimen (table 1). *C. pyloridis* was not detected in 43 (27%) of the patients. The histological diagnosis of active chronic gastritis, as defined by Whitehead [19] and Marshall and Warren [2], was made in 107 patients, and *C. pyloridis* was detected in all of these patients. Chronic gastritis without activity was present in 31 patients, and in 10 (32%) of these, *C. pyloridis* was detected. There were 22 patients with normal histology of the gastric mucosa, and in none of these was *C. pyloridis* detected.

ELISA results. In our polyclonal ELISA test,

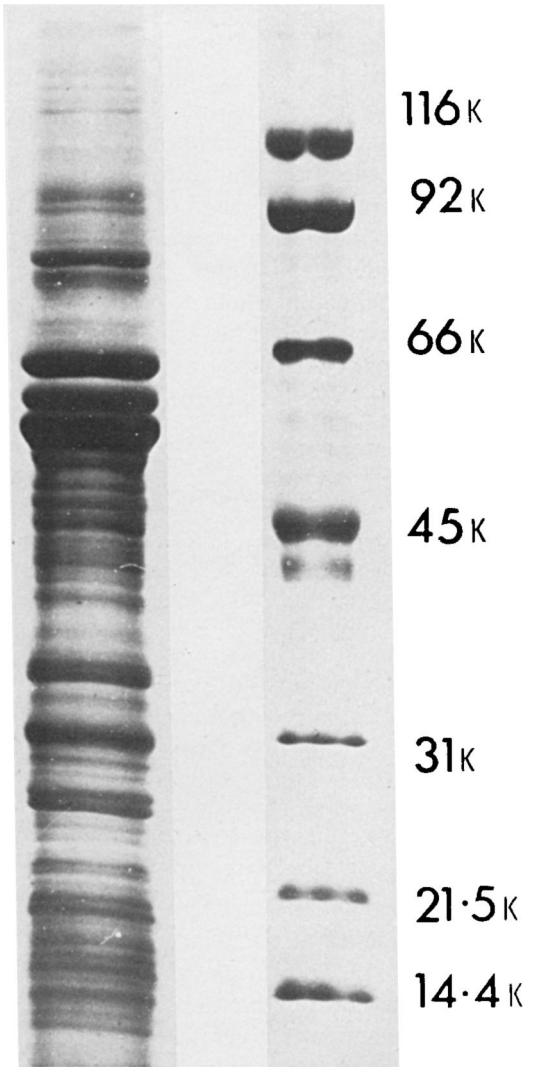

Figure 3. SDS-PAGE profiles of the acid-washed preparation used in the ELISA.

there was a linear relation between the reciprocal serial dilutions of the highly positive pooled serum and absorbance (figure 1) in the region from 200- to 6,400-fold serum dilutions. We found that serum IgA was not detected by our antigen; sera sent to us by Dr. Kaldor (Fairfield Hospital for Communicable Diseases, Melbourne), which apparently containing high titers of IgM antibody, had low titers, whether tested unfrozen or frozen.

ELISA titers of serum antibody. The ranges of titers in patients in whom *C. pyloridis* was or was not detected are shown in figure 4. There is a highly significant statistical difference between these two

Table 1. Histological and endoscopic diagnoses related to detection of *C. pyloridis* and ELISA titers

Diagnoses	No. in group	No. with *C. pyloridis* detected	Median titer (EU)
Active chronic gastritis			
Duodenal ulcer	61	61	415
Gastric ulcer	20	20	685
No ulcer	26	26	965
Chronic gastritis	31*	10	150
Normal histology	22†	0	110

NOTE. Among patients with active chronic gastritis and a duodenal ulcer, none had <150 EU and 41 had >300 EU. Among patients with active chronic gastritis and a gastric ulcer, none had <150 EU and 15 had >300 EU. Among patients with active chronic gastritis and no ulcer, none had <150 EU and 21 had >300 EU. Among patients with normal histology, 15 had <150 EU and two had >300 EU.

* Four of these patients had ulcers.

† Two of these patients had ulcers.

groups (Student's $t = 3.04$, $P < .002$). In the 43 patients in whom *C. pyloridis* was not detected, only two (5%) had a titer >300 EU, and 28 (65%) had a titer <150 EU; only three had a titer >250 EU. Of the 117 patients in whom *C. pyloridis* was detected, 86 (74%) had a titer >300 EU, 94 (80%) had a titer >250 EU, three a titer of 150 EU, and one patient had a titer of 100 EU. Thus, at a cut-off point of

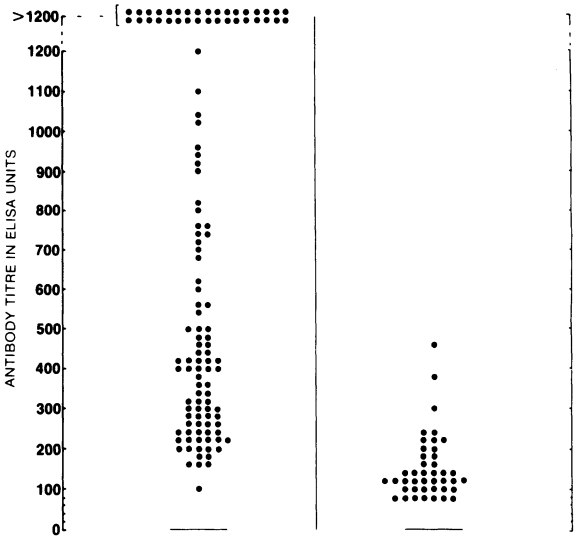

Figure 4. Serum titers of antibody to *Campylobacter pyloridis* in patients from whom a gastric antral biopsy specimen was examined for *C. pyloridis*. *Left*, titers when *C. pyloridis* was detected; *right*, titers when *C. pyloridis* was not detected.

300 EU, our assay produced very few false-positive results. At 300 EU our test had a specificity of 97%, a sensitivity of 81%, a predictive positive value of 98%, a predictive negative value of 57%, and an efficiency of 86%. At a cut-off point of 150 EU our assay produced very few false-negative results. At 150 EU our test had a sensitivity of 99%, a specificity of 78%, a predictive positive value of 91%, a predictive negative value of 98%, and efficiency of 62%. Thus at 300 EU, our ELISA test showed good specificity, and at 150 EU the test showed good sensitivity.

Relation between ELISA titers and the histological and endoscopic diagnoses. Among the 107 patients with active chronic gastritis, 61 had a duodenal ulcer, 20 had a gastric ulcer, and 26 did not have an ulcer. Among 31 patients with chronic gastritis, only four had an ulcer; among 22 patients with normal histology, only two had an ulcer. The median ELISA titers are shown in table 1. In the patients with chronic gastritis the median titer was 150 EU, which was much lower than the median titer of those with active chronic gastritis regardless of whether the patient had a duodenal ulcer, (median, 415 EU), gastric ulcer (median, 685 EU), or no ulcer (median, 965 EU). Patients with normal histology had the lowest median titer, 110 EU. There were only five titers >6,000 EU, and to avoid weighting the statistical analysis, we estimated these titers to be 6,000 EU; the titers of those with active chronic gastritis were very significantly higher than those with chronic gastritis ($t = 3.13$, $P < .002$). The titers of patients with duodenal ulcers and active chronic gastritis were significantly lower than those with active chronic gastritis but no ulcer ($t = 2.36$, $P < .02$).

Among the 64 patients with a duodenal ulcer, only one had normal histology, and two had chronic gastritis; the titers of these three patients ranged from 80 to 220 EU. Among the 23 patients with gastric ulcers, only one had normal histology, and two had chronic gastritis; the titers of these patients ranged from 110 to 555 EU.

Discussion

A serological method to detect the presence of *C. pyloridis* should not give a high-positive result when *C. pyloridis* cannot be detected. Before a serological method is used for surveys it should be checked by the means we have used in this study, namely the analysis of gastric biopsy specimens for the presence of *C. pyloridis*. Apparently healthy volunteers have been found to have histological gastritis in the stomach, and *C. pyloridis* has been detected [14]. Therefore, the definition of a healthy individual must be made on histological and microbiological grounds and not on the absence of symptoms. Patients with nonulcer dyspepsia [5] have seen a doctor and been assured that they have no organic disease, but frequently are found to have gastritis and *C. pyloridis* infection.

Among the patients in whom *C. pyloridis* was not detected, the upper limit of two standard deviations above the mean of their titers was 300 EU; in patients with a titer >300 EU, *C. pyloridis* was nearly always found in the gastric antrum. When the titer was <150 EU, *C. pyloridis* was rarely found. With regard to titers between 150 and 300 EU, some patients may have falling titers because of the natural healing process [20], or healing may have been aided by undeclared antibacterial treatment such as bismuth tablets (bismuth is effective against *C. pyloridis* [4]). Some patients may have a relatively low titer if their infection has only just begun. In this study, there were nine patients with titers between 250 EU and 300 EU, and in only one of these was *C. pyloridis* not detected. Thirty-four (21%) of our 160 patients had a titer between 150 and 250 EU, and in 24 (71%) of these thirty-four patients, *C. pyloridis* was detected. There were two patients with a titer >300 EU in whom we could not detect *C. pyloridis*; they were each a close relative of a patient with peptic ulcer and *C. pyloridis* in the stomach. Among the patients who were excluded from the study because of prior antibacterial or antipeptic ulcer treatment, there were two patients who had apparently received cimetidine or sucralfate. In the patient who received cimetidine, *C. pyloridis* was not detected, but he had an antibody titer of 5,750 EU. In the patient who recieved sucralfate, *C. pyloridis* was also not detected, and the antibody titer was 1,030 EU.

We investigated the possibility that the higher ELISA titers in our patients would be associated with the most-severe inflammation histologically. However, among the 107 patients with active chronic gastritis, severe inflammation was found in the biopsy specimens of patients with low ELISA titers, between 200 EU and 300 EU, and some patients with high titers had only mild or moderately severe inflammation in their biopsy specimens.

An acid-glycine extract of *C. jejuni* has been found to be more reliable than are other antigenic preparations for the serological diagnosis of infection with

that organism [6–8]. Despite the evidence that antibody to *C. pyloridis*, as measured in our ELISA, did show some evidence of cross-reactivity with the extracts of *C. jejuni*, *C. fetus*, and *E. coli*, there were nevertheless very few false-positive ELISA results.

Other antigens and methods have been reported for serological tests for *C. pyloridis*. A passive hemagglutination test with sonicated *C. pyloridis* as the antigen has been reported [10], but we have found that unsensitized erythrocytes hemagglutinated as readily as sensitized erythrocytes. An ELISA method with formalin-killed whole bacteria as the antigen [12, 13] detected high titers in apparently healthy contacts [12]. A CF method was used with an antigen that was "a thick suspension of organisms" harvested from a three-day culture [11]; a CF method was also used with sonicated *C. pyloridis* as antigen, but 12 of 78 patients negative for *C. pyloridis* were found to have a high titer of antibody [21]. The antibodies to *C. pyloridis* detected in healthy contacts of patients with peptic ulcer may be protective antibodies; immunoblot analysis may reveal whether different antigens are involved in the serological response of healthy contacts compared with patients with *C. pyloridis*.

A serological test can be made more reliable by using stringent controls. We used an additional control to those of Blaser and Duncan [8], namely a calibration serum on each plate, and for the plate to be accepted, a correlation coefficient >0.980 was required. This serum allowed us to record our results in a standardized fashion rather than directly as absorbances (OD).

The ELISA titers among our patients mirrored closely the histological diagnosis of active chronic gastritis, chronic gastritis, or normal histology. Table 1 shows that among the 31 patients with chronic gastritis the median titer was only 150 EU, whereas among the three groups with active chronic gastritis the median titers were 415 EU, 685 EU, and 965 EU. Among those with normal histology the median titer was only 110 EU. We regard these results as confirmation of the significance of the histological diagnosis of active chronic gastritis in relation to *C. pyloridis* infection. Price et al. [22] stated that in their study the incidence of *C. pyloridis* was similar in patients with chronic or active chronic gastritis. We question this observation and suggest that these authors have set their normal range for neutrophil infiltration too high, so that they relegated patients with active chronic gastritis to their chronic gastritis group. When active gastritis is absent in the gastric mucosa, neutrophils are almost completely absent. These authors may not have obtained biopsy specimens from sites similar to ours, or their selection of patients may have been different.

In addition to the presence of specific serum antibody in patients in whom *C. pyloridis* can be detected, the presence of local antibody in biopsy specimens would confirm that *C. pyloridis* is a genuine pathogen and not merely a commensal. Wyatt et al. [23] have reported that in vivo coating of *C. pyloridis* by host IgA was present in all 83 cases of active gastritis, and coating of bacteria with IgG or IgM was significantly correlated with activity of the gastritis and was rarely seen in the absence of neutrophil infiltrate.

Our conclusions from this study are that symptomatic patients who have a titer <150 EU will be highly unlikely to have *C. pyloridis* in the stomach, and patients with a titer >300 EU are very likely to have the organism in the stomach. As with any serological test there will be patients with intermediate results, and it is particularly these patients who will need an endoscopic examination to determine whether *C. pyloridis* is present.

References

1. Anon. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 17. International Journal of Systematic Bacteriology 1985;**35**:223–5
2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;**1**:1311–5
3. Marshall BJ, Royce H, Annear DI, Goodwin CS, Pearman JW, Warren, JR, Armstrong JA. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. Microbios Letters 1984:**25**:83–8
4. Marshall BJ, McGechie DB, Rogers PA, Glancy RJ. Pyloric campylobacter infection and gastroduodenal disease. Medical Journal of Australia 1985;**142**:439–44
5. Goodwin CS, Armstrong JA, Marshall BJ. *Campylobacter pyloridis*, gastritis and peptic ulceration. J Clin Path 1986;39:353–65
6. Svedhem Å, Gunnarsson H, Kaijser B. Diffusion-in-gel enzyme-linked immuosorbent assay for routine detection of IgG and IgM antibodies to *Campylobacter jejuni*. J Infect Dis 1983;**148**:82–92
7. Kosunen TU, Rautelin H, Pitkänen T, Pönkä A, Pettersson T. Antibodies against an acid extract from a single campylobacter strain in hospitalized campylobacter patients. Eur J Infect 1983;**11**:189–91
8. Blaser MJ, Duncan DJ. Human serum antibody response to *Campylobacter jejuni* infection as measured in an enzyme-linked immunosorbent assay. Infect Immun 1984;**44**:292–8

9. Kaldor J, Tee W, McCarthy P, Watson J, Dwyer B. Immune response to *Campylobacter pyloridis* in patients with peptic ulceration. Lancet 1985;-921
10. Marshall BJ, McGechie DB, Francis GJ, Utley PJ. Pyloric campylobacter serology. Lancet 1984;**2**:281
11. Jones DM, Lessells AM, Eldridge J. Campylobacter like organisms on the gastric mucosa: culture, histological, and serological studies. J Clin Pathol 1984;**37**:1002-6.
12. Hutchinson DN, Bolton FJ, Hinchcliffe P, Holt AV. Distribution in various clinical groups of antibody to *Campylobacter pyloridis* detected by ELISA, complement fixation and microagglutination tests [abstract no. 188]. In: Pearson AD, Skirrow MB, Lior H, eds. Campylobacter III, Proceedings of the Third International Workshop on Campylobacter Infections. London: Public Health Laboratory Service, 1985:185
13. Rathbone BJ, Wyatt JI, Worsley BW, Trejdosiewicz LK, Heatley RV, Losowsky MS. Immune response to *Campylobacter pyloridis*. Lancet 1985;**1**:1217
14. Langenberg M-L, Tytgat GNJ, Schipper MEI, Rietra PJGM, Zanen HC. Campylobacter-like organisms in the stomach of patients and healthy individuals. Lancet 1984;**1**:1348
15. Goodwin CS, Blincow ED, Warren JR, Waters TE, Sanderson CR, Easton L. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. J Clin Pathol 1985;**38**:1127-31
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;**193**:265-75
17. Laemli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-5
18. Kurstak E. Progress in enzyme immunoassays: production of reagents, experimental design, and interpretation. Bull WHO 1985;**63**:793-881
19. Whitehead R. Gasritis. In: Major problems in pathology. Vol 3. Mucosal biopsy of the gastrointestinal tract. Philadelphia: WB Saunders, 1985;41-58
20. Goodwin CS, Armstrong JA. Will antibacterial chemotherapy be efficacious for gastritis and peptic ulcer? J Antimicrob Chemother 1986;**17**:1-4
21. McNulty CA, Crump B, Ghearty J, Davis M, Donovan IA, Melikian V, Wise R. The distribution of and serological response to *Campylobacter pyloridis* in the stomach and duodenum. In: Pearson AD, Skirrow MB, Lior H, eds. Campylobacter III. Proceedings of the Third International Workshop on Campylobacter Infections. London: Public Health Laboratory Service, 1985;174-5
22. Price AB, Levi J, Dolby JM, Dunscombe PL, Smith A, Clark J, Stephenson ML. *Camplyobacter pyloridis* in peptic ulcer disease: microbiology, pathology, and scanning electron microscopy. Gut 1985;**261**:1183-8
23. Wyatt JI, Rathbone BJ, Heatley RV. Local immune response to gastric campylobacter in non-ulcer dyspepsia. J Clin Pathol, 1986;**39**:863-70