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SCANNING ELECTRON MICROSCOPY IN THE STUDY OF CAMPYLOBACTER PYLORI ASSOCIATED GASTRITIS

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Abstract

The close association between Campylobacter pylori (CP), gastritis and peptic ulcer is now well established. Moreover increasing evidence has been collected of a major etiological role of CP in type B chronic gastritis. For this reason, searching for CP is essential in all patients with upper gastrointestinal symptoms.

Scanning electron microscopy (SEM) is a most reliable technique for studying the distribution of microorganisms and their relationship to the gastric mucosal surface.

The aim of this paper is to compare SEM to other routine methods of detection for CP, such as Giemsa staining on histological sections and Urease Microtiter Test (MT) on fresh tissue and to investigate the surface morphology of gastric mucosa colonized by CP and to correlate it with the histopathological picture. Thirty-seven biopsies taken from the gastric body and the antrum of 22 patients were used for each type of determination. The different parameters were graded semiquantitatively. Histology showed a normal mucosa in 4 cases , chronic superficial gastritis in 12 and chronic atrophic gastritis in 21 cases. SEM was more sensitive than histology and Urease MT in detecting Campylobacter pylori. This is due to the patchy distribution of this bacterium on gastric mucosa. For this reason SEM should always be performed when routine tests are negative. The presence of CP correlated significantly ($p<0.001$, Spearman Rank Correlation Test) with the neutrophilic infiltrate, thus with the "activity" of the gastritis. The CP associated gastritis has no distinctive surface features other than the presence of the bacterium . SEM morphology of surface gastric mucous cells suggests that CP does not damage the lining epithelium directly. Neutrophils and inflammatory mediators could be involved in the production of the mucosal lesions.

Key Words: gastric mucosa, gastritis, Campylobacter pylori, scanning electron microscopy, Giemsa staining, Urease Microtiter Test

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Introduction

In the last four years increasing evidence has been collected that a strict correlation between Campylobacter pylori, gastritis and peptic ulcer exists (3, 6, 9, 18, 23). This represents a turning-point in gastroenterological research, questioning the most well-established etiopathogenetic theories. The organism presumed to be responsible is not a recent discovery. As far back as 1893, Bizzozero (2), a pathologist from Turin, identified CP in the stomach of the dog and described its spiral morphology. Since then, various other authors have observed spiral bacteria but considered them harmless commensals or contaminants. In 1975 Steer and Colin-Jones (26) redirected attention towards the possible pathogenicity of bacteria observed by light microscopy and transmission electron microscopy in patients with gastric ulcers. However, Pseudomonas aeruginosa was isolated from the cultures of the biopsy fragments. In view of current knowledge, it is thought that this bacterium was an endoscopic contaminant, and that the bacteria observed on the gastric mucosa were in fact Campylobacter pylori. These bacteria were subsequently isolated in 1984 (16) by Warren and Marshall who proposed anew (31) the likely pathogenic role of spiral bacteria in particular in chronic gastritis.

The name Campylobacter pylori was coined on the basis of the various morphological and microbiological affinities with the Campylobacter species and from their site of colonization in the antrum. From here the bacteria subsequently invade the body and the fundus of the stomach. The external sources from which the CP originate are not known. It is presumed that they cross the mucus layer covering the stomach, on account of the extreme mobility provided by the flagella, and, protected by this mucus, thrive on an alkaline milieu on the superficial and foveolar mucous cells.

Factors in favour of the etiological role of Campylobacter in diseases of the gastric mucosa include:

- the already-mentioned association between Campylobacter pylori (CP) and type B chronic

gastritis (non-autoimmune). There is also a close association with gastric and duodenal ulcers but this seems to be attributable to the fact that these diseases always have a background of gastritis;
- the presence, in patients with gastritis, of anti CP antibodies the levels of which decrease after eradication of the bacteria, with concomitant clinical and histopathological improvement of the gastritis (23);
- the fact that Campylobacter pylori led to acute gastritis with a tendency to become chronic, in volunteers who ingested it (17, 19). This gastritis was accompanied by a transitory alkalinity of the gastric juice. This is because of the fact that Campylobacter pylori is able to split the urea in ammonium due to an endogenous urease activity which is a hundred times greater than that of other urealytic bacteria (e.g., Proteus vulgaris).

The majority of studies concerning CP associated gastritis are based on histological techniques using light microscopy.

Particular effort was made to evaluate the type of inflammatory infiltrate present in the stomach, and thus to better understand the host response to "infection". The results are controversial. Several authors, in fact, found a correlation between CP presence and neutrophilic granulocytic infiltrate (1, 12, 13, 16, 27). On this basis, Hazell et al. (12) considered the presence of Campylobacter pylori as a marker of active chronic gastritis, using the presence of the neutrophilic infiltrate as a sign of activity of the gastritis. In our previous paper on 155 biopsies of the gastric antrum and body, we made a semiquantitative evaluation of the infiltrate of the lamina propria in relation to the presence of Campylobacter pylori (7). Our results indicated that the highest correlation was between CP and neutrophilic infiltrate in the antrum of patients with chronic atrophic gastritis. Other authors did not find any correlation between the presence of the CP and the type of infiltrate (14, 15, 22). Few ultrastructural studies have been reported on the gastric mucosa colonized by Campylobacter pylori (5, 20, 21, 25, 28, 29).

Scanning electron microscopy (SEM) is undoubtedly the best technique for studying the distribution of micro-organisms and their relationships with the mucosal surface. To investigate these aspects in greater detail we used scanning electron microscopy to study the gastric mucosa of patients referred to us for upper gastrointestinal tract symptoms. In this paper, we made a more detailed study of the morphological aspects of the gastric mucosal surface exposed to the bacteria and we tried to correlate those with the histopathological findings, in particular with the inflammatory infiltrate. We also aimed to compare SEM to other routine methods of detection for Campylobacter pylori (Giemsa staining on histological sections and Urease Microtiter Test)(11, 23) to determine the contribution and possible clinical application of this ultrastructural morphological technique.

Materials and Methods

The study includes twenty-two patients (15 females and 7 males) aged between 20-77 (mean 51 years) who underwent endoscopy for upper gastrointestinal symptoms . In 15 patients, three adjacent biopsies were taken from both the gastric body and the antrum, and in 7 patients from the antrum only. One biopsy was utilized for the Urease Microtiter Test (MT), the other two for the histopathological and ultrastructural studies, respectively. For each type of determination, 37 biopsies were used.

Specimens for Urease MT were put in sterile wells of a microtiter tray containing urea, a bacteriostatic agent and phenol red as an indicator of pH change. The urease activity was graded on the basis of the reaction time, indicated by the change of colour from yellow to dark pink: 0 = absent; 1 = low (2h-12h); 2 = medium (30 min - 2h); 3 = high (within 30 min).

Specimens for histopathological study were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin for the histopathological diagnosis and with undifferentiated Giemsa for the detection of Campylobacter pylori (Fig.1). The neutrophilic infiltrate was graded from 0 to 3 as follows: 0 = absent; 1 = rare neutrophils, not seen in every high power field; 2 = intermediate between 1 and 3; 3 = numerous neutrophils also infiltrating surface and crypt epithelium. The presence of Campylobacter pylori on Giemsa stained sections was graded from 0 to 3 as follows: 0 = absent; 1 = rare CP not visible in all high power fields; 2 = intermediate between 1 and 3; 3 = CP covering more than 50% of the surface and/or foveolar epithelium.

Specimens for scanning electron microscopy were rinsed in saline, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 3 h at 4°C. After washing in the same buffer, the specimens were placed overnight in a solution of 1% HCl, then vortexed for 5 min. Dehydration in ethanol and critical point drying were subsequently performed. The specimens were then coated with gold by sputtering and observed in a Philips 505 SEM in a range of 5-15 kV. The presence of Campylobacter pylori on SEM specimens was graded as follows: 0 = absent, 1 = rare CP, not present in all 1000x fields, 2 = intermediate between 1 and 3, 3 = CP surrounding at least 50% of the surface epithelial cells or covering them also sparsely for at least 1/3 of the mucosal surface.

Some SEM specimens were also reprocessed for transmission electron microscopy (TEM) using the technique described by Versura and Maltarello (30). Semithin sections were stained with toluidine blue. Thin sections were counterstained with uranyl acetate and lead citrate and observed in a Jeol 100 B TEM at 80 kV.

The data obtained with the grading of the various parameters considered were analyzed statistically by means of the Spearman Rank Correlation Test and the Fisher Exact Test.

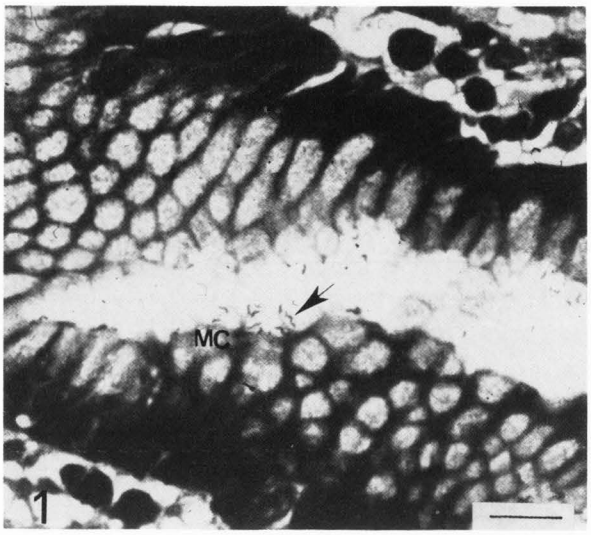


Fig.1. Gastric mucosa (antrum): surface epithelium. Campylobacter pylori (arrow) are visible free in the lumen and on the surface mucous cells (MC). Giemsa stained histological section. LM, bar = 20 μm

Results

Detection of CP: comparison between scanning electron microscopy, histology and Urease MT. SEM detected CP in 24 out of 37 biopsies , histology in 23 out of 37 , and Urease MT in 19 out of 37 . There was a high concordance between the three methods in CP detection ($p<0.0005$, Fisher Exact Test). The sensitivity of histology and Urease MT with respect to SEM was, respectively, 95.8 % and 79.2 % . With respect to endoscopic diagnosis, CP was found in 1 out of 2 patients with normal mucosa, 0 out of 1 patient with gastric ulcer, 1 out of 2 patients with duodenal ulcer, 6 out of 6 patients with erosive antritis, 1 out of 2 patients with erosive duodenitis and 8 out of 9 patients with chronic gastritis. All patients, including the one with normal endoscopy, had histological gastritis.

Bacterial colonization, graded by SEM, histology and Urease MT, was significantly correlated ($p<0.001$, Spearman Rank Correlation Test) with neutrophilic infiltrate of gastric mucosa as graded on histological sections (Table 1).

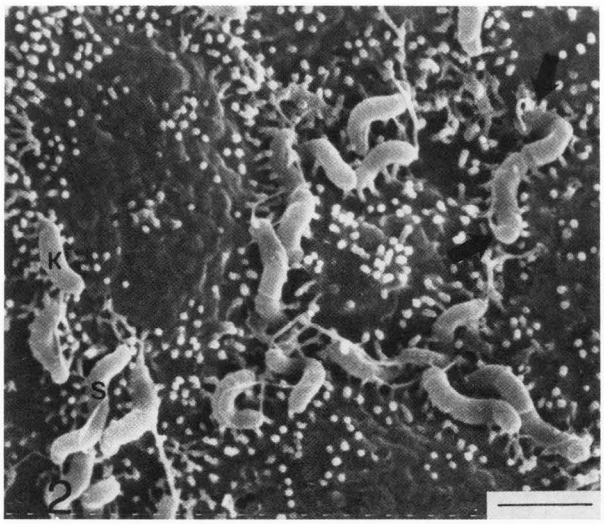
SEM of Campylobacter pylori and its relationship to the gastric mucosa . Campylobacter pylori presents a spiral (1-2 turns) or kidney-shaped morphology. The length varies from 1 to 4 microns (2 micron average) with a diameter of 0.5 micron (Figs. 2, 3, 4, 5). In the majority of the bacteria that were observed, the presence of a polar flagellum was visible; sometimes more than one was observed (Figs. 2-4). Bipolar flagella were observed in dividing bacteria (Fig.2). It was possible at times to observe flagella adhering to the apical membrane of the mucous cells (Fig.4). CP flagella show bulbous tips (Fig. 5).

In the majority of specimens the bacteria were disposed circumferentially around the intercellular junctions of adjacent epithelial mucous cells (Fig.2). The organisms often appeared adherent to microvilli (Fig.3), their intimate relationship to the glycocalyx being best revealed by TEM (Fig.3, insert). Occasionally the bacteria penetrated the intercellular spaces (Fig. 6), but only rarely were they seen on the luminal membrane of epithelial cells lying between protruding mucus granules (Fig.7).

TABLE 1 - CAMPYLOBACTER PYLORI COLONIZATION

			Grading		
				Campylobacter p.	
n.	biopsy	diagnosis	neutrophils	Giemsa	SEM	UMT
1	body	N	0	0	0	0
2	antrum	N	0	0	0	0
3	body	CSG	1	1	3	3
4	antrum	CAGM	1	1	3	3
5	body	N	0	0	0	0
6	antrum	N	0	0	0	0
7	body	CSG	0	0	0	0
8	antrum	CAGS	0	0	0	0
9	body	CSG	0	0	0	0
10	antrum	CAGS	0	0	0	0
11	body	CAGm	2	1	2	2
12	antrum	CAGm	1	1	2	2
13	body	CSG	1	1	2	3
14	antrum	CAGM	1	1	2	3
15	body	CSG	0	1	2	3
16	antrum	CAGm	1	2	3	3
17	body	CSG	0	0	0	0
18	antrum	CSG	0	0	0	0
19	body	CSG	0	0	0	0
20	antrum	CSG	0	1	1	0
21	antrum	CAGS	1	2	3	3
22	antrum	CAGM	2	1	2	0
23	body	CAGm	1	1	2	3
24	antrum	CAGm	1	1	2	3
25	antrum	CAGS	2	0	0	0
26	body	CAGM	1	2	2	2
27	antrum	CAGM	1	1	2	2
28	body	CAGS	2	3	3	3
29	antrum	CAGS	2	3	3	3
30	body	CAGM	0	0	3	0
31	antrum	CAGm	1	1	3	1
32	antrum	CAGm	1	1	2	3
33	body	CAGS	2	2	3	3
34	antrum	CAGS	2	2	3	3
35	antrum	CSG	0	1	2	0
36	antrum	CSG	1	1	2	0
37	antrum	CSG	0	0	0	0

N = normal human gastric mucosa
CSG = chronic superficial gastritis
CAG = chronic atrophic gastritis of mild (CAGm), moderate (CAGM) and severe (CAGS) grade.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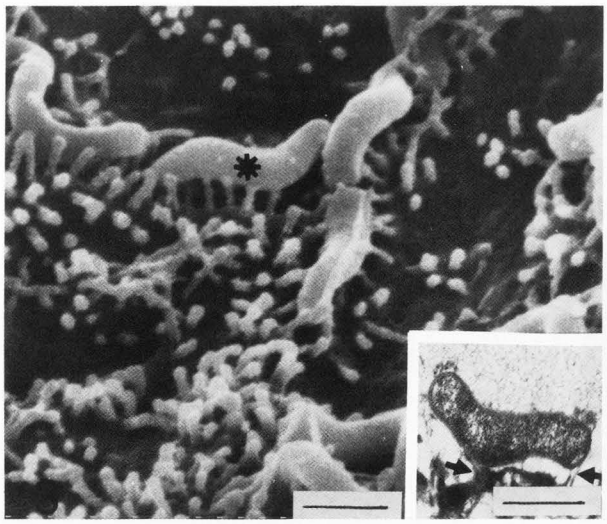

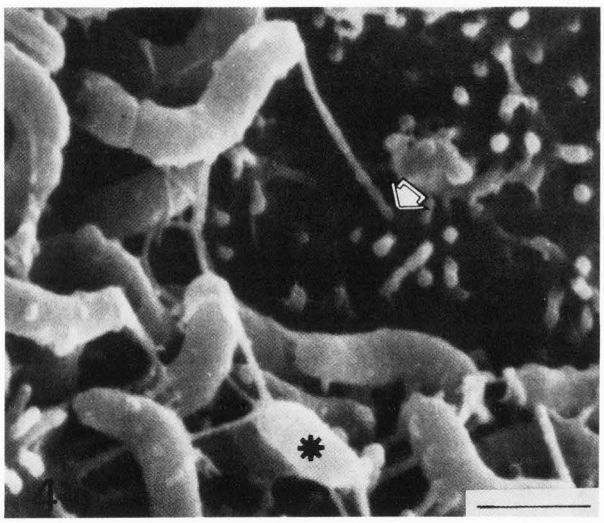
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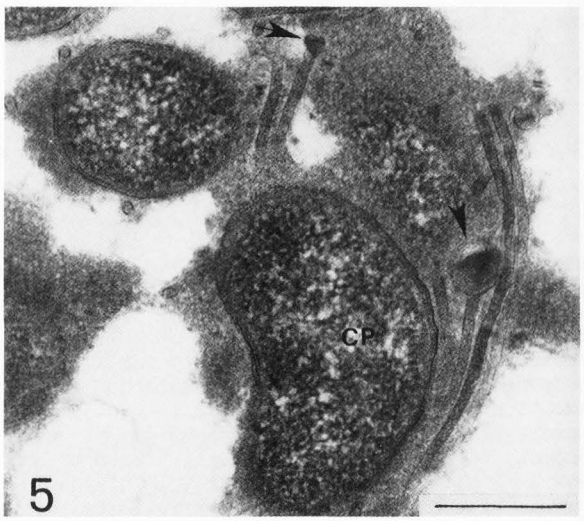
CP
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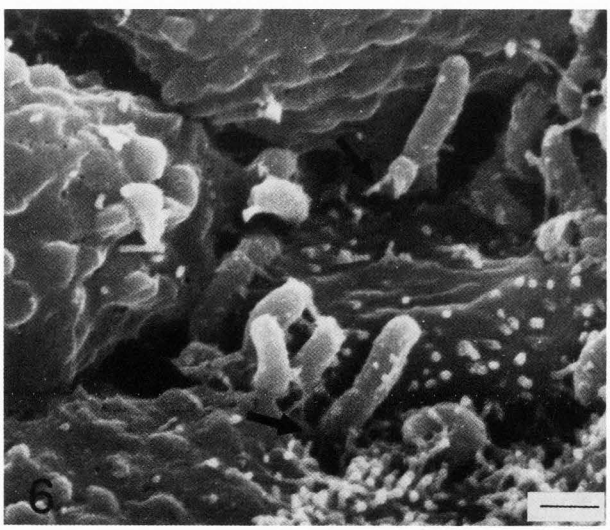
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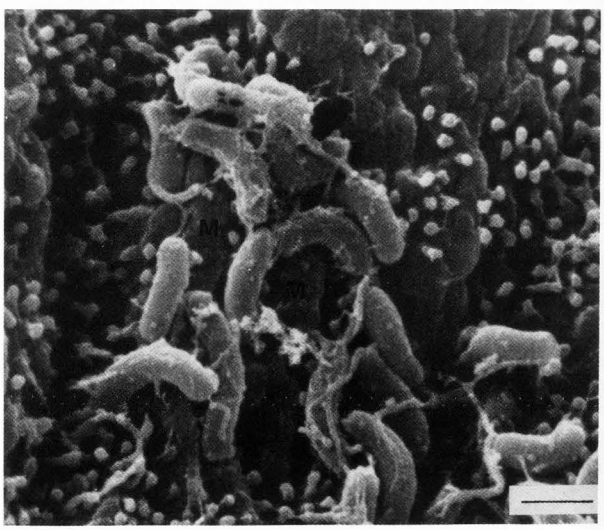


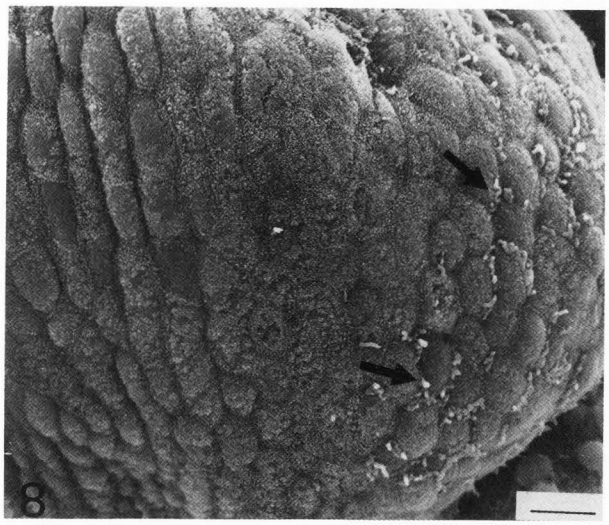


Fig.2. Gastric mucosa (antrum): surface mucous cells with few microvilli. Spiral shaped (S) and kidney shaped (K) Campylobacteria are visible at the intercellular junctions. Arrows indicate bipolar flagella in a dividing bacterium. SEM, bar = 2 μm.

Fig.3. Gastric mucosa (antrum): surface mucous cells. A Campylobacter pylori is adherent to microvilli (asterisk). SEM, bar = 1 μm
Insert: reprocessed specimen for TEM showing a bacterium attached to the glycocalyx of mucous cell microvilli (arrows), bar = 1 μm

Fig.4. Gastric mucosa (antrum): surface mucous cell. A Campylobacter pylori flagellum adherent to the cell apical membrane (arrow).
Asterisk indicates CP with multiple polar flagella. SEM, bar = 1 μm.

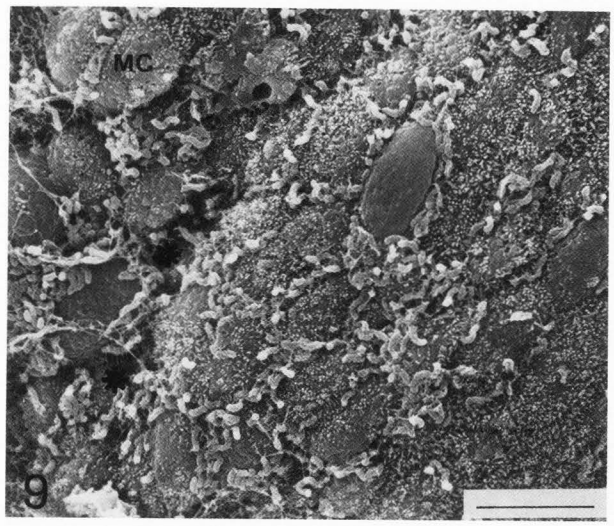
Fig. 5. Campylobacter pylori (CP) in the gastric lumen. Arrows indicate the bulbous tips of flagella. TEM, bar = 1 μm

Fig.6. Gastric mucosa (antrum): surface mucous cells. Campylobacteria penetrate between the intercellular spaces (arrows). SEM, bar = 1 μm.

Fig.7. Gastric mucosa (antrum): surface mucous cell. Campylobacteria lie between the protrusions of mucus granules (M) on the apical surface. SEM, bar = 1 μm

Fig.8. Gastric mucosa (antrum): patchy distribution of Campylobacter pylori (arrows) on the mucosal surface. The polygonal profiles of surface mucous cells are visible.
SEM, bar = 10 μm.

Fig.9. Same specimen of fig.8. Campylobacteria have an intercellular localization. Mucous cells with swelling of the apical surface (MC) and aspects of cell loss (asterisks) are visible. SEM, bar = 10 μm



There was a patchy distribution of the bacteria on the biopsy fragments of gastric mucosa (Fig. 8). In our cases the predominant localization pattern was intercellular (Fig. 9). In fact, only in two cases was it possible to observe bacteria covering mucosal areas more or less extensively. Figures 10 and 11 show the mucosal surface of a biopsy taken from a patient with chronic superficial gastritis in whom histology and Urease MT were negative for CP. Bacterial sheets cover an extremely limited area of the mucosal surface which shows only mild abnormalities compared with control biopsies (Fig.12).

The epithelium of the areas colonized by bacteria with an intercellular localization was normal in most cases or showed cells with a slight apical swelling and some aspects of cell loss where the bacteria were more numerous (Fig.9).

The cells adjacent to the areas covered by sheets of bacteria did not show any particular alterations either (Fig.11).

SEM of Campylobacter pylori associated gastritis. The histopathological findings of the biopsies examined by light microscopy were indicative of normal mucosa in 4 cases (all CP negative) and of chronic gastritis in 33. The chronic gastritis patients included 12 superficial type (4 CP positive) and 21 atrophic type (20 CP positive) cases.

Figures 12-15 show the SEM features of normal biopsies taken from body or antrum. The body mucosa is flat and small rounded holes open onto the surface relative to the gastric foveolae (Fig.12). The antral mucosa is raised in anastomosing ridges which mark the limits of deeper and more irregular foveolae (Fig.13). Figures 14 and 15 show typical surface mucous cells which present two different surface patterns:(i) those covered by a number of gastric type microvilli which are short, knob-like stumps (Fig.14) characteristic of cells not in the secretory phase, and (ii) those with microvilli towards the periphery of the cell surface, the apical membrane raised by underlying mucus granules or cavitated by recently-discharged granules (Fig.15) - a feature indicating a merocrine secretion (4).

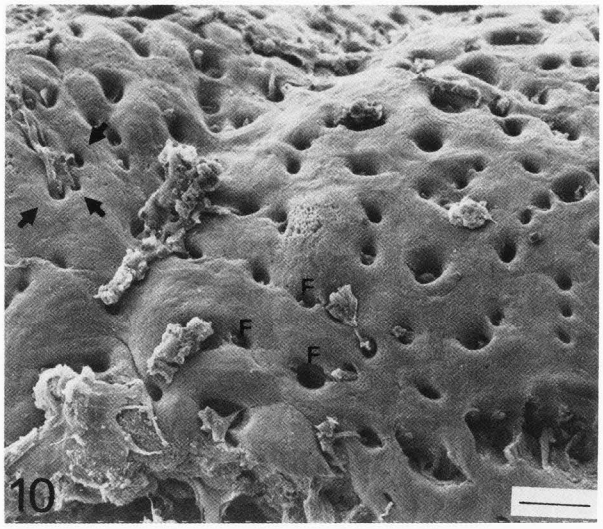


Fig.1Ø. Gastric mucosa (body) in chronic superficial gastritis which was CP negative on histology and Urease MT. Slightly irregular distribution of the foveolar openings (F). Arrows delimit the small area in which Campylobacteria were found . SEM, bar = 1ØØ µm

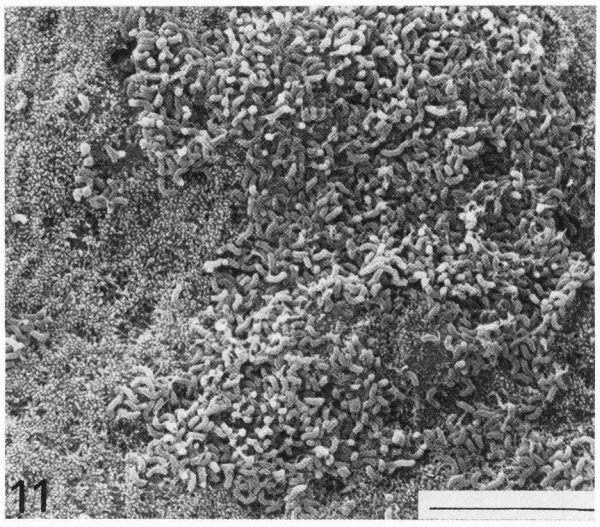


Fig.11. Detail of fig.1Ø: a "tapetum" of Campylobacter pylori is visible. The cells not covered by the bacteria have normal features. SEM, bar = 1Ø µm

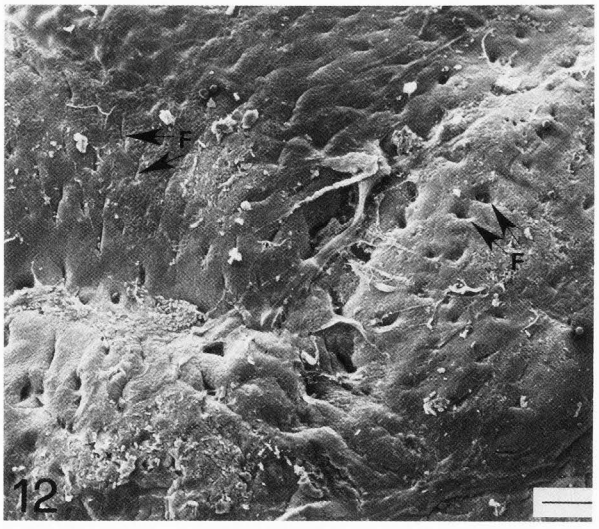


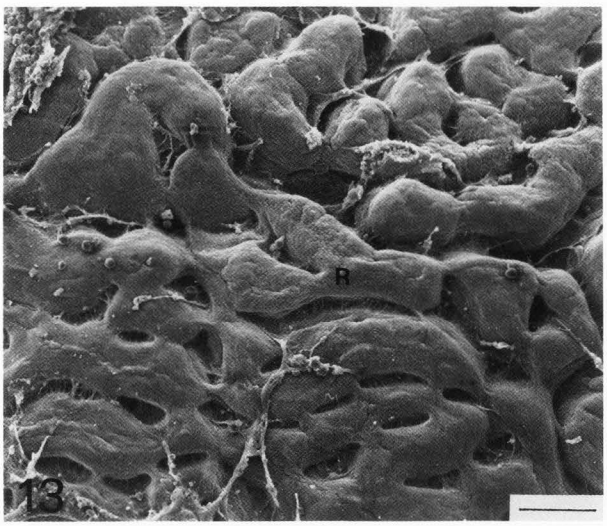
Fig.12. Gastric mucosa (body) : normal appearance of the mucosal surface where the foveolae open (F). SEM, bar = 1ØØ µm

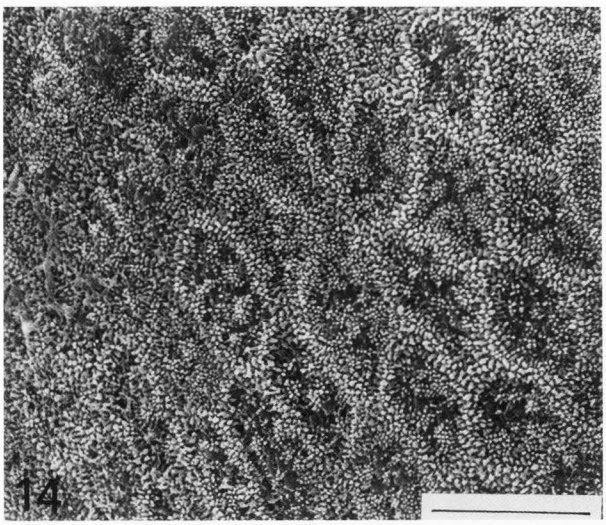
Chronic superficial gastritis. Biopsies with chronic superficial gastritis had only a slightly altered mucosal architecture with no difference between CP positive and CP negative (Fig. 16). No alterations were detected at the level of the lining epithelium (Fig. 17).

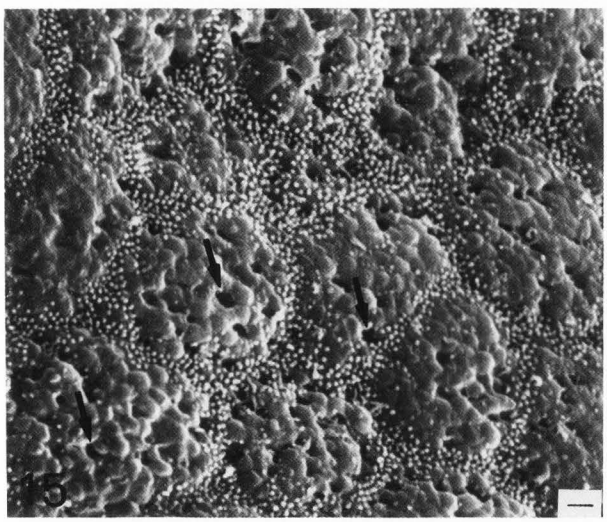
Chronic atrophic gastritis. Biopsies with chronic atrophic gastritis revealed greater variations in surface architecture , including areas of intestinal metaplasia (Figs. 18, 19, 2Ø). An important feature demonstrated by SEM was that such islands of intestinal metaplasia were never colonized by CP, even though adjacent areas of gastric mucosa might be heavily colonized by CP (Fig. 19). The lining epithelium varied from normal to areas with swelling and exfoliation (Figs. 21, 22, 23). These features were independent of CP and of the relationship of the bacteria with the cells, as demonstrated by Figs. 24 and 25 which show, respectively, Campylobacter positive chronic atrophic gastritis epithelium with normal cells and CP negative chronic atrophic gastritis epithelium with clearly altered cells. The mucosal alterations were related above all to the activity of the gastritis.

Discussion

The data of this study indicate that scanning electron microscopy is a more sensitive technique than histology or urease MT for the detection of Campylobacter pylori on gastric biopsies. The greater advantage of SEM over histology is based on (i) its extensive surface surveying capacity and (ii) the patchiness in the distribution of CP as demonstrated herein. SEM permits an extensive 3-D view of mucosal biopsy, while its high resolving power makes it possible to study surface details. Since Campylobacter pylori distribution is extremely patchy and the bacterial load varies within the same fragment, it is logical to expect that even multiple histological sections may fail both in detecting the bacteria and in determining the true extent of the bacterial colonization itself. In the same way, even if scattered throughout the fragment, isolated bacteria may not be seen with bidimensional







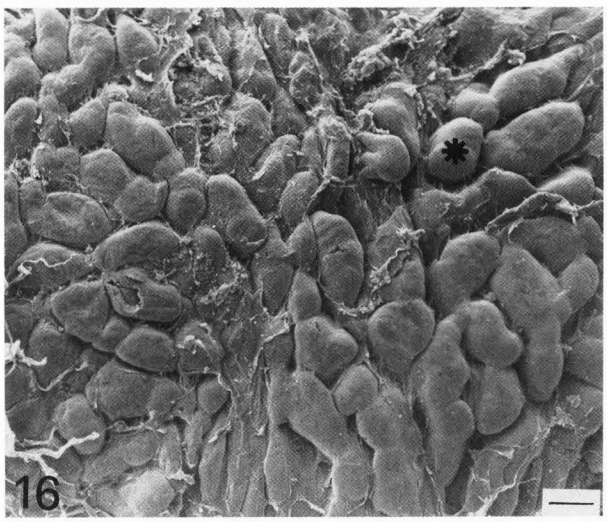


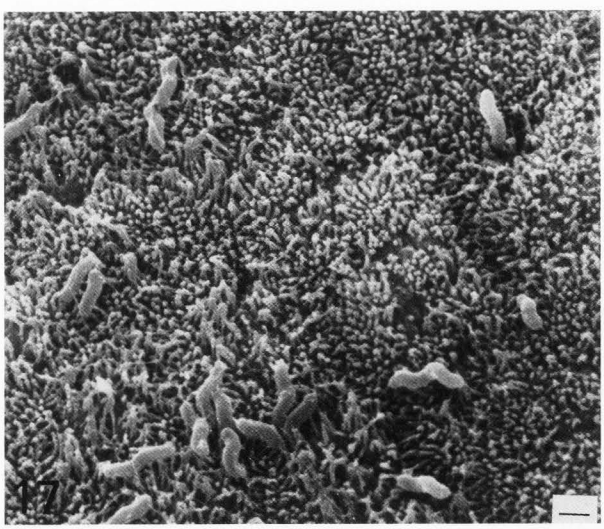
Fig.13. Gastric mucosa (antrum): normal appearance of the surface showing anastomotic ridges (R) limiting the foveolar openings. SEM, bar = 100 µm

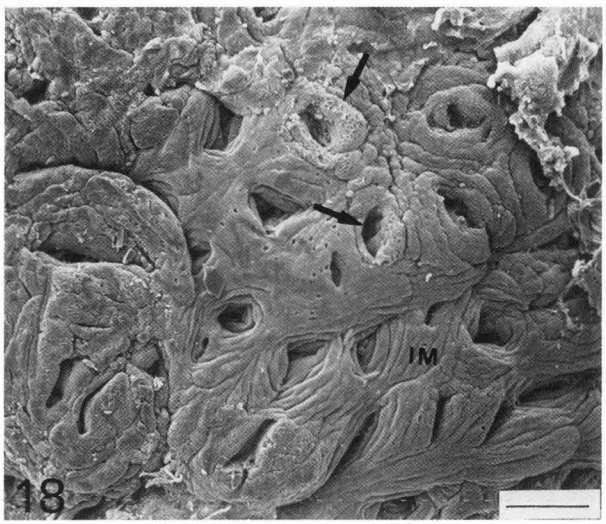
Fig.14. Gastric mucosa (antrum): surface mucous cells covered by microvilli. SEM, bar = 10 µm

Fig.15. Gastric mucosa (antrum): surface mucous cells with peripheralized microvilli, bulging of the apical membrane which has the impressions of the underlying mucus granules. Arrows indicate holes left by secreted granules. SEM, bar = 1 µm

Fig.16. Gastric mucosa (antrum) in case of chronic superficial gastritis: slightly irregular surface architecture with some mucosal structures resembling stumpy villi (asterisk). SEM, bar = 100 µm

Fig.17. Detail of fig. 16. Campylobacteria are visible between unaltered surface mucous cells . SEM, bar = 1 µm

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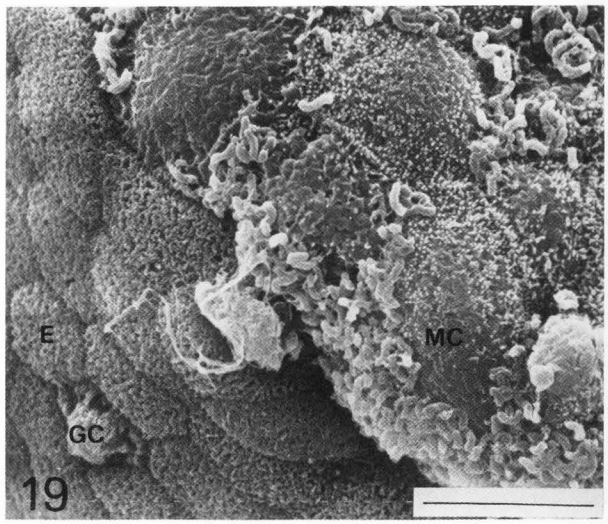
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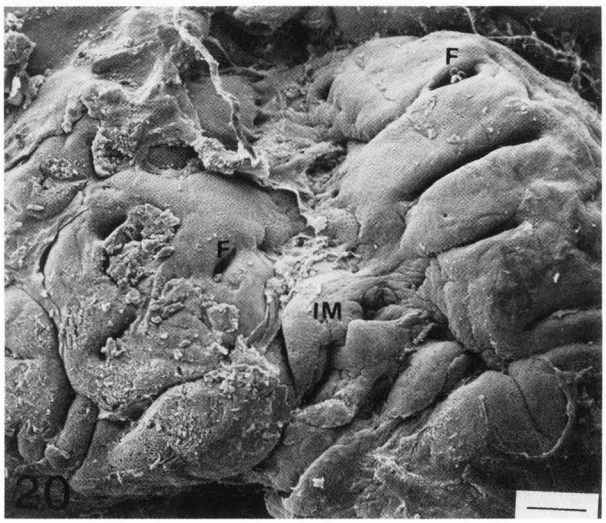
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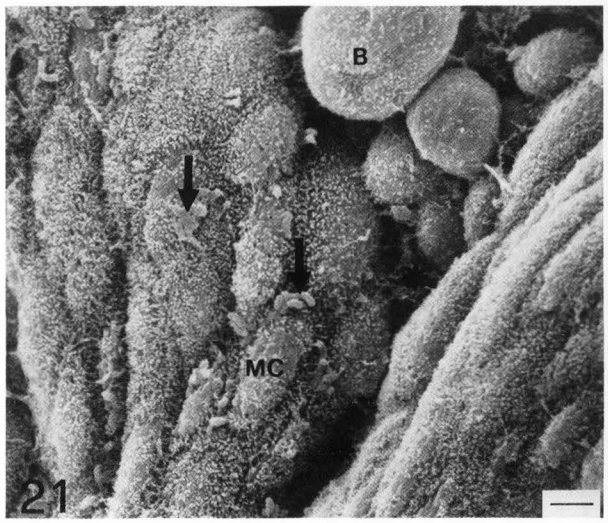
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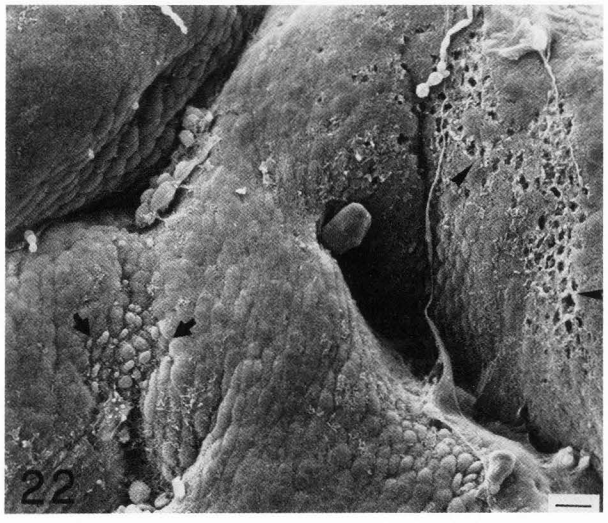
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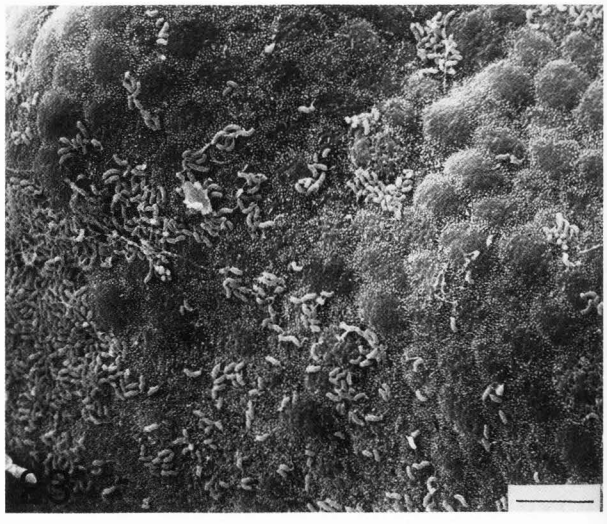


Fig.18. Gastric mucosa (antrum) in case of chronic atrophic gastritis. Small areas of gastric epithelium (arrows) are visible close to large areas of intestinal metaplasia (IM). SEM, bar = 100 µm

Fig. 19. Detail of fig. 18 showing a clear-cut separation between enterocytes (E) free from Campylobacteria and gastric mucous cells (MC) encircled by numerous bacteria. GC = goblet cells. SEM, bar = 10 µm

Fig. 20. Gastric mucosa (antrum) in chronic atrophic gastritis. The surface mucosal architecture is quite irregular. F = foveolae, IM = intestinal metaplasia. SEM, bar = 100 µm

Fig. 21. Detail of fig.20. Anisocytic mucous cells (MC), ballooning mucous cells (B) and aspects of cell loss are visible (asterisks). Arrows indicate Campylobacteria. SEM, bar = 2 µm

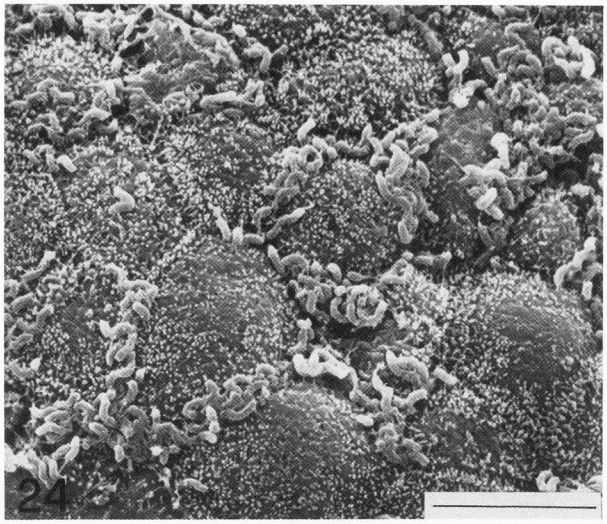
Fig. 22. Gastric mucosa (body) in case of chronic atrophic gastritis. Irregular surface mucosal architecture. Aspects of swelling of the apical surface of mucous cells (arrows) and cell loss (arrowheads). SEM, bar = 10 µm

Fig. 23. Detail of fig. 22 showing quite normal surface mucous cells and many Campylobacteria. SEM, bar = 10 µm

morphological methods; these also may be inadequate to produce sufficient urease activity detectable by current tests. We believe it is important to find even only one CP. In fact, even if isolated, a bacterium may be a sign of a more extensive bacterial infection in mucosal areas other than in the biopsy fragment that is examined. With regard to the specificity of scanning electron microscopy, we believe that the numerous studies comparing morphological, biochemical and cultural techniques have created a definitive identification of the bacteria.

The only morphological aspect of the bacteria which is slightly controversial is the number of the flagella which have been described as multiple unipolar sheathed flagella (8, 16) or paired, bipolar (20). We observed bipolar flagella in bacteria at the time of dividing. We could not identify the exact number of flagella within the tissue limits; in fact, some flagella encase the body of adjacent bacteria or adhere to the mucosal surface making themselves indistinguishable. For the same reason, it is often not possible to distinguish by SEM the bulbous tip described at the extremity of the flagella (8).

Our results are in agreement with the previously reported close association between CP and chronic gastritis type B (16, 18). They also confirm the correlation between CP and the activity of the gastritis reported by various authors (1, 12, 13, 16, 27) and by ourselves in a previous study, although denied by others (14, 15, 22). We believe, also in agreement with what was suggested by Hazell et



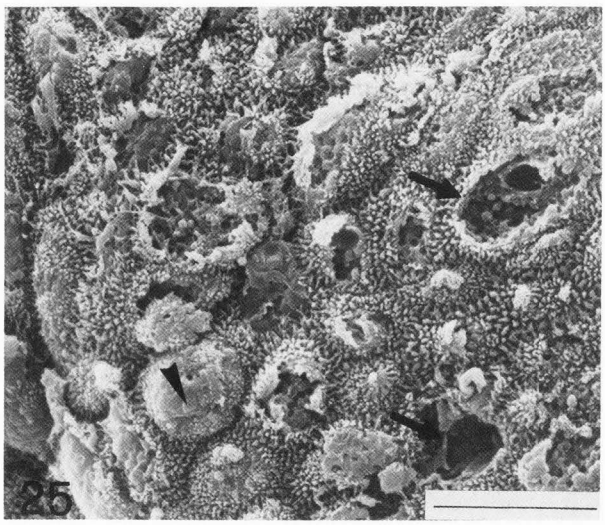


Fig.24. Gastric mucosa: surface mucous cells in CP positive chronic atrophic gastritis. The cells are quite normal. SEM, bar = 10 µm

Fig. 25. - Gastric mucosa: surface mucous cells in case of CP negative chronic atrophic gastritis. Anisocytosis, swelling of the apical surface (arrowhead), and cell loss (arrows). SEM, bar = 10 µm

al. (12), that the diversity of results depends on the patchy distribution of the bacteria and thus on the bacterial detection techniques. Our data demonstrate that the presence of Campylobacter pylori is underestimated and that the studies on the prevalence of the bacteria in the various diseases should be critically reviewed. Future studies should not leave SEM out of consideration in CP research, especially when other techniques have given dubious or negative results.

As far as the CP and gastric mucosal

surface relationship is concerned, our observations by means of SEM, correlated with the histopathological findings, seem to indicate that the bacterium has no direct lytic action on the cells. The fact that the bacteria frequently localize at the level of the intercellular spaces was interpreted on the basis of the presence of factors favouring the growth of the bacteria (urea, hemin) in these areas (10). Some TEM studies have reported breaks in intercellular junctions, penetration of the bacterium also within the surface mucosal cells and even in the oxyntic cells (5). On this basis it has been suggested that CP produces a cytolytic toxin (24). In our fragments reprocessed from SEM for TEM we did not detect these aspects.

The association of the bacteria with the neutrophilic infiltrate suggests that it is the bacterium which causes this inflammatory response in the host. Steer and Colin-Jones (26) demonstrated the presence of neutrophils phagocytosing these bacteria . As yet unidentified, other inflammatory mediators are likely to be involved.

Another interesting aspect, also, confirmed by our study by SEM is that CP is never present on cells which are not gastric mucous cells, i.e., not on intestinal metaplastic cells. On the other hand, some authors have reported their presence at the duodenal level on gastric metaplastic cells (28). The close relationship between the bacterial wall and the microvillous glycocalyx of the surface mucous cells observed by TEM suggests the presence of receptors for these bacteria.

In conclusion, CP associated gastritis has no particular nor distinctive superficial connotations other than the presence of the bacterium. The increasing evidence of the etiological role (3, 6) of the bacterium with regard to gastritis and peptic ulcer, of which gastritis probably constitutes the substrate, mean that it is indicated to look for CP in patients with upper gastrointestinal symptoms and in patients with peptic ulcers. In fact, in the latter it has been seen that antibacterial treatment reduces the rate of recurrence of the disease (18). In our opinion, it is, therefore, of great interest in clinical practice, to include the study of gastric biopsies with a technique such as SEM, which should reduce the number of false negatives.

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Discussion with Reviewers

M.N. Marsh: CP organisms were detected in 24/37 biopsies. Does this suggest a failure rate of 13/37 or 35%? Since SEM picked up only one more example of CP than routine histology, are you really entitled to say that SEM is much more effective, and if so why? Furthermore, in commenting thereon, consider that (i) many hospitals may not have immediate access to an SEM, (ii) that, given the high volume of gastric biopsies being provided on a weekly basis from most busy GI units, would an SEM lab be prepared to undertake such a regular, heavy commitment on time and energy, and (iii) whether such usage could be justified in the face of a 13/37 failure rate?

M.N. Marsh: How do you account for your failure to pick up CP in all your sample cases? Was this in any way related to technique, such as washing and swirling in all fluids associated with tissue processing, or were the organisms just not present?

Authors: Our results on the prevalence of CP colonization in chronic gastritis (40% CP positive biopsies in chronic superficial gastritis, 90% CP positive biopsies in chronic atrophic gastritis) are in agreement with those of the literature obtained by applying single or combined invasive and non invasive tests (Text reference n.16). On this basis we do not consider that negative cases represent a pick-up failure. In particular, histologically normal biopsies are expected to be CP negative, and in our series (4 normal, 12 CSG, 21 CAG) the overall expected prevalence was 26/37. Therefore the results of 24 CP positive biopsies suggest a SEM pick-up rate of 92.5%.

The gold standard technique for the detection of CP has still to be defined. Invasive or non invasive tests are chosen on the basis of what one is looking for. Non invasive tests (serum antibodies, ^{13}C-^{14}C urea breath tests) are indicated for screening purposes, while invasive tests for the diagnosis of gastric mucosal lesions associated with CP infection. Recently severe gastric dysplasia has been described in CP colonized mucosae as well as the resolution of this lesion after CP eradication (41). Among the invasive tests the histological study of the mucosal biopsy is recommended on the basis of its high sensitivity and specificity in detecting CP, particularly when appropriate stains are used (38). However the examination of a number of serial sections is needed to avoid false negative results due to the patchy distribution of the bacteria. SEM is a useful complementary technique which should be reserved to verify negative cases. Undoubtedly the application of this relatively simple, low cost and non time consuming technique requires a good multidisciplinary connection.

M.N. Marsh: It is necessary to express positive CP pick-up rates not only in terms of numbers of biopsies analyzed, but in terms of numbers of patients. This leads onto the important question as to (i) what extent the use of multiple biopsies improves CP pick-up rate and (ii) what is the least number of biopsies required to reach a positive detection rate per patient. With these data, it should then be possible to state whether, because of its enhanced field of view, SEM reduces the number of samples necessary to detect CP relative to (i) routine histology (ii) Urease MT testing. This then leads to another important consideration i.e., whether SEM in the long term is the most cost-effective method of diagnosis. Have the authors thought in this way?

F. Al-Bagdadi: Do you think that the SEM test for CP should replace the routine histological tests? Do you recommend that Urease MT should be dropped as a test for detection of CP?

Authors: We do not think that SEM would reduce the number of biopsies necessary to detect CP positive patients. SEM enhances the diagnostic sensitivity of mucosal biopsy. Moreover this technique could add useful information about the mechanism of CP mucosal colonization, in particular as concerns the possible hypothesized existence of ulcerogenic strands. Histology is always necessary to know the mucosal inflammatory reaction to the bacteria. The Urease test, while less sensitive in our experience, represents a very quick and simple method of detection which can be applied directly in the endoscopy room.

We did not express CP positivity or negativity in terms of patients because the study of CP prevalence in different conditions was beyond the scope of this paper. Other reports in literature deal extensively with this problem (42,45).

M.N. Marsh: The use of high power fields to quantitate cellular infiltrate is morphometrically inappropriate. In specimens with gastritis in which neutrophils are predominant, the inflammation may increase the volume of lamina propria: in such circumstances, use of high-power fields would bias the counts in a downward direction, resulting in lower values than might actually be present within each mucosal biopsy.
Authors: We applied a semi-quantitative evaluation using the same criteria of other authors who studied the infiltrate of gastric mucosa colonized by Campylobacter (Text reference n.12). We examined the entire section by 400 X magnification.

M.N. Marsh: Details about recent medications taken by the patients immediately leading up to endoscopy should be given. It is rare for patients with "dyspeptic" symptoms not to have been given some kind of symptomatic relief by their personal physicians (General Practitioner) before referral to hospitals. Can the authors supply details and whether antibiotics had been used, particularly with reference to negative CP, positive gastritis patients?
Authors: The patients under investigation did not take antibiotics in the month preceding endoscopy and biopsy. Antacids, H2-antagonists and metochlopramide were discontinuously used by some. Antacids are presumed by some authors to reduce CP colonization (32), while H2-antagonists do not seem to modify the mucosal environment for the attachment of CP (39).

M.N. Marsh: As I see it, the relevance of this work (apart from any intrinsic scientific merit) impinges directly on clinical practice. In these terms, the sample of patients described in this paper is very small. A far greater body of information needs to be built up as time proceeds. Thus at this stage, a carefully planned prospective study should be organized and designed to address questions raised by the work as it stands to date.
Authors: We will take into account Dr. Marsh's suggestions.

J.R. Poley: There is a preponderance of females, any reason why?
Authors: No reason.

J.R. Poley: Was there a macroscopic/endoscopic correlation between surfaces colonized/not colonized by Campylobacter? Would one find Campylobacter predominantly in areas of chronic/atrophic/erosive gastritis?
Authors: First of all a correlation between endoscopy and histopathology was not found in our cases, as shown by other authors (36). For this reason it is difficult to correlate the endoscopic aspect with the presence of CP. The endoscopist has to biopsy also in areas of macroscopically normal mucosa to pick up all CP positive patients.

J.R. Poley: Is there a pH optimum for the growth of Campylobacter pylori?
Authors: CP grows in an alkaline milieu under the mucus coat and so is protected from the gastric juice. Hypo-achlorhydria seems not to affect CP colonization as well as hyperchlorhydria (34).

J.R. Poley: Would you comment on the observation of lymphocytic gastritis in relationship with your observation of neutrophilic infiltration?
Authors: In our study we did not find any case of lymphocytic gastritis, a rare and distinct form of chronic gastritis characterized by a marked infiltration of the surface and pit lining epithelium by T lymphocytes. In this type of gastritis a low prevalence of CP (41%) was found in gastric mucosa (35). However high levels of IgG antibodies against CP were found also in negative patients. This supports the hypothesis that CP plays a role also in lymphocytic gastritis. In fact this histological entity could be the result of an abnormal immune response to some CP antigens after the first step of neutrophilic infiltration.

S. Siew: Does Campylobacter pylori colonize the gastric mucosa of any species other than the human stomach?
Authors: CP was also found in the stomach of primates like baboons, Rhesus monkeys, Macaca nemestrina, and of pigs (40).

S. Siew: Your work gives a clear demonstration that Campylobacter pylori colonizes gastric mucous cells, with a special predilection for the intercellular junctions.
Has any work been done on in vivo cultures of Campylobacter pylori to compare the effects of the addition of gastric mucus, gastric mucosal cells to the culture medium?
Authors: Not at our knowledge.

S. Siew: You have stated that the greater concentration of urea and hemin in the intercellular junctions may explain the concentration of bacteria in those areas. Both of these metabolites are increased in hemorrhage, and, more particularly urea, in renal failure. Is there any evidence of an increase of Campylobacter pylori in such cases?

Authors: Hazell et al. (Text reference n.10) hypothesized that the environment of intercellular spaces could favour the growth of CP, but other factors may be involved.

As concerns uremic gastritis, no difference was found in the prevalence of CP with respect to other conditions (44).

S. Siew: On the basis of your findings and conclusions, it would appear that there is not a direct causal relationship between Campylobacter pylori and peptic ulceration, as you have stated that its cytolytic action has not been proved. As the presence of gastric mucous cells is a prerequisite for the colonization by Campylobacter, these organisms would not be able to colonize areas denuded of mucosa. Would you agree, then, that the association of Campylobacter pylori with peptic ulcer is more an association of it with the concomitant chronic gastritis? (In instances of chronic peptic ulcer with hemorrhage there would be an increase of hemin and non-protein nitrogen, which may form a suitable nidus for the bacteria).
Authors: In our study we did not observe relevant damage on mucous cell surface. A direct cytotoxic effect was demonstrated only in vitro. The prevalence of CP is lower in gastric ulcer with respect to gastritis and duodenal ulcer. This could be due to the mucin depletion and intestinal metaplasia mostly occurring in gastric ulcer.

F. Al-Bagdadi: Do you believe the SEM technique reveals in depth information on the relationships of CP and the gastric mucosal surface?
Authors: It is widely accepted that SEM is a reliable technique for studying the distribution of microorganisms on mucosal surfaces. Undoubtedly it has to be correlated with other morphological techniques to which SEM is complementary.

M.S. Al-Tikriti: Were CP bacteria found in the lumen of pyloric glands?
Authors: Yes, in some instances by LM.

M.S. Al-Tikriti: How did you determine whether apical cell loss was due to bacteria or to normal wear and tear, fixation artifact, or precipitated mucus?
Authors: In normal mucosa we did not find any of the described alterations.

M.S. Al-Tikriti: Which came first, bacterial infection or gastritis and/or the peptic ulcer?
R.W. Henry: Is there evidence that these CP are a causative agent and not just opportunistic?
Authors: The etiological role of CP in gastritis is now established (45). Evidence is still lacking as concerns peptic ulcer even if clinical therapeutic trials sow a striking reduction of ulcer recurrence in patients in whom CP infection had been eradicated (37).

M.S. Al-Tikriti: You mention SEM is more sensitive than histology and other techniques in detecting CP. Is the spiral shape and flagellum specific features (diagnostic) for this bacterium? Are there other bacteria that have such features?
Authors: In cultures CP shows distinct morphology from other Campylobacter species so that another taxonomical classification has been proposed. Moreover CP can assume a coccoidal form in older culture plates and after therapy. In vivo it is not always easy to detect CP features. For this reason cultural studies may be useful for clinicians when the presence of therapy-resistant CP-strains is suspected.

R.W. Henry: Why was Campylobacter pylori never observed in areas of intestinal metaplasia?
Authors: CP was never found in intestinal metaplasia nor in duodenum except in areas of gastric metaplasia. This could be due to the presence of a glycoprotidic receptor on gastric mucous cells (33).

R.W. Henry: Why does Campylobacter pylori correlate with gastritis when swollen exfoliated areas were often observed without the presence of bacteria?
Authors: Surface mucosal changes are correlated with the histological changes of gastritis. The etiology of CP negative gastritis has still to be clarified.

R.W. Henry: Why is it important to search out even only the presence of one bacterium?
Authors: One bacterium is likely to be the expression of a wider colonization of adjacent areas not picked-up by the biopsy. This is due to the patchy distribution of CP in the stomach (43).

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