HELICOBACTER – THE EASE AND DIFFICULTY OF A NEW DISCOVERY

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by

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PREFACE

This is the story of my discovery of *Helicobacter*. At various times I have been asked: did I steal the discovery; did I find it by accident; did it follow some brilliant research work; or was it serendipity. My answer to most of these is a definite "No." Obviously, as with any new discovery, there is an element of luck, but I think my main luck was in finding something so important. I think the best term is serendipity; I was in the right place at the right time and I had the right interests and skills to do more than just pass it by. First, let us examine this.

Before 1970, well-fixed specimens of gastric mucosa were rarely seen in clinical practice. Biopsies, taken with the rigid gastroscope or the suction method, were very uncommon. Gastrectomy specimens are clamped at each end, with the contents inside. They fix slowly from the outside. Meanwhile the mucosa autolyzes and any organisms disappear. Autopsy specimens are even worse. Most surgical specimens were taken to remove tumours or ulcers and pathology descriptions centred on this rather than the fine histology of the mucosa. If they described gastritis at all, pathologists gave it names such as 'superficial' or 'atrophic,' which showed little real relationship to the histology.

Since the early days of medical bacteriology, over one hundred years ago, it was taught that bacteria do not grow in the stomach. When I was a student, this was taken as so obvious as to barely rate a mention. It was a "known fact," like "everyone knows that the earth is flat." Known facts can be dangerous; to quote Sherlock Holmes (Conan Doyle, *The Boscombe Valley Mystery*) "There is nothing more deceptive than an obvious fact." As my knowledge of medicine and then pathology increased, I found that there are often exceptions to "known facts." In the stomach, organisms, usually yeast or fungus, often grow in the necrotic debris in ulcers or tumours. Unusual infections sometimes do involve the gastric wall. Once I saw tuberculosis. Bacteria, floating above the mucus layer on the epithelium, are often seen in gastric biopsies. They appear to be mixed varieties, probably just passing through, dead, or contaminants; they are relatively sparse in cultures.

The introduction of the flexible endoscope changed all this. It enabled gastroenterologists to biopsy many of their patients. Small biopsies, placed

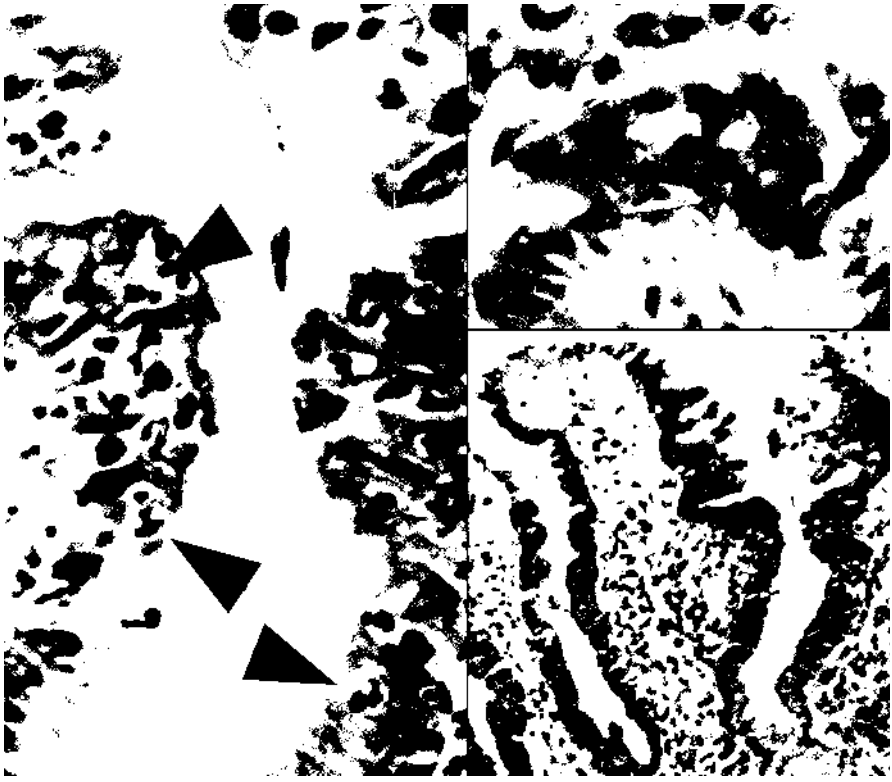


Figure 1. Whitehead's illustration of active change shows gross distortion of the superficial epithelium (above) and intra-epithelial polymorphs in the neck of a gland (arrowheads). (Whitehead R. Mucosal Biopsy of the Gastrointestinal Tract, 1st edition, figures 15, 16, 17, pages 20–22. © 1973 Elsevier Inc., reprinted with permission.)

immediately into formalin, fixed well. Instead of rare, these became some of our most frequent biopsies. Whitehead accurately described them in 1972. He described 'active' changes, which become important in my story. His pictures of this (figure 1) show intraepithelial polymorph infiltration in the necks of the gastric glands and a remarkable distortion of the foveolar (surface) epithelium. These features proved to be quite common and easy to diagnose. They were remarkably consistent in appearance, although often much more focal or mild than in the original illustrations (figure 2 and 3). The changes were superficial, usually involving only the epithelium.

Whitehead devised a classification based on the features he actually saw and described. Most of these features are mentioned in the diagnosis. This allows any associations between histology and other clinical features to be noted. I was very impressed with Whitehead's work. I simplified his classification for my own use (table), and the pathology of the stomach suddenly seemed to make sense. The diagnosis describes in one short line the features actually seen.

Microbiological stains are excellent for staining bacteria in smears, espe-

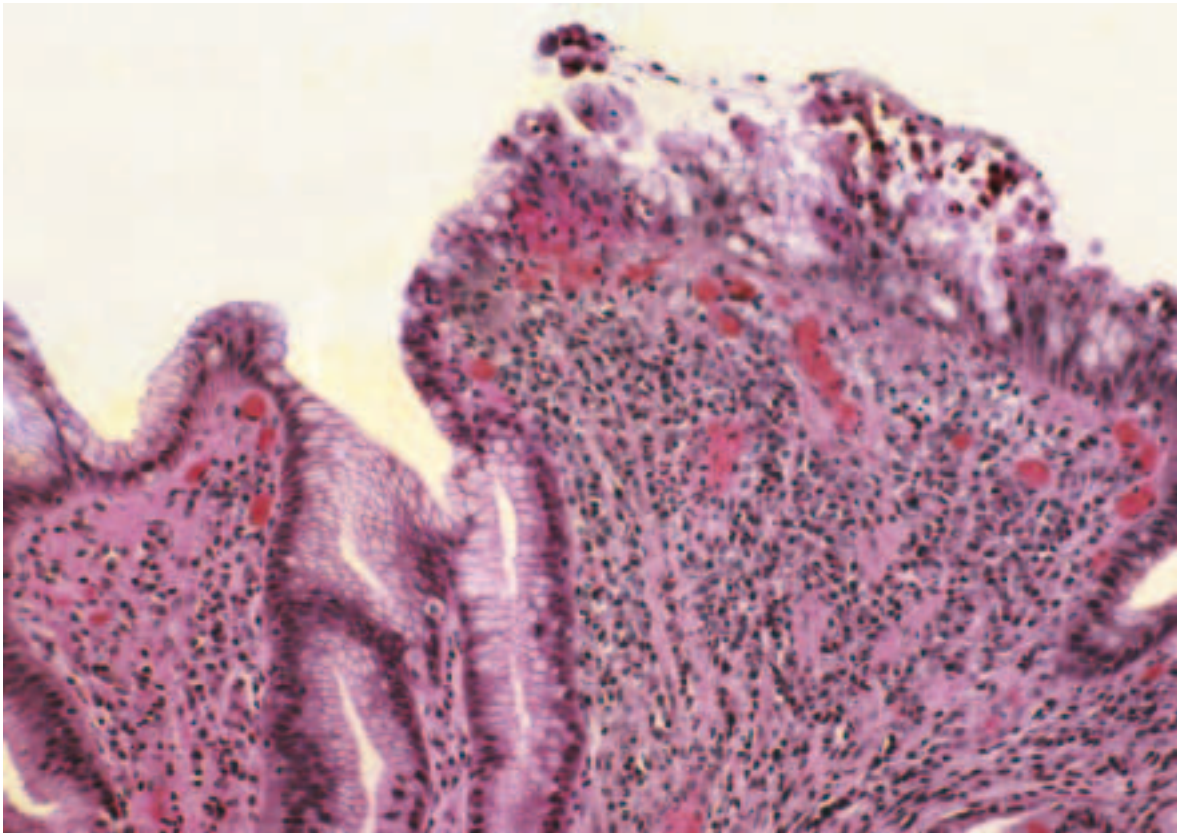


Figure 2. The surface (foveolar) epithelium to the right shows a focus of gross epithelial irregularity, of the type described by Whitehead. Elsewhere the epithelium shows only mild non-specific changes. In many biopsies the changes are often much milder than shown here (H&E x100).

cially from a clean culture. However, histology shows a complex mass of tissue structures that also stain. To see bacteria, it is necessary to contrast them with the tissue. Gram positive organisms and acid fast organisms contrast with tissue sections. Warthin-Starry silver stain of tissue shows spirochaetes (in

Table. My simplification of Whitehead's Classification of Gastritis

Pathology	Description
Severity	Mild, Moderate, Severe
'Active'	Active (if present)
Type of Inflammation	Acute, Chronic etc.
Other features present	Atrophy, Metaplasia, Dysplasia, Reduced mucus secretion

Using this table, the diagnosis may be written as a single line. In the following example, replace the headings (in brackets) with the appropriate descriptive terms.

Diagnosis: (Severity) – (Active?) – (Type) – gastritis – (with any other features).

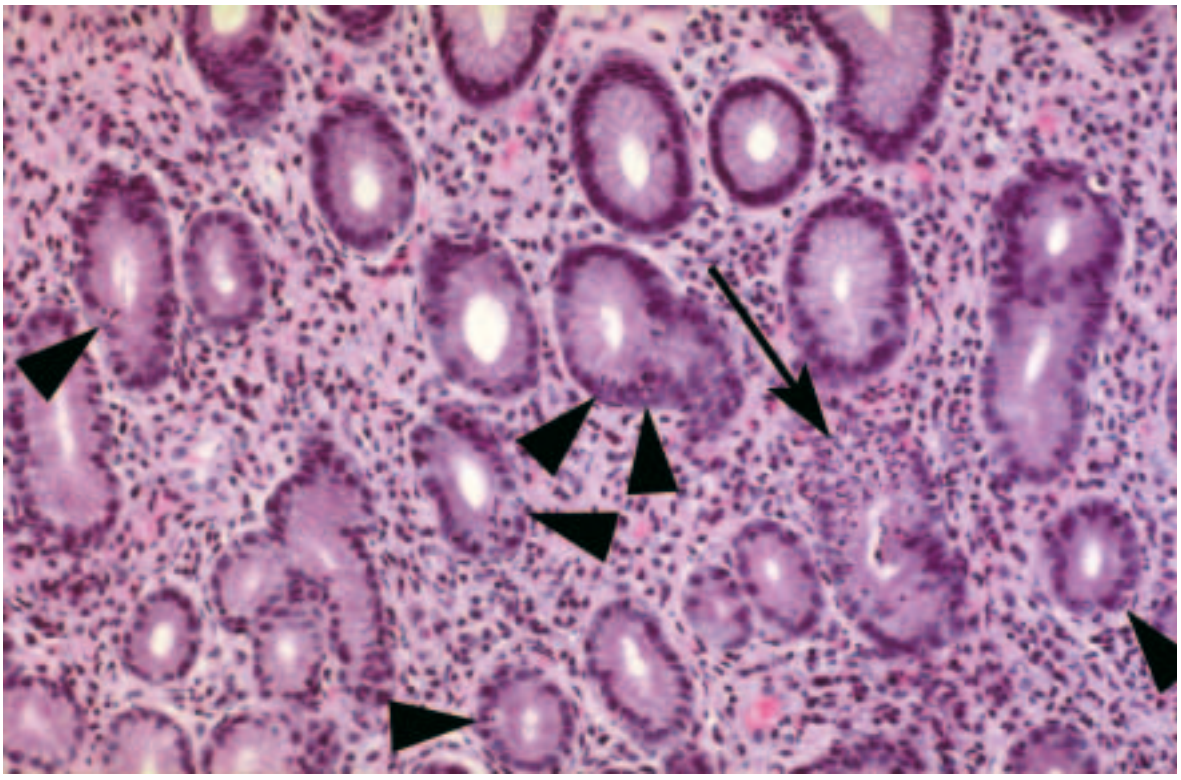


Figure 3. The section is cut obliquely through the necks of the gastric glands. This shows numerous gland necks in transverse section, lined by foveolar type epithelium. Glands are visible in the lower area, lined by smaller mucus-secreting cells. Polymorphonuclear leucocytes infiltrate the epithelium of the neck of one gland (arrow). There are also individual PMN's in other gland necks (arrow heads). Sometimes a few of these is all that is found, and the infiltration is often focal, as shown here (H&E x100).

syphilitic chancres) and bipolar Donovan bodies (the Gram negative bacilli in granuloma inguinale). I was interested in microbiological stains. After seeing several cases of granuloma inguinale in which the bacteria were clearly visible with the silver stain, I was experimenting with this stain on other Gram negative organisms, with variable success.

Thus, I was a young pathologist when high quality gastric biopsies became frequent. By 1979, I had a particular interest in gastric pathology, based on Whitehead's work and, in particular, his description of active gastritis. I was interested in bacterial stains, especially the use of silver stains for Gram negative bacilli. In addition, electron microscopy had recently started in our department. I found this interesting, giving another dimension to histology. Finally, I was interested in drawing specimens, and also in photography, both of which helped me to discern detail.

DISCOVERY: THE EASY PART

My adventure with *Helicobacter* began in June 1979. A routine biopsy showed severe active chronic gastritis (figure 4). The epithelium showed gross cob-

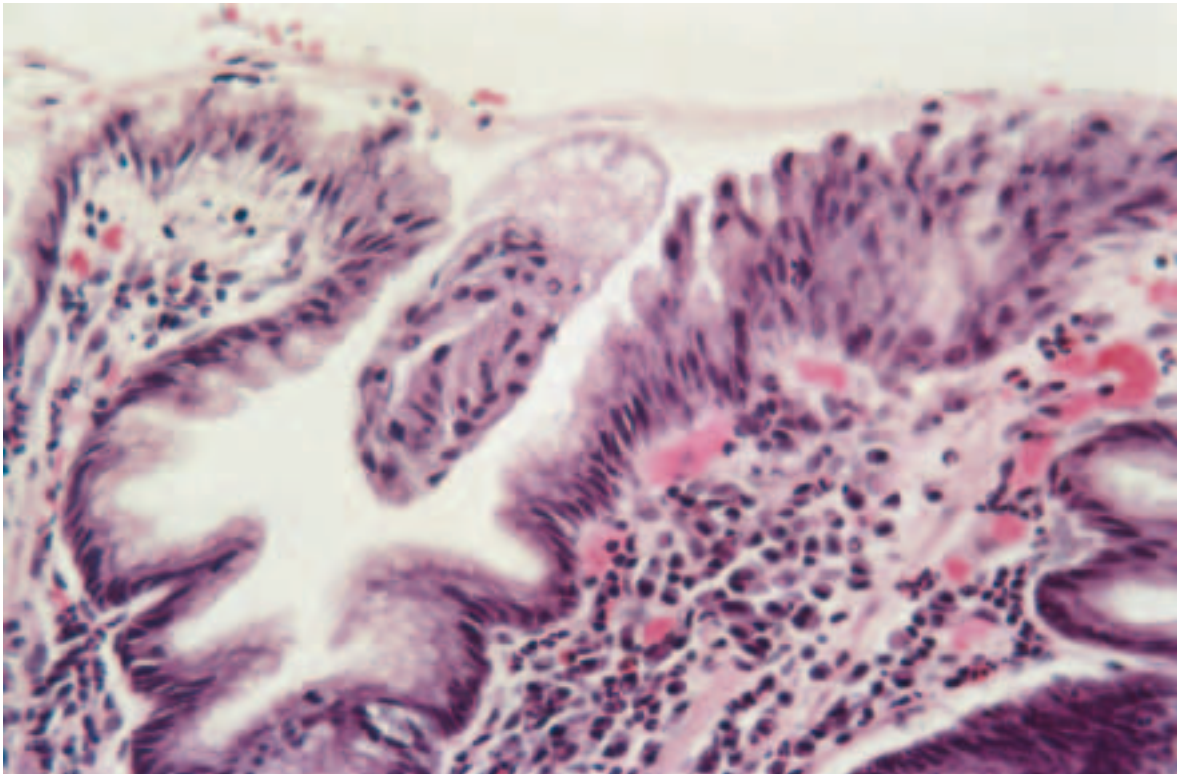


Figure 4. My first case. The epithelium shows gross cobblestone change, most marked to the right, resembling Whitehead's 'active' change. A thin blue line on the surface shows bacteria at high power (H&E x100).

blestone change, very similar to Whitehead's description. Nuclei were out of alignment. Mucus secretion showed a marked patchy reduction. Focal intraepithelial polymorphonuclear leucocytes were present (figure 5). There were numerous lymphocytes and plasma cells in the stroma. A thin blue line was visible on the surface, which on high power I thought consisted of numerous bacteria. My colleagues could not see them, so I stained them with the Warthin-Starry silver stain and numerous bacteria were easily visible at low power. At high power (figure 6), they were obviously small curved and spiral bacilli, closely applied to the epithelial surface and often arranged in palisades.

I took tissue from the wax block used for standard histology and obtained the electron microscopy. The images were of good quality and showed the bacteria well (figure 7). There were small curved bacilli closely applied to the surface. Some were attached to microvilli. The top of the cells bulged out. Mucus secretion was reduced. Bacteria were infiltrating between the bulging tops of the cells. They were not obviously penetrating past the cell junctions; however they may do so, because occasional bacterial fragments were present in the superficial stroma.

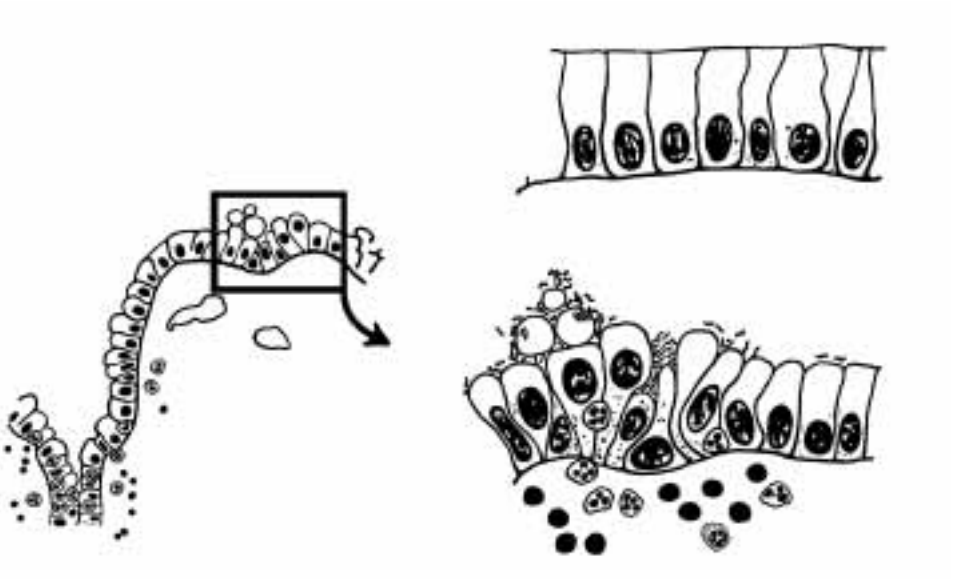


Figure 5. Diagram from my first case shows active changes in the infected epithelium (below). Normal (above) shows a flat surface and well aligned basal nuclei.

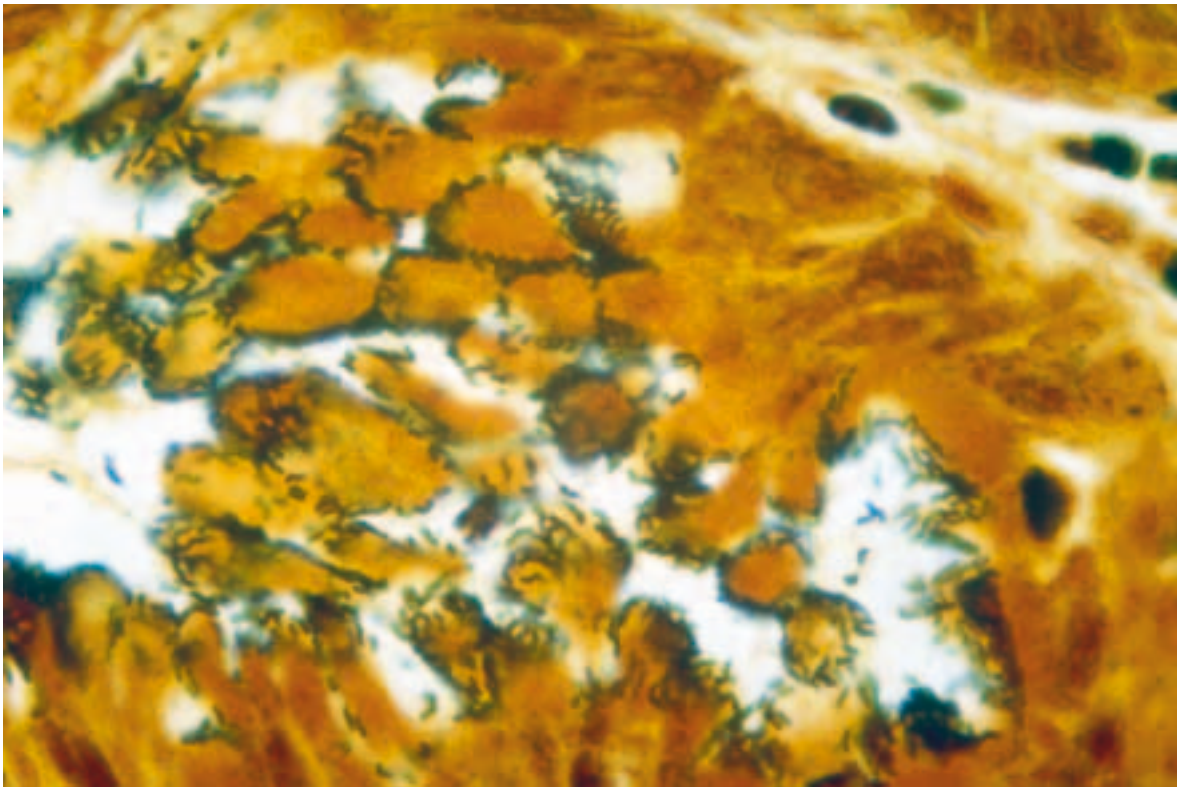


Figure 6. My first case. High power view with the silver stain shows numerous curved bacilli on the distorted epithelium (Warthin Starry x 1000).

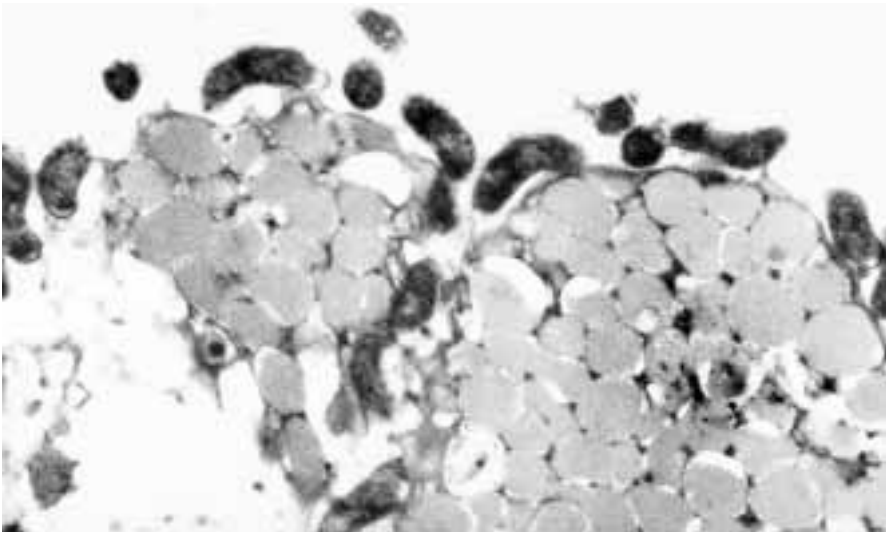


Figure 7. My first case. High power electron microscopy shows the top of two epithelial cells bulging out, with small curved bacilli closely applied to the surface. Few microvilli are seen.

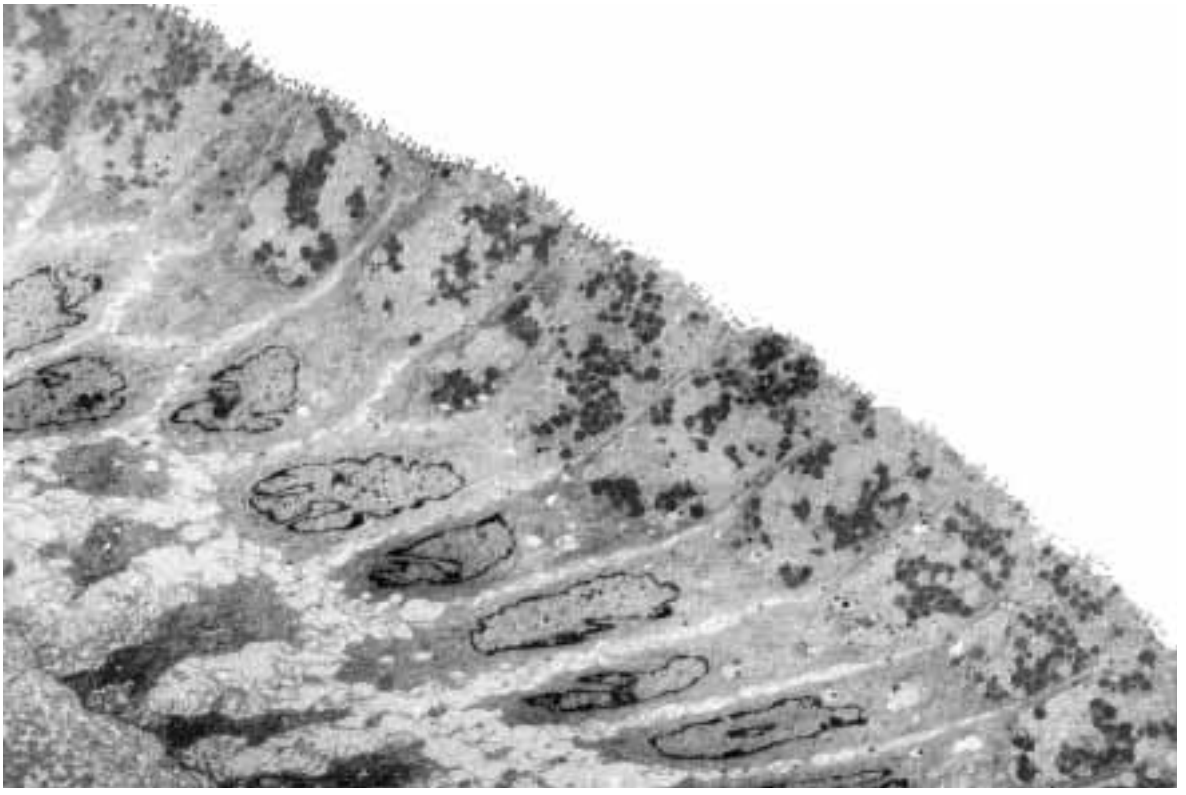


Figure 8. Electron microscopy, low power, of normal foveolar epithelium shows a flat surface with numerous tiny microvilli just visible.

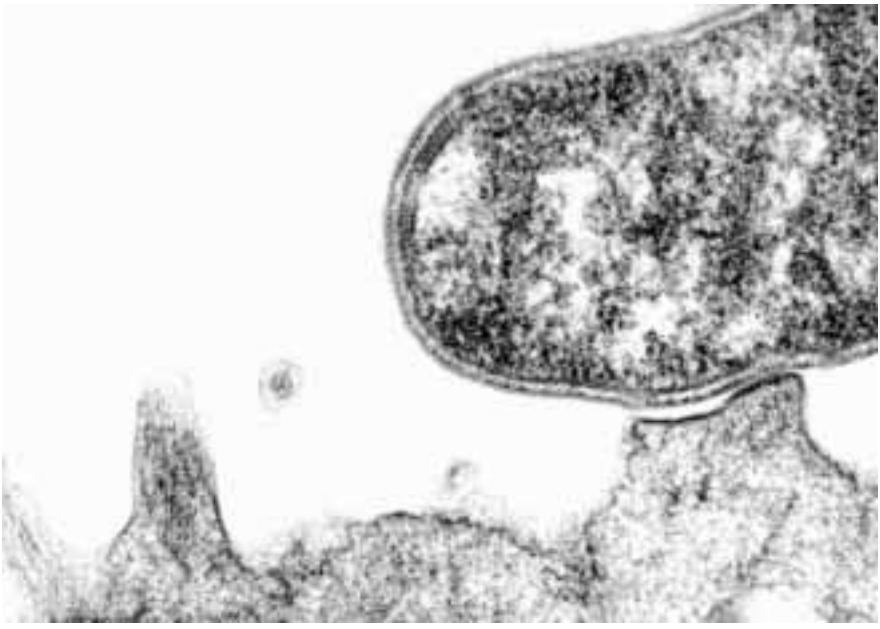
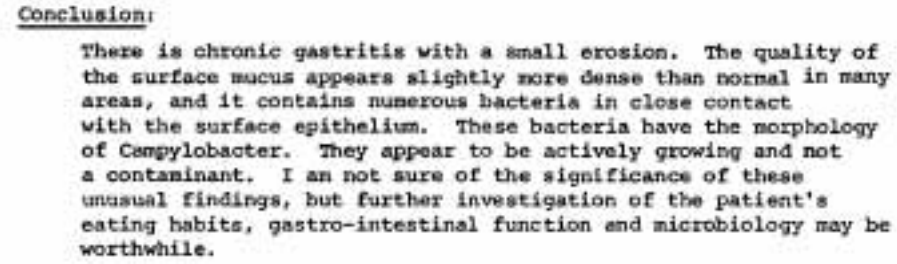


Figure 9. Very high power electron microscopy shows how the bacteria attach to the surface microvilli and flatten them. Bundles of filaments are visible within the microvilli to the left.

Electron microscopy demonstrates the normal anatomy of the columnar (foveolar) epithelium and the mechanism of the active change. The normal epithelium shows a flat surface, but there are numerous tiny microvilli (figure 8). The microvilli contain bundles of filaments that attach to the top of them. These filaments normally extend through the cells and attach to the cell base, giving the cells a rigid structure. This fixes their shape and also maintains their internal architecture, with basal nuclei and superficial mucus secretion. The normal columnar epithelium can be scraped from the mucosal surface, smeared onto a glass slide and still retain its columnar structure on cytological examination. *Helicobacter pylori* attach to the microvilli (figure 9) and often flatten and destroy the microvilli. The filaments become detached and the cells loose their structure. They behave in an amoeboid fashion, with nuclei floating through the cytoplasm and the surface bulging out.

My colleagues finally believed the bacteria were there. However, they doubted their importance, and challenged me to find any more cases. I thought they were worthy of further study (figure 10) so I continued to search and, to my surprise, I found them in quite a significant number of biopsies. The number increased with experience. Many cases showed only mild pathology, but the basic changes were still present. Eventually I was finding them in about a third of the gastric biopsies.

Another interesting feature gradually became apparent as my experience increased. I found the bacteria were easily visible on many surgical specimens. They were only seen along the cut edge of the specimens, where a nar-


Conclusion:

There is chronic gastritis with a small erosion. The quality of the surface mucus appears slightly more dense than normal in many areas, and it contains numerous bacteria in close contact with the surface epithelium. These bacteria have the morphology of Campylobacter. They appear to be actively growing and not a contaminant. I am not sure of the significance of these unusual findings, but further investigation of the patient's eating habits, gastro-intestinal function and microbiology may be worthwhile.

Figure 10. My original conclusion when I first reported the bacteria.

row strip of mucosa came into rapid contact with the formalin fixative. In addition, they were often mixed with a variable number of spherical organisms, particularly slightly further (2–3 mm) from the cut edge. It soon became apparent that the spherical organisms were the degenerating form of *Helicobacter*. This strip of 'mixed' organisms, only seen along the cut edge of the specimen, probably helps explain the absence of past reports. They would undoubtedly be seen as contaminants. We found these specimens a very useful source of positive control specimens when performing the bacterial stain.

DIFFICULTIES

I was unable to convince the clinicians of the importance of the organisms. Generally, they did not believe they were there at all. 'Everybody knows the stomach is sterile'. Gastritis was not considered to be of much significance anyway. Most thought that if the bacteria were there, they were just secondary to the gastritis. The histology suggested the opposite to me, but it was hard to prove. Another common question was 'If they are there, why has not anyone described them before?' At that stage I did not know why I had not seen them, let alone no one else.

It has become apparent over the years that gastric bacteria have been described many times over the last 100 years (ref 4). However, these descriptions were not generally known. Most of them were either veterinary biopsies or from research animals, which provided well fixed specimens without regard for 'patient' well-being. Most descriptions were looked on as peculiarities, of no particular importance, even by their authors. The apparent absence of any previous report was given to me as one of the main reasons why they could not be there at all.

I worked in a laboratory, without patient contact. Although the tissue quality was far better than it had been before the flexible endoscopes, most gastric biopsies were taken from visible lesions such as ulcers, to diagnose or exclude carcinoma. As a result, the histology often showed the effects of the nearby lesion. I needed biopsies from apparently intact antral mucosa, to show the effects of the bacteria without the competing effects of other

lesions. The idea of taking gastric biopsies for culture was considered ludicrous. The patient's well-being was the prime consideration.

Acute inflammation in the stroma is not specific for *Helicobacter* infection, and is often due to nearby ulceration. As might be expected with a surface infection, only superficial polymorphs within the epithelium are closely associated with the infection. Flattening of the foveolar epithelium is often due to the healing edge of an ulcer, particularly when associated with grossly reduced mucus secretion, gland atrophy or stromal fibrosis and polymorph infiltration. *Helicobacter* is often rare in such areas, even when it is plentiful on nearby intact mucosa.

After two years I had collected many cases and was almost ready to publish my findings. Then Barry Marshall, the new gastroenterology registrar, came to my room and asked to see my work. He had been told to find a research project, and since he did not like the one suggested, his superiors sent him to me. He was the first person to show any interest in my work, so I showed him. He did not seem impressed at first, but he agreed to send me a series of biopsies from apparently normal gastric antrum, to see if the same findings were present. He soon became more enthusiastic, and I finally had a clinical collaborator.

SUCCESS

In 1982, we obtained biopsies for culture and histology from 100 consecutive outpatients referred for gastroscopy. Most of them complained of peptic symptoms or pain, so this could not be investigated. They all completed a detailed clinical protocol that listed every symptom Barry could think of.

The results were totally unexpected. First, the bacteria were not related to any significant symptoms, only bad breath and burping. The gastroscopy reports were surprising. They showed that the gastric infection was most closely related to duodenal ulcer. Most gastric ulcers were associated with the infection, but every patient with a duodenal ulcer was infected. "Gastritis," as observed on gastroscopy, was not related to either the histology or the bacteria.

At first, no bacteria were cultured. Finally, plates incubated for five days over the Easter holiday showed a culture of a new type of bacteria, not described previously. The microbiology technicians had previously treated our research culture plates as routine cultures and discarded negative plates at 48 hours. After this, the plates were allowed to mature, and several more cultures were obtained. The bacteria showed many features of *Campylobacter*, but they were unusual and were eventually considered to be a new genus, now termed *Helicobacter*.

I sent a letter to the Lancet in 1983, a summary of the work I had done before I met Barry (ref 1). Barry sent an accompanying letter describing our joint work. He also presented our findings at the Brussels Campylobacter conference. Martin Skirrow, who chaired the conference, was very impressed with our work.

We sent our definitive paper to *the Lancet* in 1984 (ref 2). Although the

editors wanted to publish, they were unable to find any reviewers who believed our findings. Our contact with Skirrow became crucial here. We told him of our trouble, and he had our work repeated in his laboratory, with similar results. He informed *the Lancet* and shortly afterwards they published our paper, unaltered.

I continued as a clinical pathologist, with an interest in *Helicobacter*. The subject rapidly expanded throughout medicine over the next decade. The original methods for diagnosis and treatment were all suggested by Barry. I was involved with the pathology from: two attempts to fulfil Koch's postulates; the development of the breath test for diagnosis; improved methods of culture; studies of duodenal ulcer.

Helicobacter patients show considerable variation. I was involved with these early examples.

- Barry gave himself a severe active gastritis, to the disgust of his wife, in an attempt to fulfil Koch's postulates.
- Morris, in New Zealand, gave himself chronic gastritis and took years to cure it.
- My wife developed arthritis and as soon as she took NSAIDs she developed severe epigastric pain. Stopping the NSAIDs reversed this. And again. I sent her to Barry, who found *Helicobacter*, treated it and she was able to take the NSAIDs. Do not take it for granted that NSAIDs are the only guilty party.
- Most patients are symptomless. This was actually one of our major difficulties. I was an example. After she was treated, my wife complained that I had bad breath. I was positive for *H pylori* and after treatment marital bliss returned.

ACTIVE GASTRITIS

In 1986, we undertook a double blind trial to find the effect of treatment of *Helicobacter pylori* infection on ulcer relapse (ref 3). All patients received treatment for their ulcers. They received antibacterial therapy or placebo for *Helicobacter* infection. All were examined by multiple gastroscopies and biopsies for 12 months and again after 7 years. This provided me with excellent material for the study of the pathology related to *Helicobacter* and, also, the pathology of duodenal ulcers.

I quantified the grade of gastritis on a 0–36 scale by giving a value 0–9 for each of the main four features seen with active gastritis: intraepithelial polymorphs; typical epithelial distortion; reduced mucinogenesis in the foveolar epithelium; increased stromal lymphoid cells (a non-specific change seen with all chronic inflammation). This gave easily obtainable and remarkably consistent grades of gastritis for each case. From these results I made a histogram to show the grades of inflammation before and after eradication of *H pylori* (figure 11).

The grade of gastritis when *Helicobacter pylori* was present was usually above 20. This includes all patients in the study, including pre-treatment biopsies of

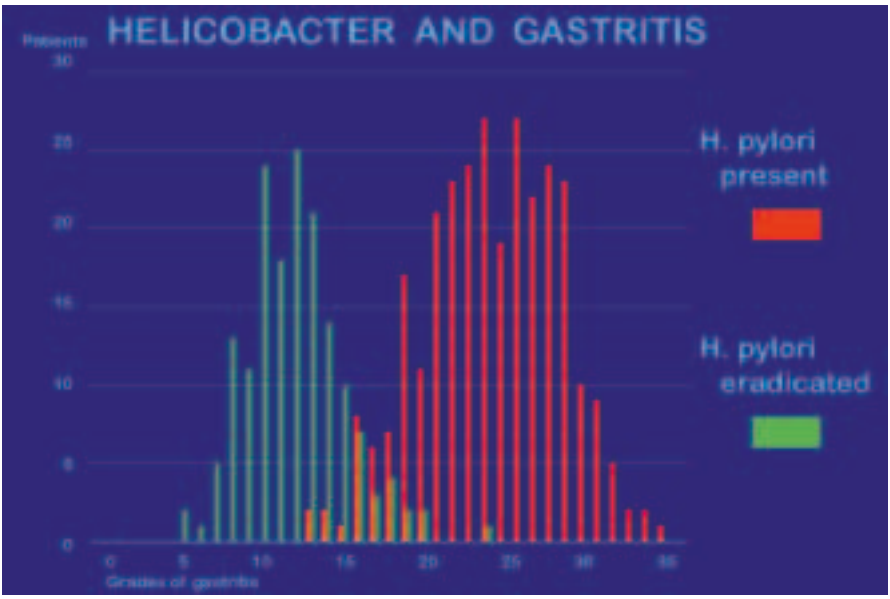


Figure 11. Histogram, comparing gastritis before and after eradication of *H pylori*. The normal range is (0–14), in the absence of pre-existing disease or infection.

those in whom the bacteria were later eradicated. Biopsies were taken 2 weeks after treatment. After successful eradication of *H pylori*, the active changes disappeared very quickly, and the grades in the histogram for these patients were mainly below 20. The true normal range is 0–14, but our cases show treated active gastritis, many biopsies taken only 2 weeks after treatment, not random normal samples. The stromal lymphoid cell infiltration disappeared more slowly, over about twelve months or more.

The absolute difference between the two groups is very impressive. There is some overlap, but the difference in the gastric pathology with and without *Helicobacter pylori* is incontrovertible (figure 11). One interesting feature was the consistency of the results over time. Repeated biopsies from each patient showed remarkably constant histological features throughout the 7 years of the study, as long as the bacteria remained. The active changes vanished as soon as the bacteria were eradicated, within weeks. This strongly suggests the bacteria caused these changes. 'Active' changes are almost never seen in the absence of *H pylori*. Other changes remained longer, particularly structural damage such as scarring, and epithelial changes such as gland atrophy, metaplasia and dysplasia.

DUODENAL ULCER

We were surprised to find duodenal ulcer so closely related to *Helicobacter*. However, further investigation shows that most duodenal ulcers can be viewed as distal pyloric ulcers. They are in the duodenal cap and the pyloric

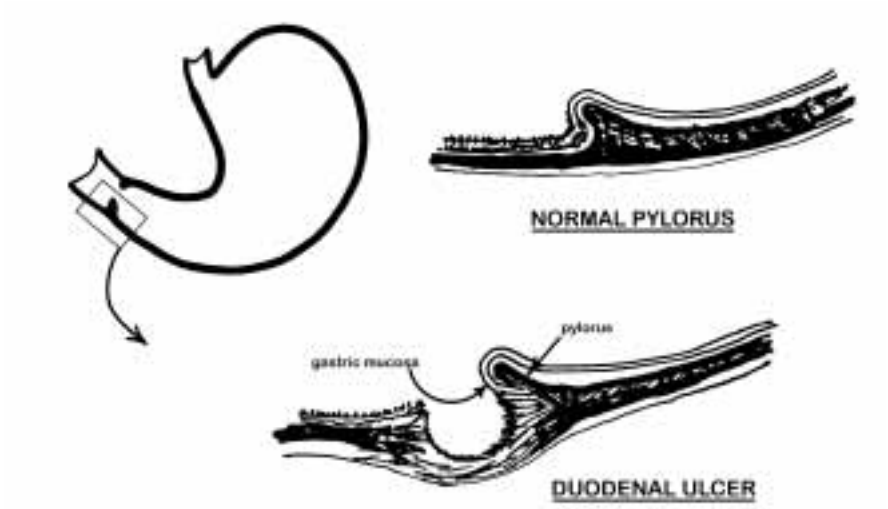


Figure 12. Diagram of the pylorus; the gastric mucosa normally extends into the proximal duodenum, and forms the proximal border of most duodenal ulcers.

mucosa normally extends through the pylorus (figure 12). Biopsies from the proximal border of all duodenal ulcers in this study showed either gastric mucosa or scarred mucosa, consistent with a gastric origin and with no apparent Brunner's glands, as seen in duodenal mucosa.

The pyloric mucosa is very mobile and easily moves some distance through the pylorus. When the stomach contracts, a mixture of food fragments and corrosive gastric juice squirts through the pylorus. Perhaps it is not surprising that ulcers are so common here, especially when the epithelium is damaged by infection and active inflammation.

CONCLUSION

Now, the importance of *Helicobacter* is generally recognised, particularly with regard to duodenal ulcer. As a pathologist, I am disappointed that active gastritis is not considered worthy of treatment. I see it in all infected stomachs, although often mild. Unfortunately, it does not cause many symptoms and nobody is interested. In conclusion, we now know that *Helicobacter* had been seen and largely ignored for over 100 years. I saw them 25 years ago and linked them with active gastritis. Barry Marshall and I cultured the bacteria and linked them to duodenal ulcer. In various different ways over the next few years we proved these relationships.

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Portrait photo of J. Robin Warren by photographer U. Montan.