Leading article

Helicobacter pylori: 10th anniversary of its culture in April 1982

The body is not made up of one part, but of many.
(I Corinthians Chapter 12, Verse 14).

Teamwork, between four departments, was the secret of the first successful culture, in Royal Perth Hospital, Western Australia, of human gastric spiral bacteria, now called *Helicobacter pylori*. Credit has rightly been given to Marshall and Warren for their publications, but the first culture was made by others; and the eminent electronmicroscopist, Armstrong played a seminal role at all stages.

Histologists have described spiral organisms in the human stomach since 1874,^{1,2} Others described 'spirochaetes' in animals,³ but these were unlikely to have been *H. pylori*. Probably they were '*Gastrospirillum hominis*',⁴ which has not been cultured nor characterised, and so has not been officially named. Others who described spiral bacteria in man and animals usually also failed to distinguish the shorter, gently spiralled *H. pylori* from the longer, tightly spiralled '*Gastrospirillum hominis*'.^{5–7}

Detailed histological and ultrastructural studies on human *H. pylori* were done first in Southampton by Steer. In 1975 he noted that spiral bacteria were closely apposed to the mucus secreting cells, and the bacteria possessed at least one flagellum.⁸ He emphasised that polymorphonuclear leucocytes migrated through the gastric mucosa, presumably in response to the bacteria. Endoscopic biopsy specimens were cultured but yielded only *Pseudomonas aeruginosa*⁹; this is not a spiral organism. In May, 1984, his scanning electron microscopic pictures of curved and spiral bacteria in large numbers on the surface of gastric epithelial cells appeared.¹⁰ Independently, in Birmingham, from 1981 Rollason *et al* had observed gastric spiral bacteria.¹¹

In Perth, Western Australia, at the Royal Perth Hospital, histological and ultrastructural studies of the gastric mucosa had been published in 1979¹²; spiral bacteria were seen, but because they did not invade the mucosa were thought to be irrelevant. The histopathologist, Warren, took more notice of these bacteria and correlated them with the presence of polymorphonuclear leucocytes.¹³ He emphasised that the stomach must not be viewed as a sterile organ.

In 1981, Marshall was in the middle of a three year internal medicine training programme, and for six months was learning gastroenterology. With Warren he reviewed case notes of patients in whom large numbers of gastric spiral bacteria had been seen.¹⁴ One of the patients, with severe epigastric discomfort, had been treated fortuitously with tetracycline; the symptoms resolved, and subsequent endoscopic biopsy showed that the antral gastritis had also resolved.¹⁴

It was most fortunate that by then Armstrong had come from Mill Hill to head the Electronmicroscopy Unit at Royal Perth Hospital. Marshall asked him for assistance, and in 1981 he and his assistant Wee obtained high magnification electronmicrographs of the spiral bacteria in endoscopic biopsy specimens. When Warren and Marshall could not agree on the wording of a joint letter to the *Lancet* in 1983, Armstrong advised them to write separate letters.^{13,15}

As Head of the Microbiology Department, in late 1981 Marshall asked me for microbiological assistance. We agreed

on a protocol (which I still possess); gastric biopsy specimens would be obtained from 100 consecutive patients during routine endoscopy, by the consultant gastroenterologists Waters and Sanderson, and these would be processed by Gram stain and culture, and by the histopathology department. The project started in March 1982, and I asked my microbiologist colleague, Pearman, to supervise the project. The technologists involved were Kosaras and Royce¹⁴; these with Pearman, carefully reviewed each microscopy and culture result. Among the first 34 specimens, spiral bacteria were seen in the Gram stain in six. In spite of frequent variations of media, and temperatures of incubation, from these 34 specimens, however, spiral bacteria were not cultured, because incubation was limited to 48 hours. The 35th culture was left incubating during the Easter holiday, which in Australia lasted for five days. When the plates were finally viewed, a pure growth of 1 mm transparent colonies were seen. *H. pylori* had been finally cultured! The date was 14 April 1982.¹⁶ Subsequent specimens were therefore incubated for at least four days, and although, in some cases, heavy growths of contaminants obscured the cultures of *H. pylori*, 11 isolations of this new organism were achieved. Among the 100 specimens, spiral bacteria were seen in the Gram stain in 34. Histologically, spiral bacteria had been seen in 58 patients.

Gram stain of the colonies showed only slightly curved organisms, not spirals as in the smear of the specimen, and Marshall doubted whether we had grown the correct organism. At this time we all became indebted to the skills of Annear, the senior scientist in our microbiology department, who successfully maintained these cultures. From his broth cultures Armstrong and Wee produced electronmicrographs that revealed that the bacteria were spiral, and had five sheathed flagella, which proved that they were not *Campylobacter* spp. Annear also achieved lyophilisation of several cultures of *H. pylori* in May 1982. The two earliest isolates are now NCTC 11637 and NCTC 11638.

Marshall read widely and came to realise that gastritis had an extremely high association with duodenal ulcer and only slightly less so with gastric ulcer. In our study, 100% of patients with duodenal ulcer and 80% of those with gastric ulcer had these spiral bacteria.¹⁴ In 1983, he moved to Fremantle Hospital, and worked part time in the microbiology department. From there he wrote the first published description of the culture.¹⁵ His letter contained Armstrong's electronmicrographs of *H. pylori*, both in vivo and in vitro.

Other early published references to the culture of *H. pylori* were a 1983 editorial on the Second International Workshop on *Campylobacter* infections,¹⁷ and the *Proceedings of the Workshop* which record the suggestion by Skirrow of the name *Campylobacter pyloridis*,¹⁸ and a preface written in 1983.¹⁹ The name *C. pyloridis* was formally proposed by us in 1984.¹⁶ The rules of Latin grammar, however, required us to change it to *C. pylori*.²⁰

During 1983 I was on sabbatical leave from Perth, but in January 1984 I inserted *C. pylori* into our study of cellular fatty

acids, and was thrilled to discover a unique profile which indicated a new genus. Armstrong and I discussed this when we published these findings with his ultrastructural studies.²¹ By 1988 our Perth microbiology team, and international colleagues had accumulated sufficient evidence to justify a new genus, for which I devised the name *Helicobacter*.²² So we now have *H pylori*, which is unlikely to change yet again!

In the *Lancet* of 16 June 1984 appeared Marshall and Warren's article on the first culture of 'unidentified curved bacilli',²³ and also a letter from Amsterdam detailing the powerful urease enzyme of this organism.²⁴ Marshall was undoubtedly the catalyst who initiated and maintained the momentum of our team effort that resulted in the first culture; and he rapidly realised the significance of bacterial gastritis particularly in relation to duodenal ulceration. I hope my article, however, now gives due recognition to the other actors in this drama. During 1983 Marshall devised selective media for primary isolation, and discovered the sensitivity of *H pylori* to bismuth and metronidazole; in 1985 he reported this in a detailed study of 267 patients.²⁵

With Armstrong and Marshall, I started our review in 1986 with the words 'Until a microbe is cultured and characterised, histopathological observation of the new organism remains tantalisingly incomplete.'²⁶ The culture of *H pylori* was not an accident, as stated by some; but we were fortunate in Perth that a five-day Australian holiday occurred during our project, and so proved that 'chance favours the prepared mind.'

Many gastroenterologists and histopathologists still disregard the clinical significance of *H pylori*, although logical answers to most questions have been published.^{27 28}

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