

Adventures with spiral bugs and *Helicobacter*

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These adventures began in 1967 when I was lucky enough to get a post-doctoral fellowship at the Rockefeller University in New York. This was in the laboratory of René Dubos, a distinguished microbiologist who was the first to systematically find an antibiotic, to pioneer the investigation of *Mycobacterium tuberculosis*, but who recently had become interested in the microbial flora of the intestinal tract (Moberg, 2005). Indeed, Dubos really can be considered to be the Father of the gut microbiome, which is currently all the rage, sixty years later (Prescott, 2017). His group worked with germ-free (GF) and specific-pathogen-free (SPF) mice, studying the bacteria in their intestinal tracts and the impact of factors such as nutrition, stress, maternal care, housing conditions, social interactions and sanitation on immune functions and health over the lifespan of the mice.

With colleague Russell Schaedler, Dubos was the first to consider the digestive tract as an ecosystem. In their words: “Recent studies have revealed that there exists in normal animals an abundant and characteristic microflora, not only in the large intestine but also in all the other parts of the digestive tract ... they become so intimately associated with the various digestive organs that they form with them a well-defined

ecosystem in which each component is influenced by the others and by the environmental conditions.” (Dubos & Schaedler, 1964).

So there I was, poised to work with Schaedler and Dubos on their SPF mice convinced that “the indigenous flora is responsible in some part at least for a number of physiological and immunological traits both favorable and unfavorable which are commonly assumed to have a genetic basis.” By chance, I was there at the beginning of the gut microbiome. How right their hypothesis turned out to be.

What was I to do? It was clear that many of the bacteria in the mouse intestinal tract had never been grown. Under the microscope, the dominant microorganisms were pointed or fusiform-shaped bacteria, which had certainly never been cultured.

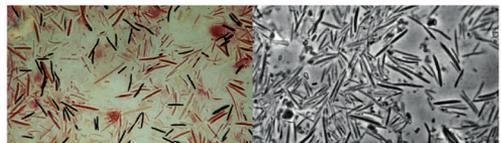


Figure 1: Mouse faeces with fusiforms

That was my task, to grow these fusiforms. Using one of the first ever anaerobic chambers, we succeeded, and to my delight my first publication was in the journal *Nature* (Lee et al., 1968).

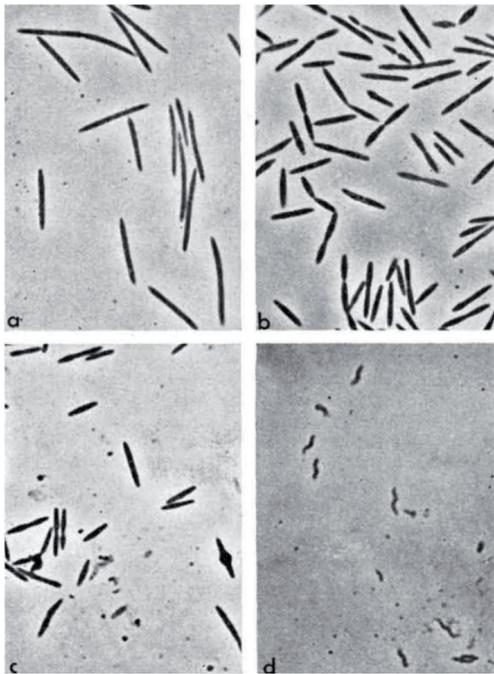


Figure 2: The culture published in *Nature*

Growing gut bugs became my passion for the next forty years. Arriving at UNSW in 1968, a special focus became the bacteria that lived in intestinal mucus, particularly those with a spiral morphology. They packed the intestinal crypts of most animals. One fascination was: why were they spiral? We grew them early on, our favourite being a beautiful organism we called “Stubby,” and tried to convince the NHMRC that these bacteria were worth studying (Leach et al., 1973; Lee & Phillips, 1978; Phillips & Lee 1983). This was made much easier by the discovery of a major diarrheal pathogen, also a spiral organism, *Campylobacter jejuni*. In mouse experiments, we showed that this organism also colonized intestinal mucus (Lee et al., 1986).

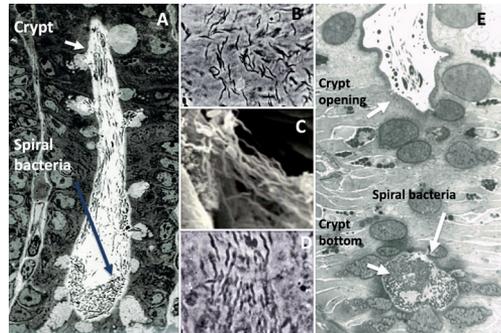


Figure 3a: Spiral bacteria in mouse

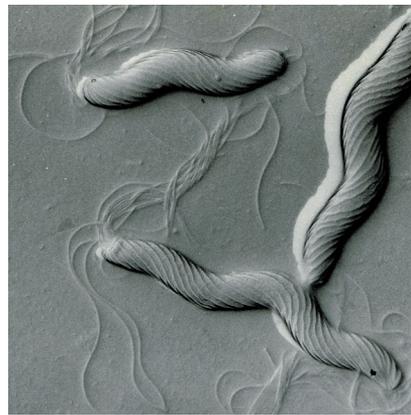


Figure 3b: Stubby: an early cultured bacterium

This work took us to Brussels in 1983 to the Second International Workshop on *Campylobacter* Infections, where we attended a presentation by another Australian that was to change my life. A Barry Marshall was presenting a paper on a bug he had grown from the human stomach and he was suggesting it could play a role in gastritis and duodenal ulceration. I had not met Barry then but had had contact with him. He had written what turns out to be an historic letter from Port Headland to one of my PhD students, Michael Phillips. He wanted to know what we thought about his stomach organism. He had used the conditions we had used to grow Stubby to grow it.

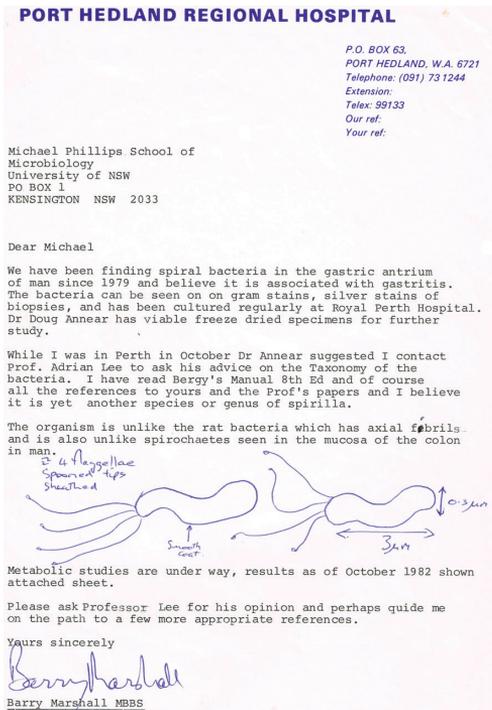


Figure 4: The Port Hedland letter

By this time, he was trying to get the world to take Robin Warren's and his discovery seriously. He submitted the work to the Gastroenterological Society of Australia for their February 1983 meeting. It was rejected!! "I regret that your research paper was not accepted for presentation — the number of abstracts we received continues to increase for this meeting. Sixty-seven were submitted and we were able to accept fifty-six."

Fortunately for Barry, he had submitted the same work to the *Campylobacter* Meeting. Bacteriologists had not grown up with the gastroenterological dogma of the stomach being sterile and we're always fascinated by the growth of a new organism. In particular Martin Skirrow, who was the leading *Campylobacter* researcher, was intrigued. It was due to him that the work did indeed

get international exposure by a paper in *The Lancet* following their two initial letters (Warren & Marshall, 1983; Marshall & Warren, 1984).

The medical world now became divided into two camps, the Believers and the Non-Believers. The work was eventually presented to the Gastroenterological Society of Australia in 1984 where one delegate reflected "Too Gung Ho. No controls had not even thought of them." Barry did not endear himself to his critics by being convinced he was right and amazed that "they had missed it all these years." Now in 1984, NSW enters the fray. There had been one believer at Barry's talk in Hobart: a Bill Hennessey, Chairman of the Department of Gastroenterology at St Vincent's Hospital, Sydney. He felt it was the "best lecture he had ever heard." He forgave Barry his brashness and felt "he was on to something." Two days after returning to Sydney, he said to microbiologist Jock Harkness "we must do something about this." Jock introduced Bill to me and it all snowballed from there.

At that time a student, Stuart Hazell, was deciding what topic to select for his PhD with me. Given our focus on spiral bacteria, we wanted to examine why they preferentially inhabited intestinal mucus. We decided to compare three spiral organisms *Campylobacter jejuni*, Stubby, and this new bug. Marshall was now calling the *Campylobacter*-Like-Organism (CLO). Stuart started to learn how to grow a culture of CLO that Barry Marshall had sent him. Initially, he had some trouble, but one anaerobic jar with a crack in it seemed to work well. This got us thinking about gas mixtures and so we removed the catalyst from the anaerobic jars we were using and lo and behold Stuart could grow the bug

well. This was good timing, as this was when he went to Bill Hennessey for clinical specimens. They took 10 gastric biopsies and grew the organism from all 10! This got Bill very excited and Stuart started regularly working at St Vincent's. The other two spirals were discarded and studying the CLO became his sole PhD topic. He was remarkably productive, making major discoveries¹ on what was now *Campylobacter pyloridis*. This included the first studies explaining the mechanism of colonization, and he devised one of the first urease tests for diagnosis. Stuart was probably the first person world-wide to get his PhD studying this gastric pathogen.

Also, around this time another PhD student, Hazel Mitchell, was working with material from St Vincent's, looking at the epidemiology of *Campylobacter pylori*. (Another name change!) She devised one of the first serology tests for the organism.² One has to realise that at that time I was young and inexperienced in the commercial world of microbial diagnosis. I refused to let Stuart and Hazel publish on the urease or serology tests until they had many cases, and the idea of patenting anything did not enter my head. Thus, future friend Cliona McNulty published on the urease test on 112 biopsy specimens and beat us to it. Barry patented his CLO test. Likewise, Hazel was pipped to the post. They do still talk to me!

This is where Tom Borody enters the story. He will tell his part in an accompanying article, but it is important to acknowledge the serendipity of the pathway that led Tom

into *Helicobacter* and his major role in world-wide acceptance of *Helicobacter pylori*, as it was finally called, as the cause of peptic ulcer disease. The interest in the organism became so great that a dedicated journal "*Helicobacter*" was born a few years later. Tom's role in the story is summed up by the following comment from David Graham, the editor, in his introduction to the journal (Graham, 1996): "The triple therapy introduced by Tom Borody (Borody et al., 1989) provided a therapy that for the first time could cure the infection reliably." Barry had used bismuth and metronidazole with some success but had not achieved adequate cure rates.

Stuart was in the St Vincent's tea room one day when I walked young gastroenterologist, Tom Borody, fresh from the Mayo Clinic in the USA, to work in the Department. Bill Hennessey had encouraged them to meet and discuss the new research that was going on with the CLO. Tom was intrigued and never looked back. In his enthusiastic, entrepreneurial way he started working with ulcer patients, trying to work out a cure. He even offered to fund the development of Stuart's urease test, which was now being routinely used, and suggested he patent it. Unfortunately, we did not take him up on that!

For the next twenty years, Tom and I researched *H. pylori*, mainly independently, and travelled the world trying to convince the non-believers.

My first big symposium presentation was at the World Congress of Gastroenterology in 1990 (Tytgat et al., 1990) — me talking on the microbiology, David Graham on the epidemiology, Barry on the pathogenesis, friends Mike Dixon on the pathology, and Tony Axon on treatment. An audience of thousands. Heady days for a humble microbiologist. I was flown

1 Hazell et al., 1986; Hazell & Lee, 1986; Hazell Borody et al., 1987; Hazell Hennessey et al., 1987; Lee & Hazell, 1988.

2 Mitchell et al., 1988; Mitchell et al., 1989; Mitchell et al., 1992.

all over the world to talk. My rationalization was that my job was to convince clinicians to take this organism seriously. It was remarkable how certain companies worked against accepting antimicrobial therapy for ulcers and how long before this became accepted practice. Now most ulcers have disappeared in the developed world except those caused by NSAID-induced lesions. But that is another story. What I would like to do now is describe the research we carried out over those years and what a fun and rewarding journey it was.

I loved trying to grow spiral bacteria. Having noted that nearly all animals were heavily colonized in their stomachs with spiral bacteria, we used the helicobacter growth conditions and successfully grew these beautiful organisms from cats. Identification techniques revealed, not surprisingly, that they were helicobacters and we named this organism *Helicobacter felis* (Lee et al., 1988; Paster et al., 1991).

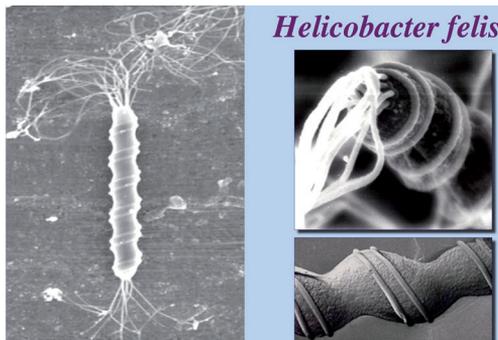


Figure 5: *Helicobacter felis*

There was no convenient animal model of helicobacter infection. It colonized pigs, but this is not very practical. We wondered whether it was worth using *H. felis*. A happy meeting of Jim Fox at MIT provided the opportunity to test this hypothesis. Jim was Head of the Department of Comparative Medicine at MIT and an expert on animal

models, with brilliant facilities in his lab. So it was off to Boston for a sabbatical with a culture of *H. felis* in my shirt pocket to keep it warm. Proper documentation, of course! We put the culture into GF mice, together with another animal helicobacter we had cultured from an infected human.

We hit the jackpot! I vividly remember the first time I looked at sections of the stomach of infected mice. The gastric crypts were packed with the helicobacters and there was clear evidence of inflammation. We had our animal model, which was subsequently used around the world (Lee et al., 1990; Dick-Hegedus & Lee, 1991).

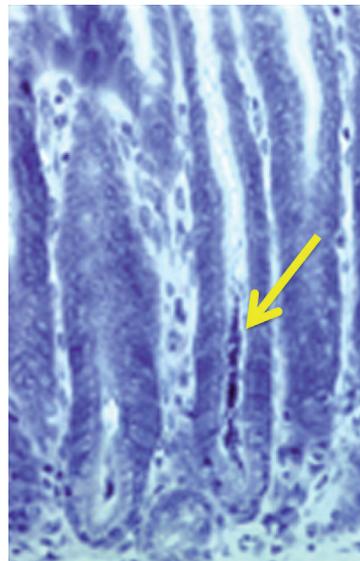


Figure 6: *H. felis* gastritis in GF mice

This allowed us to visualize the answer to that question “Why are they spiral?”

Examination of scrapings of *Helicobacter*-infected mice under phase contrast microscopy revealed spiral-laden crypts full of mucus. The bacteria could be seen boring through the mucus, confirming what culture results in viscous methyl cellulose had showed us. The spiral morphology gives the

bacteria torque in the viscous environment and allows them to move more easily than rod-shaped organisms (Lee et al., 1993).

While the *H. felis* mouse model was successful it was not *H. pylori*. Thus another step forward was when Jani O'Rourke isolated a culture of *H. pylori* from a gastric biopsy that would colonise mice. Patriotically we called it the Sydney strain and so another mouse model was found, again used by many around the world (Lee et al., 1997). One of our most exciting uses of these models was when we proved we could successfully immunize mice against helicobacter infection and even demonstrated a therapeutic effect (Chen et al., 1992; Doidge et al., 1994; Lee & Chen 1994). Our letters to *The Lancet* were noted by CSL, and so began a major seven-year collaborative project seeking a vaccine against *H. pylori*.³ We even got the US patent for therapeutic immunization (Doidge et al., 1995).⁴ Up to fifty million dollars was spent on this project until it was dropped, as there was not considered to be likely profit in immunisation in the developing world. Of all the diseases I could have tried to produce a vaccine on, I would choose a disease that was disappearing from the developed world! The other vaccine project CSL was working on at the same time was Ian Frazer's Human Papilloma Virus vaccine, which was a lot more successful.

Barry Marshall and Robin Warren had proposed that *H. pylori* infection could cause cancer. With our mouse model we showed that long-term *H. felis* infection caused gastric lymphoma, indistinguishable to the disease found in some *H. pylori*-

infected humans (Enno et al., 1995). Others later showed that gastric adenocarcinoma also occurred in long-term infection in our mouse models (Rogers & Houghton, 2009). This work resulted in me being one of the six helicobacter researchers on the panel of eighteen of the International Agency for Research on Cancer (IARC) Working Group on the Evaluation of carcinogenic risks to humans: Shistosomes, liver flukes and *Helicobacter pylori* (IARC, 1994). During this week-long meeting we rigorously reviewed the published literature on each agent and voted on our conclusions. Having only a third of the panel with expertise on each agent reduced the chance of bias in the voting. It was a vigorous and exciting process with the final evaluation concluding that "There is sufficient evidence in humans for the carcinogenicity of infection with *Helicobacter pylori*." A very far-reaching conclusion world-wide.

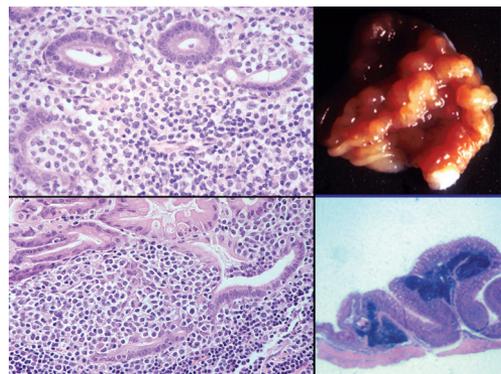


Figure 7: MALT lymphoma lesions

There are many other results of our endeavours over the years, but there is not time to describe them here. But it worth describing the tongue-in-cheek hard-luck story of my research career we described in a book Barry Marshall edited called *Helicobacter Pioneers* (2002). Barry gathered together chapters

³ Vaccine project goes commercial, *Uniken*, 25 October 1996, p.3.

⁴ US Patent WO 95/03824 CSL/UNSW Licensee AstraZeneca.

from all those who had a claim to being the first discoverers of *H. pylori*. This book should be compulsory reading for all those interested in the serendipity of science. Our chapter was called “We grew the first *helicobacter* and did not even know it.”

Strike 1: When Barry sent us that letter from Port Hedland in 1983 asking if we thought his new bug was the same as Stubby, we said: “It is possible that your isolate may belong to a new genus ... I am sorry we cannot be of more help, however the taxonomy of the spiral organisms associated with the gastrointestinal mucosa is, as you will be aware, very poorly understood.” What we should have said was, “We are fascinated by this very interesting and important bacterium. Please send us one of Dr Annear’s cultures to us so we can use our considerable experience with this type of bacterium to help you identify it quickly”!

Strike 2: We were convinced Stubby was a new Genus and submitted a paper proposing the name *Mucospirillum ileocryptum* General nov., sp. Nov. The name was rejected, as we only sent in two cultures to the journal. It turned out later that Stubby is in fact a *Helicobacter*, *Helicobacter muridarum*. Due to the taxonomic rule of precedent, if they had accepted our name, we would now all be talking about *Mucospirillum pylori*! (36).

Strike 3: Remember that very first *Nature* paper in 1968 where we were all excited we had grown the fusiform bacteria? Inspection of the reprints thirty years later revealed an image I had taken no interest in at the time. The fourth panel of images of our cultures clearly showed a pure culture of spiral bacteria. We now know, based on all our work on these organisms since, that they

were almost certainly a *Helicobacter* species. We really had grown the first one and had not known it!

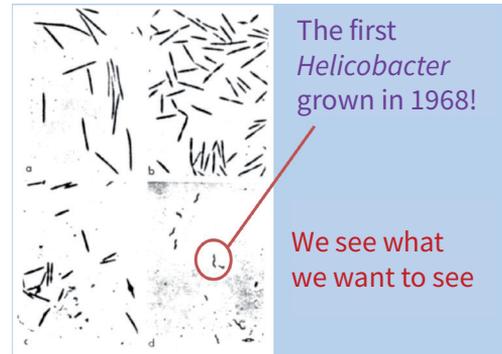


Figure 8: The first *Helicobacter* in 1968

But all was not bad. There were many rewards over the years. We did in fact grow a new spiral genus which we got to name and could honour my Rockefeller mentor Russell Schaedler. We named it *Mucospirillum schaedleri* (Robertson et al., 2005). Recently this organism was shown to have a role in protection against salmonella infection in mice, which is exciting (Herps et al., 2019).

We travelled to some wonderful places, including Rome in 2000 where we were presented to the Pope in front of half a million in St Peter’s Square. Barry and Robin both invited my wife and me to the Nobel Ceremony in Stockholm on 10 December 2005, which turned out to be a pretty special way to close down my research career, as I had moved to the dark side: university administration.

The *Helicobacter* story is a wonderful example of the need to keep an open mind in science. Indeed, for a number of years I told the story to medical students in their first lecture, telling them, “During your

medical course and beyond you will be exposed to, and will discover, the current medical dogmas in many facets of your work. Wonder at how far we have come but be prepared to challenge, disprove, and even discover the next paradigm shift.”

Thank you. It is now time to hear Tom Borody’s story of the NSW *helicobacter* days.

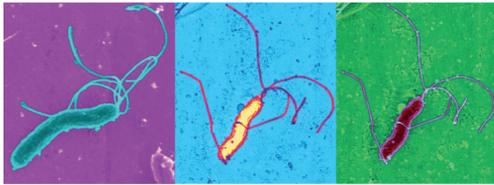


Figure 9: *H. pylori* coloured

Acknowledgements

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