



Adventures with mucus and Stubby: Life amongst the helicobacters

The following article is based on the Rubbo Oration that I was honoured to present at the 2002 Annual Scientific Meeting in Melbourne.

Having worked as a medical microbiologist at the University of New South Wales for 30 years but having now moved on to the executive of the University as Pro Vice Chancellor (Education), I took the opportunity to reflect on my life and discoveries in a way that hopefully opened up a new field to the audience unfamiliar with the interesting world of gastroenterology and served as an example of the serendipity of science.

I also wanted to make a statement to Government that, while it may appear logical to demand of us that we work solely on targeted research aimed at the national good, science is just not like that and that basic curiosity-driven research can still turn up important and commercial findings that simply could not have been anticipated. Thus I have taken the liberty of writing this article in the first person, as it is a personal story albeit about a neglected area of microbiological research.

The Rubbo Legacy

Given that I started my career in microbiology as a student in the Department of Microbiology at the University of Melbourne under the leadership of Syd Rubbo, it is appropriate to include some words on this remarkably influential microbiologist in whose honour the oration is dedicated.

Sydney Dattilo Rubbo graduated in a combined science-pharmacy degree at the University of Sydney. He did a Dip Bact and PhD in London before taking up a senior lectureship in 1937 in the Department of Microbiology at the University of Melbourne. He was a dashing figure and, being the son of two

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artists, it was not surprising that he had artistic flare. His father, well-known Sydney artist Datillo Rubbo, was the first to encourage modernism amongst Australia's young artists including Grace Cossington Smith, Donald Friend and Lloyd Rees. Like his father, Syd Rubbo was very active in the Dante Alighieri Society.

While teaching microbiology at the 'Bugs School', Rubbo completed a medical course and became Professor of Bacteriology in 1945 at the very young age of 33. His specialist interest was sterilisation and disinfection and, together with Joan Gardner, he wrote the definitive reference/textbook on that topic.

One of Syd Rubbo's great strengths was that he recognised talent. I was fortunate to be taught by the following academic staff who since that time have had a major impact on microbiology and science in general throughout Australia: Nancy Millis, Jim Pittard, Bruce Holloway, Doris Graham, Frank Gibson, Ian Holmes, David White, David Gray, Geoff Cooper, Rose Mushin and Joan Gardner.

The wonder of that first discovery

The advantage of being in the Bugs School with Syd and his staff was that the Department had a great reputation and this meant they could arrange some great postdoctoral positions. I was lucky enough to have two years at the Rockefeller University in New York with one of the grandfathers of medical microbiology, Rene Dubos, and his colleague Russell Schaedler. These were two of the first scientists to

realise the benefit of the normal intestinal flora on the host and they determined to undertake the first systematic study of its composition. For convenience they choose the mouse as the first animal to study¹.

If one looks at a specimen of mouse stools under phase microscopy, it is impressive that all that can be seen are bacteria. No debris in faeces, just living bacteria; we now know there are 10¹¹ bacteria per gram and at least 200 different species. This is the classic microbial ecosystem.

The 'simple' task allocated to me for my postdoctoral project was to grow the dominant bacteria seen under microscopy. They were easily recognised by their pointed (fusiform) shape. Schaedler and I reasoned that these bacteria might be oxygen sensitive because of the highly reduced anaerobic environment that the gut bacteria were known to live in. Thus, I constructed one of the world's first anaerobic glove boxes out of a rigid plastic safe handling cabinet. To remove the oxygen, an atmospheric research balloon was blown up and down a number of times replacing the air with nitrogen that had been passed over a primitive column of heated copper filings to remove the oxygen. Final traces of oxygen were removed by letting mung beans germinate in the cabinet. This method worked, although the smell in the cabinet when it was opened was putrid!

I will never forget the excitement as I looked at the culture plates that I had inoculated in the anaerobic atmosphere of the glove box with fresh mouse faeces under a plate microscope and saw a number of wonderful purple speckled colonies and realised I was the first in the world to ever have seen them. This was Science! I have grown new bugs for much of the rest of my life and the same thrill of



being first to make a scientific observation, no matter how big or small, is still the same.

We published our mouse fusiform results in *Nature* which was very exciting as a first paper for a young Aussie². The original figure from this paper is shown as Figure 1. Remember this picture, as it will return 30 years later in this story.

The *Nature* paper helped me get a job as lecturer in medical microbiology at the University of New South Wales in 1968 where the newly appointed Professor Geoff Cooper, my PhD supervisor, had set up a new department to teach medical students microbiology. I continued to work on the normal flora of the mouse gut but my focus changed. I convinced the NHMRC that I was studying these bacteria as understanding the normal would help us understand disease. In reality I just loved looking at new bugs. I now considered myself a microbial ecologist³.

Mucus: a neglected niche

The ecological niche that everyone had neglected was the mucus that lined the intestinal tract to protect the mucosa and to act as a lubricant for digesting foodstuff as it passed along the tract⁴. The bacteria that have adapted to the intestinal mucus are very different from those in the lumen. In fact you can't even see them in faeces. Remarkably, very different populations of spiral-shaped bacteria inhabit the mucus in the different areas of the mouse gut. Figure 2 shows the different areas of the mouse lower bowel and shows the different bacterial morphologies that live there and how the organisms pack the mucus! Figure 3 shows these bacteria more clearly.

The intestinal tract must have been one of the original complex microbial ecosystems with spiral bacteria adapting to the very different viscous mucus environment. All animal species have these bacteria inhabiting their intestinal mucus; we have seen them in dogs, pigs, and even kangaroos. Fixed specimens although giving the impressive pictures seen in Figure 3 gives you no idea as to what this mucus niche really looks like. It

is only if you see these bacteria live that you get a true sense of why I became fascinated by them.

If you ever want to get a student interested in bugs in the gut, take one mouse, remove the large bowel, slit it open, wash vigorously and then scrape off the mucus, put on a slide, place a cover slip on top, press hard, and let them look under phase microscopy. A whole new world will be opened up to them. It certainly does not look like you expect. Short digital videos of these bacteria moving in mucus are available for any interested reader on a CD Rom and may be obtained by contacting the author.

The next scientific challenge was to try and grow these spiral bacteria. Thus with Jani O'Rourke, who has worked with me at UNSW for 25 years, we started growing^{5,6}. This resulted in the isolation of many new species and even a new genus of bacteria.

Figure 1. My first discovery: culture of fusiform bacteria from the mouse intestinal tract. a-c Fusiforms d Spiral-shaped bacteria.

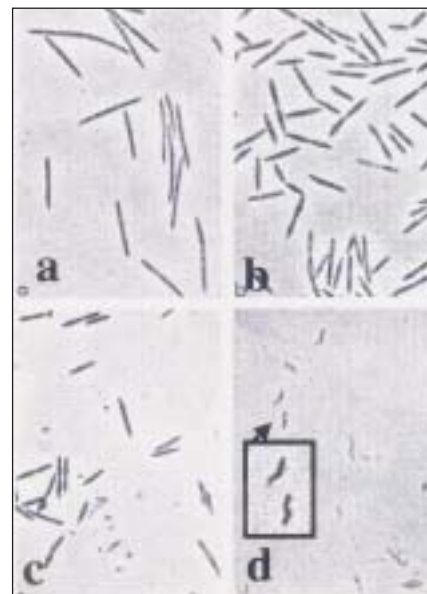
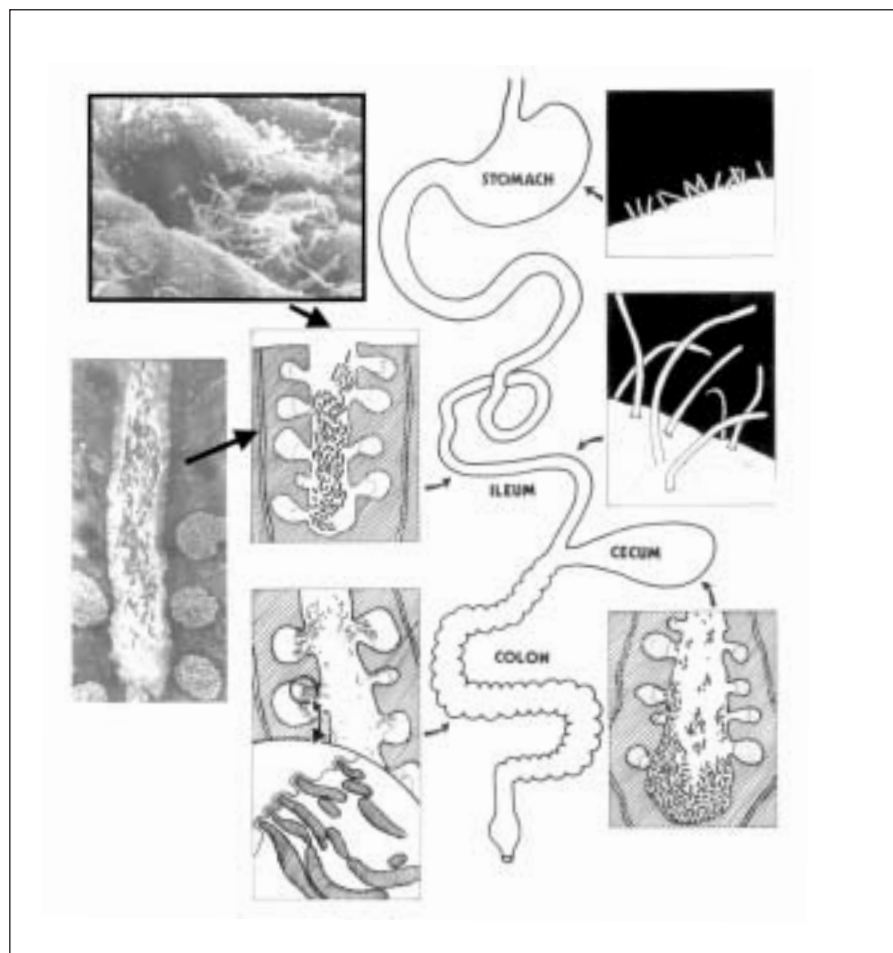


Figure 2. The mucus-associated bacterial flora of the normal mouse intestine. Note the different populations inhabiting the different ecological niches along the tract. But they are all spiral shaped⁴.



This is where 'Stubby' the organism mentioned in the title comes in. It was the first of the spiral/helical shaped bacteria we grew from the mouse intestine and remains our favourite^{7,8}. This is due to the quite beautiful and distinctive morphology.

Life then changed because a spiral-shaped gut pathogen came on the scene⁹. The bacterium was *Campylobacter jejuni*, the major cause of bacterial-induced

diarrhoea in a country like Australia. Michael Phillips, an honours, later to be graduate student conducted an experiment that showed why a spiral shape gave these bacteria an advantage in mucus. It was because it allowed the organism to cope with viscosity. The motility of *C. jejuni* and a number of other bacteria was measured in differing viscosities of methylcellulose. At a viscosity, around 10 centi poises, campylobacters are still busily swimming

around while other motile bugs are slowed down. We also put *C. jejuni* into mice and showed it colonised the mucus filled crypts beautifully confirming our hypothesis as to the benefits of a spiral shape.

This began a great scientific debate with those who believe adhesion is essential for colonisation of the gut surface. Anyone who has seen the movement of these bacteria under phase microscopy scurrying up and down the mucus strands would not support the adhesion only lobby.

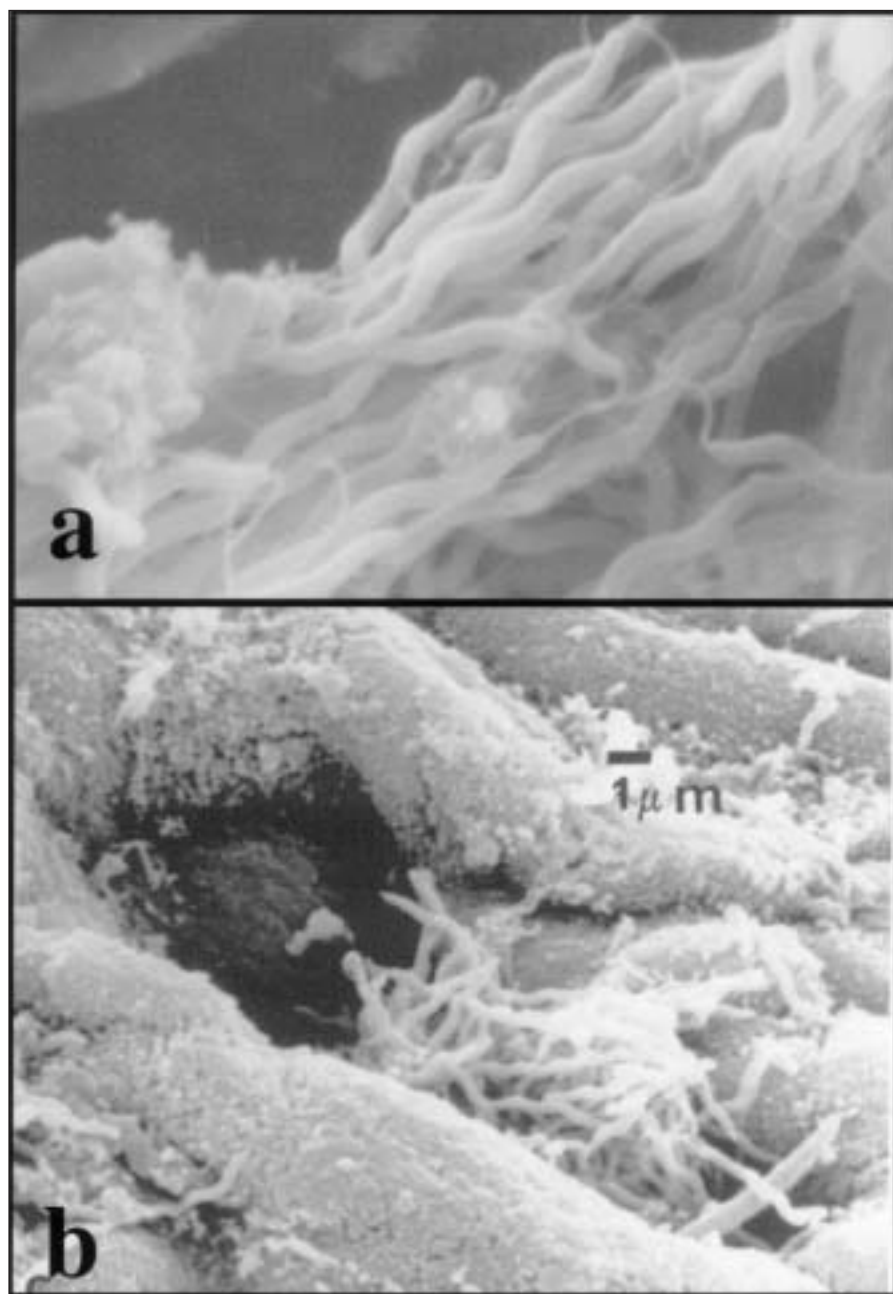
A change in direction: The birth of *H. pylori*

A much more dramatic life-changing event then happened in Perth is 1982. *Helicobacter pylori* was born^{10,11}. Due to the perseverance of a pathologist Robin Warren and the young intern Barry Marshall, a spiral-shaped bacterium was grown from the stomach mucosa and identified as *H. pylori*. The story of this discovery is told elsewhere and Barry Marshall, now a Macfarlane Burnet Professor, was the Rubbo Orator two years ago¹². *H. pylori* has now conclusively been shown to cause most duodenal ulcers, a majority of gastric ulcers and gastric cancer.

We were interested in this organism very early on because here was yet another spiral-shaped bacterium inhabiting mucus. What was especially interesting was that it was in gastric mucus which was always thought to be sterile. It was a great pleasure to work on this discovery of Marshall and Warren's that lead to such a paradigm shift in medicine and the management of gastroduodenal disease. Suddenly, I had to learn a lot about stomachs and work with the gastroenterologists.

My advice to any young microbiologist working in any microbial disease is that you become a complete expert in everything there is to know about the disease and the anatomical location in which pathology appears. This has turned out to be of enormous benefit to my career.

Figure 3. Lower bowel spiral-shaped bacteria (*beliobacters*) in their natural habitat of intestinal mucus
a within a mucus strand
b the entrance to an intestinal crypt.



Stuart Hazell joined me to do a PhD on this spiral bacterium recently discovered in Perth. He was extremely successful and indeed at the last Sydney ASM meeting gave the Fenner Lecture. Together we kept the ecological theme to this research. Hazell showed that the Campylobacter Like Organism (CLO), as *H. pylori* was then called, preferentially colonised the mucus near intracellular junctions and even more convincingly showed how the bacterium coped with a viscous environment. He published the first paper in the US on the pathogenicity of *H. pylori*¹³.

Jani O'Rourke and I kept growing spiral bugs, some of which allowed us to develop mouse models of helicobacter infection, that made possible in depth studies into the ecology of gastric infection⁶.

It is time to introduce *Helicobacter felis*¹⁴; a bacterium initially grown from the cat

stomach but which, to our delight, colonised the mouse stomach very well and over time developed a pathology¹⁵. Again the organism had a striking and, to us, aesthetically pleasing morphology (Figure 4). Movies of this organism moving in the gastric mucus of our animal model demonstrate most convincingly exactly how the helical shape allows helicobacters to cope with a viscous environment. Again these videos can be viewed on the CD Rom.

The mouse models

Initially, we had to use *H. felis* as the mouse model of *H. pylori* infection because human isolates would not colonise the mouse stomach. After trying with hundreds of isolates from patients, we were successful in isolating a human strain of *H. pylori* that did colonise well¹⁶.

All my best ideas come in the shower, and one of the best was to call the mouse

colonising isolate the Sydney Strain, SS1. Another piece of advice to new players is that if you discover a new bug, the name counts. Another good decision I made was not to be possessive. I made the Sydney strain available to all who wanted it, no strings attached. SS1 is now used all over the world. Great for citations!

As an ecologist an important feature of SS1 is that its pattern of colonisation mirrored the human pattern (Figure 5). The colonisation level is very good and persists for the life of the animal. My last graduate student Lucy Thompson has just isolated an even better coloniser with another shower-inspired name: the Olympic strain-Sydney 2000 or SS2000!

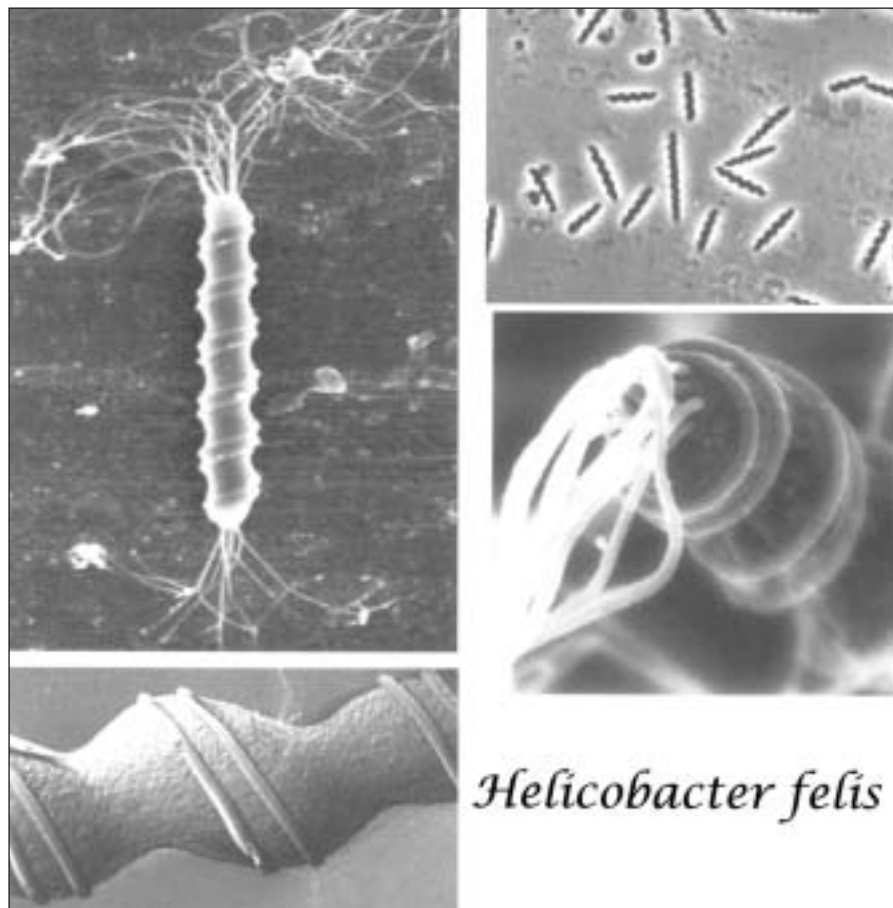
The mouse models have allowed us to do many things for which the group has become well known. For example, we proved the principle of immunisation against *H. pylori* is possible and even showed you could cure infection with immunisation^{17,18}. This was the result of a very productive project with CSL Ltd in Melbourne where Chris Doidge and myself were successful in gaining the American patent for therapeutic immunisation against *H. pylori*. Another Rubboite, Trevor Trust, now Director of AstraZeneca Boston, has been involved in this project.

Another important result with the animal models has been the finding that *H. felis* infection causes lymphomas in the mouse stomach just as *H. pylori* does in the human stomach^{19,20}. This has great potential and in cooperation with Stan Falkow, yet another Rubbo Orator we are using DNA microarrays to explore the factors influencing tumour induction.

The ecology of *H. pylori* infection: solution of a great enigma

This has been great for getting grants but, still the microbial ecologist at heart, I think what has excited me most has been to explain a great mystery of gastroduodenal disease by understanding the subtlety of colonisation of the gastric mucosa by the helicobacters.

Figure 4. *Helicobacter felis*, the bacterium that produced our first mouse models of *Helicobacter* infection showing aspects of its remarkable, but beautiful, morphology.



A great enigma in gastric diseases is that there are two different pathways of disease with very different outcomes and it is clear that both these pathways are definitely a consequence of *H. pylori* infection.

Patients infected with *H. pylori* all have inflammation of the stomach; this is called gastritis. In some patients, the gastritis progresses to a duodenal ulcer which occurs just outside the stomach opening into the duodenum while in others the infection results in an ulcer in the stomach proper that is called a gastric ulcer. If conditions, e.g. a high salt diet, are present, the gastric ulcer patient may progress to gastric cancer.

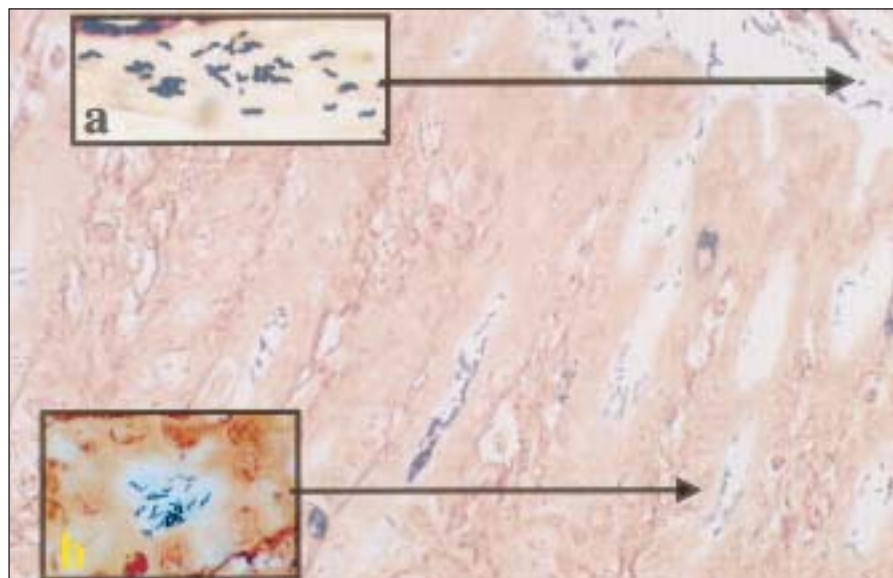
Another feature of these patterns of disease is that the duodenal ulcer pathway is more common in countries with higher standards of living while the gastric ulcer/gastric cancer scenario predominates in societies with a low socio-economic status. What is not understood is the conditions that result in these different pathways.

I am convinced we have the answer but it is very hard for gastroenterologists to understand this concept^{21, 23}. I have to also say this has been hard for some NHMRC reviewers to understand! Below is an attempt to explain this hypothesis

Figure 5. The Sydney strain of *Helicobacter pylori* colonising the antrum of the stomach of a mouse.

Insert a: in the gastric mucus.

Insert b: deep in a gastric crypt.



allowing for the reader who may have little knowledge of gastroenterology but a good understanding of ecological principles.

Firstly we need to understand how *H. pylori* can colonise the harsh gastric environment. The most distinctive feature of the stomach is that it is a bag full of acid. Due to this acid-rich environment, microbiologists and gastroenterologists alike always considered this to be 'sterile'. This is why gastroenterologists never wore gloves when they endoscoped patients²⁴.

Most bacteria do indeed die once they have entered the stomach. However, *H. pylori* has evolved to survive in this environment by acquisition of the enzyme urease which breaks down endogenous urea to produce alkaline ammonia which can neutralise the acid²⁵. The simplest test to diagnose infection devised by Barry Marshall is the CLO test²⁶. The biopsy is put on urea agar with indicator. If urease is present the ammonia turns the indicator red. Urease negative mutants of *H. pylori* will not colonise the animal models.

Next we need to learn more about the stomach. The organ is divided into two main sections. Firstly, the non-acid secreting antrum, the area closest to the

entry from the stomach into the duodenum. Secondly, the acid-secreting body of the stomach, a tissue rich in acid-producing parietal cells. Ulcers occur in the duodenal bulb or around the transitional zone between the antrum and the body.

Our animal models have allowed us to show how the actual area of colonisation by helicobacters is dependent on the local acid conditions. If one suppresses the acid output in the stomach, then the area of colonisation moves to a different area of the stomach. This was first shown by a then graduate student Stephen Danon²⁷. In mice infected with *H. felis*, the organism normally colonises the antrum as does *H. pylori* in the human. When acid suppression is induced in the mouse by administration of the proton pump inhibitor, omeprazole, the bacteria move from the antrum to the body of the stomach.

The organism needs some acid in the local environment but not too much. Thus in the acid-suppressed animals, the lack of acid in the environment makes the antrum too alkaline and less hospitable for the helicobacters and the numbers decline. In contrast, there is now less acid in the body of the stomach but enough for the organisms to thrive. Indeed some bacteria are seen to travel deep into the crypts even down into the parietal cells themselves. This could never happen if the cell was producing acid.

The subtlety of the impact of the local acid environment was shown in parallel experiments in which we used ranitidine, an acid inhibitor that is not as effective as the proton pump inhibitors. In the ranitidine-treated animals there were certainly more bacteria in the body than in the normal control infected mice. However, the bacteria only go part way down the crypts in comparison. This was the first evidence of just how subtle the pH effect is.

It is now known that reduced acid has exactly the same effect on colonisation in *H. pylori*-infected humans. An early treatment of ulcers was selective vagotomy where the nerve signalling



production of acid was surgically cut. The gastritis was noted to move from the antrum to the body. Likewise the number of *H. pylori* in the antrum declines.

The basic message from our animal experiments and the histological changes observed in patients on acid suppression is that if you change the acid output of the stomach and the local environment changes, the bacteria will thrive in different parts of the stomach. It is this phenomenon that results in differences in disease.

Recent advances on the biology of the acid-protective urease enzyme system of *H. pylori* gives us an understanding of how local acid changes result in different behaviour of the organism²⁸. Remember how bacteria generate their energy. ATP-generating enzymes are inserted in the inner periplasmic space where there is a potential difference across the membrane. ATP is generated as hydrogen ions, protons, pass down the enzyme complex. An increase in acid in the environment of the organism means an overwhelming increase in protons, the charge differentiation cannot be maintained, no ATP is generated and the bacterium dies.

Originally, it was thought that the urease enzyme was stuck on the outside of the cell of *H. pylori* and simply produced a cloud of neutralising ammonia. In reality, the mechanism is much more subtle than this as you would expect with a highly-evolved ecological adaptation. The work of the brilliant physiologist George Sachs and his team at UCLA have worked out the mechanism²⁹. Sachs was the scientist who discovered the proton pump of the parietal cell and explained how omeprazole worked.

The urease gene cluster has a critical gene, *ureI*, that codes for a transmembrane protein that is in fact a urea transporter. It opens in the presence of acid via a protein rearrangement in the transmembrane domain, i.e. urea moves into the cell when it is needed. The urease binds to part of the *ureI* protein so it is located exactly where the enzyme action is

needed. The ammonia is produced and the periplasmic space pH is maintained at a level that maintains the proton motive force. Very clever.

However, this adaptation to acid resistance is finally balanced and obviously there is a fairly narrow range over which the urease protects and allows optimum growth of the bacterium. *H. pylori* does not like too much acid but also it does not like too little. In the stomach it finds its optimum pH for growth.

Another factor in this complex story is to appreciate that it is the gastritis or inflammation of the stomach mucosa that is the essential precursor lesion for symptomatic disease.

Two aspects of gastritis impact on the disease that it induces. Firstly, the intensity of the inflammation. This is dependent on a pathogenicity island (PAI) possessed by *H. pylori* that codes for a type IV secretion system. Following contact with the mucosal cells, the PAI coded syringe injects a gene product, Cag A protein, into the cell and sets off a cascade of intercellular reactions resulting in the production of the pro-inflammatory cytokine IL8^{30, 31}. Inflammatory cells are recruited to the site of infection and the gastritis is more severe. It is interesting that the *H. pylori* codes for a system that brings in more supposedly defensive cells. Here is another aspect of adaptation and evolution, but that is another story. Secondly, the location of the gastritis is critical. This is the aspect we have been interested i.e. the location of the gastritis and its impact on disease.

Let's put all this together. *H. pylori* in the acid-secreting body mucosa is restricted to the mucus layer. The acid comes up as protons are secreted by the parietal cells at the bottom of the crypt. The local environment is too acid and so the bacteria cannot swim down the pit and are not as close to tissue. There is little contact with the tissue, IL8 is not induced and thus inflammation is minimal. In the antrum where the acid is not produced in the crypts the acid comes from the lumen. The bacteria can and do swim

down the crypts, make more close contact with the tissue and the inflammation is greater. In the body when the parietal cells stop making acid, the local environment more closely resembles the antrum. The *H. pylori* can now get closer to the body tissue and so gastritis is induced.

So these are the differences that result in the different patterns of disease. The duodenal ulcer patient makes more acid. Antral gastritis predominates. The inflammation is not across the transitional zone where gastric ulcers occur and so duodenal ulcers are caused because of the excessive acid coming out of the stomach and impacting on the duodenal mucosa. The bacterium actually colonises pieces of gastric-like tissue in the duodenum. Gastric ulcer patients make less acid, thus the bacterium survives throughout the stomach. Inflammation across the transitional zone results in a gastric ulcer.

The reason for the location of ulcers at the transitional zone is explained by this ecological dependence of *H. pylori* colonisation on acid. At the junction of the non-acid secreting antrum and the acid-secreting body mucosa there is a transitional zone where the pits have more and more parietal cells. Thus there is a gradation of local acid. *H. pylori* finds the optimum pH to cause most damage. The damage reaches a threshold where the mucosa is no longer protected from the gastric acid and an ulcer forms³².

So now the enigma is explained. Different populations make different levels of acid. For example, wealthy people in more developed countries make more acid. Hence they have duodenal ulcer disease as the manifestation of *H. pylori* infection. In the developing world, less acid is made and so the ecological consequence of *H. pylori* infection is the gastric ulcer/gastric cancer pathway²³.

How could it be that acid output has increased as a society develops? One of the most effective anti-secretory molecules is the cytokine IL1. IL1 is increased with nearly all infectious



diseases thus as microbial infection is rife in the developing world more infection means more IL1, which means less acid. As a society has less and less infectious disease due to immunisation and improvements in hygiene, IL1 levels go down and acid output goes up. There are also probably dietary impacts on acid secretion. Also it is known that acid output correlates with height, which will increase in well-nourished populations.

This also explains another enigma. That is, that the pattern of disease has changed as countries develop. The change occurred in the 1920s in the US and Australia and is happening in Japan now. A long time ago ulcers were rare and mainly gastric. The gastric ulcer and cancer were dominant. Then duodenal ulcers came up and gastric ulcers went down. Now the populations are losing their *H. pylori* and so duodenal ulcers are disappearing. Reflux disease begins to increase.

The very best papers written are those that confirm your hypotheses! Imagine my delight when a wonderful paper was published in the journal *Gut* showing that in young Japanese, acid output has gone up over the past 20 years³³. I rest my case!!

One of the frustrations of science is that even when the evidence to confirm your hypothesis is overwhelming, it takes so long to get the ideas accepted. Look at what happened to Barry Marshall and *H. pylori*. Thus, despite the concepts above, there are still many many scientists using sophisticated genetic techniques to look for the elusive ulcer causing strain of *H. pylori* as compared to a cancer causing *H. pylori* strain. If one understands what I have described above, this is a nonsensical goal and these strains could not possibly exist.

A key to success for the young microbiologist: learn the biology!

This is probably my most important lesson to the young researchers who may be reading this article and which I foreshadowed above. Before you start doing your clever mutations and

microarray experiments, learn the biology! I put a major effort into learning the gastroenterology of the stomach and understanding its biology. If you don't put this effort in to think more broadly than your own narrow discipline, then you will ask the wrong questions and waste a lot of time.

Thus, at the moment there are a large number of studies looking at gene expression of *H. pylori* in acid conditions and drawing huge conclusions. Based on the biology above much of this is probably artefactual. There have been tons of artefactual papers on *H. pylori* written simply because people did not understand the biology. Rule one. Learn everything about your disease before you do your first experiment.

How I could have been famous but blew it!

Now for some reflections on the serendipity of science and what makes a great discovery. Last year was the 20th birthday of the birth of *H. pylori* in an incubator in Perth over an Easter long weekend. The fourth Western Pacific Helicobacter Conference was held in Perth as a celebratory meeting. To coincide with this meeting, Barry Marshall published a fascinating book entitled *Helicobacter pioneers: firsthand accounts from the scientists who discovered helicobacters, 1892-1982*³⁴.

You are strongly encouraged to read this book and gain some insights into the ups and downs of science. The importance of the Warren and Marshall discoveries is immense. Both these Australians have won many awards and a Nobel prize is a definite possibility. It is amazing how many people now claim, after the event, that it really was them that discovered it.

Many of the stories are told in this book. Some serious, others tongue in cheek. I hope my chapter is taken as the latter. We called it *We grew the first helicobacters and did not even know it*³⁵. All true. Here are three situations where if I had known better I could have been famous. Yet we blew it all. Firstly, Stubby was a *Helicobacter*!

Apparently Barry Marshall used some of the growth conditions we used to grow Stubby to grow the early isolates of *H. pylori*. He wrote to my then graduate student Michael Phillips in November 1982 (the year of the discovery!) asking if we thought his Perth bug was like Stubby. We said no! And Michael replied thus:

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, as you will be aware, is 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.

We blew it and so lost our first chance to be famous! As it turns out we had blown it way before *H. pylori* was identified. We submitted a paper in 1983 on the identity of Stubby to the *International Journal of Systematic Bacteriology* claiming it belonged to a new genus and proposing the name *Mucospirillum ileocryptum*. The editors rejected the manuscript because we had only described one isolate of the bacterium St1 Stubby 1 ATCC49282. If we had only sent in two more cultures they would have accepted our proposal and today we would have been talking about *Mucospirillum pylori* instead of *Helicobacter pylori*. Strike two! Our story of woe and lost fame gets worse.

Remember I said we would come back to Figure 1. If you look closely, the fourth panel in the figure shows that bacteria we had grown in large numbers were spiral! I was so focussed on the fusiforms that I had been charged with growing that I was not the slightest bit interested in these spiral organisms. Indeed, I did not even remember them. Hence the near heart attack only 3 years ago where I was



looking through my early papers and saw them. Based on what we know now this was definitely a helicobacter and we had grown it in 1968!. Great discoveries need a prepared and open mind, which was what Warren and Marshall had.

As I move on to my new life on the dark side of university administration, I bequeath a few PhD topics for those who simply like to understand. Remember you never know when the results will be useful. This is the joy of basic science.

The following two topics are 'lay down misere' PhDs. Firstly, we still don't know all about how these helicobacters colonise the gastric niche. In an experiment where we dually infected mice with the Sydney strain of *H. pylori* SS1 and *H. felis*, *H. felis* wins hands down and excludes the human organism. Why? More amazingly, if you look at conventional mice infected for a long time with *H. felis*, Stubby starts to appear in the stomach having come up from the intestine. In such huge numbers it completely excludes *H. felis*. I think the *H. felis* infection suppresses acid and that Stubby, *H. muridarum*, can now colonise the stomach. But how does it happen?

My next PhD offering is to understand and compare the motility of the helicobacters. It is obviously important and helps the colonisation of mucus but the processes are very different. Those interested can request the CD Rom of videos and they will see a remarkable section where a single helicobacter works hard for 40 minutes to extricate itself from a tight situation. Why is the organism so determined where does it get the energy? Given there are now microarrays with the whole genome on them there are some wonderful experiments to be done. To prospective supervisors that want to know more about these projects let's talk!

Life goes around: back to the bowel

Life has a habit of coming full circle. Before I left the wonderful world of helicobacteriology, I had moved away from the stomach and gone back to all

those spirals I had seen in the lower bowel of mice and had worked on through the 1970s. As you may have guessed by now, they are all helicobacters. The mouse gut is colonised by all of the following: *Helicobacter muridarum*, *Helicobacter trogonum*, *Helicobacter rodentium*, *Helicobacter hepaticus*, *Helicobacter bilis*, *Helicobacter typhlonius*, *Helicobacter muricula*, *Helicobacter ganmani*. All animals have these bacteria in their lower bowels and my great friend and collaborator Jim Fox at MIT in Boston finds almost a new helicobacter a month³⁶.

But the big discovery of Fox, and confirmed by many others, is that these helicobacters cause inflammatory bowel disease (IBD) in immunocompromised mice. IBD, including Crohn's disease and ulcerative colitis, is the next great frontier of gastroenterology, as we still do not understand the cause and these are diseases that are increasing in the developed world³⁷.

One of the last experiments I was involved with was done by Stephen Danon. Using the methods we had developed to prevent gastric infection by helicobacter species we managed to protect mice from helicobacter-induced IBD by immunisation. An exciting finale and one being continued by Associate Professor Hazel Mitchell at UNSW who has some very exciting projects for APA students who would like to take their scholarships with them to UNSW!

A lifetime of science is also a lifetime of wonderful honours and PhD students and colleagues. I would like to pay a special thanks to: Jani O'Rourke, Stuart Hazell, Hazel Mitchell, Richard Ferrero, Stephen Danon, Minhu Chen, Bronwyn Robertson, Kylie Fischer, Fiona Buck, Fiona Radcliffe, Chris Doidge, Cora DeUngria, Tassia Kolesnikow, Takashi Sakagami, Angelina Enno, Jenny Vella, Phil Sutton, John Wilson, Sander van Zanten, Lucy Thompson, Michael Phillips and Elisabeth Dick.

Now to a new life

And so after more than 30 wonderful years with mucus, Stubby, and much more, I have moved on to a new career in university administration with the specific goal of improving the student experience in large research intensive universities. I like to think my interest in teaching started with Syd Rubbo who was an inspirational teacher and he recruited great teachers. In academia, teaching is as important as research. We must all strive to teach well. To me the answer, illustrated by Rubbo so many years ago, is to create a learning experience that is student-centred, interesting, challenging, relevant, practical and fun. Do this and you will not fail.

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Web Review



*Contributions listing interesting sites or short reviews are welcome.
Please send to <pbishop@nursing.usyd.edu.au>.*

Probiotics

http://www.fao.org/es/esn/food/foodandfood_probio_en.stm

FAO/WHO Expert Consultation. Evaluation of health and nutritional properties of powder milk with live lactic acid bacteria and guidelines for the evaluation of probiotics in foods.

Public health

The September-October 2002 edition of the NSW Public Health Bulletin is now available from the NSW Health website at:

<http://www.health.nsw.gov.au/public-health/phb/phb.html>

* FactSheet : Shigellosis

http://www.health.nsw.gov.au/public-health/phb/HTML2002/sept_Oct02html/factsheetshigellosis.html

Infectious diseases

<http://www.eurosurveillance.org>

The Eurosurveillance Project is funded by the European Commission with the aim to promote the diffusion and exchange of information on communicable diseases.

It includes a monthly publication, *Eurosurveillance*, and a weekly bulletin *Eurosurveillance Weekly*.

<<http://www.promedmail.org>>

<<http://www.isid.org>>

ProMED-mail is a program of the International Society for Infectious Diseases.

Two recent items of interest:

- Glycopeptide Intermediate Resistant *Staphylococcus aureus* (GISA) death in Scotland.
- *Staphylococcus aureus* (MRSA), community acquired: California, USA.