Gastric spirochaetes: 100 years of discovery before and after Kobayashi

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Abstract. The discovery of *Helicobacter pylori*, by Warren and Marshall in 1982, was preceded by nearly 100 years of inconspicuous publications relating to spiral bacteria, achlorhydria, gastritis, gastric urease, and antimicrobial therapy for ulcers. Japanese investigators, notably Kasai and Kobayashi, should be acknowledged for their pioneering work showing that spiral bacteria could infect many animals, could cause haemorrhagic erosions, and would be effectively cured with various antimicrobials.

Key words: Helicobacter, gastritis, arsenicals, bismuth, urease

It must have been very difficult for gastroenterologists and physicians to accept the proposal of two unknown investigators, Marshall and Warren, who stated in 1983 that spiral bacteria in the human stomach were likely to be the cause of peptic ulcers.[1] Not only did these two have no track record, but also they seemed to be supremely confident that their hypothesis was correct.

This is where the relevance of the history of *Helicobacter pylori* becomes evident. The key to Marshall and Warren's confidence was the historical literature on gastric bacteria, including the reports of Kasai and Kobayashi.

In their review of the medical history of spiral bacteria, Marshall and Warren detected four clues to *H. pylori*. These were:

1. Spiral bacteria in the stomach.
2. Epidemic hypochlorhydria (the acute infection with *H. pylori*, usually seen in children).
3. Gastric urease (by which *H. pylori* releases ammonia and survives in the acid-secreting stomach).
4. Descriptions of antibiotic therapy for gastric diseases particularly peptic ulcer.

Spiral Gastric Bacteria

In 1892, the Italian anatomist Bizzozero described gram-negative spirochetes within parietal cells of the dog stomach. Bizzozero postulated that either these bacteria must have been acid tolerant, or, alternatively, they could turn off acid secretion.[2] We now know that both of these facts are true of *H. pylori*, as it exhibits a degree of acid tolerance, as well as being able to induce achlorhydria.

Table 1 Sterilization Effects of Acid Salvarsan Solution on the Spirochaete *in vivo*

Mouse number	Dilution	Spirochaete
1,2,3	1:100	–
4,5,6	1:200	–
7,8,9	1:300	–
10,11	1:500	+
12,13	1:1000	+
14,15	1:2000	+
16,17,18,19	Control	+

A decade later, Kasai and Kobayashi at the Kitasato Institute studied the spiral bacteria in several mammals.[3] As shown in Table 1, they then used Arsaminol (the Japanese equivalent of Salvarsan – an arsenical) to eradicate the bacteria from mice stomachs. It is interesting to note that they always used controls in their experiments, and that they documented that the drug was effective at 1:300 dilution (3 mg/ml).

As shown in Table 2, Kobayashi found that dogs, cats and monkeys all had the organism, but that other species – rabbits, mice, voles and guinea pigs – did not[3]. These results have certainly been verified in modern studies. Many years passed before mice were successfully colonised by *H. pylori*.[4] However, there is now a mouse model, although it is less than ideal since the mouse stomach is so different from the human stomach.

Lytic Actions of Saponin, Sodium Taurocholate and Bile on the Spirochaete

In the days before antibiotic therapy, Kobayashi was interested in natural compounds that could inhibit the

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Table 2 Distribution of Spirochaetes in Animals

Species	N	No. infected %	Comment
Dogs	49	43 (88)	Absent from young puppies 2–3 weeks post weaning
Cats	13	8 (62)	Absent from some young cats
Monkeys	13	13 (100)	
Wild rats	38	1 (3)	
White rats	10	0	
Rabbits	20	0	Inoculated with virus fixe of rabies
Lab mice	15	0	
Field voles	15	0	
Guinea pigs	15	0	

spiral bacteria. Saponin, a plant glycoside extract, damaged the bacteria with changes visible at 1–3 hours (Table 3). Taurocholate, a component of bile was also effective, as was bile itself, with both compounds completely lysing the organism by 1 hour. As with all their experiments, Kasai and Kobayashi included data from a control animal.

Result Obtained by the Inoculation of the Virus Fixe Into the Canine-strain Bearing Rabbit

It now appears that, as far as could be done at the time, Kasai and Kobayashi fulfilled Koch's postulates for gastric spirochaetes and erosive gastritis. A rabbit-adapted spirochaete strain could be shown to have no effect when spirochaetes alone were instilled into the stomach (Table 4). However, usually the infection would not establish, but in the presence of a rabies infection, rabbit-adapted strains caused heavy colonisation. Following this, marked haemorrhagic changes were seen in the gastric mucosa. One can only speculate as to why rabies potentiated the spirochaetes' pathogenicity. Probably gastric hypersecretion occurred as part of the sympathetic overdrive seen in CNS conditions

Table 4 Result Obtained by the Inoculation of the Virus Fixe Into the Canine-strain Bearing Rabbit

Rabbit no.	Generation	Interval between transmission and inoculation	Spirochaetes	Gastric mucosa
1	1	0*	++	−
2	1	0	−	−
3	1	0	−	−
4	1	1	−	−
5	2	3	+++	+
6	2	3	−++	++
7	3	4	−++	+++
8	3	4	−++	+++
9	4	3	−++	+++
10	4	5	−++	+−
11	5	2	++	−
12	5	***	−	−
13	6	6	*	+++

and this favoured the growth of the bacteria in some way. One wonders whether Kobayashi was really aware of similar gastric lesions that occurred in humans. Of course he had no access to endoscopy, but it is surprising that he did not discuss human disease parallels in his paper.

In summary, Kasai and Kobayashi observed that the spiral organism lives in the fundic glands of the stomach. It is present in cats, dogs, and monkeys, but not in rabbits, mice, guinea pigs or voles. It is soluble in saponin and taurocholate and bile. It is susceptible to salvarsan in the stomach and parenterally. It is easily transmitted to rats and mice, but not easily to rabbits or guinea pigs. After infection with the spirochaete, a rabbit infected with virus fixe of rabies would develop gastric haemorrhagic erosions. Quite clearly, Kobayashi considered the idea of an infectious agent causing stomach disease. He was also the first person to consider treating gastric disease with arsenic as a specific antimicrobial.

Table 3 Lytic Actions of Saponin, Sodium Taurocholate and Bile on the Spirochaete

Chemicals	15 Mins	30 Mins	1 Hr	2 Hrs	3 Hrs
Saponin	Spirochaetes became swollen and stained unfavourably. Some in process of degradation.	Staining very faint, and spirals irregular and indistinct	Almost complete degradation	Almost complete degradation	A very few organisms remaining
Sodium Taurocholate	A few degenerated organisms remaining	Complete degradation			
Bile	A few spirochaetes in the process of degradation	Degenerated forms unlike the spirochaete seen very rarely	Complete degradation		
Control	No change	Degenerated forms unlike the spirochaete seen very rarely	Complete degradation	Almost complete degradation	A very few degenerated organisms remaining

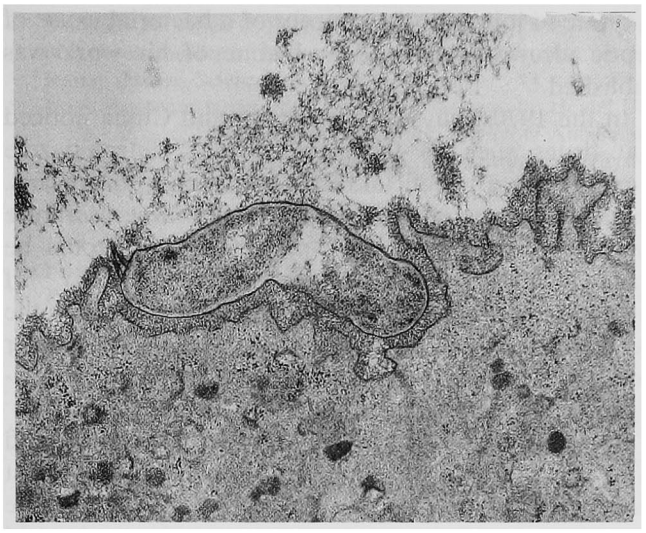

Fig. 1 *Helicobacter pylori* illustrated by S. Ito in 1967[5]. The biopsy specimen was taken from his own stomach.

A second Japanese investigator appeared in the *Helicobacter pylori* story in 1967. Susumu Ito, a well-known anatomist from Boston, had illustrated a perfectly formed *Helicobacter pylori* organism within the canaliculus of a parietal cell (Fig. 1).[5] In fact, this organism was taken from Ito's own stomach. He swallowed a biopsy tube and removed a gastric suction biopsy in which he discovered his own *Helicobacter* species. He did not think it was important because he had seen similar bacteria in cats. Ito, a Japanese-born American, carried *Helicobacter pylori*, as did most Americans and Japanese born before 1930.

Professor Ito, who is now emeritus Professor of Anatomy at the Harvard Medical School, is also famous for being in the highly decorated all Japanese division of the United States Army, which fought in Europe in 1944. In a last ditch effort, the 442nd – known for its toughness – had been sent to tackle the Nazis. Despite heavy artillery barrages and hidden machine gun nests, the 442nd managed to carve through Nazi lines. But the four-day battle was so deadly that when the 442nd's "I" company finally broke through, only 27 soldiers were left alive out of an initial 200. The 442nd lost a total of a thousand soldiers in rescuing the 175 Texan survivors.

Although others studied spiral bacteria between 1920 and 1979,[6] there was reduced interest in the field as the pathologist Edward Palmer stated in 1954 that "infestation is an agonal or post-mortem process". This implied that the spiral bacteria did not cause disease in life.[7]

The Acute Infection

The second relevant historical description of *H. pylori* infection concerns the clinical syndrome of acute *Helicobacter* gastritis. Since most adult persons with peptic ulcer disease already have the organism, it must have been acquired some time after birth. Like most infectious diseases, it is likely that children first acquire the infection, but unlike other infections, the organism is unable to be eradicated by the host's defences. In the literature, there were several descriptions of individuals or even epidemics in which gastritis had developed and Marshall and Warren were able to study the histology of these publications.

Epidemic Hypochlorhydric Gastritis

The great Canadian physician, William Osler, described a syndrome of children in which vomiting without acidity was a feature. He stated "examination of the vomitus reveals as a rule, absence of hydrochloric acid and sometimes the presence of volatile fatty acids."[8] We now know that in the absence of acid, other putrefying bacteria can live in the stomach, generating fatty acids such as butyric and isobutyric acid, and possibly also allowing nitrosation in the stomach, thus causing a cancer risk. This acute *Helicobacter pylori* infection was well known to Osler in 1910, but it was subsequently forgotten and disappeared from the medical texts.

In 1978, an epidemic of approximately 30 individuals in Dallas, Texas was described.[9] These persons were undergoing acid secretion studies and, one by one, they developed abdominal pain, nausea, vomiting, and lost their ability to secrete acid under the stimulus of histamine or pentagastrin. Those individuals biopsied were found to have a neutrophilic gastritis that persisted for many months, as did the hypochlorhydria. Unaware of the spiral bacteria, the investigators were unable to find a cause. That *H. pylori* could cause achlorhydric gastritis was proven by self-experimentation in 1984 in which Marshall drank *H. pylori* and developed this same syndrome.

Gastric Urease

The third big clue to the presence of *H. pylori* was gastric urease. In 1950, two Irish physicians, Fitzgerald and Murphy, published a two-volume thesis on gastric urease in humans, which described the physiology and biochemistry of gastric urease in great detail.[10]

Fitzgerald and Murphy assumed that gastric urease was either a safety mechanism to rid the body of excess nitrogen, or, by generating alkaline bicarbonate and ammonia in the gastric mucosa, it acted to protect the epithelial cells from acid attack. Oliver Fitzgerald later became the president of the British Society of Gastroenterology and died in 1983.

In 1957, Charles Lieber and his colleague LeFevre in

New York City, were interested in hyperammonemia as a cause of hepatic encephalopathy. Besides the colon, they knew that the gastric mucosa generated ammonia by means of urease. They observed that before therapy with antibiotic, most of the nitrogen present in the gastric juice was there as ammonium. Subsequently, after antibiotics, most of the nitrogen was present as urea, thus showing that urea breakdown was prevented by antibiotic treatment.[11] Lieber concluded that gastric urease must have been due to bacteria, but his more senior colleagues generally did not believe him. He then repeated the experiment with other antibiotics, and again showed that gastric ammonia production ceased. Lieber and others then suggested that antibiotics such as oral neomycin would be good treatment for hyperammonemia since they interfered with both gastric and colonic urease activities and did not have systemic toxicity because they were not well absorbed.

Therapy

Treatment of gastric spiral bacteria has been carried out for more than 100 years and several groups used antibiotics to treat ulcers even before *H. pylori* was discovered. The forerunner antimicrobial, similar in structure to Kobayashi's Arsaminol, was bismuth. In 1900, a suspension of bismuth subsalicylate compound called Bismosal was made by the Norwich Company in Connecticut, USA. This was used for the treatment of infantile cholera. We now know that infantile cholera (spiral organisms present in the stool of infants) was probably a *Campylobacter jejuni* infection. *Campylobacter* is one of many organisms that are killed by bismuth, so Bismosal was probably a very effective therapy. In later years, bismuth subsalicylate, bismuth subcarbonate and bismuth subnitrate were all used as oral treatments for gastric upset and gastric ulcers. These compounds were never studied in proper clinical trials because before 1940, x-ray barium meals and endoscopy were difficult to obtain. In addition, bismuth salts were thought to be antacids, rather than antibacterial agents, so their use in many countries was phased out once more powerful antacids such as magnesium and aluminium hydroxide were available. In Germany, however, where there was a tradition of bismuth treatment, bismuth compounds remain in many gastric medications to the present day.

Antibiotic treatment for ulcers was actually pioneered by a Greek physician called John Lykoudis. He became famous between 1960 and 1970 after he treated thousands of patients in a special clinic in Athens. He gave a combination antibiotic therapy that included non-absorbed quinolones. Although he presented his ideas to the drug companies and attempted publication in refereed journals, the concept of a bacterial cause of peptic ulcer was ignored and none of his work was published.[12]

In the 1970s, physicians in Spain and China noticed that drugs such as furazolidone and metronidazole seemed have a beneficial effect on patients with ulcers. One interesting statement from the Chinese literature was that the diagnosis of a malignant ulcer could be made using a barium meal in the following manner.[13] If the patient was given antibiotics for two weeks, then the swollen and inflamed margin of the benign gastric ulcer would diminish in size, proving that the ulcer was benign rather than malignant.

So in summary, we can say that before Marshall and Warren made their discovery, it was known that most animals had spiral bacteria in their stomach. These bacteria resembled spirochaetes or spirillae and could even live in the canaliculi of the parietal cells. The bacteria were there in very large numbers so they could not have been contaminants. In conjunction with these bacterial infections, large amounts of gastric urease accumulated which therefore caused significant ammonia production in infected persons. The enzyme associated with these bacteria, urease, could be shown to be absent from germ-free animals and to disappear when humans were treated with antibiotics. Humans were commonly infected with these spiral bacteria, particularly persons with a gastric ulcer in whom the infection was present in about 80%, but the bacteria could also be seen around the borders of duodenal ulcers. The acute infection associated with these bacteria caused many months of hypochlorhydria and severe neutrophilic gastritis, although many persons seemed to have the infection without symptoms. Finally, the decision to use heavy metals and antibiotics to treat gastritis and gastric ulcers could possibly be explained by the inhibitory effect of these compounds on the bacteria. Thus, it gives me great pleasure to acknowledge the initial historic work done by several Japanese investigators, defining a tradition of excellence that continues to this day.

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