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A Century of *Helicobacter pylori*

Paradigms Lost – Paradigms Regained

Key Words

Acid
Bacteriology
Gastritis
Helicobacter
Peptic ulcer
Urease
Warren

Abstract

The investigation of gastric bacteria properly began in the latter half of the nineteenth century when microscope resolution had sufficiently advanced. Whilst a bacterial etiology was demonstrated for dysentery, tuberculosis and syphilitic ulcers, problems in the isolation and culture of pure strains circumvented a role for bacteria in gastric pathology. Furthermore, dogma and the intellectual chorus were in harmony advocating that gastric acid was critical in ulcer disease. The consideration of a role for a pathogen or pepsin was regarded as whimsical in the context of mucosal ulceration. Indeed, the effects of acid inhibitory agents were held as gospel truth whilst the use of antibiotics or metallic ions were deemed to be quackery or at least ill judged. Nonetheless, spiral-shaped bacteria had been identified in both mucosa and gastric contents of patients as early as 1889. Elegant studies had documented the infectivity of these organisms, and suggested but not proven a causative role in gastric disease. The prescient identification by Doenges of organisms associated with gastritis in both man and monkey, was buried by the observations of Palmer, and an opportunity for early progress lost. It required two decades and Antipodean pathological perspicacity to elucidate the warren of previous archaic gastric bacterial misinformation. The subsequent marshalling of clinical and pathological data established the fatal flaw in the mucosa to be bacteria and not only acid on the mucus shore. It is now widely apparent that *Helicobacter* is ubiquitous, pathological and, a century after its initial discovery, still remains a paradox of as yet incompletely determined biological consequence. It is of note that an organic helical configuration has twice in this century provided biological information of unique import.

Introduction

Although *Helicobacter pylori* was discovered by John Robin Warren and cultured by Barry Marshall in 1982 in the Antipodes, its historical origins are firmly

rooted in the myopia of the latter half of the European nineteenth century (table 1). As noted so elegiacally by Paul de Kruif – those were indeed halcyon days for microbe hunters [1]. It was during this period that the eminent German bacteriologist Robert Koch proved

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0012-2823/98/0591-0001\$15.00/0

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Table 1. Observations on gastric infection

Year	Individual	Observations
1875	G. Bottcher/M. Letulle	demonstrated bacteria in ulcer margins
1881	C. Klebs	bacterial colonization and ‘interglandular small cell infiltration’
1888	M. Letulle	experimental induction of acute gastric lesions in guinea pigs (<i>S. aureus</i>)
1889	W. Jaworski	spiral organisms (<i>Vibrio rugula</i>) in gastric washings
1893	G. Bizzozero	identified spirochetes in gastric mucosa of dogs
1896	H. Salomon	spirochetes noted in gastric mucosa and experimentally transferred to mice
1906	W. Krienitz	spirochetes in gastric contents of patient with gastric carcinoma
1908	F.B. Turck	induced gastric ulcers in dogs by <i>Bacillus (Escherichea) coli</i>
1916	E.C. Rosenow	described streptococcus induced gastric ulcers
1917	L.R. Dragstedt	identified bacteria in experimental ulcers, no significant role identified
1921	J.S. Edkins	experimental physiology of <i>S. regaudi (H. felis)</i>
1924	J.M. Luck	discovered gastric mucosal urease
1925	B. Hoffman	described <i>B. Hoffmani</i> – putative ulcerous agent
1930	B. Berg	partial vagotomy inhibits secondary infections in ulcers
1938	J.L. Doenges	spirochetes/inflammation in <i>Macacus</i> monkey and man
1940	A.S. Freedberg/L. Barron	identified spirochetes in man – no etiologic role
1940	F.D. Gorham	postulated gastric acidophilic bacteria as an etiologic agent in ulcer disease
1954	E.D. Palmer	no spirochetes detected using HE in 1,180 suction biopsies
1975	H.W. Steer	polymorphonuclear migration in ulcers – isolated <i>Pseudomonas aeruginosa</i>
1983	J.R. Warren	identified <i>Campylobacter (Helicobacter) pylori</i> in human gastritis
1983	B. Marshall	isolated and cultured <i>H. pylori</i>
1985–1987	B. Marshall/A. Morris	ingested and proved the infectivity of <i>H. pylori</i> (Koch’s 3rd postulate)

scientifically that bacteria were the cause of certain diseases. Almost simultaneously, the Frenchman Louis Pasteur, having been galvanized by Koch’s contributions, was in the process of developing vaccines against the microbes causing cholera and rabies. In Sicily, in a small home-made laboratory in Messina, an émigré Russian, Elie Metchnikoff, had discovered phagocytosis thus initiating an entirely new vista of biological investigation: host defense mechanisms. Fortunately, the stomach was not neglected during this period of enthusiastic research, and in this vignette is illuminated some prescient scientific observations which might otherwise be condemned to an obscurity, quite undeserved given the richness of their thought:

‘For thee, who mindful of th’ unhonored dead
Dost in these lines their artless tale relate
If chance, by lonely contemplation led,
Some kindred spirit shall inquire thy fate ...’ [2]

The Stomach up to the Nineteenth Century

Alterations in gastric physiology and the associated pathology documented had been noted in detail by the last quarter of the nineteenth century. The elucidation of

biology had, however, been tardy, and many theories, often fanciful, abounded in regard to the etiology of the disease. The recorded history of gastric disturbances dates back at least to Hippocrates, to whom we owe the first description of purely gastric symptoms, which included epigastric burning and aerophagia [3]. A thousand years later, Avicenna or Abu’Alia A’-Husayn Ibn-Sinn (980–1037), the great Arabic physician, who, unlike his contemporaries, possessed a wealth of personal experience, was the next to note the relationship between gastric ulcer pain and mealtimes [4]. He suggested that the pain, pyrosis and an acute thirst, could all be related to the presence of a gastric ulcer. Probably the first recorded case of gastric ulcer was described by the Italian physician, Marcello Donati (1538–1602), who reported a case in 1586 [5]. However, further understanding of the complexity of gastric diseases only became available when experimental methodologies became more widespread. Stahl, in 1728, was probably the first to use the term ‘gastritis’ in his ‘Collegium Practicum’ [6]. In this work he also put forward his hypothesis that some episodes of fever could be related to superficial inflammation of the gastric mucosa, especially in those cases which had a tendency to develop ulcers. Forty years later (1765), the anatomopathological studies

Fig. 1. A. Leeuwenhoek (1632–1723), the enthusiastic pioneer of the biological microcosmos, was the first to see gastrointestinal bacteria (mouth, colon), but due to his lack of medical training ascribed no pathologic importance to this new world. Once microscopes had sufficiently evolved, order was brought to the vague Linnaean genus of ‘Chaos’ by the morphological classification of bacteria by F. Cohn (1828–1898). Cohn’s protégé, R. Koch (1843–1910), one of the founders of modern bacteriology, was also first to develop the correct theory of species specific infectious diseases.

of the Italian pathologist G. Morgagni (1682–1771) of Padua described, for the first time, the pathology of this process. In ‘De sedibus et causis morborum’ he described the erosions and mucosal flattening as signs of gastric inflammation [7]. The first clear description of the symptoms and morbid anatomy of gastric ulcers can be attributed to the English physician, Matthew Baillie in 1793 [8], who also described the transposition of the abdominal viscera, and ulceration of Peyer’s patches in typhoid fever. Some two decades later, the Napoleonic surgeon, F. Broussais (1772–1838), during the retreat from Moscow in 1812, had astutely observed the tendency of acute gastritis, if left untreated, to become chronic [9]. In 1821, Nepveu published a book firmly stating his belief that chronic gastritis evolved into gastric cancer [10]. Twenty years thereafter, in 1842, Jean Cruveilhier (1791–1874), the doyen of French pathology, distinguished two types of ulcer, a benign, chronic ulcer as well as a cancerous one [11]. Ammonia was noted in gastric juice as early as 1852 [12], and, by 1880, the connection between gastric atrophy and pernicious anemia had been well established [13]. As of 1823, the seminal observation of the English physician-chemist, Prout, in regard to the fact that the stomach produced hydrochloric acid and not lactic acid had become an accepted fact in almost all the world except for Paris and Boston. Nevertheless, theories about the precise origins of digestive complaints and their underlying pathologies were both varied and exotic, resembling the earlier speculations of the ancients in regard to the spiritual nature of digestion. A variety of proposals included: mechanical causation (irritation), excessive nervousness, alterations in vascularization, the atmosphere and the nature of ingested waters, foods and agents such as bacteria, yeast and fungi [14].

Bacteriology in the Nineteenth Century

Although Leeuwenhoek was probably the first to see both gastrointestinal and oral bacteria, it was O.F. Muller (1730–1784) of Copenhagen who provided the first definitive observations and descriptions of microorganisms; he also coined the terms ‘bacillus’ and ‘spirillum’ [15]. The initial problems with identification of microorganisms in the late eighteenth and early nineteenth centuries was compounded by the low resolution of the microscopes. Thus, at higher magnifications fuzzy images with distorting halos as well as spherical aberrations confounded scientists. It required solution of chromatic and achromatic distortion as well as the development of medically orientated bacteriology before advances could occur.

Ferdinand Cohn (1828–1898) from Breslau, the botanist now regarded as one of the founders of bacteriology, could not find tenure in any German university and therefore followed his teacher, Purkinje (1787–1869) to Breslau where he was offered the chair of Botany. In 1853, he classified microorganisms into 3 groups: bacterial (short, cylindrical cells), bacilli (longer cells) and spirilla (wavy or spiral forms) and noted the fixity of bacterial species. Thus, even under varying conditions he was never able to obtain cocci from bacilli and vice versa. In 1870, Cohn established his own journal, ‘Beiträge zur Biologie der Pflanzen’ and in this communication hosted most of the original, classical bacteriology papers, authored by both himself and his young protégé, Robert Koch (fig. 1). The classic postulates of the latter would subsequently form the logical basis for the investigation and identification of the disease-causing potential of bacteria. In 1872, Cohn, published his mature exposition on bacteriology entitled ‘Untersuchungen über Bacterien’ [16, 17] wherein he fur-

ther adumbrated on the classification of bacteria into genera and species. He suggested an expanded classification into four groups: sphaerobacteria (cocci), microbacteria, desmobacteria (bacillus and vibrio), and spirobacteria (spirillum and spirochete). This work was well received and became so popular that it was reprinted in 1875 and released once again in 1876.

Apart for establishing the foundations of modern bacteriology, Cohn deserves considerable credit for both his recognition and mentorship of Koch (1843–1910) who proved scientifically for the first time that bacteria were the cause of anthrax, cholera and tuberculosis. In 1878, the term ‘microbe’ was introduced by C.E. Sedillot (1804–1883), a French surgeon who was responsible for undertaking the first gastrostomy and may have most likely unwittingly happened upon the organism. He proposed this term, derived from the Greek for ‘small life’, with the caveat that such ‘small lives’ must have the special ability to cause fermentation, putrefaction or a disease process [18]. A proposal much favored by T. Schwann (1810–1882), who had himself not only discovered pepsin in 1834, but written extensively on the role of fermentation, as well as the single cell theory of disease [19].

Early Investigators

The widespread but often unpopular use of the microscope by early investigators allowed for the identification of a role for bacteria, yeast and fungi in gastric pathobiology. Careful analysis of gastric contents revealed that under fasting conditions the normal stomach contained mucus, a few bacilli and some yeast cells, whilst in stagnant gastric contents, obtained from patients with gastric disease, bacilli, micrococci, yeast and fungus could readily be seen. Such early observations supported speculations regarding a putative causative role for these ‘foreign bodies’ in gastric pathology. It was, however, unclear to these early, eager, gastric bacteriologists whether a specific organism was the cause of a gastric disease entity or whether it was simply an abnormal accumulation of organisms in the stomach itself which culminated in gastric disturbances.

One of these first gastric bacteriologists was the German, G. Bottcher, who along with his French collaborator M. Letulle (1853–1929), could demonstrate bacterial colonies in the ulcer floor and in its mucosal margins. His convictions in regard to the disease-causing potential of ingested organisms, were that ardent that by 1875 he had attributed the causation of ulcers to the bacteria which they could demonstrate [20]. However, this was not a pop-

ular point of view and in spite of an 1881 report by the pathologist, C. Klebs, of a bacillus-like organism evident both free in the lumen of gastric glands and between the cells of the glands and the tunica propria with corresponding ‘interglandular small round cell infiltration’ [21], the ‘bacterial hypothesis’ fell into disuse. Bottcher was, however, probably the first to report the presence of spiral organisms in the gastrointestinal tract of animals, although spiral organisms were already well known and had been described as early as 1838 by Ehrenburg [22]. The pathological properties of these particular organisms had similarly been recognised by Obermeier of Berlin, who in 1872, could demonstrate their presence in the blood of patients with relapsing fever. It is of considerable interest that an examination of the report of Klebs indicates that he had noted the presence of an inflammatory infiltration, although he made no specific comments in regards to its significance. However, could this have been the first notation of if not *H. pylori* infectious gastritis then at least lymphoid tissue in the gastric mucosa?

In 1889, Walery Jaworski, professor of Medicine at the Jagiellonian University of Cracow, Poland, was the first to describe in detail spiral organisms in the sediment of washings obtained from humans [23]. Amongst other things, he noted a bacterium with a characteristic spiral appearance which he named *Vibrio rugula*. He suggested that it might play a possible pathogenic role in gastric disease. Jaworski supposed that these ‘snail’ or ‘spiral’ cells were only to be found in rare cases. However, I. Boas, already a luminary for his gastrointestinal contributions and for the discovery of the ‘Oppler-Boas’ lactobacillus, found these cells quite constantly in all ‘fasting’ gastric contents containing hydrochloric acid (fig. 2). Further detailed analysis by Boas’ assistant, P. Cohnheim, indicated that such ‘cells’ could be induced by the reaction of bronchial or pharyngeal mucus and hydrochloric acid. This led to the suggestion that Jaworski had consistently observed acid altered myelin and that similar secondary structures, threads and small masses could also be induced by these simple chemical reactions [24]. Cohnheim and Boas therefore inferred from their experiments that Jaworski’s cells were most probably the product of gastric mucus and acid chyme. Jaworski’s original observations were published in Polish and possibly because of both this and the negative response of the Teutonic thought leaders of the time, failed to significantly impact on either Europe or America.

The observations of Bottcher and Letulle had suggested a causative bacterial agent in ulcer disease and by 1888, Letulle was actively searching for this postulated

W. Jaworski

I. Boas

Gastric contents

Oppler-Boas

Fig. 2. W. Jaworski discovered and postulated a pathogenic role for the spiral organisms (*Vibrio rugula*) he found in gastric contents (f). I. Boas could generate similar morphological forms chemically and suggested that Jaworski's cells were the result of the inter-reaction of gastric mucus and acid. Boas was responsible for the co-discovery of the Oppler-Boas bacterium, which he thought was implicated in the etiology of gastric carcinoma.

entity. A few years earlier in 1881, the Scottish surgeon and bacteriologist, Alexander Ogston (1844–1929) had identified *Staphylococcus pyrogenes aureus* both in acute and chronic abscesses [25]. Noting the similarity of this bacterium to their postulated entity, Letulle, in the time-honored tradition of his day, undertook a classical experiment. He used two modes of administration to guinea pigs: intramuscular injection of Ogston's pure, cultured *Staphylococcus* or oral intake of the agent. Not surprisingly, this resulted in the formation of acute gastric lesions perfectly consistent, at least to him, with the predictable mode of generation of gastric ulcers [26]. Matters were, however, somewhat complicated by the fact that he obtained similar results with dysentery organisms and with

Fig. 3. G. Bizzozero, A. von Kolliker and C. Golgi in Pavia, 1887. Bizzozero had a profound interest in the gut and inspired Golgi to study the canalicular apparatus of the resting and stimulated gastric gland. von Kolliker was later instrumental in securing the Nobel for Golgi and Ramon Y. Cajal.

pyrogenic *Streptococci*, Letulle was never able to experimentally discriminate between these different agents and was therefore not able to conclusively prove a role for bacteria in ulcer disease. Nevertheless, the experimental work of Letulle inspired a number of other scientists to follow his lead and similar results were attained with *Lactobacillus* [27], diphtheria toxin [28], and *Pneumococcus* [29]. Thus, by the turn of the century, experimental results appeared to confirm the hypothesis that a bacterial infection might be, if not an occasional cause, at least an accessory requirement for the development of gastroduodenal ulcers. Thus, although a pathological role for bacteria in the stomach appeared to have been established, the precise role of the spirochete organisms remained to be further evaluated.

In a time frame contiguous to these sophisticated experiments, the Italian anatomist G. Bizzozero (1846–1901), was busy engaged in the extensive study of the comparative anatomy of vertebrate gastrointestinal glands with his adept and capable pupil Camillo Golgi (fig. 3). In the specimens of the gastric mucosa of 6 dogs, Bizzozero noted the presence of a spirochete organism in the gastric glands and both in the cytoplasm and vacuoles

Fig. 4. H. Salomon extensively studied *H. felis* in domestic animals. In this article, published in 1896, he described his unsuccessful attempts both at culturing the bacteria in vitro, and at establishing the mode of transmission of the organism. Nonetheless, after failing in frogs, rabbits and pigeons, he succeeded in infecting white mice with the bacterium. He also noted the invasion of the glands as well as the close association with parietal cells. His studies refuted the then current hypothesis that parietal cells were guards at the entrance of the stomach, and acted to limit the entrance of microorganisms into the gastrointestinal tract.

of parietal cells [30]. He commented that this organism affected both pyloric and fundic mucosa, and its distribution extended from the base of the gland to the surface mucosa. Although he neglected to ascribe any clinical relevance to these observations, he did, however, remark upon their close association with the parietal cells.

Three years later, in 1896, in a paper entitled 'Spirillum of the mammalian stomach and its behavior with respect to the parietal cells'. H. Salomon reported spirochetes in the gastric mucosa of dogs, cats and rats, although he was unable to identify them in other animals, including man [31]. In this early paper, Salomon undertook a series of somewhat bizarre experiments in which he tried to transmit the bacterium to a range of other animal species by using gastric scrapings from dogs. He failed to transmit it to owls, rabbits, pigeons and frogs; however, the feeding of gastric mucus to white mice resulted in a spectacular colonization within a week, as evidenced by

the series of drawings of infected gastric mucosa reproduced in the original paper (fig. 4). The lumen of the gastric pits of the mice were packed with the spiral-shaped bacteria and invasion of the parietal cells was also noted. Almost two decades later, in 1920, Kasai and Kobayashi [32] successfully repeated these experiments, and using spirochetes isolated from cats, demonstrated pathogenic results in rabbits. Histological examination indicated both hemorrhagic erosion and ulceration of the mucosa in the presence of masses of spirochetes.

The Turn of the Century

By the beginning of the twentieth century physicians involved in the treatment of gastrointestinal disease were generally familiar with some infective processes of the digestive tract: the ulcerative processes of typhoid fever, a

variety of dysenteric conditions and tuberculosis. Kiyoshi Shiga had discovered a bacteria, erroneously recognised as *Shigella dysenteriae* in 1898 [33], and an unspecified type of upper gastrointestinal (gastric) bacterial infection, not accompanied by signs of active inflammation, and designated as 'bacterial necrosis' had also been annotated and was described in detail in Hemmeter's text of 1902 [34]. This pathology was characterized by the invasion of bacteria, usually into the lower depths of the mucous membrane, followed by bacterial growth and subsequent tissue necrosis. A thoughtful suggestion at this time was that the ensuing ulceration could be caused by, or augmented by, the action of gastric juice, particularly gastric acid. In this context, an observation of considerable interest and possible relevance was the report that bacteria were identifiable in the cells around and beneath the floor of these ulcers, notwithstanding the very high level of acidity. This bacterium was tentatively identified as a bacillus resembling anthrax. However, since it could not be obtained in pure culture, further experimental investigations were precluded.

A paradigm which would dominate gastric speculation, nonetheless, had become entrenched in the studies of gastric ulcer development. J. Cohnheim (1839–1884), Professor of Pathology at Kiel, who, as early as 1880, had prophesied that the young Koch would surpass all others in the field of medical bacteriology, had suggested that the formation of ulcers depended on chemical factors. Shortly thereafter, F. Reigel attributed hyperchlorhydria to the development of chronic ulcers [35]. The scientific foundations for the recognition of the role of gastric juice (acid) in the genesis of ulcer disease were laid firstly by A. Kussmaul (1822–1902), who had in 1869 developed a method of intubation of the stomach and secondly by the creation of the experimental gastric pouch preparation by I.P. Pavlov (fig. 5). The major opinion of this time could be summed up in the quote of the famous Yorkshire surgeon of Leeds, Sir Berkely Moynihan: He opined

'It is not improbable that the gastric ulceration is the primary condition, duodenal ulcer being secondary, and caused, it may well be, by the digestion of the duodenal mucosa by the hyperacid gastric juice.' [36]

Small wonder that for decades after, the condition was known in England as Moynihan's disease.

However, the adage of Schwartz: 'no acid, no ulcer' was by no means uniformly accepted and sporadic pathological observations in regard to the bacterial origin of gastric ulceration continued to be published [37]. In 1906, Krienitz identified spirochetes in the gastric contents of a

Fig. 5. J. Cohnheim (1839–1884) postulated that chemical factors played the critical role in the etiology of ulcers. The foundation for the role of acid in the genesis of this disease were thereafter laid by A. Kussmaul (1822–1902) who developed a method for intubating patients and, who also advocated the use of bismuth subnitrate for the treatment of ulcer disease. A further contribution was by I.P. Pavlov (1849–1936) who popularised the experimental gastric pouch and was awarded the Nobel Prize in 1904 for his elucidation of the critical role of the vagal nerves in regulating secretion. L.R. Dragstedt (1893–1975) initially investigated the role of bacteria in experimental ulcer disease but thereafter turned to the vagus and its resection as a means to physiologically understand and treat the disease.

patient with a carcinoma of the lesser curvature of the stomach and commented that upon microscopic examination, three types of spirochetes, including *Spirochete pallidum*, could be identified [38]. He did not address the question of etiology. Spirochetal dysentery, as well as the presence of spirochetes in the stool of healthy individuals were known, and Muhlen and independently, Luger and

Neuberger, had all reported these organisms to be evident in the stomach contents of patients with ulcerating carcinomas of the stomach [39]. The latter authors also noted the rarity of these organisms in the gastric mucosa and gastric juice of healthy individuals. Experimental biology, however, dominated gastric research and in the same year. Turck had undertaken an experiment in which he fed broth cultures of *Bacillus coli* to dogs for a number of months. This resulted in the development of chronic gastric ulceration [40]. In an attempt to establish cause and effect, he thereafter cultured *B. coli* from the feces of ulcer patients, which were then injected intravenously into dogs, without effect. However, when the animals ingested the microorganism, every single dog reacted with a spectrum of nonspecific gastric and duodenal alterations, which Turck [41] loosely called 'ulcers'. When Gibelli [42] attempted to repeat this work, he could not confirm the results obtained by Turck.

In Cincinnati, Ohio, the American bacteriologist, E.C. Rosenow, over a decade from 1913 to 1923, vehemently maintained that ulceration of the stomach could be reproduced in laboratory animals by *Streptococcus* [43–45]. He isolated this bacterium from foci of infection in humans with ulcer disease and injected the culture into a wide range of animals including rabbits, dogs, monkeys, guinea pigs, cats and mice. A higher incidence of experimental lesions were identified using this particular inoculum than from cultures isolated from foci in other patients. Of additional interest was that *Streptococci* isolated from jejunal ulcers in Mann-Williamson operated dogs, also caused acute gastritis and duodenal ulcers which were limited to the upper gastrointestinal tract in experimental animals. Based upon these observations, Rosenow postulated that 'gastric ulcer producing *Streptococci*' had a selective affinity for the gastric mucosa and produced a local destruction of the glandular tissue. He further proposed that consequent upon such damage ulcers would thereafter form given the autolytic capacity of gastric acid. Rosenow thought that the reservoir for these bacteria were carious teeth, and advanced the idea that a hematogenous bacterial invasion would result in the formation of an ulcer. These experiments were continued by Hardt in dogs [46], and later by McGown in guinea pigs [47], with analogous results.

L.R. Dragstedt (1893–1975), initially trained as a physiologist in the Hull Physiological Laboratory of A.C. Carlson in Chicago, before becoming a surgeon. One of his early scientific interests was the causation of gastroduodenal ulceration, although he would subsequently (1943) achieve renown as the surgeon who established the 'physi-

ological' rationale for vagotomy as a treatment for duodenal ulcer disease. This acid reducing procedure had previously been published by Latarjet of Lyon some twenty-three years earlier [48]. Nevertheless, as early as 1917, as a young physiologist, he had attempted to define the different mechanisms by which gastric juice could affect healing of acute gastric and duodenal ulcers [49]. Aware of Rosenow's work, and the question of the importance of the virulence of different bacterial strains in determining the chronicity of ulcers, he attempted to isolate and culture any bacteria he could find in the silver nitrate induced ulcers of five experimental Pavlov pouch dogs. Bacteriologic examination revealed *Streptococcus*, *Staphylococcus* and *Bacillus* species, which were similar to those types of bacteria isolated from clinical ulcers in man. Dragstedt concluded that these bacteria colonised the damaged mucosa following ulcer formation and proposed that they had migrated up from the alimentary tract. He did not believe that they played a substantial role in the etiology of the disease, and did not pursue these studies further, choosing to rather focus on the role of vagal innervation in acid induced ulceration. Fifteen years later, at the Mount Sinai Hospital, B. Berg, utilized partial vagotomy to reduce 'secondary' infections in ulcer margins [48]. Soon thereafter, however, he turned his attention to the colon and along with his collaborator Crohn became more famous for his role in the discovery of the aetiology of this disease.

The Role of J.S. Edkins

J.S. Edkins (1863–1940), of London, had made a significant contribution to the elucidation of gastric physiology by the discovery of gastrin. The scientific doyens of his time declared it to be humbug, although time would vindicate Edkins [50]. Motivated by his previous disappointment incurred in the investigation of gastrin, Edkins still maintained his enthusiasm for the exploration of gastric pathophysiology (fig. 6). In contrast to the inoculation mode of experimental studies, he proceeded to investigate how the host itself might affect the prevalence and location of the spirochete organisms in different parts of the stomach [51]. The organisms were named *Spirochete regaudi*, after Regaudi who considered that the organisms of the gastric mucus layer of cats was morphologically analogous to the syphilis spirochete. Using the Giemsa stain to identify the organisms in stomach sections, Edkins identified them in both the fundus and the antrum, and noted specific invasion of the epithelial cells of the

Fig. 6. J.S. Edkins (1863–1940) is best known for his controversial (at the time) discovery of gastrin in 1905. What is less appreciated is his work on a spiral organism, most probably *H. felis*, in the stomach of cats. Apart from his critical contributions to the field of croquet Edkins deserves credit for these two seminal observations in the field of gastroenterology.

On the Chemical Mechanism of Gastric Secretion.
By J. S. EDKINS, M.A., M.B. Cantab., Lecturer on Physiology in the Medical
School of St. Bartholomew's Hospital, London.

SPIRELLA REGAUDI IN THE CAT¹.

By J. S. EDKINS.

(From the Physiological Laboratory, Bedford College.)

fundic glands. It was also evident that the organism appeared to have a preference for the surface epithelium, or for thick mucus of the feline experimental model. Of particular note was the demonstration of organisms not only in the subepithelial lymphoid tissue, but even located within the phagocytic cells. In the next phase he evaluated the effects of acid and alkalis on the organisms and demonstrated that at both pH extremes (1 or 12) there were no substantial effects on organism motility. It was however evident that after prolonged exposure to a pH of 12.6, the motion of the organisms became 'irregular and convulsive, and less purposeful', which he interpreted as an obvious prelude to death. He also described a 'beaded form' of the organism in fasting cats, an observation consistent with the discovery of sporulation bodies. Gastric secretory activity did not appear to be compromised when the organisms were present and abundant, and, indeed, there appeared to be a parallelism between the degree of acid and the abundance of the organisms. It is noteworthy that R.K.S. Lim, who was involved in the controversy regarding 'Edkins' hypothesis' (gastrin), also studied spirochete organisms in cats, although he minimised their pathological significance by characterizing them as a local laboratory infection [52].

In general, however, experimental studies continued with the effective isolation of bacteria from peptic ulcers or from foci of infection in ulcer patients. Thus, in 1925, Hoffman [53] investigated whether the causative agent of ulcer disease was a member of the bacillus family by the injection of 5 cm³ of gastric contents from a peptic ulcer patient into guinea pigs. He successfully produced gastric ulcers from which he recovered gram-negative, fine slender rods which when inoculated into another guinea pig once again produced the same lesions. He modestly

named his organism '*Bacillus Hoffmani*', but it was evident after further study that the lesion-producing capabilities of this bacterium were nonspecific. In 1930, Saunders [54] demonstrated that the streptococcus organism isolated from peptic ulcers in humans was of the alpha variety, and identified specific antibodies against this agent in serum from patients. However, he was not able to produce ulcers in animals by injecting the inoculum and proposed that laboratory animals do not spontaneously form gastric ulcers, since they exhibited an innate resistance to this organism. A strange conclusion, in view of the accumulated information of the previous 50 years of research.

Macacus and the Man from Missouri

Based to a certain extent on the recognition of the widespread scourge of luetic disease, at around the beginning of the second world war, spirochetes returned to gastric prominence. J.L. Doenges, observed the organisms to invade the gastric glands of every single one of the *Macacus rhesus* monkeys he studied and to be present in 43% of human gastric autopsy specimens [55]. In contrast to the monkey, the organisms appeared to be difficult to identify in human gastric mucosa and only 11 of the 103 specimens showed appreciable numbers. Doenges specimens, however, were autolytic which precluded the attachment of any major significance to his observations. Of special note, however, was his observation that the organism was restricted to the gastric mucosa and not evident in the intestinal mucosa. These reports prompted Freedberg and Barron [56] in 1941 to investigate the presence of spirochetes in the gastric tissue of patients who had undergone

partial resection surgery. Both authors were familiar with the methods of identifying the organism, and used the silver staining method of DaFano, which they had previously successfully used (but not published) to identify spirochetes in dogs. In spite of such expertise, they were not easily able to identify the organisms, although they could demonstrate that spirochetes were more frequently present in ulcerating stomachs as compared to nonulcerated stomachs (53 vs. 14%). Based upon their own difficulties with adequate identification, and the apparent histological differences noted in Doenges' observations in the *Macacus* mucosa, they concluded that no absolute etiopathologic role for these organisms could be predicted. One may smile sadly to read that in the report of the discussion of this paper, Frank D. Gorham, a specialist of Internal Medicine, of St. Louis, Missouri, noted:

'I believe that a further search should be made for an organism thriving in hydrochloric acid medium (and variations of hydrochloric acid are normal in all stomachs) as a possible factor of chronicity, if not an etiologic factor, in peptic ulcer.' [56]

Of interest is that Gorham also wrote that he had, over the previous 10 years, successfully treated patients who had refractory ulcer disease with intramuscular injections of Bismuth! Although Gorham may have seemed to be ahead of his time, as early as 1868, A. Kussmaul had advocated the use of bismuth subnitrate for the treatment of gastric ulcer. In fact, the oral use of bismuth for gastrointestinal symptoms was well accepted, and as early as the late 18th century, reports of the therapy had begun to appear in the English literature. The antibacterial properties of bismuth, which may or may not have been known to Gorham, had already been successfully exploited by R. Sazerac and C. Levaditit in 1921, who used it to cure experimental syphilis in rabbits [57]. Gastric syphilis had also been described, the ulcers associated with this disease were well known [58], and the infective agent, *Spirochete pallida*, had been successfully isolated and cultured from syphilitic abscesses [59].

The negative results of Freedberg and Barron and the ambivalent results of Doenges subsequently prompted E.D. Palmer, in the early fifties, to investigate spirochetes in human gastric samples. He obtained gastric mucosal biopsies from 1,180 subjects using a vacuum tube technique, but using standard histological techniques failed to demonstrate either spirochetes or any structures resembling them [60]. Although Palmer did not attempt to identify the organisms with the more reliable silver stain, he concluded (confidently) that the results of all previous authors could be best explained as a postmortem coloni-

zation of the gastric mucosa with oral cavity organisms. He also postulated that spirochetes were normally occurring commensals of the mouth. Palmer's work may thus be credited with the envious distinction of setting back gastric bacterial research by a further 30 years.

Gastric Ammonia and Urease

Whilst ammonia was noted in gastric juice as early as 1852 [12], it was not until 1924 that Luck [61] discovered gastric mucosal urease. His subsequent work and the work of others, especially the Dublin biochemist, E.J. Conway, confirmed the ubiquity of gastric urease in a number of mammals [62–64]. Histochemical studies demonstrated that enzyme activity appeared to be concentrated in the surface layers of the mucosa, in close conjunction with oxyntic cells [65, 66]. In addition, tissues surrounding gastric ulcers were found to be particularly rich in urease [65], whilst cancerous or achlorhydric stomachs were devoid of urease activity. These observations, as well as the longstanding observation of ammonia in gastric juice, prompted the proposal that urease activity was somehow coupled to hydrochloric acid secretion. This hypothesis was however swiftly refuted upon the demonstration that the mucosa could secrete acid in the complete absence of urea [65]. Nevertheless, a clinical role for urea in gastric physiology was postulated by O. Fitzgerald (Conway's medical colleague), who postulated that gastric urease functioned as a mucosal protective agent by providing ions to neutralize acid [67]. This led to a number of studies (usually on medical students) in which the ingestion of urea containing solutions was utilized to alter histamine-stimulated gastric acid secretion [64, 68]. Notwithstanding the unpleasant side effects of this administration (diarrhea, headache, polyuria, painful urethritis), Fitzgerald further applied his hypothesis by treating ulcer patients with this regimen in 1949 [69]. Although he charitably summarized his results as 'in general, satisfactory', no further therapeutic studies were undertaken with this particular agent.

Within 5 years, investigators of gastric urease-containing tissue suspensions were also able to demonstrate the presence (contamination) of urea-splitting organisms [65, 66]. This led to the suggestion that gastric urease might actually be of bacterial origin. Preliminary feeding of antibiotics (penicillin and terramycin) to animals resulted both in reduced expiration of $^{14}\text{CO}_2$ from intraperitoneally injected ^{14}C -urea, as well as the abolition of urease activity in mucosal homogenates [65, 66]. Similar studies

with analogous results were also performed in controls and subjects with uremia [70]. These experiments and observations, whilst establishing that gastric urease was of bacterial origin, failed to initiate an investigation of the relationship between urease-containing bacteria and ulcer disease [71]. Indeed the prevailing notion by the end of 1955 was that neither the bacterial gastric urease nor the bacteria played any essential role in gastric physiology [65]. Interestingly, at the time, however, the clinical information derived suggested to some that antibacterial therapy could be beneficially utilized in patients with liver disease and elevated gastric ammonia levels. Antibiotic therapy was noted to reduce gastric urea and ameliorate the associated encephalopathy [72–74].

Paradigms Regained

Interest in bacteria, however, remained but shifted to the effect of bacterial extracts on gastric function. These studies reflected an interest in evaluating the effect of pyrexia on acid secretion [75, 76], and were conducted by intravenous injection of lipopolysaccharides obtained from gram-negative bacteria. These experiments demonstrated that acid secretion could be inhibited in both dogs and rats, but the observations failed to make a significant impact on ulcer research. Rather, the innumerable studies by illustrious proponents of the proton and their corporate partners precluded further serious consideration of anything but acidity as the cause of peptic ulcer disease [77]. Indeed, the literature was deluged with an acid rain of publications on the pathogenesis of ulcer disease in the light of various perturbations of the secretory process of acid and pepsin. Nevertheless, in 1975, Steer [78], while studying polymorphonuclear leukocyte migration in the gastric mucosa in a series of biopsy material obtained from patients with gastric ulceration, identified bacteria in close contact with the epithelium and suggested that white cells migrated in response to the bacteria. In this seminal contribution, he not only clearly demonstrated bacterial phagocytosis, but provided electron-microscopic images consistent with ingestion of a *Helicobacter*. Steer also attempted to isolate and culture the organism, but being unfamiliar with micro-aerophilic techniques, succeeded only in growing and identifying *Pseudomonas aeruginosa*.

By 1980, reports concerning an ‘epidemic gastritis associated with hypochlorhydria’ [79, 80] had been published. These observations coupled with Steer’s findings of an apparent association between ‘active gastritis’ and a

gram-negative bacteria [81] suggested that the simultaneous occurrence of a bacteria in the stomach and peptic ulceration might represent more than a correlatable epiphenomenon. Robin Warren, a pathologist at the Royal Perth Hospital (fig. 7), had for many years observed bacteria in the stomach of people with gastritis. Although he was convinced that they somehow played a role in gastric disease, in the light of the prevailing dogma of acid-induced ulceration and the scepticism of his colleagues, he had been reluctant to discuss this controversial observation in the wider gastroenterological community. In 1982, a young gastroenterology fellow, Barry Marshall, was desperately looking for a project to complete his fellowship. The iconoclastic hypothesis of Warren attracted Marshall, who persuaded Warren to allow him to investigate this further in the appropriate clinical setting. Later in the year, Marshall submitted an abstract detailing their initial investigations to the Australian Gastroenterology Association. It was flatly rejected, along with a handful of other abstracts. Young, and unfazed, and seeking an alternative audience for the work, Marshall submitted the same abstract to the International Workshop of Campylobacter Infections, where it was accepted. Although the audience was sceptical of Marshall and Warren’s results, some members became interested enough to attempt to repeat some of the Antipodean observations. Soon after the meeting, both Warren and Marshall published their initial results as two modest letters in the *Lancet* (fig. 8). In the introduction to his seminal article on an S-shaped campylobacter-like organism in 1982 [82], Warren noted both the constancy of bacterial infection, as well as the consistency of the associated histological changes, which he had identified in 135 gastric biopsy specimens studied over a three year period. He commented that these microorganisms were difficult to see with hematoxylin and eosin, but stained well in the presence of silver. Furthermore, he observed the bacteria to be most numerous in an ‘active chronic gastritis’, where they were closely associated with granulocyte infiltration. It is a mystery, he wrote, that bacteria in numbers sufficient to be seen by light microscopy were almost unknown to clinicians and pathologists alike! He presciently concluded: ‘These organisms should be recognised and their significance investigated.’

Koch’s second postulate states that ‘the germ should be obtained from the diseased animal and grown outside the body’. In the very same issue of the *Lancet*, Marshall [83] described the conditions necessary to fulfill this requirement. Utilizing the knowledge that these bacteria resemble the species of campylobacters rather than spirochetes, he used campylobacter isolation techniques (microaero-

Fig. 7. The pathologist J.R. Warren (right) and his clinical colleague, B.J. Marshall (left). Their Antipodean discovery helped solve a problem which numerous investigators had grappled unsuccessfully with for the best part of a century.

J.R. Warren

THE LANCET, JUNE 4, 1983

**UNIDENTIFIED CURVED BACILLI ON GASTRIC
EPITHELIUM IN ACTIVE CHRONIC GASTRITIS**

**UNIDENTIFIED CURVED BACILLI IN THE
STOMACH OF PATIENTS WITH GASTRITIS
AND PEPTIC ULCERATION***

BARRY J. MARSHALL

J. ROBIN WARREN

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B.J. Marshall

Fig. 8. J.R. Warren observed the presence of a proliferating bacteria on the gastric mucosa from mucosal biopsies and established its close relationship to active chronic gastritis. B.J. Marshall successfully collaborated with Warren, resulting in the culture and classification of this new (old) gastric pathogen.

philic conditions) to successfully grow isolates on moist chocolate agar. It is interesting to note that no organism growth was detected after 2 days' culture in the first 34 endoscopic biopsies Marshall tried to grow. The 35th plate, however, was left to culture over the long (6-day) Easter weekend. It is somewhat ironic, therefore, that serendipity played such an important role, both in the discovery of this organism, as well as in the family of compounds (penicillin) used to treat it.

In order to substantiate that the microorganism was actually a disease-causing agent, it was necessary to demonstrate that it could colonize normal mucosa and induce gastritis (Koch's third and fourth postulates). To prove pathogenicity, Marshall looking back in time for guidance, decided to be his own guinea pig [84]. Marshall who had a histologically normal gastric mucosa and was a light smoker and social drinker, received, per mouth, a test isolate from a 66-year-old nonulcer dyspeptic man. Over the next 14 days a mild illness developed, characteristic of an acute episode of gastritis, and was accompanied by headaches, vomiting, abdominal discomfort, irritability and 'putrid' breath. The infectivity of the agent was then successfully confirmed, when after 10 days, histologically proven gastritis was endoscopically documented. The disease process later resolved on its own accord by the fifteenth day. A fellow Australasian, Morris, later followed Marshall's lead, and in a similar experiment ingested the same inoculum of *H. pylori*. Although this did not establish, a repeat challenge of the mucosa with a different, local (New Zealand) inoculum was more successful [85]. In fact, so successful that a 2-month treatment of an anti-bacterial agent and bismuth was required to 'eradicate' the organism. Morris and Nicholson [85] established a direct effect of infection on acid secretion, but unfortunately for Morris, who had residual gastritis, a relapse was inevitable. Five years after the initial experiment Morris was finally cured. There has been no recorded third experiment.

Conclusion

In spite of the twists and turns of gastric science and ritual dogma, the *Helicobacter* species has now attained the prominence it deserves (fig. 9). Fifteen years of research have revealed the *H. pylori* to be only one member of a whole family of bacteria which infect the gastrointestinal tract of both humans and animals. Investigation of the organisms and the use of animal models have provided clues both to the mechanisms by which *H. pylori*

Oral spirillum 1683

H. felis 1896

H. felis (EM)

H. pylori (EM)

Fig. 9. Evolution of the depiction of bacterial spiral forms from Leeuwenhoek to the present day. Leeuwenhoek's drawings of an oral spirillum in 1683 (G), Salomon's drawings of *H. felis* in domestic animals (1896), an electron micrograph of a negatively stained preparation of the same organism showing the characteristic tufts of polar flagellae and a scanning-electron-microscopic view of *H. pylori*.

survives in the acidic stomach and induces gastric pathology. The possibility that alimentary *Helicobacter* species may play a role in human gastrointestinal pathology remains to be completely elucidated. It is of note that an organic, helical configuration has twice in this century provided biological information of unique import.

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